USE OF SELECTED ORNAMENTAL PLANTS (*TAGETES PATULA* AND *CALENDULA OFFICINALIS*) TO REMOVE LEAD AND CADMIUM FROM SOIL

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Dedication

This study is a tribute to my hardworking, devoted, and loving parents, whose perseverance made my goal of earning this degree a reality. I can't begin to describe how grateful I am to them in words.

"O My Sustainer, bestow on my parents your mercy even as they cherished me in my childhood".

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Abstract

Macro- or micro-elements that are known to harm plants and other living things if they exceed their allowed limits in soil are referred to as potentially toxic elements. Lead (Pb) and Cadmium (Cd) are two hazardous metals whose widespread use in many regions of the world has resulted in significant environmental contamination and health issues due to food chain contamination. This study aims to screen ornamental plant species exposed to Pb and Cd in spiked soils for determination of their phytoremediation potential in order to investigate approaches to repair contaminated soils with minimal impact on the environment and expenses. Two ornamental plant species (*Tagetes patula* and *Calendula officinalis*) were chosen to evaluate their capacity for Pb and Cd accumulation. For that purpose, pot experiments were carried out to assess the accumulative characteristics of the plant species in unspiked control (Pb=0, Cd=0) and spiked soils with various amounts of Pb (500, 1000, 1500, and 2000 mg/kg of soil) and Cd (50, 75, 100 and 125 mg/kg of soil). The maximum growth of *Tagetes patula* and *Calendula officinalis* was observed in the control after 10 weeks of exposure, and it decreased when the Pb concentration in the soil increased from 500 to 2000 mg/kg. The maximum growth of *Tagetes patula* and *Calendula officinalis* in the presence of Cd was likewise noted at the control, and it declined as the quantity of Cd in the soil increased from 50 to 125 mg/kg of Cd. At the soil contaminated with 1000 mg Pb/kg of soil, *Tagetes patula* roots and shoots showed the maximum Pb uptake, measuring 1101.7 and 206.3 mg Pb/kg, respectively. *Calendula officinalis* roots and shoots observed the highest uptake of 1411.2 and 592.4 mg Pb/kg, respectively in soil containing 500 mg Pb/kg. Against Cd exposure, both the plants showed the highest uptake in soil that was contaminated by 100 mg Cd/kg. *Tagetes patula* roots and shoots depicted the highest Cd uptake 1235.5 and 905.7 mg Cd/kg, respectively. *Calendula officinalis* roots and shoots showed the highest Cd uptake of 852.8 and 34.5 mg Cd/kg, respectively. Moreover, in *Tagetes patula* and *Calendula officinalis* bioconcentration factor (BCF) was ≥1 in all samples containing Pb, translocation factor (TF) ≤1 and enrichment factor (EC_f) ≤.1. For Cd exposure, bioconcentration factor (BCF) of both plants was ≥1, translocation factor (TF) ≤1 and enrichment factor (EC_f) ≥1. The results are promising, showing the potential for development of an integrated phytoremediation strategy using the selected plants.

Chapter 1

Introduction

1.1. Background

Many nations flourished with the rise of globalization from the 1980s to modern civilization, yet with this globalization came certain new concerns for the developing nation, such as pollution. The primary factor, industrialization, has caused the large discharge of numerous anthropogenic pollutants entering the environment (Kamran et al., 2013). Hydrocarbons are the primary contaminant, insecticides, and heavy metals (solvents and salts), which have caused specific environmental and health issues to be reproduced. According to previous findings, these pollutants have a higher degree of pollution, are poorly soluble in biota, and are extremely harmful by nature. They can also have a variety of negative effects as mutagenic and cancer-causing chemicals (Kamran et al., 2013; Malik et al., 2009; Muhammad et al., 2011).

1.2. Heavy Metals

One of the primary environmental issues facing the world is heavy metal poisoning of soil, which is considered to be one of the many global difficulties (Qadir et al., 2008). Every country in the globe has been impacted by the problem of heavy metal contamination of soil. Heavy metals have the potential to harm an ecosystem's capacity for structure and function because of their tenacity in the environment and detrimental effects on soil and water ecosystems (Malik et al., 2009). Both natural and human-made processes, such as weathering and erosion of parent rocks, mineral deposits, mining, smelting, intensive farming, energy, electroplating, fuel generation, wastewater irrigation, power transmission, and the disposal of dust and sludge, are sources of heavy metals (Muhammad et al., 2011; Alaribe and Agamuthu 2015). The heavy metals enter our environment through emissions from the combustion of waste that contains them (Chen et al., 2015). Regular irrigation with domestic and commercial wastewater may cause extremely unfavorable and phytotoxic levels of HMs contamination in the soil, according to prior study. Industrial effluents not only decreased the fertility and structure of the soil, but they also accumulated in plants and eventually made their way into people through the food chain, having a detrimental impact on their health (Kamran et al., 2013; Muhammad et al., 2011).

They are regarded as the most problematic category of pollutants due to their persisting toxicity, lack of biodegradability, widespread distribution, and bioaccumulation in the food chain (Li et al., 2015). HMs pose a technological obstacle to reusing the soil due to their long-term persistence and habitation in the soil (Salazar and Pignata 2014).

Those macro or micro elements known to be harmful to plants and other living beings if they exceed their acceptable limits in soil are classified in the form of PTEs or potentially hazardous elements (Antoniadis et al., 2019). Most PTEs, including Cu, Fe, Mn, Mo, Ni, and Zn, are recognized as essential nutrients. Even though they are preferred by plants in tiny concentrations, some PTEs, including as Co, Cr, Se, and V, play significant roles in plant physiology yet are not regarded as essential nutrients. On the other hand, the PTEs As, Cd, Pb, Sb, and Sn are exceedingly dangerous even at very low doses (Palansooriya et al., 2020). PTEs in soil could have developed naturally or as a result of human activity (Shaheen et al., 2020). Heavy metals with densities more than 5g/cm^3 include copper, cadmium, lead, mercury, zinc, and nickel (Chen et al., 2015). Unlike soil pollution, which is dependent on the quantity of contamination, these heavy metals' extreme persistence makes them the most dangerous to the environment (Zhou et al., 2014). Several disorders are correlated with exposure to hazardous metals like Pb and Cd. Additionally, their sources of entry into soil may vary (Norton et al., 2015).

Zn, Cu, and Ni are considered to be important heavy metals in trace amounts since they are essential micronutrients for human health. While even minute level of other HMs, like Cr, As, Pb, and Cd, can cause cancer (Li et al., 2015). Less than 1% of Pb is detected as mobile, which is extremely low. This obstacle prevents Pb phytoremediation (Sarkar et al., 2008). Due to the toxicity brought on by the insolubility and immobilization of Pb, it may also be observed that plant biomass and growth are diminished (Mani et al., 2016; Chen et al., 2015).

1.3. Phytoremediation

If soil contains heavy metals, it is challenging to remove them, and returning soil to its natural state is similarly challenging. In such contaminated soil, plants experience stress as well. The soil is recovered as well as the heavy metals are collected up plants that remediate the soil. In essence, soil provides a place for heavy metals to be stored, traded, and ingested into the food chain. Negative impacts on people and animals may occur when heavy metal-contaminated soil interacts with air, water, and rocks (Obiora et al., 2016).

It is crucial to find a beneficial and environmental responsible method for cleaning up water and soil contamination. This process of using plants is called phytoremediation (Chen et al., 2015). Even on a big scale, it is seen as a suitable replacement to current soil cleansing methods (Bauddh et al., 2015; Chen et al., 2007). With this method, soil contaminants are not only avoided and remedied but also degraded, stabilized, and eliminated. The performance of phytoremediation is enhanced when combined with enhancing agents by the usage of plants (Vigliotta et al., 2016). The phytoextraction process, in which metals are absorbed by roots and then transferred to aerial parts of plants, is enabled by these boosting substances that help increase the mobility of metals in soil solutions (Paulo et al., 2015).

Phytoremediation is acknowledged as a solution for such an environmental problem for this reason. The practice of employing plants to remove or detoxify environmental contaminants is referred to as "phytoremediation" (Cunningham et al., 1993). Table 1.1 lists few phytoremediation approaches that Salt et al. (1998) previously reported and described.

Table 1.1: Phytoremediation techniques

S.NO	Techniques Name	Description	Refences
1	Phytoaccumulation or phytoextraction	Plant roots absorb metal contaminants, which are then retained in stems and leaves (harvestable regions). This technique is typically used with metals including Ni, Zn, Cu, Pb, Cr, and Cd.	(Blaylock et al., 1997)
2	Phytodegradation	Enzymes are responsible for the internal and external breakdown of organic contaminants such as	(Newman et al., 1997)

		trichloroethylene (TCE) and herbicides.	
3	Degradation of the rhizosphere	Rhizosphere microbes degrade organic contaminants.	(Schnoor et al., 1995)
4	Rhizofiltration	Pollutants are absorbed or adsorbed by plant roots. Typically, large-rooted plants are employed. Metals, agricultural runoff, radioactive contamination, and industrial waste are all regularly treated using this method. How organic contaminants travel through soil is influenced by their relative solubility in water, vapor pressure, molecular size, charge, and the presence of other organic materials in the soil.	(Raskin et al., 1997)
5	Phyto stabilization	Most organic chemical pollutants are lyophilic, which means that they are attracted to the hydrophobic surfaces of organic materials including humus, plant cell walls, and soil particles. In this aspect of phytostabilization, plants are used to reduce the bioavailability of environmental pollutants.	(Cunningham et al., 1996)
6	Phytovolatilization	Pollutants that are taken up by the roots move from the soil to the leaves and are then volatilized	(Vroblesky et al., 1999)

		through the stomata, where gas exchange occurs.	
7	Organic pumps	Poplars and cottonwoods are examples of trees with deep roots that absorb a lot of water, reducing the likelihood that surface contaminants will wash into groundwater and end up in drinking water. This is typically done to reduce agricultural runoff and landfill leaching.	(Suresh et al., 2004)

1.4. Advantages of Phytoremediation

In comparison to other procedures, the phytoremediation method has several benefits, including social and aesthetic qualities, cost effectiveness, sustainability, and environmental responsiveness (Chen et al., 2014). When native plants are employed to clean contaminated soil, its value could increase even further. Understanding the state of the plant, its capacity for biomass production, the level of metal toxicity it produces, its development potential, the organ of the plant where the metal will be gathered, and the growth cycle are necessary for a successful attempt to use this technology (Salazar and Pignata 2014).

The hyperaccumulator species allow phytoextraction, in which plants absorb pollutants from the soil in their roots before transferring them to their above-ground portions (Mahar et al., 2016). The availability of a targeted HMs in soil solution with additives, extraction in roots, and subsequent translocation to shoots determine how effectively the metal may be removed from soil (Chen et al., 2006).

The selection of plants for the phytoremediation process depends on two characteristics: the first is high biomass with rapid development, and the second is the ability to accumulate more metal (Patel and Patra 2015).

1.5. Significance of the Study

Numerous studies have demonstrated that due to human activity, the amount of HMs in soil is constantly increasing. Phytoremediation is one of the most efficient and environmentally responsible ways to remove heavy metals from soil. Because they cannot enter the food chain, ornamental plants are particularly environmentally benign.

1.6. Objectives of the Study

Keeping in view the recent work at the host institute and the background information from the literature, the objectives of present study were;

1. Growth response of selected ornamental plants (*Tagetes patula* and *Calendula officinalis*) exposed to soil contaminated with Pb and Cd
2. Post-harvest analyses of plant and soil samples for determination of heavy metals (Pb and Cd) contents
3. To ascertain phytoremediation potential of selected ornamental plants

Chapter 2

Literature Review

This chapter's main goal is to provide detailed information on the use of heavy metal remediation procedures, including information on their potential mechanisms and both positive and negative impacts on soil and the environment that have been previously studied.

2.1. Emerging Environmental Contaminants

Emerging contaminants (ECs) are a class of pollutants that are extremely harmful even at low concentrations. Due to their unique nature, these pollutants have drawn increased attention globally (Cheng et al., 2021). These are mainly unregulated compounds that are primarily produced by human activity and end up in things like water, soil, and food. Emerging chemicals are difficult to breakdown due to their high environmental persistence. They can be retained for a long time. Pharmaceuticals, surfactants, plasticizers, insecticides, and personal care items are only a few examples of significant ECs (Rout et al., 2021).

2.2. Heavy Metals

According to Antoniadis et al. (2019), potentially toxic elements (PTEs) are macro or micro elements that have a history of harming plants and other living things if their allowed limits are exceeded in soil. Most PTEs, such as Cu, Fe, Mn, Mo, Ni, and Zn, are acknowledged as essential nutrients, but others, such as Co, Cr, Se, and V, play significant roles in plant physiology but are not recognized as necessary nutrients even if they are desirable to plants in tiny levels. However, even at very low concentrations, the PTEs As, Cd, Pb, Sb, and Sn are exceedingly dangerous (Palansooriya et al., 2020). PTEs in soil could have developed naturally or as a result of human activity (Shaheen et al., 2020).

One of the major global environmental issues, especially in the light of multiple global challenges, is heavy metal contamination of soil (Qadir et al., 2008). Due to their toxicity, these heavy metals can affect the ecosystem's ability to operate and to remain structurally stable (Malik et al., 2009). According to previous finding, the routine use of home and industrial wastewater for irrigation may result in extremely unfavorable and phytotoxic levels of HMs contamination in soil. Industrial effluents not only damaged the soil's fertility and structure, but they also built up in plants and eventually reached people through food chain, leading to negative health effects (Kamran et al., 2013; Muhammad et al., 2011).

2.3. Heavy Metals' Sources

HMs mostly come from man-made sources as well, such as the disposal of dust and sludge in industrial, mining, and vehicular settings, in addition from some natural sources like erosion of rocks and weathering process, ore deposits, smelting, mining, energy, fuel production, power transmission, and intensive agriculture (Wang et al., 2020; Antoniadis et al., 2019; Bourliva et al., 2018;). The most notable instances of anthropogenic environmental metal pollution are Pb and Cd. Pb concentrations in natural soils range from 10 to 100 mg/kg, whereas industrial zones have reported Pb concentrations of up to 39,250 mg/kg (Arshad et al., 2008; Greipsson et al., 2013).

Industrialization and urbanisation have caused the release of toxic effluents that are unsuited for soil, water, and eventually crop acceptance both globally and in Pakistan (WHO, 2017). A significant issue that endangers the environment and the general public's health is HMs soil pollution. High Pb and Cd concentrations in certain industrial sectors are a result of processes like ore smelting, battery recycling, and fuel burning. High Pb and Cd concentrations were found in soils and grasses near a battery recycling facility in Hyderabad, Pakistan, according to a study by Memon et al. (2014). The study also highlighted the effects on animal health and toxicity. Afzal et al. (2014) reported similar Pb and Cd contamination in Gujranwala and the negative effects it had on the local population.

Aerial emissions from the burning of leaded fuel, battery manufacturing, herbicides, insecticides, mining, smelting, anti-spark linings, and leaded paints are a few examples of anthropogenic sources of Pb in the environment (Mahar et al., 2015; Zaier et al., 2010).

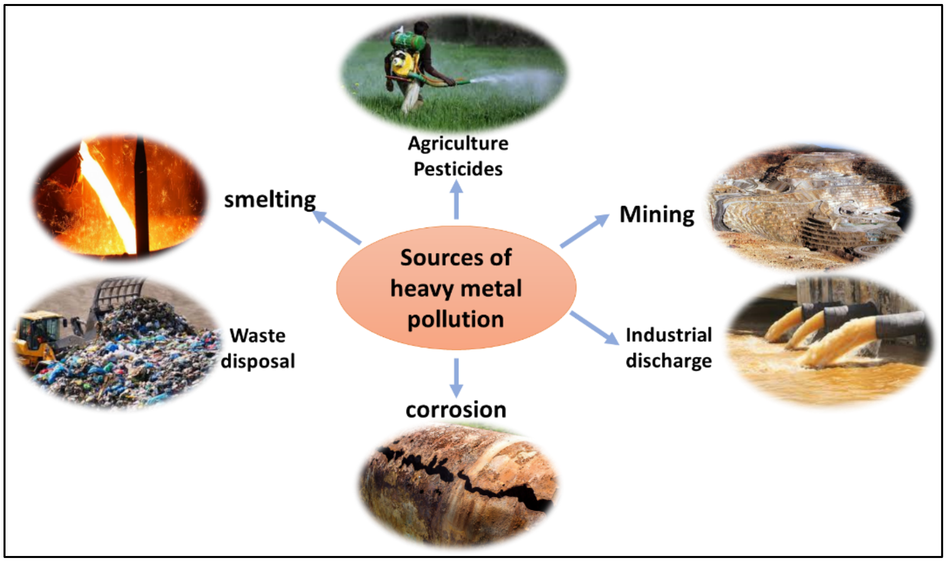

Figure 1: The sources of heavy metal pollution in the environment

2.4. Pb as Heavy Metal

As Pb is mostly associated to organic and inorganic elements or is present as insoluble precipitates in soil, heavy metals like Pb are thought to have a restricted bioavailability in soil (Sallami et al., 2013).

2.5. Cadmium as Heavy Metal

Commercially, Cd is utilized in batteries, paint pigments, cosmetics, lasers, television screens, nuclear fission barriers, galvanizing steel, and weld seals in lead water pipes before the 1960s.

2.6. Exposure Routs

Dermal, inhalation, and oral exposure are some of the numerous ways that Pb can be exposed. When exposed to a metal by any of these routes, different heavy metals have a variety of negative consequences on people (Ali et al., 2013).

2.7. Hazardous of Heavy Metals

Pb and Cd poisoning affects root and shoot length, dry mass, mineral nutrition, and cell division (Bashmakov et al., 2017; WHO, 2017; Hossain et al., 2012). The human liver and brain tissue may be harmed by certain heavy metals by entering the food chain through agricultural goods (Kushwaha et al., 2018). According to reports, Pb and Cd are the metals that are most enduring. According to Sobolev and Begonia (2008), Pb is retained in the soil for 150–5000 years while Cd is retained for 25–30 years (Genchi et al., 2020). Even at low concentrations, it may be poisonous, and levels above 400–500 mg Pb/kg and 50 mg Cd/kg are regarded as hazardous to agricultural and human health (US-EPA, 2001). Exploring remedial strategies for widespread Pb and Cd pollution is urgently necessary in this situation.

