Heavy Metal Accumulation in Selected Plant Species: Assessing Phytoremediation Potential

A Dissertation Submitted to University of Calicut in partial fulfilment of the requirement
for the award of the degree of
MASTER OF SCIENCE IN BOTANY

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DECLARATION

I hereby declare that the dissertation entitled '**Heavy Metal Accumulation in Selected Plant Species: Assessing Phytoremediation Potential**' submitted to M E S Asmabi College, P. Vemballur Kodungallur in partial fulfilment for the award of Degree of Master of Science in Botany is a *bona fide* record of the research work done by me .during the period of February 2024 to May 2024 under the supervision and guidance of Nazeema M K., Assistant professor, Research Department of botany M E S Asmabi College, P.Vemballur, Kodungallur, and Dr. S. Sandeep, Principal scientist, of Soil science Department, Kerala Forest Research Institute, Peechi and no part of this dissertation had been presented earlier for any degree/diploma/associateship or any other university or institution.

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ACKNOWLEDGEMENT

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ABSTRACT

This study conducted in Thrissur district focuses on how selected plants accumulate heavy metals in polluted areas, aiming to understand their tolerance mechanisms and potential for soil management and metal extraction. Samples were collected from waste dumping sites and industrial zones, including six plant species (*Cleome rutidosperma, Vernonia cinerea, Eleutheranthera ruderalis, Alternanthera bettzickiana, Cleome viscosa, Trianthema portulacastrum*) and corresponding soil samples. Acid digestion was employed for soil and plant sample preparation, followed by atomic absorption spectrometry (AAS) analysis to quantify concentrations of Cr, Ni, Zn, Cd, and Pb. Cleome viscosa and Eleutheranthera ruderalis showed significant accumulation of Zn and Pb. Variability in metal concentrations between Athani and Laloor sites underscored site-specific contamination levels. These findings are pivotal for assessing the phytoremediation potential of these plants and their suitability for managing polluted soils and extracting metals, emphasizing the importance of plant selection in environmental management strategies in Thrissur district.

Keywords: Heavy metal accumulation, polluted soils, phytoremediation, atomic absorption spectrometry, Thrissur district

CHAPTER 1
INTRODUCTION

INTRODUCTION

Soil, the source of infinite life is the most vital and precious natural resource ,and not renewable in short time .It is a dynamic natural body developed as a result of a pedogenic process during and after weathering of rocks ,consisting of minerals and organic constituents ,possessing definite chemical , physical, and biological properties ,having variable depth over the surface of the earth and providing a medium for the growth of the plants .A Typical surface soil has 25 % Air, 25 % water ,and 50 % solid. Solid part is made up of 45 % of the total with mineral matter and 5% of the total soil with organic matter. But the composition of subsoil and deep soil varies distinctly.

The inorganic component of soil includes both nutrient elements and heavy metals. "Heavy metals" are elements that exhibit metallic properties such as ductility, malleability,conductivity,cation stability and ligand specificity. They are characterized by relatively high density and high relative atomic weight with an atomic number greater than 20. Some heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, V and Zn are required in minute quantities by organisms.However,excessive amounts of these elements can become harmful to organisms. Other heavy metals such as Pb, Cd, Hg, and as (a metalloid but generally referred to as a heavy metal) do not have any beneficial effect on organisms and are thus regarded as the "main threats "since they are very harmful to both plants and animals.

The increasing levels of heavy metals contamination could be caused by human activity which then makes heavy metals the main sources of pollution in the environment. Heavy metals are considered as the main sources of pollution on the environment. Heavy metals are considered as a member of an ill-defined subset of elements that reveal metallic properties, which would mainly consist of the transition metals, some metalloids, lanthanides and actinides .

Soils may become contaminated by the accumulation of heavy metals and metalloids through emissions from the rapidly expanding industrial areas, mine tailings, disposal of high metal wastes, leaded gasoline and paints, land application of fertilizers, animal manures, sewage sludge,pesticides,wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition.

Nearly all human activities generate waste, and the way in which it is handled, stored, collected and disposed can pose risks to environment and public health. Solid wastes other than hazardous and

radioactive materials are often referred to a municipal solid waste. The composition of solid waste is influenced by many factors such as culture affluence and location.

Estimated municipal solid waste generated in India is around 55 million tones year and municipal solid waste dumping yards are increasing in India day by day. In this scenario of waste management and handling. Kerala is no way different from other states, with an annual estimate of waste production of 9983.80 tones day. The composition of wastes varies from biodegradable (68%) and the test, including cloth, timber, plastic, rubber, glass, syringe and brick. Improper management of solid waste is one of the main causes of environmental pollution in most of the cities. In Kerala the significant per capita waste generation varied from 0.034 kg to 0.707 kg day. On an average 50 per cent of this waste is generated from households and about 35% from various institutions (Varma, 2008). The construction sector and street sweepings together contribute 15 per cent. However, the waste is dominantly of compostable material to the tune of 72-84 per cent and is characterized by high moisture content, low calorific value and high nutrient concentration.

Municipal waste dumping sites are designated places set aside for waste disposal. As there are a variety of wastes at the dumping site, it will pollute the natural resource of the area, due to the production of toxic chemicals and pollutants like heavy metal (Loaniyan et al, 2011). Most of the cities lack solid waste management regulations and facilities for disposal of harmful waste like infectious, toxic heavy metals or radioactive substances.

OBJECTIVES

- To determine total heavy metals uptake in plants grown in polluted areas.
- To analyse the phytoremediation potential of the plants based on the bioconcentration factor (BCF) and translocation factor (TF).

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term "heavy metal," commonly used, refers to metals primarily of high density that largely belong to the group of transition elements in the periodic table (Taylor, 1964). It is widely used to collectively describe metals and metalloids associated with environmental contamination, pollution, and toxicity. Essential elements like Zn, Cu, Mn, and Fe are vital for living organisms at low concentrations and are often referred to as micronutrients in agriculture. However, when concentrations of these elements exceed critical levels, they can become toxic to plants (Scott, 1972; Bolt and Bruggenwert, 1978). The term "toxic" or "harmful heavy metal" is typically applied to non-essential elements such as Cd, Pb, Hg, and Ni, which plants absorb without any physiological function at low concentrations, yet these elements have detrimental effects on crop growth at higher concentrations (Alloway, 1990).

2.1. Sources of heavy metals in soils

All soils are found to contain heavy metals from a trace to an appreciable level being derived from various sources. Some of the important sources which contribute to the status of heavy metals in soils with emphasis on Zn, Cr, Ni, Pb and Cd are reviewed here.

2.1.1 Geogenic origin

Metals naturally present in rocks and minerals, from which soils are derived, are regarded as the principal source of heavy metals in soils (Taylor, 1964). Taylor identified that the primary factors influencing the heavy metal content in soils include the composition of the parent rock and the soil formation processes. He noted significant variability in heavy metal concentrations among soils formed from the same parent material. For example, surface soils in Tasmania originating from dolerite exhibited nickel (Ni) concentrations ranging from 5 to 140 mg kg^{-1} (Taylor, 1964).

Rose *et al*. (1979) reported mean contents of Cd, Cu, Ni, Pb, and Zn in the earth's crust as 0.1, 50, 80, 14, and 75 mg kg^{-1}, respectively. According to their findings, the primary rock types containing heavy metals as trace constituents include ultrabasic rocks (such as dunite, peridotite, and serpentinite), basic rocks (like basalt), granitic igneous rocks, and sedimentary rocks (including limestone, sandstone, and shales). These rock types serve as natural sources for the trace metals found in soils.

Lund *et al*. (1981) observed that residual soil material derived from shale parent material exhibited the highest Cd concentration, with a mean value of 7.5 mg kg⁻¹, whereas soils developed from sandstone and basalt had lower Cd concentrations, averaging 0.84 mg kg⁻¹. Alluvial soils originating from mixed parent materials had an intermediate Cd content, averaging 1.5 mg kg⁻¹. Vegetables grown in soils with such Cd concentrations may accumulate levels of cadmium that pose public health concerns.

Alloway (1990) introduced the term "geochemical pollutants" to describe toxic metals derived from parent materials. According to him, rocks with the highest concentrations of Cu, Pb, and Ni are basic, ultrabasic, and granite, while those with high concentrations of Zn and Cd are shales and clay. He emphasized that volcanoes contribute significantly to the natural input of metals into the environment and are a major source of atmospheric metal deposition. He also reported that the concentration of Cd. Cu. Ni. Pb, and Zn in air from volcanoes in Hawaii were 892, 2003000. 330.28 1200 and 1000 ng m⁻³

2.1.2 Anthropogenic origin

Heavy metals are introduced into soil through the land application of sewage sludge, fertilizers, and other waste materials (Adriano, 1986; Alloway, 1990). The extent of land contaminated with heavy metals has expanded over the last century primarily due to mining, smelting, and other industrial activities (Brown *et al.*, 1995).