2.7.1. Lead Toxicity

Pb poisoning in adults can cause miscarriages, shorter lives, higher blood pressure, neurological damage, and other problems. Children may experience effects on their brain development, decreased RBC, slowed reflexes, and sluggish learning (Ali et al., 2013;). Even at low concentrations of 400–500 mg Pb/kg of soil, Pb is carcinogenic to humans, according to the US EPA (2001).

2.7.2. Cadmium Toxicity

Epidemiological evidence points to a possible link between Cd exposure in the workplace and the environment and a number of malignancies, including those of the kidney, nasopharynx, pancreas, lung, breast, and prostate. Additionally, studies have indicated that Cd exposure from the environment may raise the risk of osteoporosis. The damaging effects of Cd are particularly vulnerable to the liver and kidneys (Genchi et al., 2020).

2.8. Heavy Metals Removals and Remediation Techniques

Heavy metals can survive in the environment because they are not decomposed by biological activities. They generated concerns about health because they remained in the soil for such a long time. Such metals accumulated and entered the food chain as a result of their presence in the environment. To repair this contamination, significant care must be used (Ali et al., 2013). These heavy metals' presence in soil not only leads to food chain buildup but also disturbs soil ecology and water quality. Heavy metals overexposure in soil leads to ecological imbalance. The need to address this problem is necessitated by the negative impacts of heavy metal contaminations of soil (Alaribe and Agamuthu 2015).

Numerous *in situ* and *ex situ* remediation methods, such as surface capping, encapsulation, landfilling, soil flushing, soil washing, electrokinetic extraction, stabilisation, solidification, vitrification, phytoremediation, and bioremediation have been developed to clean up heavy metal-contaminated sites. Through physical, chemical, biological, electrical, and thermal cleanup procedures, these remediation methods use containment, extraction, removal, and immobilisation strategies to lessen the impacts of pollution. Some of these have restrictions due to price, time commitment, logistical issues, and mechanical complexity. These methods highlight certain benefits, drawbacks, and application.

In order to cure HM-contaminated soil, a two-tiered remediation method has also been applied. The first tier sought to increase the stability of metals on soil particles *in situ* (for example, through immobilization), and the second tier sought to recover or separate metals from soil *ex situ* (for example, through washing or flotation) (Peng et al., 2009; Salomon et al., 1995; Iskandar et al. 1997).

2.8.1. *In Situ* Remediation Technology

When a bioremediation procedure is carried out at the original site of pollution, it is referred to as *in situ* bioremediation. Most often, the idea of *in situ* bioremediation is used to deal with soil and groundwater contamination. However, various factors affect the process's efficiency and rate of repair. *In situ* metal immobilisation technologies have improved greatly over the past few decades due to their lower cost and reduced impact on the hydrological systems of the environment compared to traditional *ex situ* extraction procedures. Following are some often used *in situ* remediation technologies: amendments, sand caps, and phytoremediation (Peng et al., 2009; Salomon et al., 1995; Iskandar et al., 1997).

2.8.2. *Ex situ* bioremediation Technology

Ex situ bioremediation is a process that treats pollutants elsewhere than where they were initially discovered. Contaminants are handled inside the controlled environments after being dug up or pumped out of the original site. A variety of hydrocarbons are purified using *ex situ* bioremediation. Local microorganisms are used to treat contaminated soil that has been excavated and spread out on the ground. *Ex situ* bioremediation can be handled and regulated by setting up the necessary conditions. *Ex situ* cleanup methods are typically used for soil that has only little heavy metal contamination. However, the results of their restoration can largely be disregarded for the severely polluted soil. *Ex situ* soil treatment becomes the foremost option in these circumstances (Peng et al., 2009; Catherine et al., 2009). Dredged soil can be remedied using the majority of *ex situ* procedures for soil or mineral ores. However, some methods are more expensive and complex when utilized in soil restoration because of the increased workload and various environmental variables in soil. The following introduces only those promising alternative technologies. Washing, electrochemical cleanup, flotation, ultrasonic extraction, and immobilization are some of the processes used.

2.9. Physical Remediation

Some of the physical remediation techniques incorporate soil washing, extraction of soil, solidification of soil, surface capping, encapsulation, and heavy metal stabilization in soil. It is quite expensive to physically move contaminated soil and dispose of it in landfills. The process of replacing the soil and thermal desorption is also included. Substituting clean soil with polluted soil, either entirely or in part, can reduce the number of pollutants present in a certain area (Zhang et al., 2014). This strategy was divided into three categories by Zhou et al. (2004) soil substitution, soil sweeping, as well as the importing of soil from clean areas. (1) Soil replacement comprises removing polluted soil and adding fresh soil in its place. For contaminated property in specific locations, the prior technique is ideal. Additionally, the replaced soil must be effectively treated to prevent secondary contamination; (2) Deep excavation of contaminated soil accomplishes the desired dilution and natural degradation by causing the pollutant to spread in the deep places; and (3) It is equivalent to importing new land when a large percentage of the polluted soil is replaced with clean soil. In certain ways, soil substitution lessens the environmental impact of pollutants. However, this technology is expensive, involves a lot of labor, and only works in tiny, highly contaminated areas. In order to collect mercury for on-site repairs, the USA formed commercial services and used this technology. However, the use of these devices in soil remediation is constrained by considerations including expensive devices and extended desorption times (Kuang et al., 2018).

Due to its heavy labor requirements, length of time required, and lack of economic viability, this procedure is expensive. For tiny amounts of severely contaminated shallow soil in a small region, it is doable. Additionally, the hazardous waste category frequently includes the removed contaminated soil, necessitating expensive additional management and disposal (Gong et al., 2018).

2.10. Chemical Remediation

Chemical use may reduce the amount of HMs accessible to plants. Changing the pH of the soil is one way to do this, which either causes metals to precipitate out or leads to the formation of insoluble metal complexes. Chemical remediation includes:

2.10.1. Using Vitrify Technologies

By increasing the soil's temperature to a high-temperature range between 140 and 2000 °C, the vitrification process causes organic components to disintegrate or volatilize. In this method, the pyrolysis product is recovered from the exhaust gas of a treatment system while steam is produced. *Ex situ* reclamation can be fueled in two different ways: by burning fossil fuels or by heating a room directly using a microwave, electrode, or plasma. Electrode would be used to provide heat directly to polluted soil during *in situ* treatment. This process is highly efficient at getting rid of heavy metals. Although, this approach is more challenging and takes more energy for fusion, it is costly and only has a few uses (Goswami & Das 2015).

2.10.2. Chemical Leaching-Based Remediation

Using water, chemical reagents, and other liquids or gases that can remove the pollutant from the soil, contaminated soil is washed using the chemical leaching process (Yang et al., 2010). Via precipitation, ion exchange, chelation, and adsorption, the soil's HMs were transferred to the liquid phase, where they were then detected through infiltration. Surfactants, chelating agents, and inorganic fluids make up most of the invasion. Tokunaga and Hakuta (2002) evaluated nitric acid, hydrofluoric acid, sulfuric acid, phosphoric acid, and hydrochloric acid as extractants for eliminating contaminants from the soil. They extracted metals in a variety of quantities from the soil that had been deliberately polluted.

2.10.3. By Use of Chemical Fixing

Fixation of chemical is a process of adding substances or reagents to contaminants soil to produce hardly soluble molecules that halt the spread of heavy metals to plants, water, and other environmental media, promoting regrowth of the soil (Jinadasa et al., 2016; Huang et al., 2016). As a result, fixing of chemical, as opposed to detoxification, which entails converting metal into an inert state, is used to achieve stability. The ability of apatite bone meal (Ca10(PO4).6H2O), which has been finely ground and is somewhat crystalline. The capacity of metal phosphates to immobilise metals and metal bioavailability in contaminated soil was measured (Bilgin and Tulun 2016; Hodson et al., 2000). A very high voltage is used in electrokinetic remediation technology to establish a gradient in the electric field on both sides (Luo et al., 2004). This procedure involved the electromigration, electroosmotic flow, and electrophoresis of charged contaminants to the poles (Cabrera-Guzman et al., 1990). According to Zhang et al. (2004), this technique benefits soil with low permeability since it is inexpensive, simple to install, and operates efficiently while protecting the ecotype and preserving the original soil composition (). However, because this technique couldn't regulate the soil pH, treatment effectiveness was poor (Fasani et al., 2017). The most current techniques include using an ion exchange membrane to adjust the pH of the soil to promote migration or adding a buffer solution to the soil to alter pH.

This technique has a fairly narrow range of applications, demands more fusion energy, and is expensive. However, it does not eliminate soil's heavy metals (Gong et al., 2018). As a result, it is crucial to pay attention to long-term stability. Not a long-term fix for deeply rooted crops because of the HMs into the soil under conditions that encourage weathering, the solution is not long-lasting.

2.11. Biological Remediation

The two main biological remediation methods, phytoremediation (using plant species) and bioremediation utilizing microorganisms (bacteria and fungi), can be used separately or in combination. These include techniques employed by microbes or plants (Chang et al., 2008). The following topics are covered in relation to employing microorganisms and plants for bioremediation:

2.11.1. Bacteria Use for Remediation

Instead of breaking down HMs, microorganisms change their chemical and physical properties. The remediation mechanism may include extracellular complexation, intracellular accumulation, precipitation, or the oxidation-reduction reaction. Microbial leaching is a quick and effective approach to recover metals from low-grade materials, according to study by (Galal et al., 2017). Additionally, microorganisms have the capacity to detoxify industrial waste, sewage sludge, and heavy metal-contaminated sediments and soils (Bosecker 2001). Various bacteria have different bio-sorptive capacities, therefore microbial biomass varies greatly between them. However, the experimental set-up and pretreatment employed have an impact on each microbial cell's potential for biosorption. According to Ashruta et al. (2014), bacterial consortia effectively removed Cr, Zn, Cd, Pb, Cu, and Co at a rate of roughly 75 to 85 percent in less than 2 hours of contact time, provided the necessary conditions for their growth, which might be time-consuming.

2.11.2. Use of Fungi for Remediation

Fungi are frequently utilised as biosorbents for the removal of potentially dangerous metals due to their excellent capacities for metal uptake and recovery (Fu et al., 2012). The majority of investigations revealed that the ability of inorganic compounds to adhere was significantly impacted by both active and dead fungal cells (Tiwari et al., 2013). Investigations were made into *Coprinopsis atramentaria*'s capacity to bioaccumulate 76 percent of Cd^{2+} at 1 mg/L and

94.7 percent of Pb^{2+} at 800 mg/L. The effectiveness of this plant as a heavy metal ion accumulator for myco-remediation has already been established (Lakkireddy and Kues 2017). Candida sphaerica and Aspergillus niger produce biosurfactants with removal efficiency for Zn and Pb of 95%, 90%, and 79%, respectively (Luna et al., 2016). Before separating from the soil, these surfactants might combine with metal ions to form complexes and engage in direct interactions with heavy metals. Several yeast strains, include *Rhodotorula pilimanae*, *Pichia guilliermondii, S. cerevisiae, Hansenula polymorpha,* and *Rhodotorula mucilage.*

Microbial remediation is regarded as a simple, reliable, and safe method. It benefits from minimal energy requirements, cheap operating costs, the absence of any environmental or health risks, and the potential for recovering heavy metals. Because it is a natural procedure, the public views it as a suitable course of treatment (Gong et al., 2018).

However, microbial remediation works best when the environment supports the required microbial activity and growth. It is usually necessary to add more nutrients, oxygen, and other amendments to encourage microbial activity and improve the bioremediation process. In order to speed up the remediation process and increase efficiency, it is typically necessary to combine bioremediation with physical-chemical techniques.

2.12. Phytoremediation

Several techniques collectively known as phytoremediation are used to immobilise, decompose, and decrease the environmental toxins caused by anthropogenic causes when cleaning up damaged ecosystems (Mukhopadhyay and Maiti 2010). It uses a variety of phytoremediation techniques to remove metal from contaminated environments using common plants. Studies have shown that chelating agents, fertilizers, organic additions, and pH adjustments could all help boost the bioavailability of metals and uptake of those HMs by plants. For rehabilitation of contaminated soil, phytoremediation has recently attracted a lot of attention (Huang et al., 2016). Phytoremediation is the process of using plants to clean polluted regions and eliminate contaminants. The core concept of phytoremediation is the transformation of pollutants into less dangerous substances by plant roots or their absorption and storage in plant stems and leaves (Kaur et al. 2018). Therefore, it is intended to serve as an alternative technique to eliminate or, more particularly, reduce the number of dangerous chemicals in the environment (Yadav and Srivastava 2014).

Because of its simplicity and advantages over other remediation techniques for heavy metal-contaminated soil, phytoremediation has gained attention. This method was chosen because of its effectiveness, affordability, and technology for environmental rehabilitation. This method uses the phytoextraction or phytoaccumulation process, in which metals in the soil are first taken up by plant roots and then transferred to the plants above ground portions (Aranisola et al., 2013). Through phytoremediation, not only was the soil returned to its previous state, but many hyperaccumulator species were also found and subsequently exploited for this purpose. Because of this, phytoremediation is a crucial technique for application in ongoing current research (Ali et al., 2013).

The process of phytoremediation involves using plants to detoxify, eliminate, or sequester contaminants. This technology is frequently regarded as an environmentally sound substitute for the currently used environmentally harmful chemical cleanup techniques (Peng et al., 2009;). This technology is commonly used for soil rehabilitation, and it also works well for some restoration of wetlands, shallow lakes, and rivers. This approach currently offers good immobilisation effects for Zn, Fe, Pb, and Cd in soil. Two stages make up phytoremediation the first is carried out by the plants themselves, and the second is carried out by bacteria that colonize the roots and break down the poisonous substances into even less harmful metabolites. Hydrophytes typically use phytochelatins and metal lothioneins to absorb and store various heavy metals (Peng et al., 2009; Suresh et al., 2004).

In their evaluation of the technique of phytoremediation for the reduction of HMs, Kumar et al. (2013) concluded that the employment of plants makes it acceptable to reclaim the environment by lowering the toxicity brought on by heavy metal pollution. By analyzing the potential of 15 different plants through testing to identify the hyperaccumulator specie, the effectiveness of phytoremediation was shown. The findings clearly show that most plants have a significant capacity for heavy metal accumulation. In particular, *Salvia spinosa* was recommended for phytoremediation of contaminated soil since it was thought to be a hyperaccumulator (Kazemeini et al., 2013).

Another study by Cheng et al. (2005) found that maize may be used as a bioenergy source and can also remediate soil, which verified the plants, Pb-contaminated soil's potential for phytoremediation. Different plants sections from *Magnolia grandiflora, Ligustrum vulgare,* and *Phoenix dactylifera* have demonstrated their capacity to accumulate heavy metals when used to measure the quantity of HMs (Demirayak et al., 2011).

To eliminate HMs from soil, the ability of 16 plant species to accumulate hazardous metals was investigated (Pb, Cu, Zn, Co, Ni, and Cr). It was shown that the concentrations of these plants' roots and shoots followed the patterns of Cu, Cr, Zn, Ni, Pb, and Co based on bioconcentration, translocation, and bioaccumulation. However, several were proposed or the Phyto stabilization of some HMs. No plants species were discovered to be a hyperaccumulator (Malik et al., 2009).

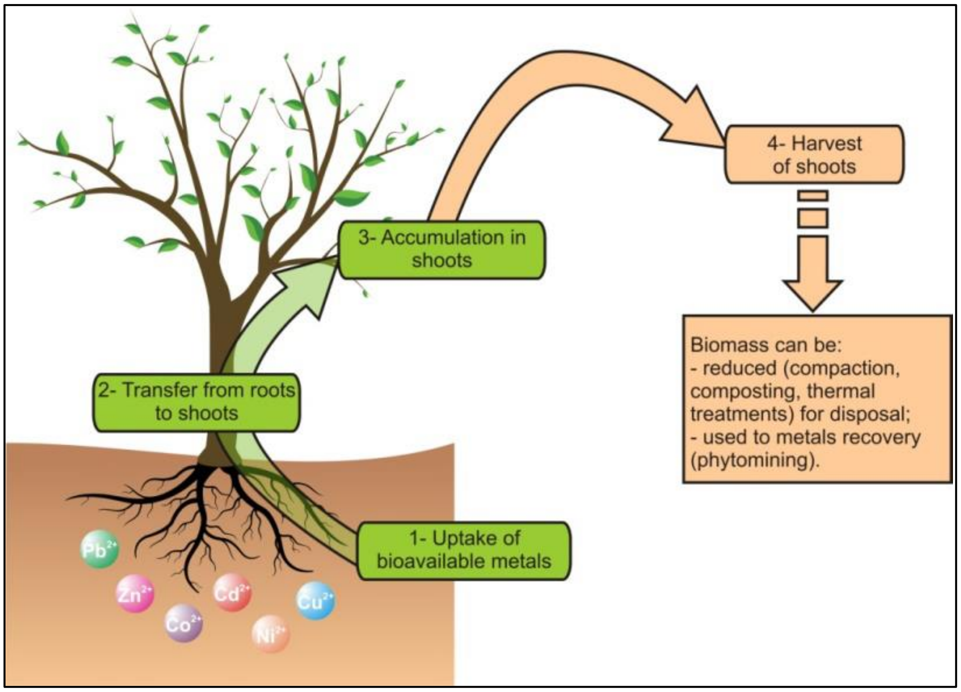

Figure 2: Illustrates the phytoextraction of HMs from soil schematically (Favas et al., 2014)

Hyperaccumulator plant species have shown the ability to build high concentrations of HMs (Memon and Schroder 2009). These plants had a remarkable capacity to absorb metal, which was necessary for phytoextraction, and they could survive high metal concentrations. For phytoextraction, a plant needs to be a hyperaccumulator, have rapid development, and be able to produce more biomass than 20 tons per hectare per year (Yadav and Srivastava 2014). Because plants may absorb HMs (50–500 times) more than typical plants can, it may be possible to genetically modify plants to transfer hyperaccumulator genes from low biomass plants to high biomass plants, such as Brassica species (Baker and Brooks 1989; Cunningham and Ow 1996). Less than 0.2 percent of all angiosperms, or 400 hyperaccumulator plants, are known.