Chaney (1973), Tiller (1989), Sakal *et al*. (1992), Norwal et al. (1994), and Rao and Shantharam (1994) have collectively reported that the long-term application of municipal, urban, and farm wastes leads to the accumulation of heavy metals in soils.

Emmerich *et al*. (1982), Logan (1985), Tiller (1989), and Mortvedt (1996) have documented significant levels of heavy metals in sewage sludges produced worldwide. The disposal of sludges on land or their application to soil as a nutrient and conditioner has resulted in the contamination and pollution of soils with heavy metals.

Singh and Kansal (1985), Gupta *et al*. (1986), Karunakaran Nair (1987), and Jayabaskaran and Sreeramulu (1996) reported very high concentrations of toxic metals such as Pb, Cd, Ni, and Cr in sewage from various cities in India.

Anderson and Nielson (1976), Azad (1981), Sharma and Kansal (1986), and Kumar (1992) have observed increased concentrations of heavy metals in industrial and domestic wastewater, sewage water, and freshwater bodies that receive industrial, municipal, and sewage effluents or wastes. They

noted that the use of these contaminated waters for irrigation or their disposal on agricultural land has led to the enrichment of soils and crops with heavy metals.

2.1.3 Inadvertent transfer

Buchauer (1973) and Lagerwerff *et al*. (1973) have reported that a wide range of industrial emissions can lead to air pollution containing heavy metals. These pollutants can subsequently contaminate soil when deposited onto the soil surface through processes such as dew or rainfall.

As suggested by Bridges (1989), industrial activities such as manufacturing pesticides, paints, coloring materials, explosives, mining, smelting, refining metals, gas and chemical works, ammunition production, oil refining and storage, pharmaceutical manufacturing, tanneries, wood preservation factories, docks, railways, asbestos production, manufacturing integrated circuits and semiconductors, scrapyards, landfills, and waste disposal sites have been identified as significant contributors to extensive soil pollution.

Recent interest in investigating how plants absorb and distribute heavy metals has increased due to documented health risks linked to their consumption in diets (Rivai *et al*., 1990; Wagner, 1993; Groten and Vanbladeren, 1994).

2.2 Status of heavy metals in soil

Merry (1989) reported that virgin soils in rural areas of Australia typically contained about 0.09 mg of cadmium (Cd) per kg in the topsoil. In contrast, soils in metropolitan areas contaminated with sewage sludge showed significantly higher levels, ranging from 1 to 10 mg Cd per kg of soil. This indicates a substantial increase in cadmium concentration due to sewage sludge contamination in urban settings compared to the natural levels found in rural soils.

Kabata-Pendias and Dudka (1991) reported that the average content of total cadmium (Cd) and lead (Pb) in the surface soils of Poland was 0.41 ppm and 18.3 ppm, respectively. They proposed reference values for evaluating soil contamination as 0.1 to 0.6 ppm for Cd and 7.6 to 22.5 ppm for Pb.

In the study by Almas *et al.* (1995), soil samples from fields in North Norway, near the Russian border, were analyzed for concentrations of Cd, Cu, Cr, Ni, Pb, and Zn to assess potential contamination from nearby Russian metal industries via atmospheric deposition. The mean concentrations of HNO_3-extractable Cd, Cu, Ni, and Zn were found to be 0.8, 44, 30.4, and 47.5 mg kg-1 soil, respectively,

indicating accumulation of these elements due to atmospheric deposition from industrial sources. The mean concentrations of Pb and Cr in the soil were reported as 9.1 and 20.4 mg kg-1 soil, respectively, which are within normal levels for these metals in Norwegian soils. Additionally, the concentration of DTPA-extractable Cd in the soils was high, ranging up to 0.58 mg kg^{-1}, further suggesting potential contamination from industrial sources affecting cadmium levels in the soil.

2.3 Heavy metal content in plants

Recent interest in understanding how plants absorb and transport heavy metals has grown due to reported harmful health effects associated with their dietary intake (Rivai *et al*., 1990; Wagner, 1993; Groten and Vanbladeren, 1994).

Allaway (1968), Haghiri (1976), and Gupta and Dixit (1992) observed a decrease in plant growth attributed to cadmium (Cd) toxicity when the concentration of Cd in plants reached approximately 2 to 3 mg kg-1. This highlights the detrimental effects of Cd toxicity on plant physiology, underscoring that higher levels of cadmium accumulation can inhibit plant growth.

Davis *et al*. (1978) suggested that Cd concentrations in plants should not exceed 15 ppm. Bowen (1979) indicated typical levels of Cd, Cu, Ni, Pb, and Zn in plants ranging from 0.1 to 2.4 ppm for Cd, 5 to 20 ppm for Cu, 0.02 to 5 ppm for Ni, 0.2 to 20 ppm for Pb, and 1 to 400 ppm for Zn.

According to Kabata-Pendias and Pendias (1984) and Nicol and Beckett (1985), concentrations above which toxic effects were likely in plants for Cd, Cu, Ni, Pb, and Zn were noted as: Cd (5-30 ppm), Cu (5-64 ppm), Ni (8-220 ppm), Pb (30-300 ppm), and Zn (100-400 ppm).

Alloway (1990) observed that the concentration of Ni in plants from non-contaminated, non-serpentinic soils typically ranged around 0.15 mg kg^{-1}. Slightly elevated levels were found in crops grown on organic soils. In contrast, plants from serpentine areas often exhibited Ni concentrations ranging from 20 to 100 mg kg^{-1}. Alloway also noted oats as particularly prone to absorbing higher amounts of Ni.

2.4 Factors controlling the heavy metals in soil

Miller *et al.* (1977), McBride *et al*. (1981), Rana and Kansal (1983), Sposito *et al*. (1982), McBride (1989), and other researchers have extensively explored the factors influencing the availability of heavy metals in soils. According to their findings, the sorption of heavy metals by soil is significantly affected by several soil properties such as pH, organic matter content, cation exchange capacity, clay content, presence of other ions, and the composition of the soil solution. A comprehensive review of the significant literature on these topics provides valuable insights into the mechanisms governing the interactions between heavy metals and soil environments.

2.4.1 pH and CEC

Chaney (1973) underscored pH and cation exchange capacity (CEC) as pivotal soil factors influencing the bioavailability of heavy metals. Iimura (1973) and Ito and Iimura (1975) specifically highlighted the impact of pH and redox conditions in rice fields on Cd availability. They demonstrated that higher pH levels enhance the stability of complex compounds with soil humus and increase adsorption by aluminum and iron hydroxides, thereby reducing Cd availability to plants. Korte et al. (1976) and Christensen (1984) further linked soil pH and clay content to the adsorption of heavy metals. These studies collectively emphasize how soil properties regulate the bioavailability and uptake of heavy metals by plants, crucial for understanding environmental and agricultural impacts.

Miller *et al*. (1977) observed that Cd uptake by plants decreased with higher soil pH and cation exchange capacity (CEC), indicating reduced availability of Cd under these conditions. Conversely, they noted that Cd availability increased with soil acidity. Harter (1979, 1983) conducted studies correlating heavy metal adsorption in soils with properties such as pH and CEC. These researchers highlighted that pH and CEC play crucial roles in influencing the adsorption and availability of heavy metals like Cd in soil environments.

Zang *et al*. (1987) and Sakal et al. (1992) both identified a significant correlation between soil pH and the concentration of heavy metals in the soil. Their studies indicated that variations in soil pH levels directly influenced the availability and accumulation of heavy metals such as Cd, Pb, and others in soil matrices. This correlation underscores the importance of soil pH as a critical factor governing the behavior and distribution of heavy metals in terrestrial environments.

Oborn *et al.* (1995) analyzed the presence of heavy metals in crops cultivated in commercial fields in Sweden. They observed that concentrations of Cd, Ni, Zn, and Cu exhibited a notable inverse relationship with surface soil pH. They concluded that if the decline in soil pH caused by diverse

acidifying processes is not counteracted by liming, it could result in heightened levels of trace elements in crops.