The availability of seeds, the plant's capacity to establish itself and thrive in polluted soil, and its capacity to remove metal from the soil and incorporate it into its root biomass are all factors in the choice of the plant. The idea of HM bioaccumulation in plant from soil and water has been considered in numerous studies. According to studies, using plants as part of phytoremediation technology is an alternative to treating heavy metal-affected areas and can also be utilised as a kind of environmental remediation (Bolan et al., 2014). While certain plants are very tolerating to HMs, others are more vulnerable to them, even different plants react differently to varied heavy metals exposures. Certain plants extract HMs from the soil because of the plant-metal interaction, which hinders their growth and development. However, certain plants can withstand heavy metal stress very well and continue to grow and thrive in such conditions. The majority of metals must exist in biological systems within a certain range (Garbisu and Alkorta 2003), but at high concentrations, they have deleterious consequences by blocking or displacing crucial molecules and functional groups. According to numerous studies, various plants, including those in the *Brassicaseae* family like *Brassica napus,* have varying tolerance levels for hazardous heavy metals and show buildup of various proportions (Bauddh and Singh 2009).

In a different study, *Brassica juncea* (Indian mustard) was shown to have the ability to phytoremediate when exposed throughout a period of 21 days at varied $CdCl_2$ concentrations of 25, 50, 100, 200, and 400 mg/kg. However, the carotenoid content, root and shoot length, tissue biomass, leaf chlorophyll, and leaf size all decreased. The results demonstrated that plants were extremely Cd resistant up to 400 mg/kg. The bud root transfer factor and the enrichment coefficient showed that Indian mustard was capable of removing Cd from the polluted soil. In *Brassica spp.* and other members of the *Brassicaseae* family, the phenomenon of hyperaccumulation was described by Yadav and Srivastava (2014). It has been discovered that sulphate absorbs more effectively, leading to a higher tolerance to Cd ions. In contrast, *Brassica juncea* was the focus of a study by Bhadkariya et al. (2014), who discovered that the plant had a high tolerance to and ability for Cd accretion. Cd was distributed in *Brassica juncea* in the following way: roots > stems > leaves. The entire plant's total Cd accumulation during the course of the 60-day development period was 89.90 mg kg^{-1}. *Brassica juncea* has therefore shown to be a successful Cd accumulator for phytoextraction from Cd-contaminated soil.

The phytoextraction capability of *Cakile maritime* (a halophyte) was assessed by Taamalli et al. (2014) and was compared to that *Brassica juncea*, which were suggested for phytoextraction of Cd. According to the findings, at all external Cd dosages, *Cakile maritime* had a larger translocation factor than *Brassica juncea*. *Brassica juncea* and *Ricinus communis*, two oil producing plants, were also tested for their Cd tolerance and phytoremedial ability in polluted soil (Bauddh and Singh 2012). *Ricinus communis* accumulated nearly twice as much Cd in its shoots and four times as much Cd in its roots as did *Brassica juncea* when the plants in this investigation were exposed to various soil Cd concentrations.

Due to its bigger subsurface and aboveground biomass, *Ricinus communis* appears to have a higher tolerance for metal contamination and a greater potential for phytoreclamation, as evidenced by the greater total elimination of metal from soil.

Sunflower, *Brassica napus*, and wheat were chosen by Moosavi et al. (2012) to examine the Phyto corrective capability of these three crops. The results showed that the fraction of seed germination and the length of roots and shoots decreased with increasing solution concentration. At a Cd level of 1000 mg/kg, no germination was seen. Applying 200 mg/kg of $BiNO_3$ increases the strength of roots and seedlings. Researchers looked into the viability of Brassica napus plant seed oil in polluted environments (Park et al., in 2012). The findings of the study of the seed's oil revealed that the trash still contains over 50% of the heavy metal. Ishikawa et al. (2006) investigated the capacity of the plant species *Brassica juncea* (L.) and a number of other species. The results showed that rice and sugar beet grown hydroponically were more effective at capturing Cd in sprouts than *Brassica juncea* grown in soil. Rice outperformed *Brassica juncea* in phytoextraction of Cd, according to the results of sequential soil extraction of Cd. According to some research, rice is more successful than *Brassica juncea* at removing Cd from contaminated soil at very low metal concentrations. Additionally, five varieties of *Brassica juncea* L. were affected by Cd application in terms of several biochemical and growth parameters (Bauddh and Singh 2009).

John et al. (2009) studied the response of *Brassica juncea* L. to Pb and Cd stress in terms of plant development, pigment content, biochemicals, and heavy metal absorption. The amount of chlorophyll, carotenoids, and growth of the plant all decreased when exposed to Cd and Pb, although Cd had a larger impact than Pb. Protein content dropped to 95% and 44%, respectively, during the flowering phase when Cd and Pb were treated. When Cd and Pb levels are low, proline content rises; when levels are high, it falls. The accumulation of Cd was found to be greater than that of Pb, however Cd absorption was hampered at higher Pb concentrations. Three Caryophyllales species were chosen by Watanabe et al. (2009) and cultivated with Cd treatment.

In comparison to *Brassica juncea*, *Amaranthus tricolour* demonstrated a higher Cd storage capacity in both soil and water culture. The findings suggested that *Amaranthus tricolour* has a stronger capacity for Cd adsorption in the rhizosphere than it does for high biomass and growth. The Cd-contaminated fields could therefore benefit from phytoextraction using *Amaranthus tricolour*. Indian mustard was tested for its capacity to absorb nickel and Cd in a controlled experiment (Tickoo et al., 2007). To do this, soils were artificially polluted with varying quantities of nickel sulphate and Cd acetate. The findings show that heavy metal accumulation is greater in shoots than in roots. *Brassica juncea* was also discovered to absorb Cd more efficiently than nickel. *Raphanus sativa* and *Brassica napus* were both grown in soil that contained various metals, were examined for their capacity to extract phytotoxins (Marchiol et al., 2004). For multi-metal soils, the phytoremedial capability of radish is minimal. It was shown that a few of Brassica species have a modest buildup of zinc and Cd. These plants were raised in pots with contaminated soil to assess the ability of selected *B. juncea*, *B. napus* L., and *B. rapa* to extract phytochemicals with *Thlaspi caerulescens*.

2.12.1. Phytoremediation Types

The process of employing plants to concentrate environmental contaminants in above-ground plant tissue is known as phytoextraction. The suitable plants for phytoextraction should be easy to harvest, able to grow outside of their collection location, create a lot of biomass, be able to grow quickly, and accumulate range of HMs in their harvestable sections (Jabeen et al., 2009;). However, it is possible to use hyperaccumulators in phytoextraction to remove metals from the soil and concentrate them in the organs above ground (Eid and Shaltout 2014).

2.12.1.1. Phytostabilization

Through a variety of mechanisms, such as root adsorption, precipitation, or complexation in the root zone, plants can be used to reduce a metal's mobility or/and bioavailability in order to prevent it from getting into the food chain or seeping into the ground water these techniques are referred to as "phytostabilization" or "phytoimmobilization" (Sarwar et al., 2016).

2.12.1.2. Rhizofilteration

Rhizofiltration is the type of phytoremediation, which is the process of remediating contaminated water by absorbing, concentrating, and precipitating pollutants using plant roots grown hydroponically (Raskin et al., 1997).

2.12.2. Application of Phytoremediation

Metal-contaminated soils can be cleaned up using a there are many different technologies, such as biological, physical, and chemical ones (Akesson and Jarup 2009). Due to lack of secondary pollution, cheap, and inability to change soil aggregation, compared to other methods, phytoremediation is the most effective method for removing or stabilising soil contaminants (Zhang et al., (2013). Modern phytoremediation technology is further divided into three types based on their uptake mechanisms: phytostabilization, rhizofiltration, rhizodegradation, and photoevaporation (Sarwar et al., 2017).

According to Sarwar et al. (2017) phytoremediation is one of the finest options for cleaning up contaminated regions of the order of hectares because it has no impact on the environment and employs the cheapest and most plentiful source of energy (solar). The bioavailability of pollutants, plant characteristics, imposed agronomic techniques, and assistance from soil additives all affect how effective phytomining and phytoextraction is (Wang et al., 2020).

In order to be compliant with this procedure, a plant must have a high capacity to take up PTEs that are enriched in the proper soil, as well as a high accumulation factor, brief lifecycle, high rate of reproduction, wide geographic dispersion, and huge aerial biomass (Visoottiviseth et al., 2002). It is typically advisable to stick with locally well-adapted plants, even if many plant species have been tested as potential choices for contaminated site phytoremediation. Native species have evolved to withstand the climatic circumstances in the study area as well as the chemical stress caused by exposure to PTEs. Numerous researchers have examined at how well native plants can absorb PTEs that have been deposited over time in highly contaminated environments (Antoniadis et al., 2021).

2.12.3. Application of Use of Ornamental Plants (OPs) as Phytoremediation

Since pollutants from the environment can accumulate and be transferred throughout plants, both in edible and inedible sections, the use of plants for remediation has drawn a lot of attention. Additionally, some studies mentioned employing trees, OPs, and grasses. It is not advisable to encourage the use of crop plants for phytoremediation. The likelihood that HMs will biomagnify in the food chain is the reason behind this, because the issue endangers both human and animal health, using aesthetic plant species is a more workable approach. Due to the large diversity and availability of several available OPs, screening the potential local OPs for the phytoremediation of HMs-contaminated areas will be advantageous. A definite advantage of adopting OPs is the simultaneous enhancement of aesthetics and remediation of the contaminated site, which may even be more acceptable for metropolitan areas and green infrastructures. In accordance with the contaminated environmental matrix, the OPs are separated into aquatic and terrestrial plants (soil, water, or air).

Following HMs phytoremediation, the OPs produced from the harvested plants and flowers can be used for practical reasons. The generated biomass can be used to make valued goods like cut flowers and potted plants. Additionally, flower cuttings can be sold in flower shops, and its aromatic wood and essential oils can be used to make perfume and air fresheners. The exposure to HMs from OPs grown on matrices contaminated with HMs has not before been reported. Based on the lack of HMs in the essential oils of medicinal and aromatic plants, it may be hypothesised that fragrances manufactured from portions of OPs using distillation methods are HM-free and can be promoted safely without compromising human health. It is nonetheless important to prove the presence of HMs in essential oils made from OPs despite the lack of any precise scientific evidence. This will only be possible after extensive testing of the OPs-HMs system. In the best-case scenario, OPs can sell cut flowers that have been exposed to and grown on HMs-containing matrices in order to gain considerable cash. There are four phases of applying OPs in the field, with important checkpoints at each stage.

Chapter 3

Materials and Methods

3.1. Source of Soil

Soil sampling was carried out in the area of National University of Sciences and Technology (NUST), Islamabad, Pakistan, to determine the levels of Pb and Cd. Three soil samples were randomly chosen from different sections of the National University of Science and Technology (NUST) and blended to generate a typical composite sample in order to measure the concentration of Pb and Cd.

3.2. Soil Characterization

The physiochemical properties of the soil that were identified included soil moisture, organic matter, pH, electrical conductivity, water holding capacity, and soil texture.

3.2.1. Soil Moisture

In order to determine the soil's moisture, a soil sample must be weighted both before and after being dried, and the difference between the two weights must be recorded. Soil moisture is the difference in soil weight (Jhonson and Wichern 1992). Wet weight of the soil sample is recorded after it is collected in a moisture container. The wet soil sample in moisture container is placed in a hot air oven at 105°C, once it is dried and a constant weight is reached, it is than recorded as the sample's dry weight.

$$Moisture\ content = \frac{wet\ weight - dry\ weight}{dry\ weight} \times 100 \qquad (3.1)$$

3.2.2. Soil Organic Matter

The amount of organic matter was measured using the dry combustion technique. Soil sample is heated at 350^{o}C for three hours, the process known as a "loss of ignition" at that organic matter is burned off, a most popular way to determine organic matter present or not in soil sample (Cheng et al. 2015). The steps of the determining organic matter content in soil samples are 20g soil was taken in China dish, was weighted in weigh balance and then placed in furnace at 550°C for 2 and half hour. At such a high temperature all the organic matter of the soil is burned, and the sample size is reduced to almost half, once again it is weighted on a weigh balance. Soil organic content is than calculated using following equation (3.2).

$$moisture\ content\ (\%) = \frac{dry\ weight - after\ burned\ weight}{after\ burned\ weight} \times 100 \qquad (3.2)$$

3.2.3. Soil pH

At the wastewater lab IESE, NUST, a pH meter was used to calculate the pH of the soil. Using the pH meter to measured soil pH, so first, collected soil samples for pH analysis. Field moist soil is weighted to 10g and placed in three cups for repeated analysis. After being weighed, cups are sealed to prevent moisture evaporation. Each cup is filled with 20 ml of deionized water using a pipet or graduated cylinder, then it is sealed and shaken for a short period of time. The cap is taken off to provide the solution at least 30 minutes to acclimate to the atmosphere. The pH meter is calibrated between pH 7 and pH 4 (Tang et al., 2014).

Figure 3: Measurement of soil pH

3.2.4. Electrical Conductivity

Preparing samples for the electrical conductivity meter is the first step in measuring the electrical conductivity of soil. Dissolve 25g of soil (around 2 mm) in 100ml of deionized water, the soil is then left for 30 minutes to determine the conductivity of the soil. An electrode was then suspended in the solution, and EC is recorded as soon as the readings become stable (Meers et al., 2005). With a direct correlation between conductivity and the concentration of ionized substances in water, which is a measure of the water's ability to carry electric current, it can be used to determine the total amount of soluble salts. EC is useful because it can be easily and precisely determined (Nathan et al., 2004).

3.2.5. Soil Water Holding Capacity

25g of soil was weighted using a weighing balance. The weighted soil sample is than placed on a filter paper set into a funnel. Precisely, 100 ml of distilled water is passed from the soil sample placed on filter paper in funnel, the filtrate of the soil is collected, and volume of filtrate is recorded (Scotter et al., 2016).

Water holding capacity =100 mL distal water – Filtrate in the cylinder (mL)   (3.3)

3.2.6. Soil Texture

Hydrometer was used to determine soil texture (Groenendyk et al., 2015). Particle size and settling velocities of silt and clay in water column are the two factors on which Hydrometer is dependent. Once the percentages of sand and silt are calculated by measuring the particle size and velocities of sand and silt in water, USDA textural triangle was used to assess the class (Barman & Choudhury, 2020). 100 g of soil was mixed in distilled water, until a clear solution is formed. The ratio of amount of water used in making the mixture to the weight of the dried soil sample determines the texture of soil. Percentage of the value is matched with the textural triangle (Jensen et al., 2014).

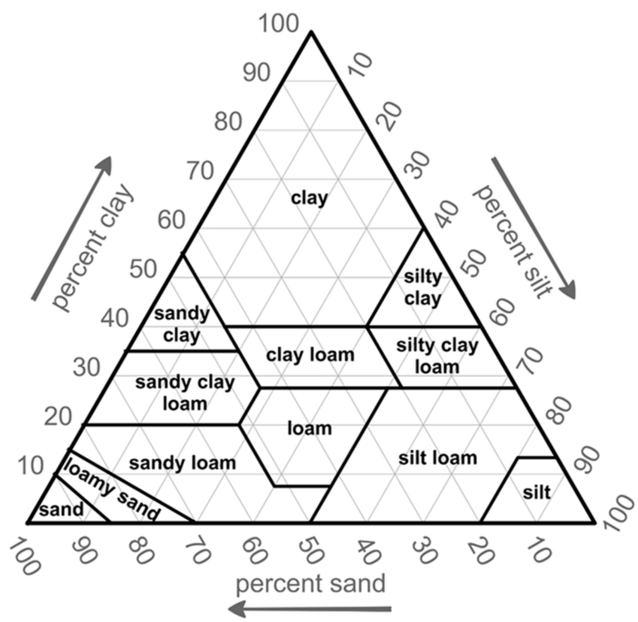

Figure 4: USDA soil texture triangle

3.3. Collection and Preparation of Soil

Uncontaminated topsoil (0-20 cm) is collected near the National University of Sciences and Technology in Islamabad, Pakistan. The soil was air dried for one week, crushed, and homogenised using a 2-mm sieve shaker (Tauqeer et al., 2016). During soil preparation process soil's physicochemical properties were determined.

Figure 5: Collection of undisturbed soil

Figure 6: Soil pass through 2-mm sieve

3.4. Artificial Contamination of Soil

For scientific testing, $Pb(NO_3)_2$ was intentionally added to uncontaminated soil. Pb concentrations in field soil samples and published data were used to determine doses. There were two types of soil prepared: control soil with no Pb (Pb=0) and soil contaminated with Pb concentrations of 500, 1000, 1500, and 2000 mg Pb/kg. In order to achieve the required Pb concentration levels, Pb was added to the soil as $Pb(NO_3)_2$, mixed, and then left for two weeks. To achieve even distribution and Pb stability. The soil was regularly stirred, and the moisture content was kept at about 60%. The analytical Pb values in each spiking level were calculated after two weeks (McLaughlin 2001).

Similarly, to how Pb soil is artificially contaminated, another group of soil was artificially added with Cd. Cadmium is added as $CdSO_4.8H_2O$. The dosages were established using the Cd concentrations in field soil samples and academic literature. There were two types of soil prepared: soil of no Cd (Cd=0 or control) and soil contaminated with Cd concentrations of 50, 75, 100, and 125 mg Cd/kg. Cd was introduced to the soil in solution form as $CdSO_4.8H_2O$, mixed, and then left to settle for two weeks in order to obtain the proper Cd concentration levels. Soil was periodically stirred in order to achieve uniform distribution and Cd stability, and soil moisture was kept at about 60%. Two weeks later, the analytical Cd levels in each spiking level were determined. After thoroughly mixing the spiked soil, it was placed in sampling bags for 7 days and labelled according to treatment (Manzoor et al., 2018).