Reddy *et al*. (1995) noted that under near neutral pH conditions, the concentration of dissolved metals in soil water extracts was primarily influenced by complexes with dissolved organic carbon (DOC). Conversely, at low pH levels, the concentration of dissolved metals in soil water extracts was predominantly in free ionic forms, with ion pairs following closely. As soil pH decreased, the availability and movement of metal ions increased due to the chemical forms in which these ions are present in the soil solution.

Lehoczky *et al.* (1996) reported that Cd availability in a soil with pH 4.2 was found to be 0.06 mg kg^{-1} soil, while in a soil with pH 6.8, it was measured at 0.08 mg kg^{-1}.

2.4.2 Organic matter

Organic matter plays a crucial role in the sorption and complexation of heavy metals in soils, affecting their availability.

Petruzzelli *et al.* (1978) suggested that metals are adsorbed by organic matter through complexation mechanisms. White and Chaney (1980) emphasized that a high organic matter content effectively reduces metal availability and restricts the uptake of Cd by plants. Furthermore, the type of soil also significantly influences the uptake of Cd and Zn by plants.

Khalid *et al*. (1981) reported that the bioavailability of heavy metals tends to decrease in the presence of dissolved organic matter (DOM), suggesting that organic matter generally has a positive effect on reducing the availability of heavy metals in soil. However, Sharma and Kansal (1986) found a positive correlation between organic carbon content and the availability of heavy metals. They suggested that an increase in organic matter could enhance the availability of these metals by forming organo-metallic complexes. These complexes can persist in the soil solution for extended periods, potentially increasing the mobility and availability of heavy metals to plants and other organisms.

In the study, the authors observed a significant increase in metal adsorption in soil. They noted that when the fields were consistently submerged throughout the growing period, the concentration of cadmium (Cd) in brown rice remained low, even when the soil Cd content was as high as 100 ppm. Specifically, it was reported to be less than 1 ppm. However, after draining the soil following the tillering stage, the Cd content in brown rice increased sharply, reaching a peak level of 5 mg kg^-1.

This suggests that the availability and uptake of Cd by rice plants were influenced by the soil's water management conditions, with submerged conditions effectively limiting Cd uptake during growth, but post-drainage leading to higher uptake levels. Khalid *et al.* (1981) demonstrated that an increase in redox potential (Eh) from 150 to 500 mV resulted in a decrease in the exchangeable cadmium (Cd) in soil.

The influence of waterlogging on the availability and uptake of Cd, Pb, Zn, Cu, Mn and Fe was studied by Bjerre and Schierup (1985) in a pot experiment using different soils. Waterlogging resulted in the release of more Cd. Pb and Zn to the inorganic reservoir. Mn and Fe concentrations increased during waterlogging. The availability of Cu was not much affected. The total uptake of all heavy metals decreased during waterlogging. The uptake of all metals except cadmium was lowest in plants grown in the organic soil under flooded conditions.

2.5 Mobility of heavy metal in soil

Taylor and Griffin (1981) conducted pot culture experiments to investigate the leaching behavior of lead, cadmium, and nickel applied to soil surfaces. They observed that lead remained localized in the uppermost layers of soil, while cadmium exhibited movement to significant depths. Nickel, on the other hand, showed a more even distribution throughout the entire soil profile. The researchers concluded that cadmium and nickel were more prone to mobility within the soil compared to lead.

Legret *et al.* (1988) reported that cadmium (Cd) showed high mobility in soil, frequently found in an exchangeable state as deep as 60-80 cm within the soil profile. Nickel (Ni) was predominantly associated with the oxidizable fraction of the soil and was observed to migrate to depths between 40-60 cm. Lead (Pb) was linked to the acid-soluble and reducible phases, reaching down to the 20-40 cm layer. Chromium (Cr) mostly remained concentrated in the uppermost layer of the soil.

In their study on the vertical migration of Cu, Zn, Ni, and Cr introduced into clay loam and sandy loam soils via urban waste compost, Giusquiani *et al.* (1992) observed higher increases in metal concentrations in the sandy loam soil. They noted that 70 to 80 percent of the water-soluble fraction of these metals remained concentrated in the upper 10 cm layer of the soils.

Swarup and Ulrich (1994) investigated the migration of applied zinc (Zn), lead (Pb), and cadmium (Cd) under steady-state unsaturated flow conditions in soil. They observed that a significant portion of the applied heavy metals remained concentrated in the top 10 cm of soil, leading to minimal recovery

of these metals in the leachate. They estimated that 87 to 96 percent of the total applied quantity was retained within the soil. Specifically, the recovery percentages of Zn, Pb, and Cd in the leachate were 12.6%, 6.0%, and 8.3%, respectively.

2.6 Effect of heavy metal on plants

Foy *et al*. (1978) highlighted the escalating use of heavy metals in diverse products and their ongoing release from foundries, smelting industries, combustion of coal, diesel, and heating oil, as well as impurities in fertilizers, contributing to the accumulation of toxic levels of these metals in soil. According to Hagemeyer *et al*. (1986), there has been increasing interest in recent years in studying the detrimental effects of heavy metals on plant growth and metabolism.

2.6.1 Growth of Plants

Shah and Dubey (1995) indicated that cadmium (Cd) in the growth medium caused a decrease in the length of roots and shoots. They also observed alterations in RNA levels and inhibition of ribonuclease activity due to Cd exposure.

Chen and Kao (1995) observed that the reduction of root growth induced by Cd was closely associated with the accumulation of proline in roots.

2.7 Methods to combat heavy metal pollution

Crooke (1956) discovered that lime application can alleviate nickel (Ni) toxicity in soils. By increasing the soil pH from 4.8 to 6.8 in a serpentine soil, the concentration of Ni in leaf dry matter decreased significantly from 188 to 77 ppm. Additionally, the application of potassium (K) was also found to mitigate Ni toxicity.

Raskin *et al*. (1994) introduced a novel approach known as phytoremediation, which utilizes plants to clean up soil and water contaminated with heavy metals. This technology is founded on the observation that specific plants possess the ability to accumulate heavy metals in their roots and shoots, often concentrating these metals to levels significantly higher than those found in the surrounding soil.

CHAPTER 3

MATERIALS AND METHODS

MATERIALS AND METHODS

This study entitled "Heavy Metal Accumulation in selected plants" was conducted at soil science department in KFRI, an autonomous institution under Kerala State Council for Science, Technology and Environment during February –May 2024.

The total and available content of Chromium (Cr), Lead (Pb), Nickel (Ni), Cadmium (Cd) and Zinc (Zn) in the samples of soils and plants collected from two different locations as indicated below were assessed by chemical analysis.

3.1 Study area

The study was conducted in Thrissur district, which is situated in the central part of Kerala lying between north latitudes 10° 31' 49.2420" and east longitudes 76° 12' 53.0244", the district is bound on north by Malappuram and Palakkad districts, east by Palakkad district, south by Ernakulam district and west by Lakshadweep Sea.

Athani and Laloor are the selected locations for heavy metal analysis.These are located in Thrissur district.

Athani in Thrissur district is a concentrated region of industrialization with several factories such as G.V. R Industries (Manufacturing of fabricated metal items), La –Famille industries de brilliante (Manufacturing of liquid detergents & cleaning chemicals), Vigneswara gases (Manufacturing of nitrous oxide), A One polimers (Manufacturing of plastic water tap),Rinoose Abrasives (Production of Aluminum abrasives).The whole area is located at 10.618015 latitude and 76.205343 longitude.

Laloor in Thrissur district is located at 10.518455 latitude and 76.184713 longitude is a residential area at a distance 5 km from the city. The entire area (3.37 ha) was considered as the centralized waste dumping site of erst while Kochi rajyam & Thrissur corporation. The waste dumping at Laloor started more than quarter century ago.
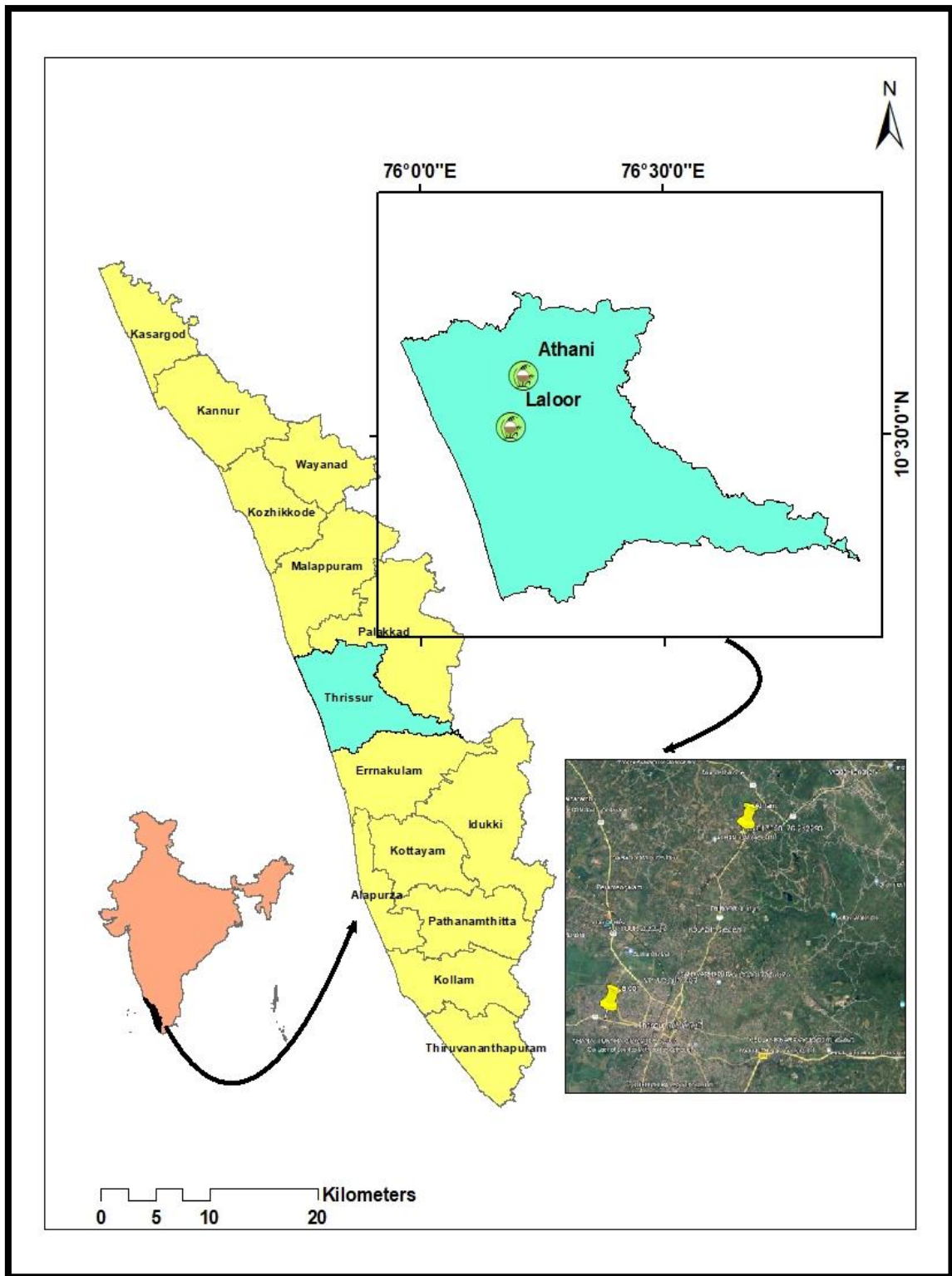
Figure 1: Location map of the study area

3.2 Soil analysis method

Soil testing is an essential component of soil resource management. Each sample collected must be a true representative of the area being sampled. Utility of the results obtained from the laboratory analysis depends very much on the sampling precision. Hence, collection of large number of samples is advisable so that sample of desired size can be obtained by sub-sampling. In general, sampling is done at the rate of one sample for every two-hectare area. However, at least one sample should be collected for a maximum area of five hectares. For soil survey work, samples were collected from a soil profile representative to the soil of the surrounding area.

Materials Required

Spade or auger, Polythene or cloth bags, a pan or bucket, Khurpi (for collecting profile samples)

Procedure of soil sample collection and method of dispatch

Scraped the surface to remove any litter and exposed the soil underneath. Used a spade to make a 'V'-shaped incision to a depth of 15cm at the sampling location. Extracted thick slices of soil from top to bottom along the exposed face of the 'V'-shaped cut and transfer them into a clean container. Thoroughly mixed the sample and eliminated any foreign materials such as roots, stones, pebbles, and gravel. Reduce the bulk sample to approximately half to one kilogram either by quartering or compartmentalization. Quartering involved dividing the well-mixed sample into four equal portions. Discarded two opposite quarters and remix the remaining two quarters; repeat the process until the desired sample size is achieved. In compartmentalization, spread the thoroughly mixed soil evenly over a clean, hard surface and created smaller compartments by drawing lines lengthwise and crosswise using fingers or small twigs. Collected a pinch of soil from each compartment. Repeated this process until the desired quantity of sample is obtained. Placed the collected sample into a clean, labeled cloth or polythene bag.

Figure 2: Showing soil Sampling process

Collection of soil samples and processing

Soil samples were collected by conducting heavy metal analysis. Four soil samples were taken from two different sites (two soil samples from each place). The two soil samples were taken from Athani industrial area with two plants with each and the two soil samples taken from Laloor with one plant with each. The sample collected from the field is air dried in shade by spreading on a clean sheet of paper after breaking the large lumps, if present, with a wooden mallet. Then the soil material is sieved through a 2 mm sieve. The material passing through the sieve was collected and stored in a clean container with proper labelling for laboratory analysis. For the determination of organic matter, it was desirable to grind a representative sub sample and sieve it through 0.2 mm (about 0.01 in) sieve.

Figure 3: Sieving the soil sample

3.3 Collection of plant sample

Whole plant samples of *Cleome rutidosperma, Vernonia cinerea, Eleutheranthera ruderalis, Alternanthera bettzickiana, Trianthema portulacastrum,Cleome viscosa* were collected from the locations mentioned under 3.1 and 3.2 above. The plants were first washed in running water and then in distilled water, pressed between folds of blotting paper and dried in shade, separated into roots and shoots, and dried in an electric oven at 40ºC.The dried samples were finally ground in an electric mixer and stored in an airtight container.

Figure 4: Cleome *rutidosperma*

Figure 5: *Vernonia cinerea*   **Figure 6: *Eleutheranthera ruderalis***

Figure 7: *Alternanthera bettzickiana*   **Figure 8:** *Trianthema portulacastrum*

Figure 9: *Cleome viscosa*

3.3 Analytical procedures and equipments

3.3.1 Soil pH

The pH of the soil is the measure of H$^+$ ion activity of the soil water system. It indicates whether the soil is acidic, neutral, or alkaline in nature. The pH is defined as the logarithm to the base 10 of the reciprocal of the hydrogen ion concentration.

$$pH = -\log(H^+) = \log 1/(H^+)$$
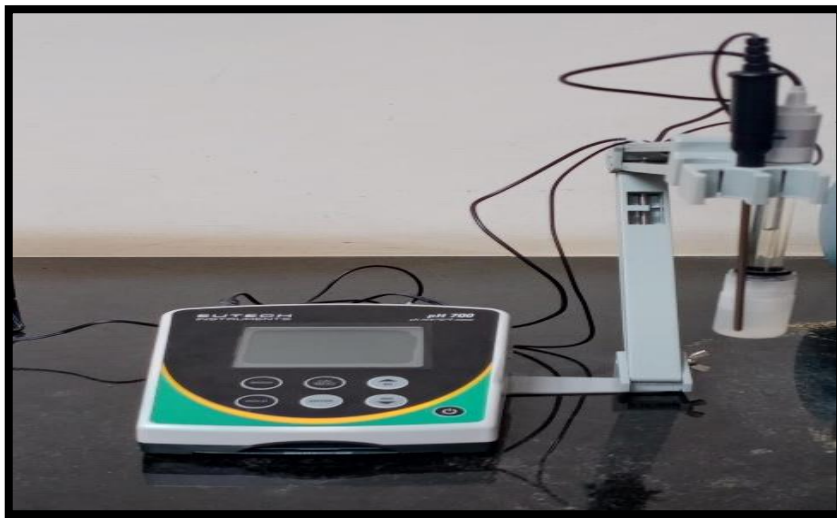
Figure 10: pH meter

Materials required

pH meter ,100 mL beakers, Glass rods, Buffer solution (pH 4.0,7.0 and 9.2)

Reagents

Standard buffer solutions:

Prepare buffer solutions of pH 4.0,7.0,9.2 using commercially available buffer tablets. Dissolve the respective tablets in freshly prepared distilled water and make up the volume to 100 mL. It is necessary to prepare fresh buffer solutions after every few days as these solutions do not keep for long.
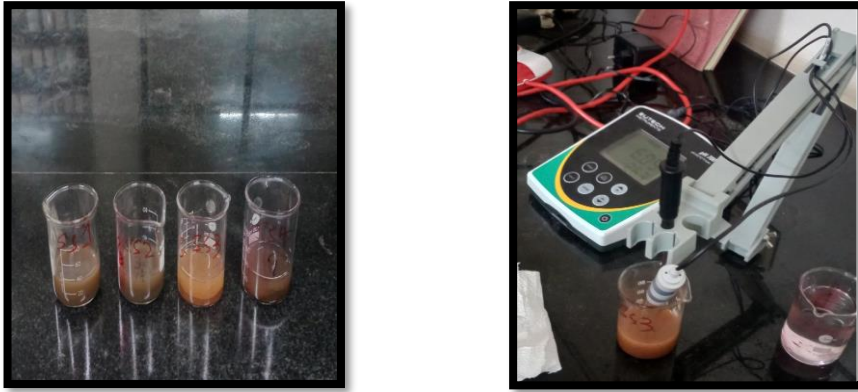
Figure 11: Steps of pH Reading

Measurement of pH

To determine the pH of soil, the pH meter was calibrated using buffer solutions. Measure out 10 grams of soil that has been passed through a 2 mm sieve and transfer it to a clean 50 or 100 mL beaker.Then, add 25 mL of distilled water.Stir the sample thoroughly using a glass rod and allow it to sit for thirty minutes.Rinse the electrodes carefully with a stream of distilled water and dry them with a piece of filter paper.Stir the soil suspension again just before taking the measurement.Submerge the electrodes into the beaker containing the soil water suspension and adjust the function switch to the pH range.Record the meter reading both in the supernatant solution and in the suspension.

3.3.2 Electrical conductivity

The electrical conductivity measurement gives the total amount of soluble salts present in the soil and is expressed as dSm^{-1} . As the number of soluble salts in a solution increases the electrical conductivity also increases. Electrical conductivity is measured in terms of the resistance offered to the flow of current using a conductivity bridge. Electrical conductivity in a soil water system is a measure of concentration of soluble salts and extend of salinity in the soil and is measured using a conductivity meter.

The clear supernatant of 1:2:5: soil: water suspension prepared for pH measurement was used for estimating EC.The conductivity meter was calibrated using 0.01N KCl solution and the cell constant determined. Then the conductivity of the soil-water suspension was determined using the instrument.

Figure 12 : Conductivity meter

Materials required

Instrument: Conductivity meter

Reagents

Standard potassium chloride solution:

Dry a small amount of potassium chloride of reagent grade at 60°C for 2 hours in a hot air oven. Weigh 0.7456 g of the dried potassium chloride and dissolve it in freshly prepared double distilled water, making up the volume to 1000 mL. At 25°C, this solution yields an electrical conductivity (EC) of 1.412 dSm^{-1}. The conductivity bridge is calibrated, and the cell constant is determined using this solution.

Procedure

To measure the electrical conductivity of the soil, switch on the conductivity bridge of the conductivity meter and wait for 10 minutes. Then check the instrument, with saturated CaSO4 solution and 0.1 N KCl solution. The EC of CaSO4 and KCl solutions should be 2.2 and 1.41 dSm-1 respectively. 10 g of soil is shaken intermittently with 25ml of distilled water in a 250 mL conical flask for 1hr and

allowed to stand. Alternatively, the clear extract after pH determination can be used for electrical conductivity measurement. The conductivity of the supernatant solution is measured with the help of a conductivity meter. Wash the electrodes carefully and immerse them into soil solution. Adjust the temperature correction. Adjust the meter knob until the magic eye of the null indicator is at the widest in width. The readings on the scale at this position indicate electrical conductivity. Multiply this by the cell constant to get specific conductivity.

3.3.3 Atomic absorption spectrometry

Heavy metals were analyzed using an atomic absorption spectrometer. Atomic absorption spectroscopy (AAS) is a spectroscopic analytical technique for the quantitative determination of chemical elements by measuring the absorption of electromagnetic radiation (light) by free atoms in the gaseous phase. In analytical chemistry, this method is employed to ascertain the concentration of an element (the analyte) in a sample. AAS is capable of determining over 70 different elements in solution or directly in solid samples.

This method determines the concentration of an element in a sample by detecting the absorption of radiation by the atomic vapor generated from the sample. This absorption occurs at a wavelength that is specific and characteristic of the element being analyzed. The fundamental principle relies on the absorption of energy by neutral atoms in their ground state when they are in the gaseous phase. The degree of absorption is directly proportional to the density of atoms present in the flame or gaseous state.

Fig. 13. Atomic Absorption spectrometer

When light emitted from an appropriate source passes through the atomic vapor of the sample, a detector measures the amount of light absorbed by comparing the intensity of radiation before and after it traverses the sample.

Samples intended for analysis are converted into atomic vapor where the majority of atoms are in their ground state. Under these conditions, atoms can only absorb radiation at specific wavelengths corresponding to narrow spectral lines. Atomic absorption occurs when the light beam passes through an atom with a specific width, typically 0.02 Å (angstroms).

Materials required

Instrument: Atomic absorption spectrometer (AAS)

Components of AAS are:

1.Radiation source 2. Atomizer 3. Monochromator 4. Wavelength 5. Burner 6. Slits and Lenses

7. Detectors 8. Phototube and amplifier

1. Radiation source

Typically, hollow cathode lamps are utilized to generate precise line sources of radiation. The hollow cathode emits resonance light. When a high voltage current is applied between the anode and cathode, the inert gas is ionized. These ions are drawn towards the hollow cathode. Fast-moving ions impact the hollow cathode, displacing metal atoms. These displaced atoms emit the characteristic spectrum of the metal. The narrow spectral lines are known as resonance radiation, which are absorbed by atoms.

2. Atomizer

The sample in the form of liquid is to be converted into vapour. For this purpose – acetylene gas is used. Air aspirates the solution into fine spray. Acetylene heats the sample. By this process the sample is converted into atomic vapour.

3.Monochromator

The monochromator should be able to pass the resonance line and filter out others. Isolates spectral region for the spectrum of the wavelength

4. Wavelength

Wavelength width is controlled by slits. These enable to get narrow light. Optimum width is 0.02 A^0

5. Burner

Rectangular and slot type. It provides a long enough light path to give a good absorption sensitivity.

6. Lenses

Gather radiation from different parts of optical system. Some instruments are double beam type. Radiation from the source is split into 3 beams of approximately equal intensity. One is reference beam, and the other is sample beam which passes through the sample.

7. Detectors

Are photomultipliers. There are separate cathode lamps for each element. The amount of absorbance by the sample can be determined by referring to the calibration curve. Solids, liquids, and gases can be analyzed. The organic substances are to be converted into inorganics. The sensitivity limit varies from 0.05 to 0.1 ppm.

8. Phototube and amplifier

The following factors are affecting the atomic absorption spectrometer. In general, an organic solvent enhances the absorption signal, and therefore, it may alter the absorption intensity: These can bond strongly with metal sand tend to reduce the signal intensity. EDTA could eliminate such an effect.

Chemicals and reagents used for Atomic absorption spectroscopy.

Nitric acid (HNO_3) and sulfuric acid (H_2SO_4) were used, and all were of analytical grade. The concentration of stock solution was 1000ppm.

Procedure

Estimation of heavy metal

Sample preparation

For the analysis, samples were ground into a fine powder and dried at 30-40°C for 6-8 hours in a controlled environment to remove moisture. Immediately after drying, 1.0g of soil and 0.5g of plant material were accurately weighed and placed in a test tube. To this, 9 mL of concentrated HNO3 and 4 mL of concentrated H2SO4 were added, and the mixture was placed in a digestion unit for 24 hours. The mixture was heated at 120-130°C for 5-6 hours until fumes ceased, resulting in a clear solution. After cooling to room temperature, the solution was filtered using Whatman filter paper No. 42. The entire filtrate was combined and made up to 50 mL in a standard flask. Each sample was analyzed twice, and the experiment was repeated 5 times.

The AAS system (AA240) utilized included both flame and graphite furnace options with a wavelength range of 185-900 nm and a photomultiplier detector ranging from 185.0 to 600.0 nm. The solvents employed in the study were of analytical grade. The concentration of the stock solutions used in AAS for elements such as copper, chromium, cadmium, lead, arsenic, and mercury was 1000 ppm.