3.5. Phytoremediation

3.5.1. Selection of Ornamental Plants

For this study, two ornamental plants were chosen: *Tagetes patula* (commonly known as Marigold) and *Calendula officinalis* (Common name Jafri).

3.5.2. Plant Growth Experiment

In culture experiments, two ornamental plant species (*Tagetes patula* and *Calendula officinalis*) were exposed to varying concentration of Pb and Cd. The growth of ornamental plants was observed in pots made from artificially contaminated soil with a control group and various Pb and Cd concentrations. For up to 10 weeks, both plants were cultivated in soil that was contaminated with Pb and Cd at varying concentrations. The freshly born plants came from a local private nursery in Islamabad, Pakistan. The newly born plants were grown in fertile soil. When choosing plants, consideration was given to (a) the literature on Pb and Cd accumulating plants and (b) appealing native and widely cultivated species.

3.5.3. Pot Experiment

Four different levels of Pb and Cd were added to the soil in containers before plants with four to five leaves were transplanted. Experimental pots with soil devoid of Pb and Cd were also put up for each plant species as a control. There were three replicates of each treatment. In a 25°C growing chamber with a 16-hour day/night cycle, the plants were grown. The pots were watered each day with distilled water, taking care to prevent heavy metal leakage, in order to keep a moisture level of 65%-70% of their water-holding capacity.

The newly germinated plants were cultivated in pots and were exposed to heavy metals throughout their lives. Plants were grown in various pots using synthetically contaminated soil containing known concentrations if HMs such as Cd and Pb. The metal concentration was administered during the active growth period of the ornamental plants under study.

(a)

Figure 7: (a) Tagetes patula grown at different Pb & Cd concentration contaminated soil

(b)

Figure 8: (b) Calendula officinalis grown at different Pb & Cd concentration contaminated soil

3.5.4. Watering Frequency

In a 25°C growing chamber with a 16-hour day/night cycle, the plants were grown, in order to keep the pots' moisture level at 65-70% of their water-holding capability, and to prevent HMs leaks, the pots were irrigated daily with distilled water.

3.5.5. Plant Harvesting

For 10 weeks, the plants had been exposed to Pb and Cd to determine their capability for phytoextraction (Thamayanthi et al. 2013). After carefully removing plants from their pots, rinsing them with distilled water, rinsed in tap water until the soil was eliminated by soaking for five minutes in 50% nitric acid to dissolve any Pb and Cd that had adhered to the surface of the root, followed by a second rinse in distilled water and a filter paper blot.

3.5.6. Plant Growth Effects

For the purpose of observing the stress on the plant's length, the lengths of the roots and shoots were also measured and recorded. After 48 hours of desiccation in a 60°C oven, the dry weight of various plant components was assessed using a weighing scale to determine plant stress, including the plant's fresh weight in its roots and shoots. In a pestle and mortar, dried samples were pulverised before being packaged in polythene.

3.5.7. Soil pH After Harvesting Plants

The pH of all sixty pots was determined using a pH meter after plant harvesting at the wastewater lab, IESE, NUST. Field moist soil was weighted to 10g in extraction cups for each pot. To prevent moisture loss, cups are capped after weighing. Each cup was filled with 20 ml of deionized water, capped, and shaken for a few seconds using a pipet or graduated cylinder. The cap was removed to allow at least 30 minutes for the solution to equilibrate with the atmosphere. The pH meter was calibrated at pH 7 and pH 4.

3.6. Sample Preparation for Heavy Metal Analysis

3.6.1. Plant Sample Preparation

A wet digestion procedure is used to examine the Pb content in the plant's root and shoots after harvesting and oven-drying it. Crushed plant matter (roots and shoots) weighed 0.5 g in total was used for digestion. In order to execute acid digestion, 0.5 g of plant material was placed in a 25 mL volumetric flask along with 10 mL of concentrated HNO_3 and 4 mL of concentrated HCl. In a fume hood, the flask was heated by being set on a hot plate. Over time, the temperature rose progressively from 50°C to 150°C (Saifullah et al., 2010). Until the sample's color turned translucent and all traces of plant matter had been fully digested, the heating procedure was repeated on the plate. Once the solution had become colorless, the sample was taken from the hot plate and filtered through Whatman No. 42 filter paper. For Pb and Cd analysis, the filtered solution was diluted to a volumetric flask volume of 50 mL with distil water and kept at 4°C.

3.6.2. Soil Sample Preparation

To do this, a diacid of HNO_3 and $HCLO_4$ was used to digest 0.5 g of each soil sample that had been spiked (4:1). A clear aliquot was obtained after the digestion had been going on for two to three hours at 150°C on a hot plate. With the use of distilled water, these aliquots were made to have a final capacity of 50 mL. Prior to metal analysis, all solutions were then filtered via Whatman# 42. The amounts of Pb and Cd were calculated in mg per kg.

3.7. Heavy Metal Analysis

Atomic absorption spectrophotometer was used for heavy metal analysis. This device works on the principle that, depending on the wavelength of electromagnetic radiation, a substance can either absorb or transmit the radiation when it is exposed to it.

In the wastewater lab of IESE, NUST, Islamabad, samples were examined using an atomic absorption spectrometer (AAS) (novAA 800D, Analytik Jena, Germany). An air-acetylene flame was used to measure the metal. The *Tagetes patula* and *Calendula officinalis* and soil samples were analysed for two HMs namely Pb and Cd.

Figure 9: Atomic Absorption Spectrophotometer (AAS) of wastewater laboratory IESE, NUST

3.8. Atomic Absorption Spectrophotometer

Using atomic absorption spectrophotometry (novAA 800D, Analytik Jena, Germany) the quantities of specific heavy metal (Pb and Cd) extracts from plant (*Tagetes patula and Calendula officinalis*) and soil samples were examined. For sample preparation and analysis, analytical-grade chemicals with a high spectroscopic purity of 99.9% (Merck Darmstadt, Germany) were employed; the analytical conditions of the instrument are listed in Table 3.2. The appropriate 1000 mg/L certified standard solutions for both elements were diluted to create standard solutions (FlukaKamica, Busch, Switzerland). The National Research Centre for Certified Reference Materials, China, provided the blank reagents and standard reference plant (GBW-07602 (GSV1)) materials, which were utilised to test the accuracy and precision of the digesting procedure. For data quality assurance, each sample batch was analysed in triplicate under ideal, controlled conditions and with a 95% confidence level. In the wastewater laboratory at IESE, NUST, samples were gathered, and the specified metals were identified.

Table 3.1. Conditions for using atomic absorption spectrometry for certain heavy metal analyses

Metal	Acetylene L/min	Air L/min	Wavelength Nm	Silt width Nm	Lamp current mA	Detection limit mg/L
Pb	2.0	17.0	217	1.2	4.0	0.013
Cd	2.0	17.0	228.8	1.2	2.0	0.0012

3.9. Bioaccumulation factor

Three variables—Enrichment Coefficient Factor (EC_f), Bio Translocation Factor (TF), and Bioconcentration Factor (BCF) can be used to describe the accumulation properties of plants. The amount of HMs (Pb, Cd) in shoots divided by the amount of heavy metals (Pb, Cd) in the soil is used to compute EC_f. TF is a measure of a plant's capacity to move metal from its roots to its aerial parts and is determined by comparing the metal content of the shoots to the soil (Aransiola et al., 2013: Zu et al., 2005). BCF is the term for a plant's capacity to sequester heavy metals (Pb, Cd) from the surrounding soil environment. Formulas were used to evaluate these parameters.

$$TF = \frac{[Metal]shoot}{[Metal]root}$$

TF = [Metal]shoot / [Metal]root. (2005) (Zu et al.) (3.4)

$$EF = \frac{[Metal]shoot}{[Metal]soil}$$

EC_f = [Metal]shoot / [Metal]Soil (Zu et al., 2005) (3.5)

$$CF = \frac{[Metal]root}{[Metal]soil}$$

BCF = [Metal]root/[Metal]soil (Yoon et al., 2006) (3.6)

3.10. Statistical Analysis

All treatments were replicated three times, and data were analysed using one-way Analysis of Variance (ANOVA) in the Statistical Package for the Social Sciences (SPSS) 16.0 programme with a significance threshold of 0.05. Excel was used to calculate the standard errors for each mean number that represents a result. Comparative graphs were made using Excel.

Chapter 4

Results and Discussion

4.1. Preliminary Study

Results of the preliminary study are shown in table 4.1. Results of all the physical parameters show that the condition of the soil is favorable for plant growth. After plant harvesting, the pH of all pots of soil was in between 7.3-7.6 that are suitable for plant growth.

Table 4.1: show he physicochemical properties of soil before plant growing

Physicochemical property of soil	**Values**	**Methods**
Moisture	3.36%	(Jhonson and Wichern 1992)
Organic matter	1.89%	Dry combustion
pH	7.6	pH meter
Electrical conductivity	224 µs/cm	EC meter
Water holding capacity	10.3	(Scotter et al., 2016)
Texture	Sandy loam	Paste method
Heavy Metals (Pb, Cd)	Not detected	AAS

The elemental composition was basically determined to find out the already present Pb in soil. Cd and Pb metal concentration data were obtained using AAS (novAA 800D, Analytik Jena, Germany). The findings demonstrated that the chosen soil has no Pb or Cd at all.

4.2. Stress on Plants Due to Heavy Metals

4.2.1. Effects of Pb on Total Average Plant Biomass

Total biomass of *Tagetes patula* and *Calendula officinalis* decreased as the amount of Pb in the soil increased (4.1). Total biomass illustrated a maximum value of 8.6g in control and a lowest value of 4.5g in *Tagetes patula* growing in soil that had 2000 mg/kg Pb treated to it. *Calendula officinalis* illustrated a maximum value 11.8g of total biomass at control and lowest value 4.6g total biomass at 2000 mg/kg of Pb in soil. The current study found that plant dry biomass reduced in Cd and Pb spiked soil by 23% and 19%, respectively, without the application of amendments respectively, compared to the reference (control). The continuous decrease in plant total biomass could be attributed to heavy metal stress as it can partially affect the microorganism's growth, as result affecting nutrient cycling and thus plant growth oxidative stress and increased membrane permeability. Manzoor et al. (2018) and Monok et al. (2018) also reported a continuous decline in plant biomass with increasing Pb concentration. *Tagetes Patula* can tolerate a low-level Pb concentration of below 500 mg/kg Pb in soil, and a continuous decline was observed at higher concentration (Monol et al., 2018). Biswal et al. (2022) reported similar results showing that heavy metal contamination significantly affected biomass yield of marigold specie, untreated soil recorded more biomass as compared to treated soil.

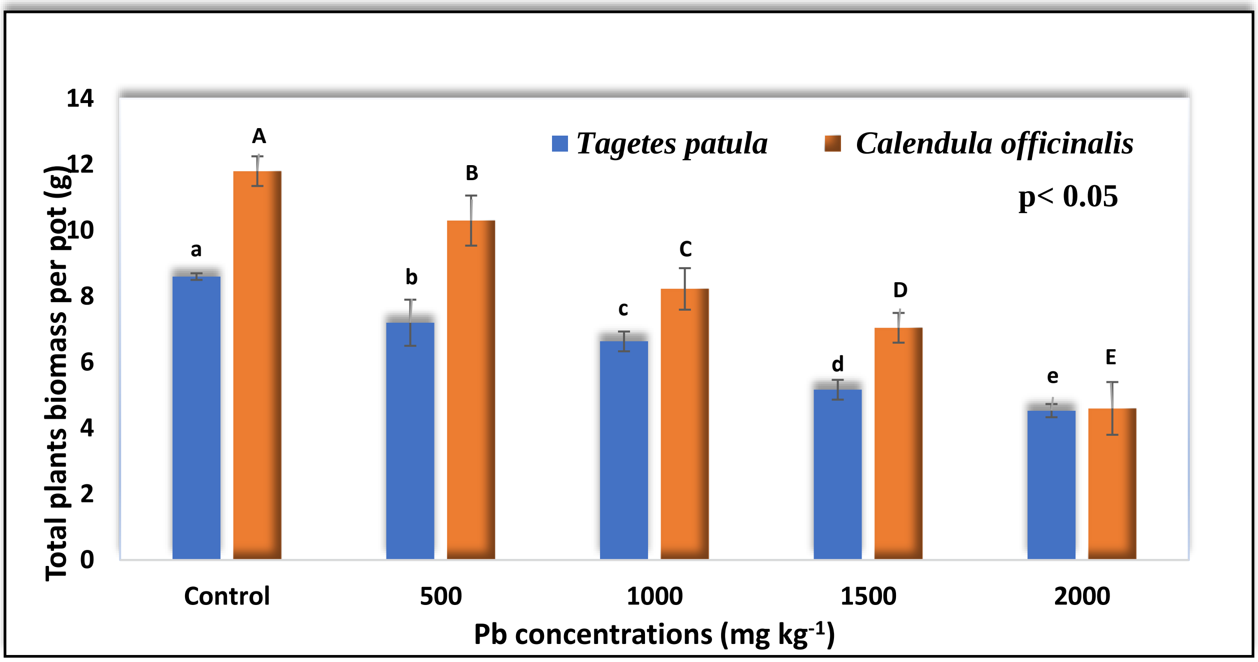

Figure 10: Total average plants biomass at different Pb concentrations

4.2.2. Effects of Pb on Plant Root Weight

The weight of *Tagetes patula* and *Calendula officinalis* roots decreased as the amount of Pb in the soil increased, from the control group to 2000 mg/kg. Maximum root weight, 2.1g was observed at the no concentration (control), while the minimum root weight was observed in *Tagetes patula* grown at the highest Pb contaminated soil of 2000 mg Pb/kg. In case of

Calendula officinalis, a maximum of 2.5g root weight was observed in control, while lowest weight of 0.8g was observed at highest Pb contamination (2000 mg Pb/kg). The trend of declining root weights in both plants is shown in figure 4.2.

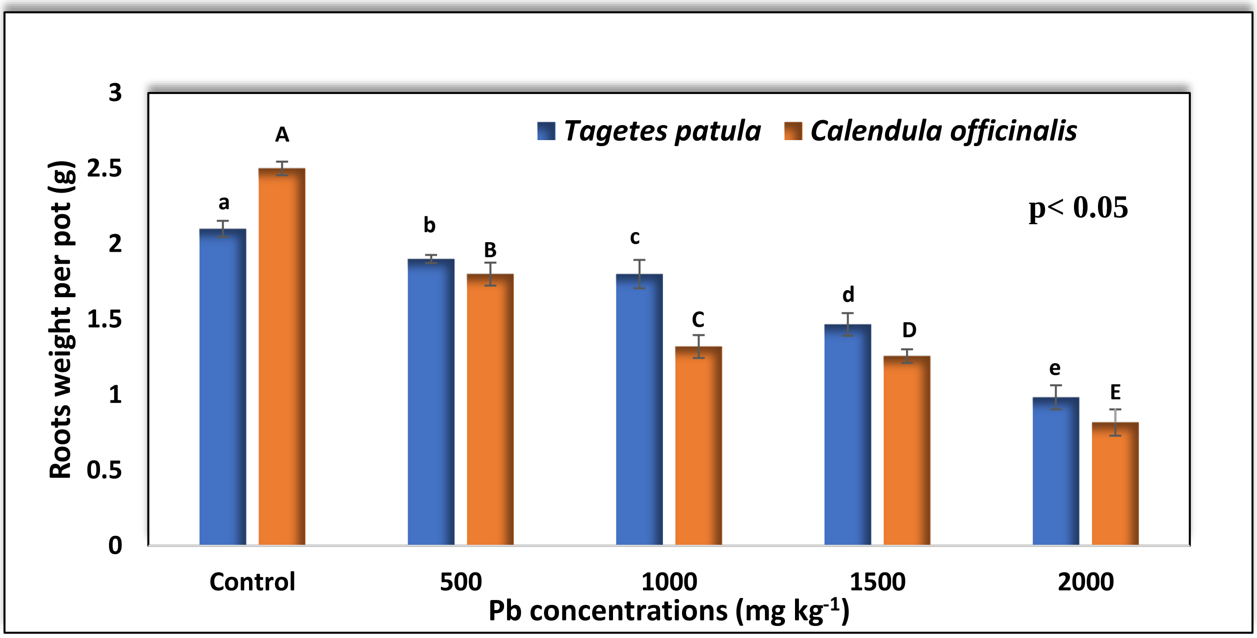

Figure 11: Total average plant root weight at different Pb concentrations

4.2.3. Effects of Pb on Plants' Shoot Weight

Shoot weight of *Tagetes patula* and *Calendula officinalis* showed decrease with an increase in the amount of Pb in soil, from control to 2000 mg/kg. Shoot weight illustrated a maximum value of 5.7g in control and a lowest value of 2.6g in *Tagetes patula* cultivated on soil that has been contaminated with 2000 mg/kg Pb. The *Calendula officinalis* illustrated a maximum value 9.2g of shoot weight at control and lowest value 4.1g shoot at 2000 mg/kg of Pb in soil. The shoot dry weights of *Tagetes patula* considerably decreased under Pb stress, according to Lu et al. (2017), which was consistent. Aghelan et al. (2021) find that in comparison to stems growing in low levels of Pb, the average fresh weight of samples of *Tagetes patula* stems decreased in soil samples with higher levels of Pb contamination.