Calculation

Heavy metal (AAS) $= Reading \times \dfrac{Made\ up\ to}{Wt.of\ soil}$
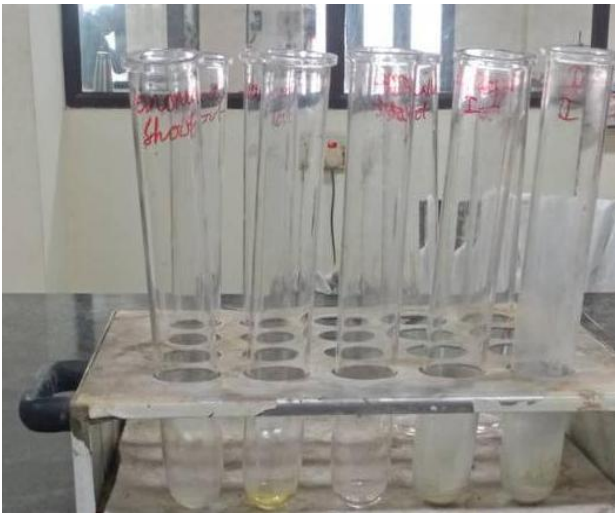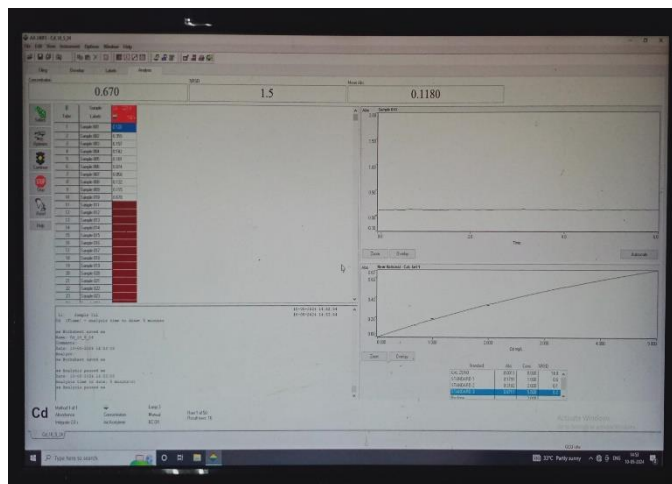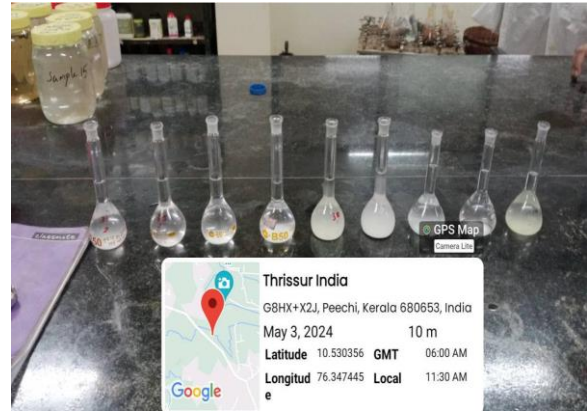
Fig 14 .Showing Steps in Heavy metal reading by using Atomic absorption spectrometer

3.3.4 Bioconcentration factor(BCF)

The bioconcentration factor evaluates the ability of plants to accumulate a heavy metal from the environment into their tissues. It is calculated as:

BCF = Concentration of heavy metal in plant / Concentration of heavy metal in.   soil

3.3.5 Translocation Factor (TF)

The translocation factor measures the efficiency of heavy metal transport from roots to above-ground plant parts.

It is calculated as:

TF = Metal concentration in stems or leaves/Metal concentration in roots

3.3.6 Organic carbon in soil

Soil organic matter is defined as the organic component of soil, comprising plant, animal, and microbial residues in various stages of decomposition, as well as the relatively stable soil humus. However, estimates of soil organic matter only include organic materials that pass through a 2 mm sieve with soil particles. Carbon is the predominant element in soil organic matter, constituting 48-58% of its total weight. Therefore, organic carbon measurements are commonly used to estimate organic matter content. For many years, the Van Bemmelen factor of 1.724 has been utilized, assuming that organic matter contains 58% organic carbon.

Organic carbon present in organic matter is oxidized by chromic acid in the presence of conc. H_2SO_4. Potassium dichromate on reaction with H_2SO_4 provides nascent oxygen which combines with carbon and forms CO_2. The HSO_4 enables easy digestion of organic matter by rendering heat of dilution. Only a certain quantity of chromic acid is used for oxidation. The excess chromic acid left unused by the organic matter is determined by back titration with 0.5 N ferrous sulphate or ferrous ammonium sulphate using diphenylamine indicator.

$2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 2KSO_4 + 2Cr_2(SO_4)_3 + H_2O + 6(O)$

$3C + 6 (O) \rightarrow 3CO_2$
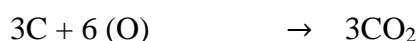
Materials required

Conical flasks 250 mL, Burette 50 mL, Pipette, Sieves, Pestle and mortar, Wash bottle with distilled water

Chemicals and reagents

Potassium dichromate ($K_2Cr_2O_7$) - 1N: Dissolve 49.04 g of reagent grade $K_2Cr_2O_7$ (dried at 105° C) in water and diluted solution to a volume of 1000 ml.

Ferrous ammonium sulphate hexahydrate solution - 0.5 N: Dissolve 197 g of reagent grade Fe $(NH_4)_2SO_4.6H_2O$ in water add 15 ml of concentrated sulphuric acid. Cool the solution and dilute it to a volume of 1000 ml. Standardize this reagent daily by titrating it against 10 ml of 1 N potassium dichromate.

O - phenanthroline - ferrous complex (ferroin) - 0.025M: Dissolve 14.85 g of O-phenanthroline monohydrate and 6.95 g of ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) in water. Dilute the solution to a volume of 1000 ml. The O - phenonthroline - ferrous complex is available under the name of Ferroin.

Sulphuric acid (H_2SO_4) concentrated: If Cl^- is present in soil, add Ag_2SO_4 to the acid @ 15 g L^{-1}

Procedure

Estimation of Organic carbon

Sample preparation

For organic carbon determination, precisely weigh 0.5 g of soil ground through a 0.2 mm sieve and transfer it to a 250 mL conical flask. Add 5 mL of 1N potassium dichromate and gently swirl the flask to disperse the soil in the solution. Then, add 10 mL of concentrated H2SO4. Swirl the flask gently immediately to mix the soil and reagents, and then vigorously for one minute. Place the flask on an asbestos sheet for approximately 30 minutes to prevent heat damage to the table surface. After 30 minutes, add 20 mL of water to the flask. Add 3-4 drops of o-phenanthroline indicator and titrate the solution with 0.5 N ferrous ammonium sulfate. Initially, the color is dull green and gradually turns to a cloudy blue as titration progresses. The endpoint is very distinct, shifting sharply to a bright green color. Concurrently, perform a blank titration (without soil) and record the volume of 0.5 N ferrous ammonium sulfate consumed for the blank solution.

Calculation

Organic carbon (%) = $10 \left(\frac{B-T}{B}\right) \times 0.003 \times \frac{100}{S}$

Where,

B = Volume of ferrous ammonium sulfate required for blank titration in mL.

T = Volume of ferrous ammonium sulfate needed for soil sample in mL.

S = Weight of the soil in gram
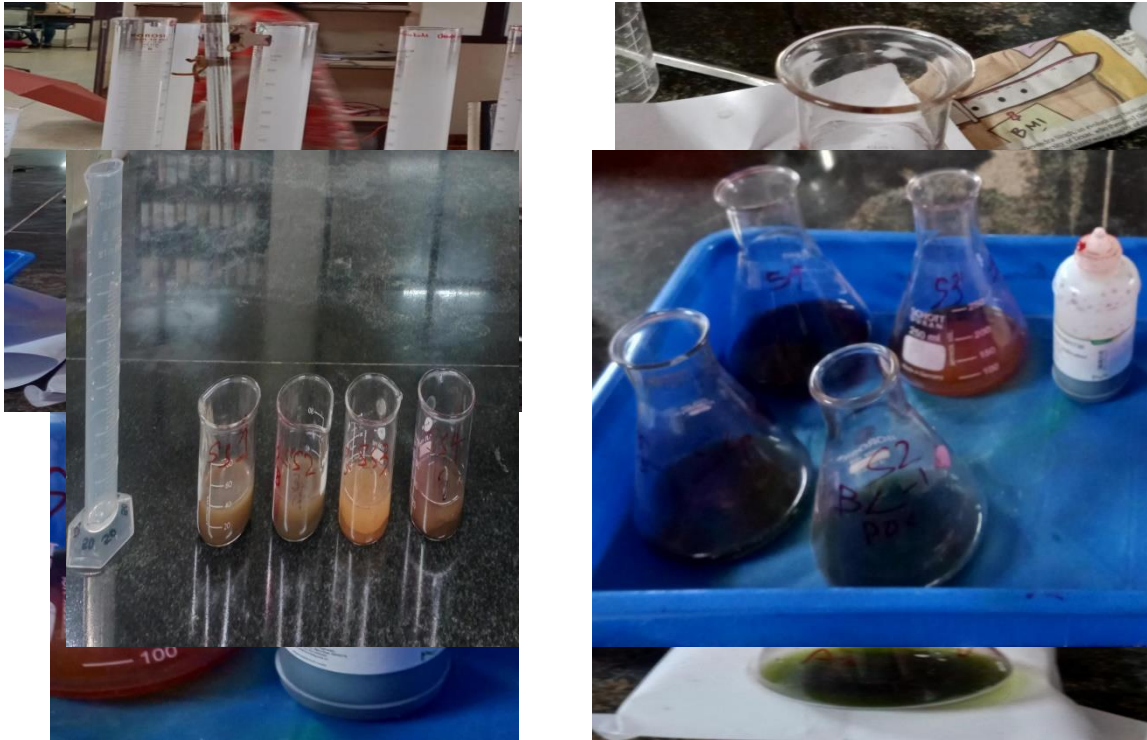
Figure 15: Showing steps in estimating organic carbon