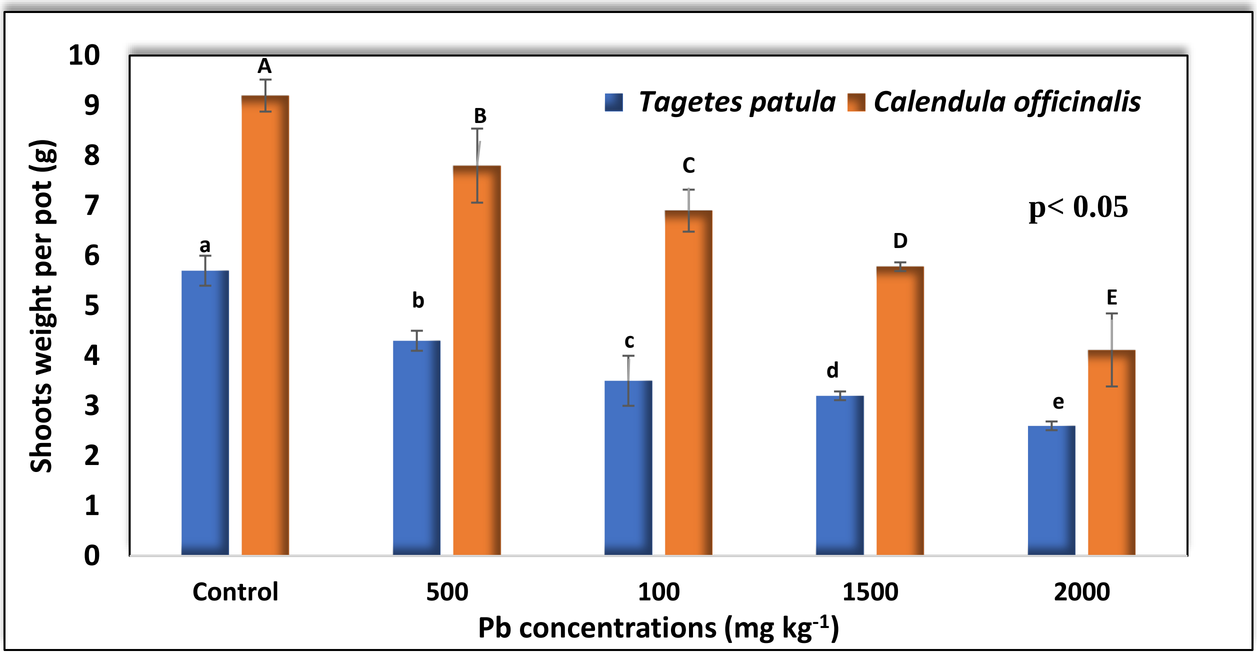

Figure 12: Total average plant shoot weight at different Pb concentrations

4.2.4. Effects of Pb on Total Average Length of Plants

Like all the results of Pb on plant biomass, root and shoot weight, length was also observed a decreasing trend from control to the highest concentration of Pb contaminated soil (2000 mg Pb/kg). Total average length of *Tagetes patula* illustrated a maximum value of 30.4 cm in control and the lowest value of 16.9 cm grown in soil spiked with 2000 mg/kg Pb of soil. The *Calendula officinalis* illustrated a maximum value 31.8 cm of total average length at control and lowest value 14.6 cm at 2000 mg/kg of Pb in soil. Fig. 4.4 shows the total average length of plant at different concentration of Pb contaminated soil. Aghelan et al. (2021) reported similar results with a decreasing trend in plant height from 400 mg Pb/kg.

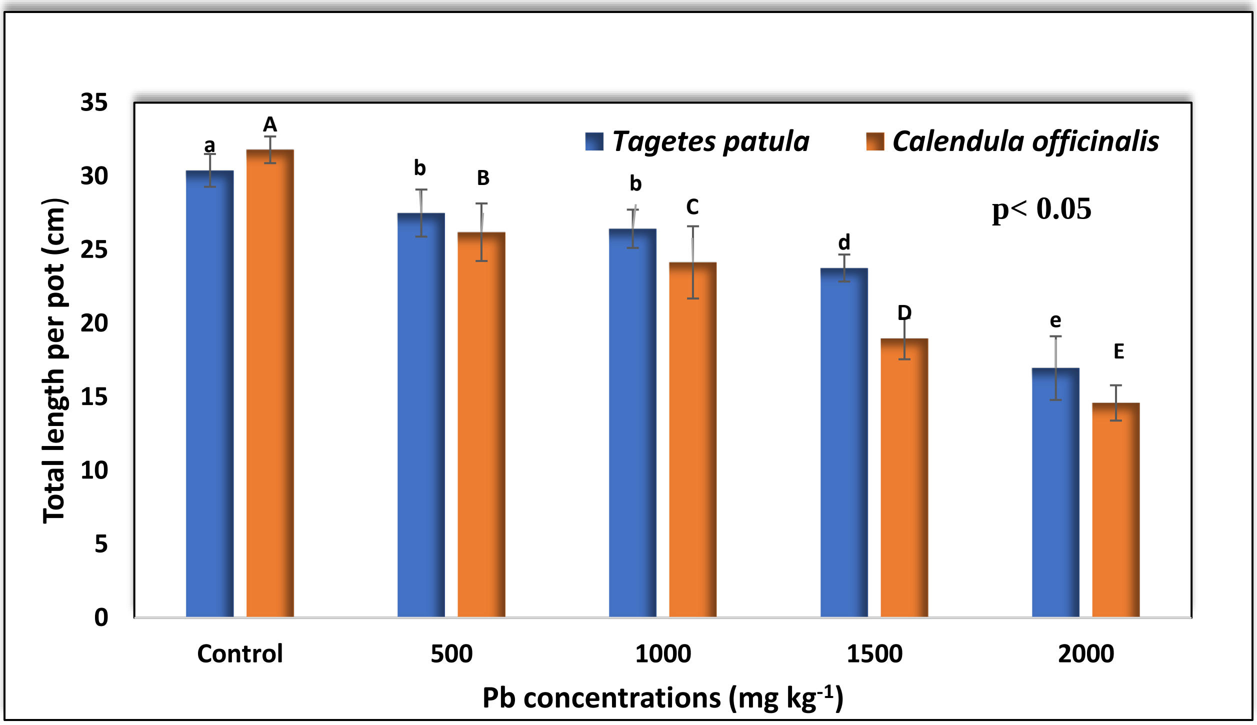

Figure 13: Total average plant length at different Pb concentrations

4.2.5. Effects of Pb on Plants Root Average Length

Root lengths of *Tagetes patula* and *Calendula officinalis* showed decrease with the increase the soil Pb concentrations, from control to 2000 mg/kg. Root length illustrated a maximum value of 7.6 cm in control and a lowest value of 4.6 cm in *Tagetes patula* growing in soil that had 2000 mg/kg Pb added to it. *Calendula officinalis* illustrated a maximum value 10.2 cm of root length at control and lowest value 3.8 cm root length at 2000 mg/kg of Pb in soil.

Aghelan et al. (2021) reported similar results showing that average root length decreased as the concentration increases and the plant cultivated on soil with no Pb concentration had the highest root length. The typical *Tagetes patula* root and stem lengths are also mentioned to reduce when the Pb dose was increased (Aghelan et al., 2021). According to the literature, heavy metals reduce the viscosity and elasticity of cell walls, which prevents roots from growing (Gul et al., 2019).

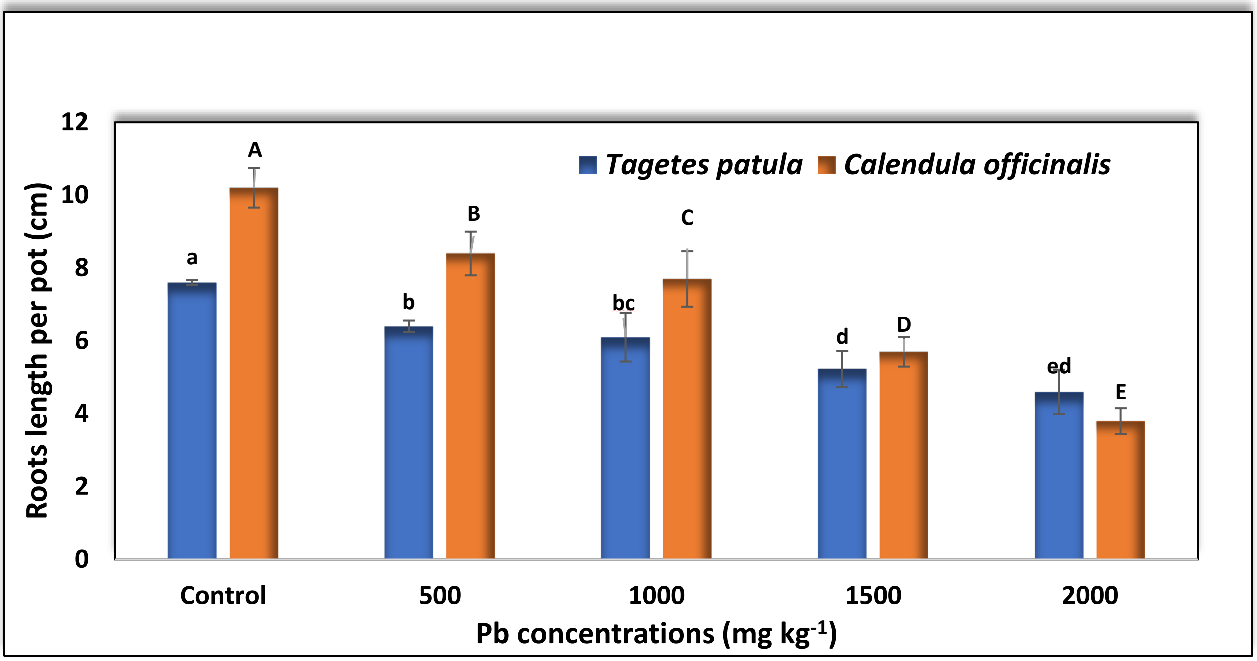

Figure 14: Plant root average length at different Pb concentrations

4.2.6. Effects of Cd on Total Average Plant Biomass

Total biomass of *Tagetes patula* and *Calendula officinalis* showed declines when the amount of Cd in the soil increased from the control to 125 mg/kg of Cd in soil (fig 4.6). Total biomass illustrated a maximum value of 11.6g in control and the lowest value of 7.5g in *Tagetes patula* growing in soil that has been 125 mg/kg Cd-spiked. The *Calendula officinalis* illustrated a maximum value 10.2g of total biomass at control and lowest value 6.7g total biomass at 125 mg/kg of Cd in soil.

Miao et al. (2022) reported that with higher Cd concentrations, the plant biomass reduced, demonstrating that Cd inhibits the growth of *T. patula*, especially when treated with 30 mg Cd/kg. This demonstrates that Cd inhibits the growth of plants (Liu et al., 2010). In response to both shoot and root growth responses, the biomass of the treatment groups CK, T1, T2, and T3 declined sequentially. *Tagetes patula* lacks the ability to phytoremediate and cannot withstand high Cd concentrations.

Shi et al. (2022) reported that when the Cd content was greater than 100 mg/kg, the biomass of *Tagetes patula* dramatically decreased. According to Liu et al. (2008), *C. officinalis* had a dry weight gain that varied depending on the treatment in particular. The dry weight of the plant tissues was 1.27 and 1.26 times higher under TS1 and TS2, respectively, than it was in the control. According to Sun et al. (2018), all cultivars showed increased fresh biomass when exposed to Cd2 and Cd10, and a negligible reduction when exposed to Cd50, demonstrating a high tolerance to Cd stress.

In the current study, the dry biomass of plants decreased in the Cd and Pb spiked soil without amendment application and the control by 23% and 19%, respectively. The biomass of *P. hortorum* was significantly decreased by 22.3% and 16.8%, respectively, at 150 mg/kg Cd and 1500 mg/kg Pb, according to Gul et al. (2019a). Increased membrane permeability, oxidative stress, and HMs stress may be to blame for this decline in biomass. According to Zhang et al. (2018), heavy metals reduce the viscosity and flexibility of cell walls, which prevents root growth.

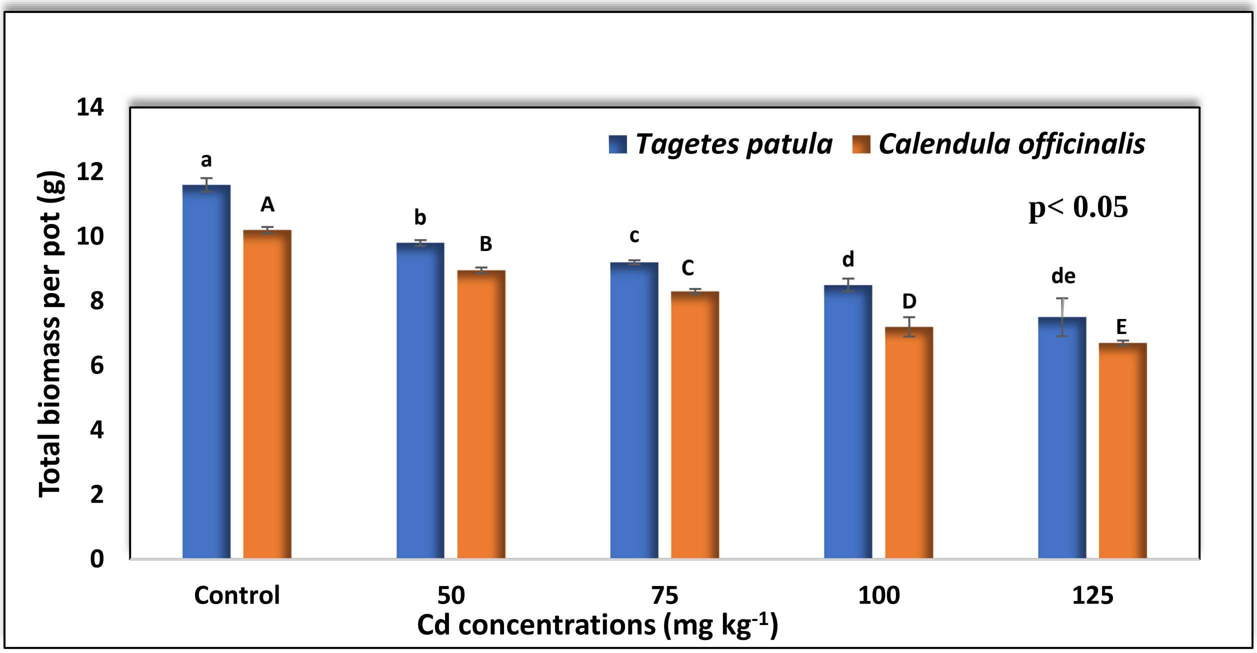

Figure 15: Total average plants biomass at different Cd concentrations

4.2.7. Effects of Cd on Plants Root Weight

In roots (fig 4.7), the weight of *Tagetes patula* and *Calendula officinalis* showed decrease with the increase in the concentration of Cd in soil from control to 125 mg/kg of Cd in soil. Root weight illustrated a maximum value of 3.03g in control and a lowest value of 1.59g in *Tagetes patula* grown in soil spiked with 125 mg/kg Cd of soil. The *Calendula officinalis* illustrated a maximum value 3.7g of root weight at control and lowest value 2.1g root weight at 125 mg/kg of Cd in soil. Following the rise in Cd levels, the dry weight of the root and shoot gradually and considerably reduced.

The biomass of the CK, T1, T2, and T3 treatment groups fell sequentially in both shoot and root growth responses, according to Miao et al. (2022). Because of this, *T. patula* lacks the ability to phytoremediate and is unable to withstand high Cd concentrations.

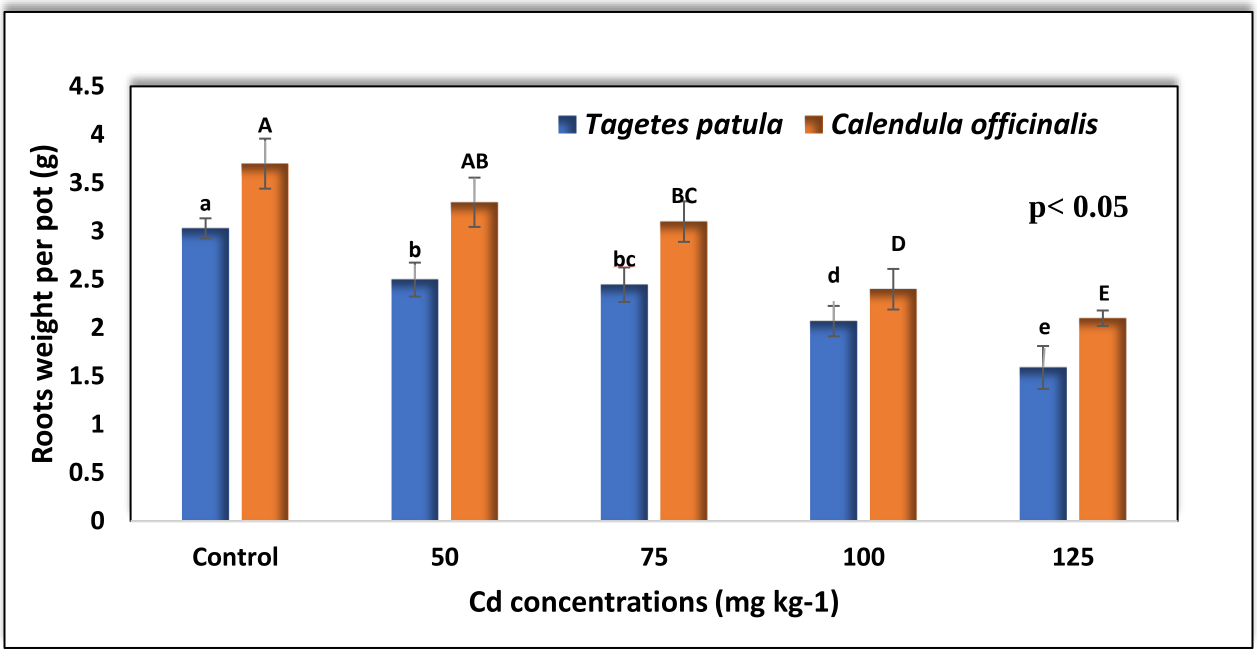

Figure 16: Total average plant root weight at different Cd concentrations

4.2.8. Effects of Cd on Plants Shoot Weight

Shoots weight of *Tagetes patula* and *Calendula officinalis* showed decrease with the increase in the concentration of Cd in soil from control to 125 mg/kg of Cd in soil (fig 4.8). Shoot weight illustrated a maximum value of 8.5g in control and a lowest value of 5.2g in *Tagetes patula* grown in soil spiked with 125 mg/kg Cd of soil. The *Calendula officinalis* illustrated a maximum value 6.8g of shoot weight at control and lowest value 4.7g shoot at 125 mg/kg of Cd in soil. Following the rise in Cd levels, the dry weight of the root and shoot gradually and considerably reduced. Similar to shoot and root lengths, lower Cd treatments had little to no impact on the dry weights of the shoot, root, and flower. However, with the greater amounts of Cd in the soil, Dry weights were reduced by 23%, 33%, and 30% for the shoot, root, and bloom, respectively, in comparison to controls at the highest soil Cd concentration (100 mg/kg). In a related investigation, Miao et al. (2022) found that the biomass of the CK, T1, T2, and T3 treatment groups declined sequentially in both shoot and root growth responses. Because of this, *T. patula* lacks the ability to phytoremediate and is unable to withstand high Cd concentrations. Lin et al. (2010) reported shoot weight of *Tagetes Patula* (10.4 g/plant).