3.3.7 Distilled water still

Every analytical laboratory needs large quantities of distilled water for daily use. This is produced with distilled water still. In this tap water or water from any other source is allowed to flow continuously at a constant rate through the heating coils. The liquid -water is converted to steam and comes at the exit leaving behind the salts. The steam is condensed and collected as distilled water.

3.3.8 Mechanical shaker

Most of estimation of soil requires constant shaking over a period and this cannot be performed manually. Hence, electrically operated shakers are used

.

Figure 16 : Mechanical shaker

3.3.9 Glass wares

Burettes, pipettes, standard flasks, measuring jar, conical flask, funnel etc.

CHAPTER 4

RESULT & DISCUSSION

RESULT AND DISCUSSION

The data on basic soil properties such as pH, Electrical conductivity, Organic carbon, and content of heavy metals viz; chromium, nickel, zinc cadmium and lead are given and discussed below.

4.1 Soil pH

The data on soil pH given in Table 1 indicated that the pH of the soil in the study area varied widely ranging from 6.79 to 8.14. This points out that the soils were neutral to alkaline in reaction. The lowest pH value (6.79) was recorded at Laloor and the highest pH value (8.14) in Athani.

4.2 Electrical conductivity

Electrical conductivity (EC) is a measure of soluble salts present in water. The conductivity of solution increases with the increase in the amount of ions. The data on electrical conductivity in the present study ranged from 0.135 µS/cm to 0.955 µS/cm and this indicated a wide range of salinity in the soil. The various polluted areas in the decreasing order of salinity were Laloor > Athani. The higher salinity is at Laloor compared to Athani.

4.3 Organic carbon

The determination of total organic carbon is an essential part of any soil characterization or ecological assessment since its presence or absence can markedly influence how chemicals will react in the soil (Schumacher,2002).

The soil organic carbon content in the study area ranged from 0.628 % (SS3 Athani) to 5.485 % (SS4 Laloor).The various polluted areas in the decreasing order of organic carbon was Laloor > Athani

4.4 Heavy metal status in soil

The soils collected for the study represent soil samples from Industrial area soils and waste dumping site soils. The data presented in Table 1 indicate the accumulation of Cr, Ni,Zn,Cd and Pb in each soil showing their total and extractable content.

Chromium concentrations in the soil samples range from 1.95 ppm to 9.9 ppm.

Nickel concentrations in the soil samples range from 24.32 ppm to 51.25 ppm.

Zinc concentrations in the soil samples range from 79.04 ppm and 322.25 ppm.

Cadmium concentrations in the soil samples range from 5.75 ppm to 33.5 ppm.

Lead concentrations in the soil samples range from 75 ppm to 156.5 ppm

SI No	Sample name / Site	Soil pH	Electrical conductivity (µS/cm)	Organic carbon (%)	Heavy metal reading AAS (ppm)				
					Chromium (Cr)	Nickel (Ni)	Zinc (Zn)	Cadmium (Cd)	Lead (Pb)
1	Soil sample 1- Athani	8.14	0.135	0.742	1.95	24.32	322.25	5.75	83.5
2	Soil sample 2- Athani	7.75	0.259	0.628	21.9	30.3	79.045	33.5	75
3	Soil sample 3 - Laloor	6.79	0.199	4.342	9.9	51.25	138.71	6.25	156.5
4	Soil sample 4 - Laloor	7.38	0.955	5.485	5.1	25.4	94.43	5.9	138.5

Table 1. pH, Electrical conductivity, Organic carbon, Heavy metal content in soil samples

4.5 Heavy metal content in selected plants

The content of heavy metals in different plants collected from two different locations of soil sample collection are presented in Table 2. Among the five elements studied Zn was the most abundant element in plants. In all cases, above ground plant parts registered much lower values of all heavy metals compared to roots.

Cleome rutidosperma

Chromium (Cr) content present in the root of *Cleome rutidosperma* was 2.75 ppm and in the shoot it is 6.35 ppm.Nickel (Ni) content present in the root is 19.25 and in the shoots it is 21.6. ppm.Zinc (Zn) content present in roots is 81.4 ppm and the content present in shoots is 83.7 ppm. Cadmium (Cd) content present in roots is 6.4 ppm and in shoots it is 17.95 ppm.Lead (Pb) content in roots is 9.2ppm and in shoots it is 88.5 ppm.

Vernonia cinerea

Chromium (Cr) content present in the root of *Vernonia cinerea* is -1.3 ppm and in the shoot it is 5.5 ppm.Nickel (Ni) content present in the roots is 27.95 ppm and in the shoots it is 27.1ppm.Zinc(Zn) content present in roots is 50.14 ppm and the content present in shoots is 45.745 ppm. Cadmium (Cd) content present in roots is 7.85 ppm and in shoots it is 7.1 ppm.Lead (Pb) content in roots is 81.5 ppm and in shoots it is 73.5 ppm.

Eleutheranthera ruderalis

Chromium(Cr) present in the roots of Eleutheranthera ruderalis is -2.95 ppm and in shoots it is -1.3 ppm.Nickel content present in roots is 32.45 ppm and in shoots it is 28.95 ppm.Zinc (Zn) content in the roots is 656.8 ppm and in the shoots is 6.395 ppm.Cadmium (Cd) present in the roots is 5.05 ppm and in shoots is 3.7 ppm. Lead (Pb) is present in the roots is a 98 ppm and in shoots it is 80.5 ppm.

Alternanthera bettzickiana

Chromium (Cr) present in the roots of *Alternanthera bettzickiana* 0.95 ppm and in the shoot it is 1.05 ppm.Nickel (Ni) present in the root is 25.6 ppm and in the shoot is 29.5 ppm. Zinc (Zn) present in the root is 57.635 ppm and in the shoot it is 38.38 ppm.Cadmium (Cd) present in the root is 2.9 ppm and in the shoot it is 6.6 ppm..Lead (Pb) present in the root is 87 ppm and in the shoot it is 69.5 ppm.

Cleome viscosa

Chromium (Cr) present in the root of *Cleome viscosa* is 10.05 ppm and in the shoot it is 26.85 ppm.Nickel(Ni) present in the root is 12.15 ppm and in the shoot it is 10.15 ppm.Zinc (Zn) present in the root is 35.45 ppm and in the shoot it is 109.15 ppm.Cadmium (Cd) present in the root is 0.55 ppm and in the shoot it is 0.75 ppm. Lead (Pb) is the present in the root is 145.5 ppm and in the shoot it is 163 ppm.

Trianthema portulacastrum

Chromium (Cr)present in the root of *Trianthema portulacastrum* is 0.25 ppm and in the shoot it is 1.5 ppm. Nickel (Ni) present in the root is 12.5 ppm and in the shoot it is 5.35 ppm.Zinc (Zn) present in the root is 127.6 ppm and in the shoot it is 46.85 ppm.Cadmium (Cd) present in the root is 4.25 ppm and in the shoot it is 2.3 ppm.Lead (Pb) present in the root is 148.5 ppm and in the shoot it is 141.5 ppm.