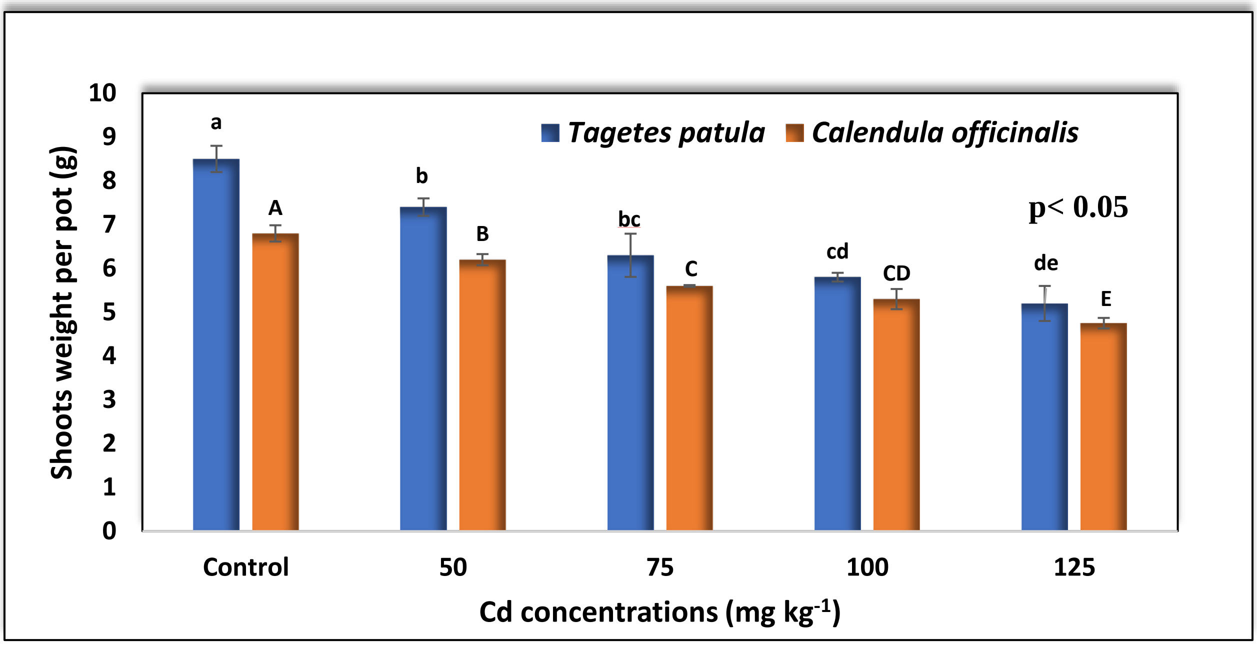

Figure 17: Total average plant shoot weight at different Cd concentrations

4.2.9. Effects of Cd on Plants Total Average Length

Total average length of *Tagetes patula* and *Calendula officinalis* showed decrease with the increase the increase in soil Cd content from the control level to 125 mg/kg (fig 4.9). Total average length of *Tagetes patula* illustrated a maximum value of 22.3 cm in control and a lowest value of 14.5 cm growing in soil that has been 125 mg/kg Cd-spiked. The *Calendula officinalis* illustrated a maximum value 21.5 cm of total average length at control and the lowest value 16.3 cm at 125 mg/kg of Cd in soil. Similarly, Shi et al. (2022) reported that *Tagetes patula* length was decreased at 150 mg/kg Cd contaminated soil.

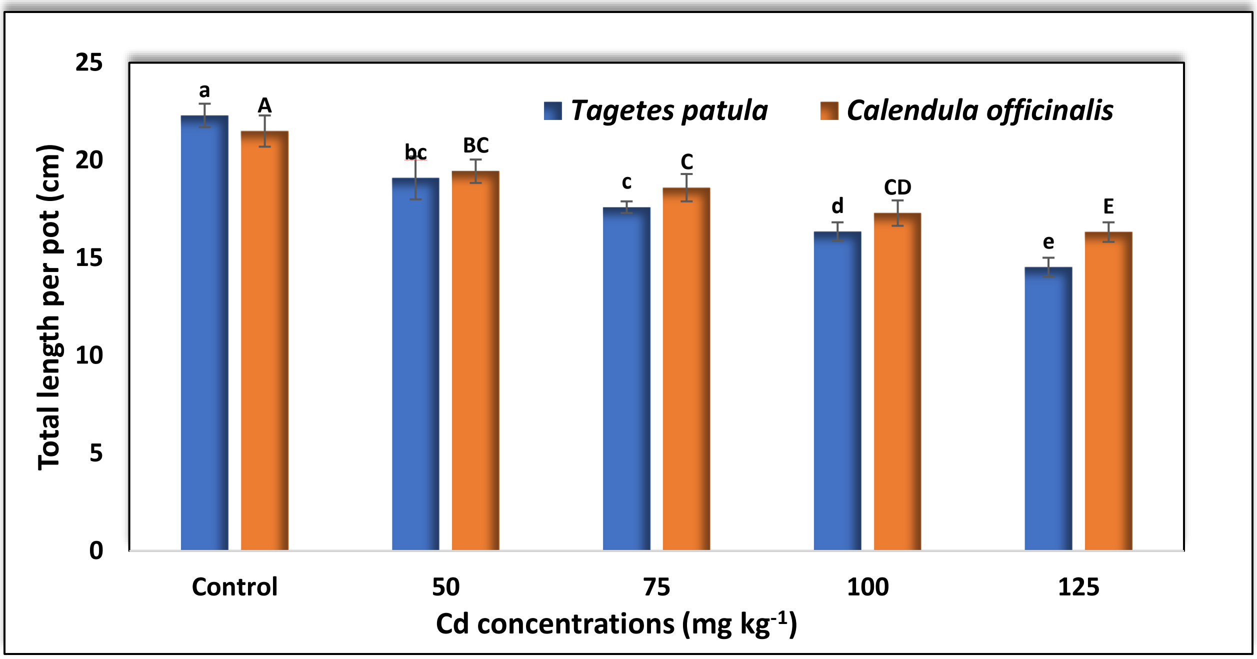

Figure 18: Total average plant length at different Cd concentrations

4.2.10. Effects of Cd on Plants Root Length

Root lengths of *Tagetes patula* and *Calendula officinalis* showed decrease with the increase in soil Cd content from the control level to 125 mg/kg (fig 4.10). Root length illustrated a maximum value of 6.4 cm in control and a lowest value of 3.7 cm in *Tagetes patula* growing in soil that has been 125 mg/kg Cd-spiked. *Calendula officinalis* illustrated a maximum value 5.8 cm of root length at control and lowest value 3.3 cm root length at 125 mg/kg Cd in the soil. Root length decreases as a result of Cd's effects on the normal production of plant growth hormones and metabolites.

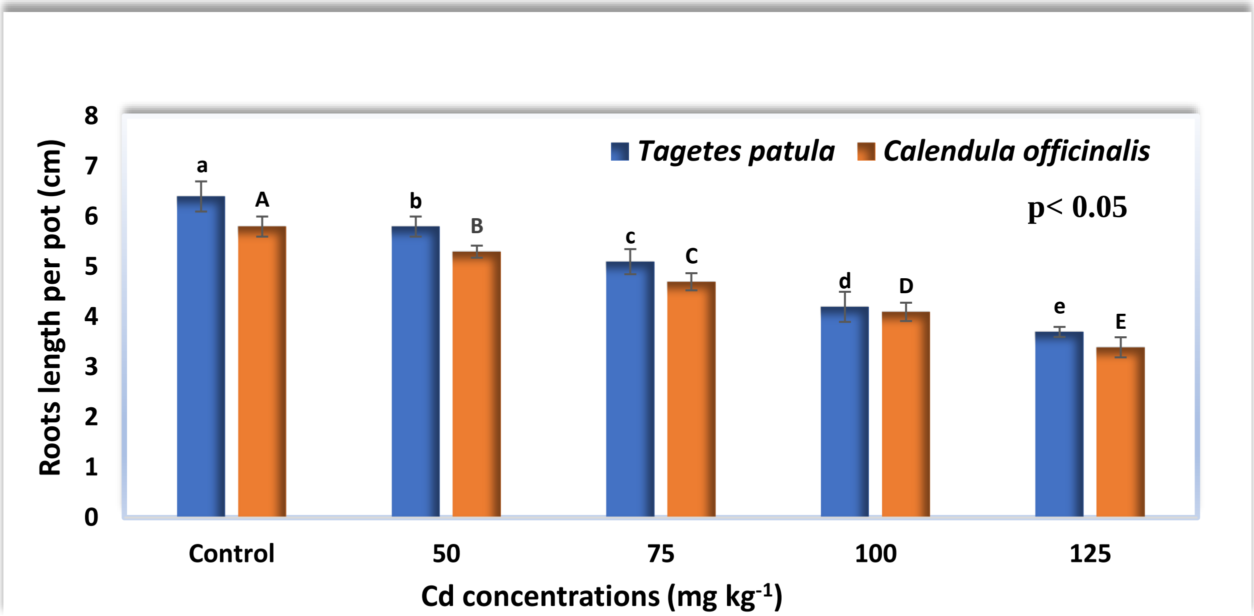

Figure 19: Plant root average length at different Cd concentrations

4.3. Lead in Plants and in Soil After Harvesting Plants

4.3.1. Pb Uptake by Plants' Root at Different Pb Concentrations

Lead uptake by *Tagetes patula* plants roots was observed the highest at 1000 mg/kg Pb, while the highest uptake of Pb in *Calendula officinalis* was observed at 500 mg/kg Pb contaminated soil. The uptake of Pb decreased as the contamination of Pb increased in soil. Manzoor et al. (2018) reported that in range of 500-1000 mg/kg Pb contamination, roots of *Calendula officinalis* showed a significant accumulation.

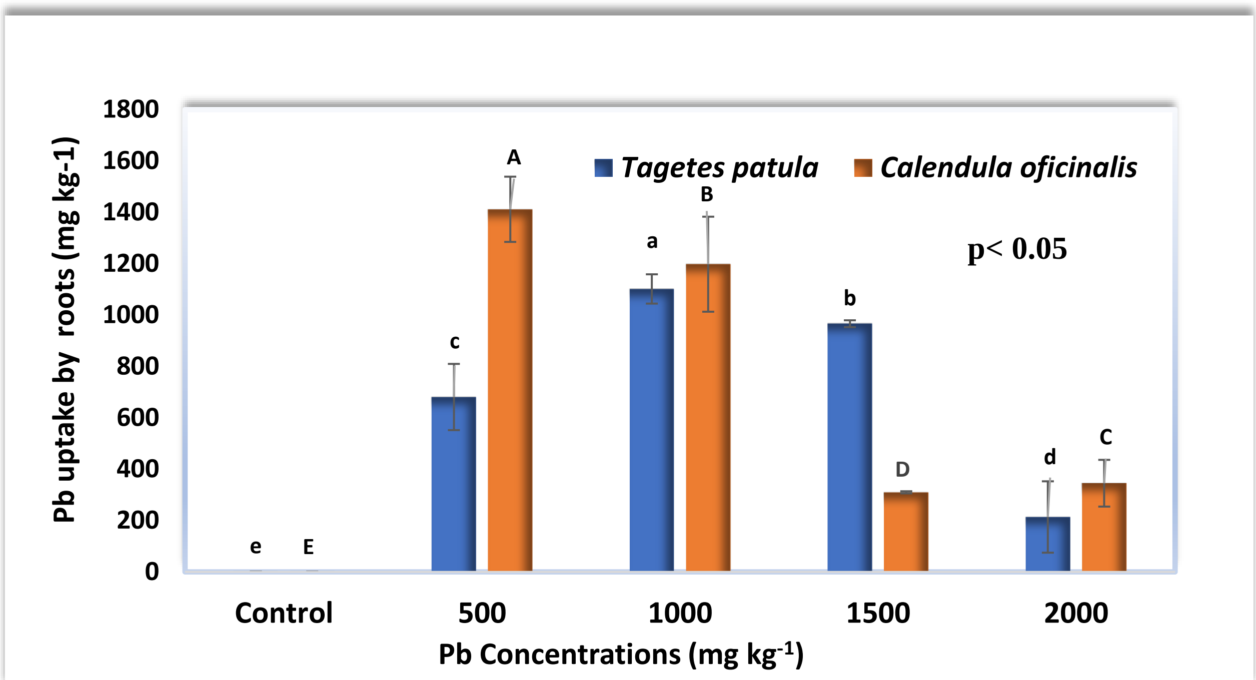

Figure 20: Pb uptake by plants root at different Pb concentrations in soil

4.3.2. Pb Uptake by Plants' Shoots at Different Pb Concentrations

The highest lead uptake in *Tagetes patula* plant shoots was 206.3 mg/kg Pb which was observed at 1000 mg/kg Pb contaminated soil, while the highest uptake Pb uptake in *Calendula officinalis* plant shoots was 592.4 mg/kg Pb which is observed at 500 mg/kg contaminated soil. The figure 4.12 shows that uptake of Pb in both plants decreased as the contamination of Pb increases in soil. That means both the plants shoots have capacity (500-1000 Pb in soil) at which show high uptake while if the Pb concentration exceed their uptake by shoots decreases. Similar findings were reported by Manzoor et al. (2018), who found that *Calendula officinalis* roots accumulated considerable amounts of Pb at soil concentrations of 500 and 1000 mg Pb/kg, i.e., 1439 and 1170 mg/kg, respectively.

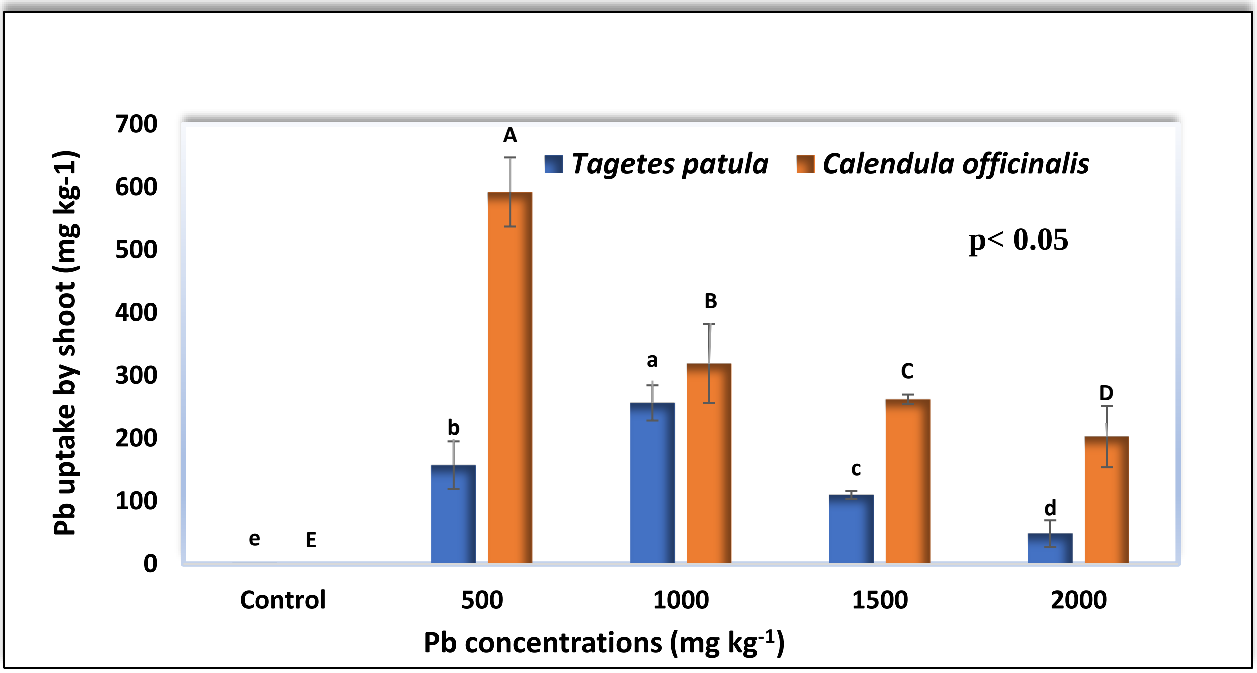

Figure 21: Pb uptake by plants shoots at different Pb concentrations in soil

4.3.3. Pb in Soil After Plants' Harvesting at Different Pb Concentrations

After harvesting of *Tagetes patula* and *Calendula officinalis* plants, Pb contamination in soil increased from 500 mg/kg to 2000 mg/kg, with an increase in the concentration of Pb. Figure 4.13 shows that the highest concentration of Pb in soil was left in 2000 mg/kg Pb contaminated soil. Which means that the least Pb was accumulated in plants grown on soil contaminated with 2000 mg/kg Pb. That means *Tagetes patula* and *Calendula officinalis* plants have low capacity to uptake Pb at high concentrations and their growth also affected.

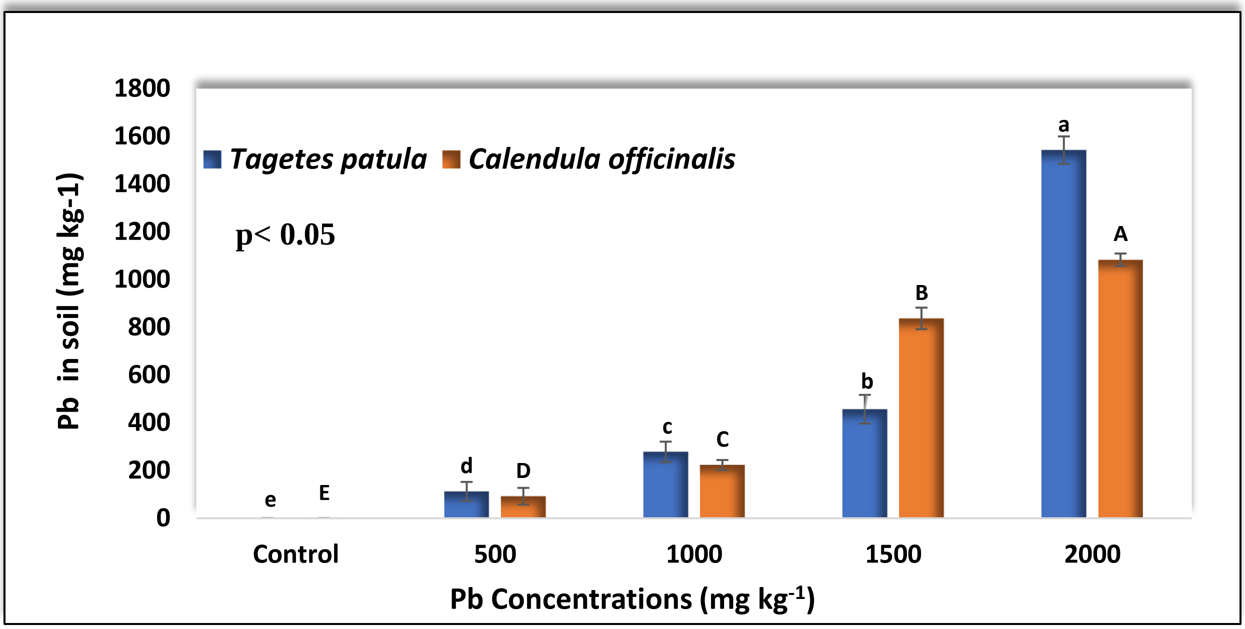

Figure 22: Pb in soil after plants' harvesting at different Pb concentrations of initial soil

4.4. Cadmium in Plants and in Soil After Harvesting Plants

4.4.1. Cd Uptake by Plant Roots at Different Cd Concentrations

The greatest Cd uptake in *Tagetes patula* plant roots was 1235.5 mg/kg Cd and was reported at 100 mg/kg Cd contaminated soil following harvesting of both plants (*Tagetes patula and Calendula officinalis*). While the highest Cd uptake in *Calendula officinalis* plant roots was 834.5 mg/kg Cd in plant roots was also observed at 100 mg/kg Cd contaminated soil. Figure 4.14 shows the uptake of Cd in roots of both plants increased from 50 – 100 mg/kg Cd contaminated soil, while the uptake was observed to be low at 125 mg/kg Cd contaminated soil.

Shi et al. (2022) reported similar Cd uptake by *T. Patula* plant as observed in current study and a similar trend of increasing accumulation up to 1000 mg Cd/kg soil. Roots accumulated a bigger amount of the Cd that plants absorbed, followed by shoots and flowers, respectively, as soil Cd levels increased.

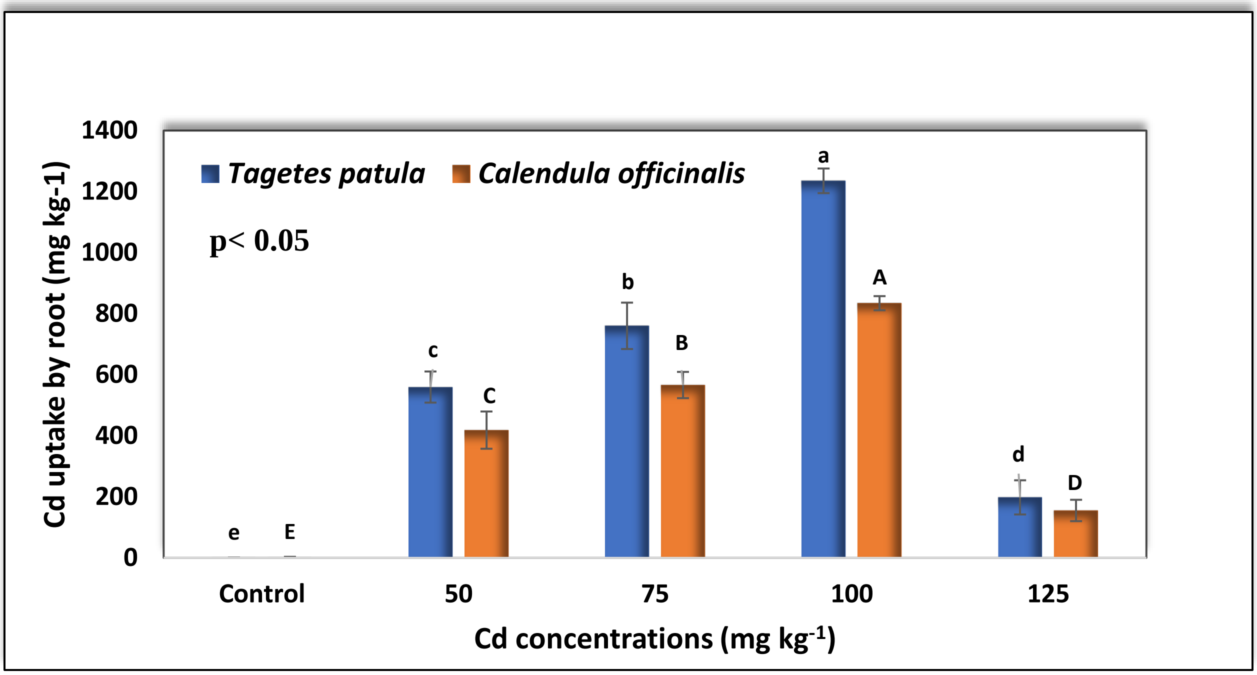

Figure 23: Cd uptake by plants root at different Pb concentrations in soil

4.4.2. Cd Uptake by Plants Shoots at Different Cd Conditions

After harvesting of both plants (*Tagetes patula* and *Calendula officinalis)* in Cd contaminated soil, the highest Cd uptake in *Tagetes patula* plant shoots was 905 mg/kg Cd in plant shoots was observed at 100 mg/kg Cd contaminated soil. While *Calendula officinalis* plant shoots had the maximum uptake of Cd at 852.5 mg/kg, Cd in plant shoots was also found in soil that was 100 mg/kg Cd polluted. Figure (4.15) show the uptake of Cd in shoots of both plants increased from 50 – 100 mg/kg Cd contaminated soil, While the uptakes was observed to be low at 125 mg/kg Cd contaminated soil.

According to Shi et al. (2022), the Cd level in *T. patula* root was often lower than that in stem during the seedling phase but was generally higher throughout the flowering and fructification phases. According to Wei et al. (2012), as compared to other treatments, soil treated with 40 mg Cd/kg and 80 mg Cd/kg soil resulted in higher Cd concentrations in *Tagetes patula* shoots. Liu et al. (2011) also observed high Cd accumulation in shoots of *Tagetes patula* at treatments of 10, 25 and 50 mg Cd/kg.

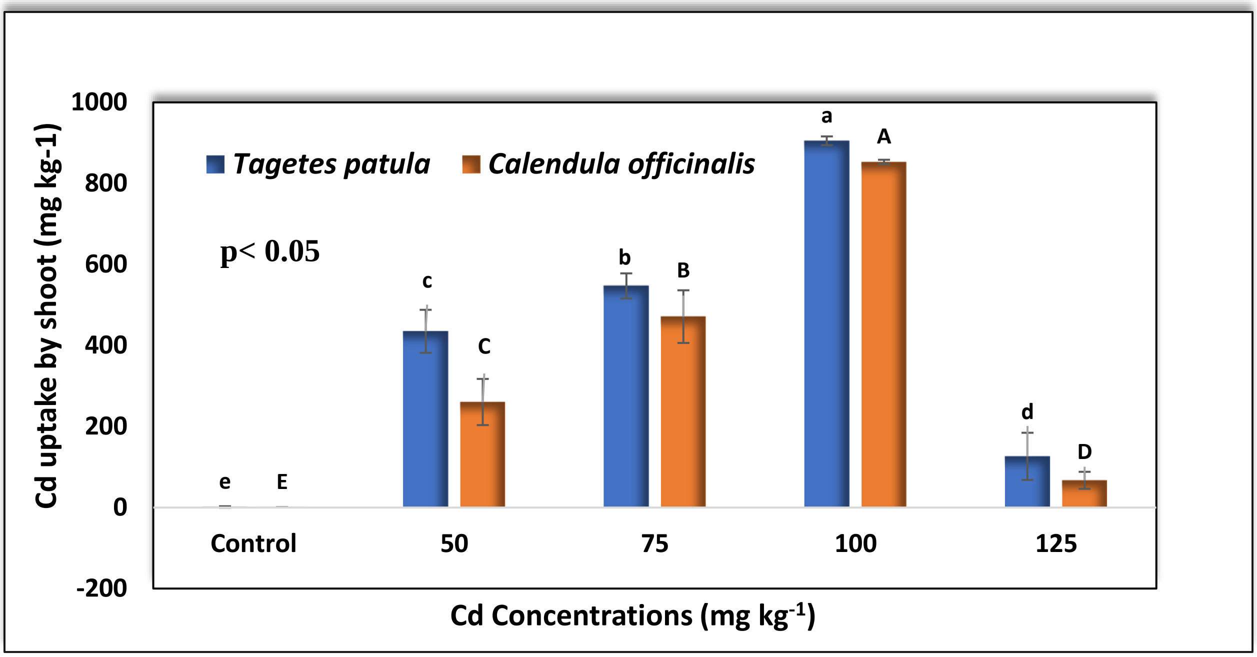

Figure 24: Cd uptake by plant shoots at different Cd concentrations in soil

4.4.3. Cd in Soil After Plant Harvesting at Different Cd Concentrations

After harvesting of both plants, the concentration of Cd in soil was observed the highest in 125 mg/kg Cd contaminated soil. Figure 4.16 showed that the concentrations of Cd in soil after harvesting was observed to be in decreasing order from 50- 100 mg/kg Cd contaminated soil. That means both the plants have capacity up to 100 mg/kg to uptake Cd but at certain limit, their uptake capacity decreased.

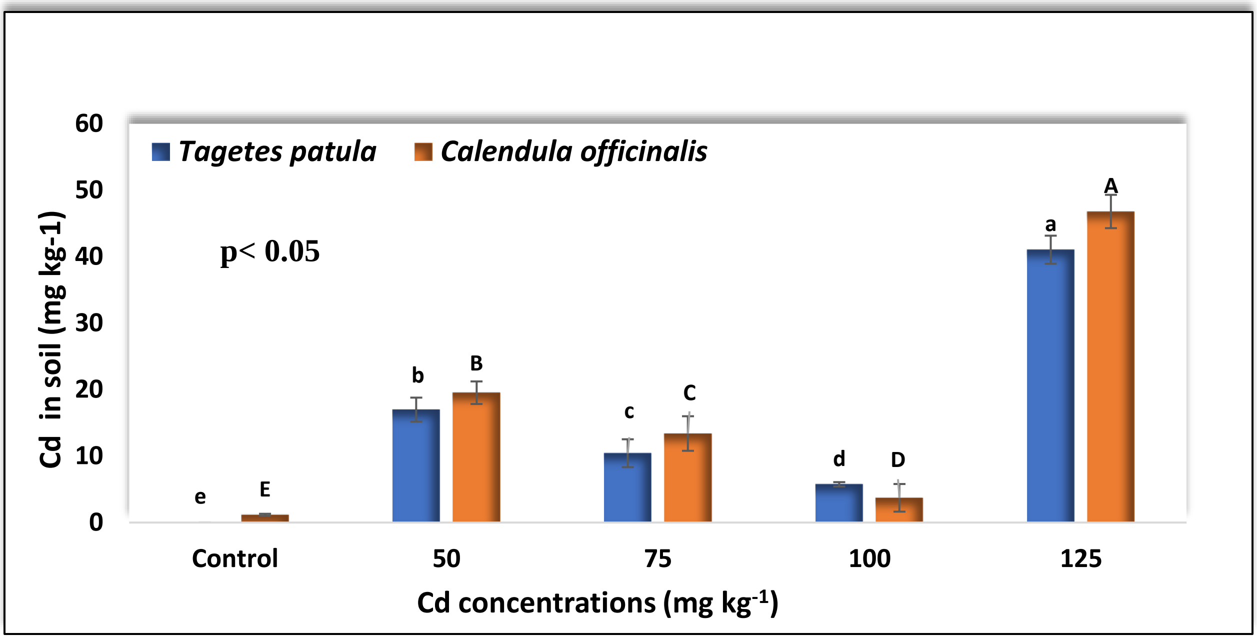

Figure 25: Cd in soil after plant harvesting at different Cd concentrations of initial soil

4.5.Bioaccumulation Factors

Excluders, excretors, and accumulators or hyperaccumulators are three types of plants based on metal-plant interactions and patterns of uptake and accumulation in various parts (Muller et al., 2000). Excluders are hypertolerant plants with a TF smaller than one but have high concentration of HMs in their roots (Boularbah et al., 2006). Excluders may thrive in highly polluted soil, and even at higher soil heavy metal concentrations, their uptake of HMs is reduced (Marchiol et al., 2004, Baker et al., 1981). Excretors are plants that can release heavy metals through salt glands or trichomes on their aerial portions, they possess the morphological and physiological traits to do this. The term for this procedure is phytoexcretion (Madanan et al.,2021).

4.6.Bioconcentration Factor

The ratio of metal concentration in roots and shoots to soil concentration is known as the bioconcentration factor.

4.6.1. Bioconcentration Factor (BCF) Against Pb

Figure 4.17 shows that *Tagetes patula* had 4.4, 3.7, 3.2 and 2.9 BCF values at 500, 1000, 1500 and 2000 mg/kg Pb of contaminated soil, respectively. While *Calendula officinalis* had 6.7, 4.1, 3.1 and 1.8 BCF values at 500, 1000, 1500 and 2000 mg/kg Pb of contaminated soil. BCF value greater than 1 show that the plant is accumulator. In this case the BCF value (>1), for both the plants**.** In case of Pb, the results indicate that both plants are accumulator. The BCF value from 500-2000 continuously decreases for both plants that means plants are good accumulator at low concentrations of Pb as compared to high concentrations.

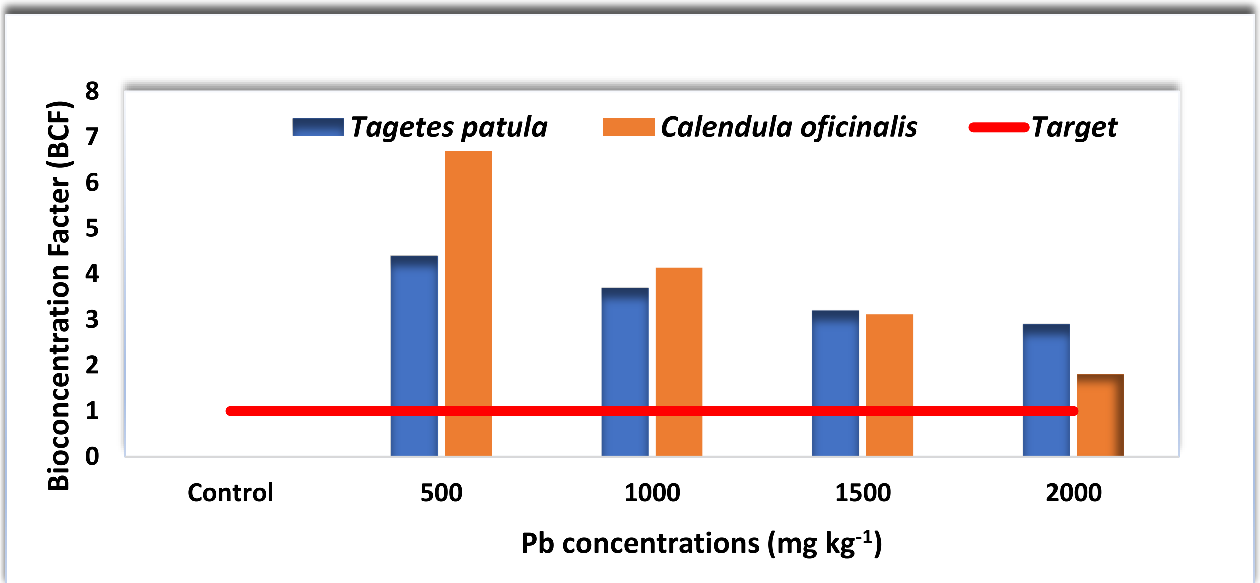

Figure 26: Bioconcentration Factor (BCF) of plants at different Pb concentrations

4.6.2. Bioconcentration Factor (BCF) Against Cd

Results show that *Tagetes patula* had 5.6, 4.3, 3.5, and 2.1 BCF values at 50, 74, 100 and 125 mg/kg Cd of contaminated soil. While *Calendula officinalis* had 3.2, 2.8, 2.6 and 1.7 BCF values at 50, 75, 100 and 125 mg/kg Cd of contaminated soil. When BCF value greater than 1 show that the plant is accumulator. In this case the BCF value (>1), for both the plants**.** In case of Cd, the results indicate that both plants are accumulator. The BCF value from 50 to125 continuously decreases for both plants that means plants are good accumulator at low concentrations of Cd as compared to high concentrations. Wei et al. (2012) reported similar results for both *Tagetes patula* and *Calendula officinalis* varied between 3.77 and 4.61 and 5.02 and 5.06 for Cd, respectively.

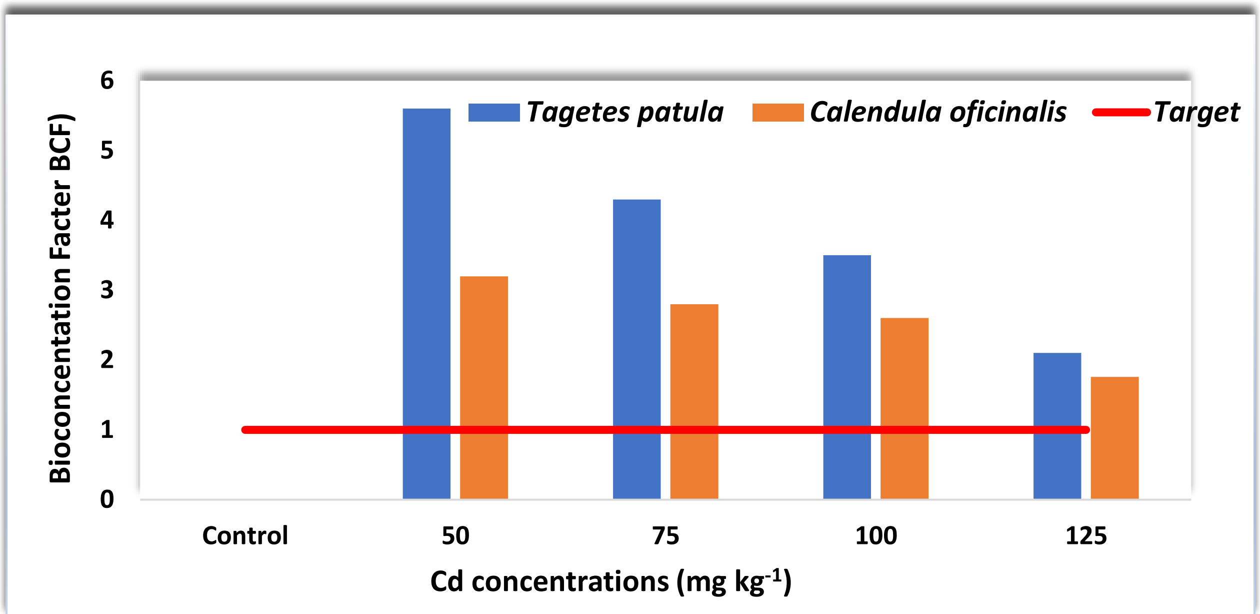

Figure 27: Bioconcentration Factor (BCF) of plants at different Cd concentrations

4.7. Translocation Factor

The ratio of the metal concentration in the root to the metal concentration in the shoot is known as the translocation factor.

4.7.1. Translocation Factor (TF) Against Pb

Figure (4.18) show that *Tagetes patula* and *Calendula officinalis* TFs value in all concentrations of Pb in soil are (TF < 1). The result of the translocation factor shows that both the plants are metal excluder and hypertolerants to Pb. *Tagetes patula* and *Calendula officinalis* store the majority of Pb in their roots. The TFs value in both plants decreases with increases the Pb concentrations in soil from 500 to 2000 mg/kg Pb of soil. Biswal et al. (2022) reported similar TF value of Pb in *Tagetes patula* was < 1.

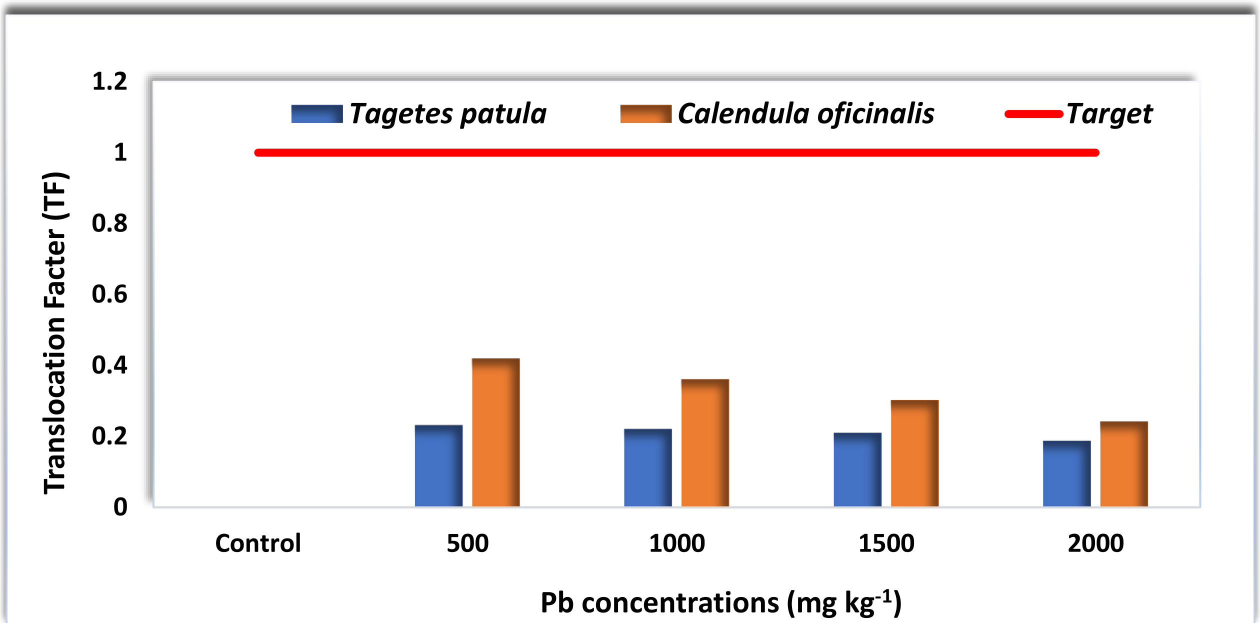

Figure 28: Translocation Factor (TF) of plants at different Pb concentrations

4.7.2. Translocation Factor (TF) Against Cd

Results show that *Tagetes patula* and *Calendula officinalis* TFs value in all concentrations of Cd in soil were TF < 1. The result of the translocation factor shows that both the plants are metal excluder and hyper-tolerant to Cd. *Tagetes patula* and *Calendula officinalis* store the majority of Cd in their roots. The TFs value in *Tagetes patula* plants decreased with increase in the soil's Cd concentrations, which ranged from 50 to 125 mg/kg Cd, while the TFs value in *Calendula officinalis* increased with the Cd concentrations in soil from 50 to 125 mg/kg Cd of soil. Biswal et al. (2022) reported similar TF value of Cd in *Tagetes patula* that was < 1. The *Calendula officinalis* is a hyperaccumulator plant for Cd elimination (Safari and Safari 2020). The rise in Cd levels was followed by a notable increase in the TF. While Wei et al. (2012) reported TF values for *Tagetes patula and Calendula officinalis* 0.59 to 0.69 and 1.01 to 1.66, respectively.

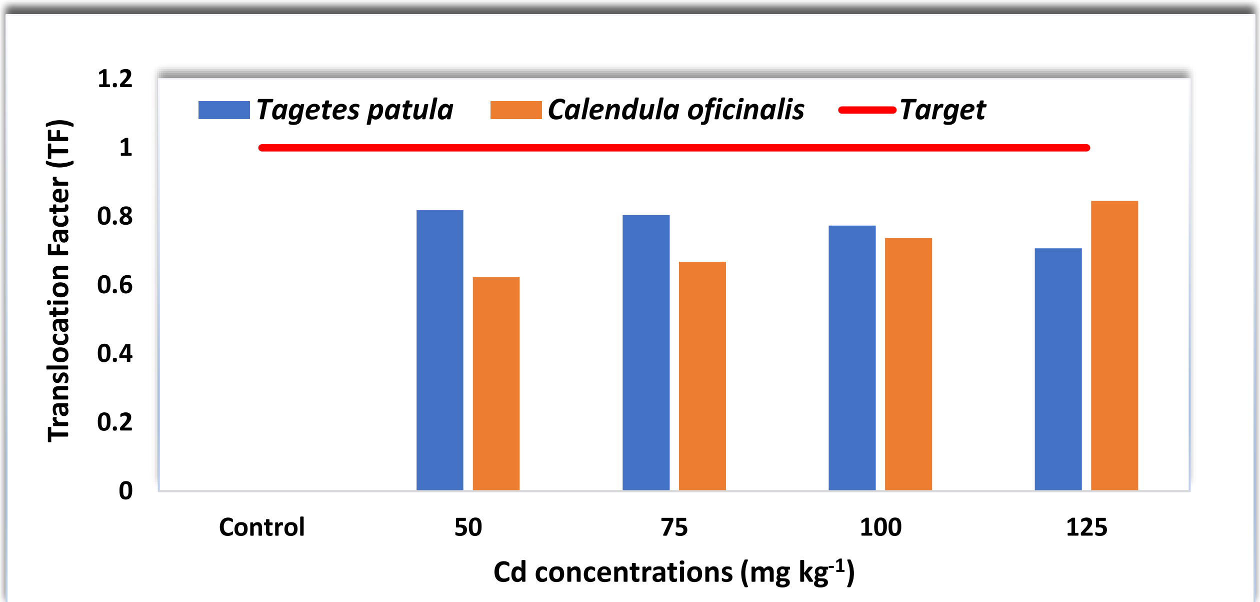

Figure 29: Translocation Factor (TF) of plants at different Cd concentrations

4.8.Enrichment Coefficient

The ratio of the metal concentration in the shoot to the soil is known as the enrichment coefficient factor. Additionally, it demonstrates the plant's capacity to store heavy metals in its aerial components.

4.8.1. Enrichment Coefficient Factor ((EC_f) Against Pb

EC_f value of *Tagetes patula* and *Calendula officinalis* decreased with the increase in the range of soil Pb concentrations, 500 to 2000 mg/kg Pb, as shown in Fig. 4.19. Results showed that *Calendula officinalis* stored metal in their aerial parts and exhibited as a hyperaccumulator at 500 mg/kg. Biswal et al. (2022) reported similar EC_f value of Pb in *Tagetes patula* was >1.

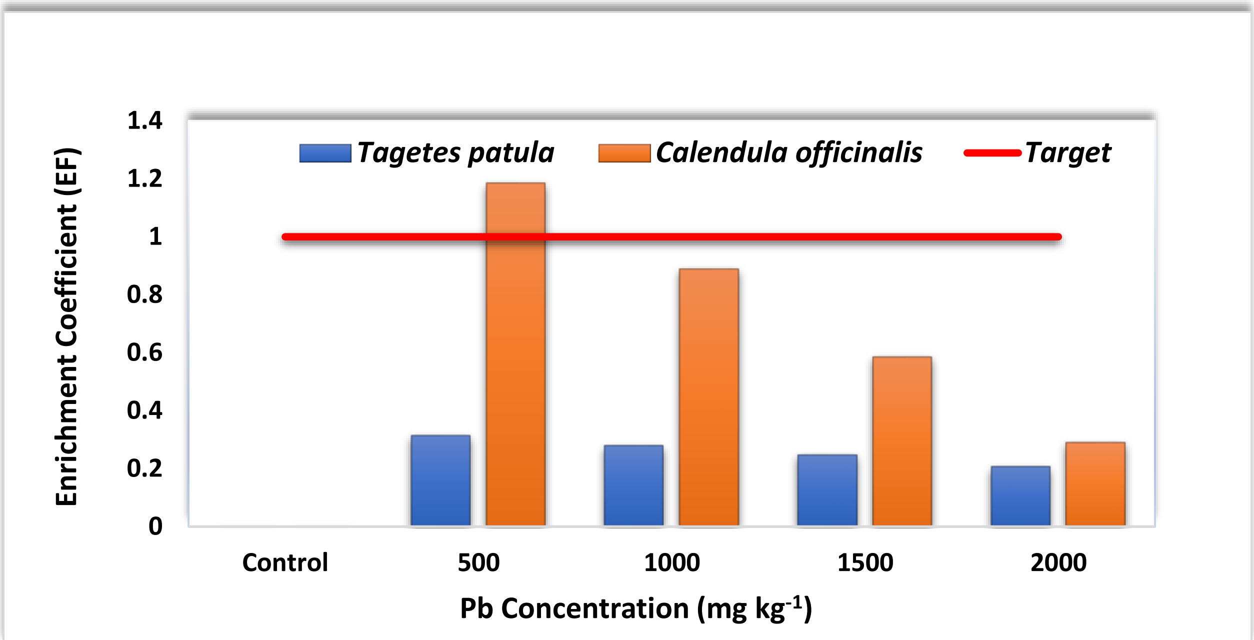

Figure 30: Enrichment coefficient factor of plants at different Pb concentrations

4.8.2. Enrichment Coefficient Factor (EC_f) Against Cd

The ratio of the metal concentration in the shoot to the soil is known as the enrichment coefficient factor. It demonstrates the plant's capacity to store heavy metals in its aerial components. Figure 4.22 demonstrates that the EC_f value of *Calendula officinalis* increased as the Cd concentration in soil increased from 50 to 125 mg/kg Cd of soil. The EC_f value of *Tagetes patula* dropped as Cd concentration in soil increased from 50 to 125 mg/kg Cd of soil. The research also demonstrates that *Tagetes patula* and *Calendula officinalis* are both hyperaccumulators of Cd at soil concentrations between 50 and 125 mg/kg. According to Lui et al. (2010), *Calendula officinalis* had EC_f values greater than 1, showing that the plant's shoot was effective at storing Cd.

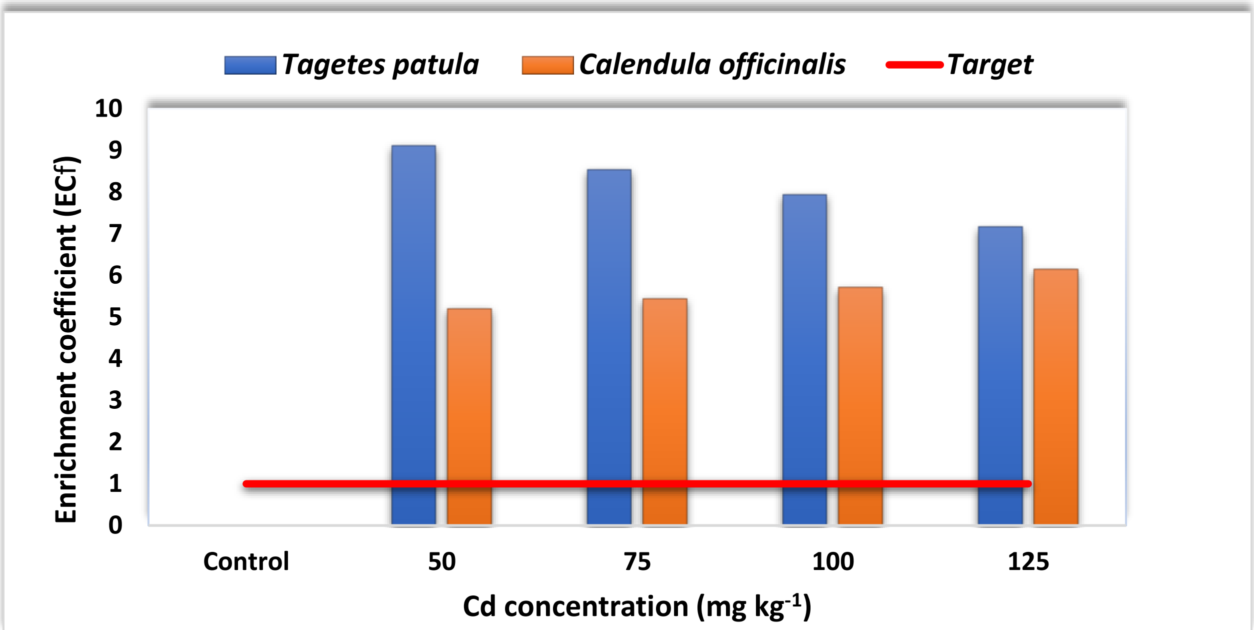

Figure 31: Enrichment coefficient factor of plants at different Cd concentrations.

Chapter 5

Conclusion and Recommendation

5.1 Conclusions

In a concentration gradient experiment, the potential for Pb and Cd hyperaccumulation in two ornamental plants was explored. Both the plants (*Tagetes patula* and *Calendula officinalis*) produced the highest biomass and length at the control and continuously decreased with increasing the concentrations of Pb and Cd in soil. This shows that as the Pb and Cd concentrations in soil increased, it may affect the plant physiological parameters, microbial activities, consequently effecting the nutrient cycle as a result of which growth of the plant is affected negatively. The highest uptake of Pb by *Calendula officinalis* was observed at 500 mg kg^{-1} Pb contamination, while *Tagetes patula* accumulated the highest at a contamination of 1000 mg kg^{-1} Pb. In case of phytoextraction, accumulation of Cd in both plants increased from 50-100 mg kg^{-1} Cd, while it reduced at 125 mg kg^{-1} of Cd contamination that means both plants have capacity to clean soil which are contaminated by Cd up to 100 mg kg^{-1} of soil. BCF > 1, for *Tagetes patula* and *Calendula officinalis* against Pb and Cd, indicated that both the plants are potential hyperaccumulators, while EC_f < 1 against Pb and EC_f >1aginst Cd in both plants, TF <1 in both the plants, highlighted that both the plants are hypertolerants and possibly store majority metals in their roots.

5.2 Recommendations

Based upon the findings of the current study, here are some recommendations for future work.

1. Despite rapid progress in the field of heavy metal phytoremediation around the world, there is requirement tremendous scientific and practical approaches for further research on this subject, particularly tapping the potential of plant biodiversity for remediation.
2. This experimental process is extremely beneficial for the discovery of new species capable of efficiently removing metals. Such exploration leads to efficient phytoremediation of contaminants; therefore, the exploration of new ornamental plants should be encouraged.
3. To reclaim the degraded land area, the phytoremediation technique must be used in areas moderately contaminated with metals and other contaminants.

4. Based on this study, a cost-benefit analysis comparing other methods could be performed. Economic analysis of experiments would facilitate the practical application of such techniques/methods.

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