SI No	Sample name		Heavy metal reading AAS (ppm)				
			Cr	Ni	Zn	Cd	Pb
1	*Cleome rutidosperma*	Root	2.75	19.25	81.4	6.4	92
		Shoot	6.35	21.6	83.7	17.95	88.5
2	*Vernonia cinerea*	Root	-1.3	27.95	50.14	7.85	81.5
		Shoot	5.5	27.1	45.745	7.1	73.5
3	*Eleutheranthera ruderalis*	Root	-2.95	32.45	656.8	5.05	98
		Shoot	-1.3	28.95	6.395	3.7	80.5
4	*Alternanthera bettzickiana*	Root	0.95	25.6	57.635	2.9	69.5
		Shoot	1.05	29.5	38.38	6.6	87
5	*Cleome viscosa*	Root	10.05	12.15	35.45	0.55	145.5
		Shoot	26.85	10.15	109.15	0.75	163
6	*Trianthema portulacastrum*	Root	0.25	12.5	127.6	4.25	148.5
		Shoot	1.5	5.35	46.85	2.3	141.5

Table .2. Heavy metal content in plant samples collected from Athani and Laloor

4.6 Analysis of Bioconcentration factor and Translocation factor in selected plants

The Bioconcentration Factor (BCF) values for heavy metals in roots and shoots of various plants from Athani indicate significant variations in metal uptake. *Cleome rutidosperma* shows high BCF values for Lead (92 in roots, 88.5 in shoots) and Zinc (81.4 in roots, 83.7 in shoots), with Chromium, Nickel, and Cadmium also displaying notable values. *Vernonia cinerea* exhibits substantial BCF values for Nickel (27.95 in roots, 27.1 in shoots) and Lead (81.5 in roots, 73.5 in

shoots), despite an anomalous negative BCF for Chromium in roots. *Eleutheranthera ruderalis* has an exceptionally high BCF for Zinc in roots (656.8) but low values in shoots, with Lead also highly concentrated (98 in roots, 80.5 in shoots). *Alternanthera bettzickiana* accumulates Lead (69.5 in roots, 87 in shoots) and Zinc (57.635 in roots, 38.38 in shoots) effectively. *Cleome viscosa* demonstrates remarkable BCF values for Lead (145.5 in roots, 163 in shoots) and Zinc (35.45 in roots, 109.15 in shoots), with the highest Chromium BCF found in shoots (26.85). Lastly, *Trianthema portulacastrum* shows high Lead (148.5 in roots, 141.5 in shoots) and Zinc (127.6 in roots, 46.85 in shoots) BCF values, indicating strong metal uptake and translocation capacities among these species.

SI No	Sample name		BCF Value of				
			Cr	Ni	Zn	Cd	Pb
1	*Cleome rutidosperma*	Root	1.4	0.79	0.252	1.113	0.11
		Shoot	3.25	0.88	2.59	3.12	1.05
2	*Vernonia cinerea*	Root	-0.66	1.14	0.155	1.36	0.97
		Shoot	2.82	1.114	0.148	1.2	0.88
3	*Eleutheranthera ruderalis*	Root	-1.34	1.07	8.3	1.442	1.3
		Shoot	-0.05	0.93	0.08	0.11	1.07
4	*Alternanthera bettzickiana*	Root	0.043	0.844	0.729	0.086	0.926
		Shoot	0.047	0.973	0.4855	1.97	1.16
5	*Cleome viscosa*	Root	1.015	0.237	0.255	0.088	0.929
		Shoot	2.68	0.198	0.786	0.12	1.04
6	*Trianthema portulacastrum*	Root	0.049	0.492	1.35	0.72	1.072
		Shoot	0.294	0.21	0.496	0.389	1.021

Table 3 Showing BCF value of selected plant samples

Bioconcentration Factor of selected plants
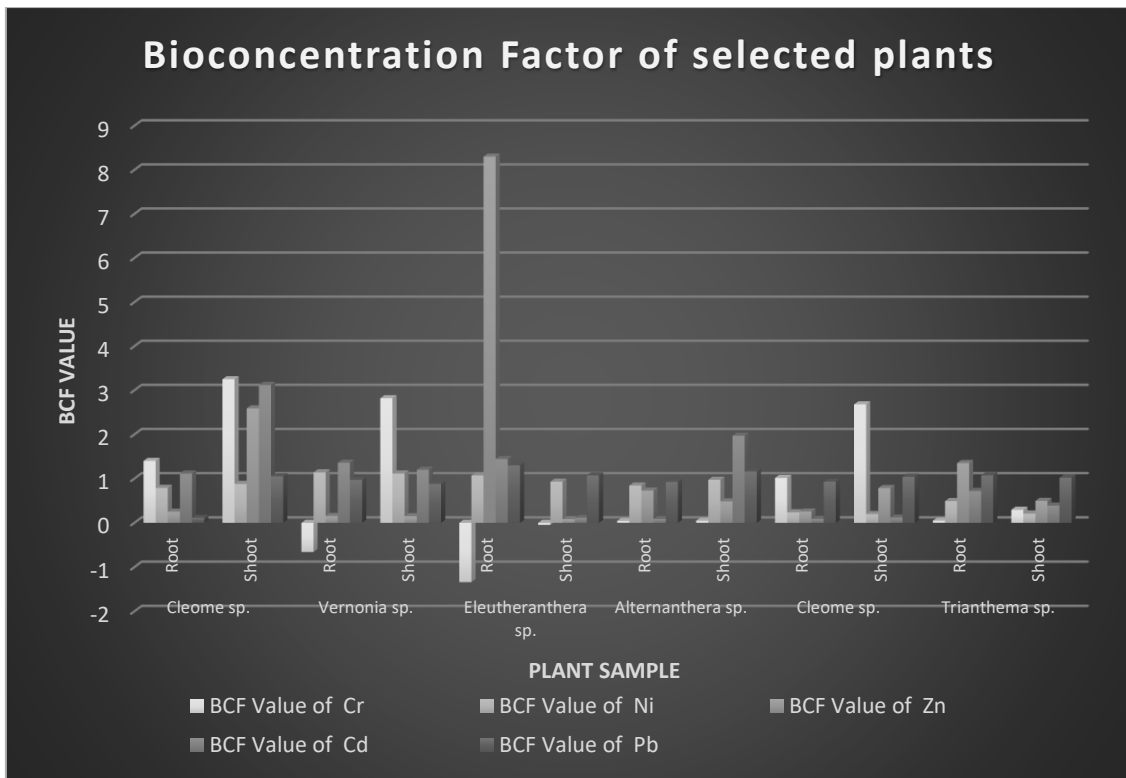
The Translocation Factor (TF) values for heavy metals in different plant species reveal their abilities to translocate metals from roots to shoots. *Cleome rutidosperma* demonstrates high TF values for Cadmium (2.80) and Chromium (2.30), indicating effective translocation, while values for Nickel (1.12) and Zinc (1.02) are moderate, and Lead is lower (0.96). *Vernonia cinerea* shows a negative TF for Chromium (-4.230), suggesting an anomaly or analytical error, with low translocation for Nickel (0.96), Zinc (0.952), Cadmium (0.904), and Lead (0.901). *Eleutheranthera ruderalis* has a high TF for Zinc (9.73), suggesting efficient translocation, but lower values for Chromium (0.440), Nickel (0.892), Cadmium (0.732), and Lead (0.821). *Alternanthera bettzickiana* displays effective translocation for Cadmium (2.275), Lead (1.25), and moderate values for Nickel (1.15) and Chromium (1.105), but lower for Zinc (0.66). *Cleome viscosa* exhibits high TF values for Chromium (2.67), Zinc (3.07), and Cadmium (1.36), with moderate translocation for Lead (1.12) and lower for Nickel (0.835). *Trianthema portulacastrum* shows a notably high TF for Chromium (6), but low translocation for Nickel (0.428), Zinc (0.367), Cadmium (0.541), and moderate for Lead (0.952). These results highlight the varying translocation efficiencies among the plant species, which is crucial for their potential use in phytoremediation.

SI NO	Plant Name	TF Value				
		Cr	Ni	Zn	Cd	Pb

1	*Cleome rutidosperma*	2.3	1.12	1.02	2.8	0.96
2	*Vernonia cinerea*	-4.23	0.96	0.952	0.904	0.901
3	*Eleutheranthera ruderalis*	0.44	0.892	9.73	0.732	0.821
4	*Alternanthera bettzickiana*	1.105	1.15	0.66	2.275	1.25
5	*Cleome viscosa*	2.67	0.835	3.07	1.36	1.12
6	*Trianthema portulacastrum*	6	0.428	0.367	0.541	0.952

Table 4 Showing TF Value of selected plant samples
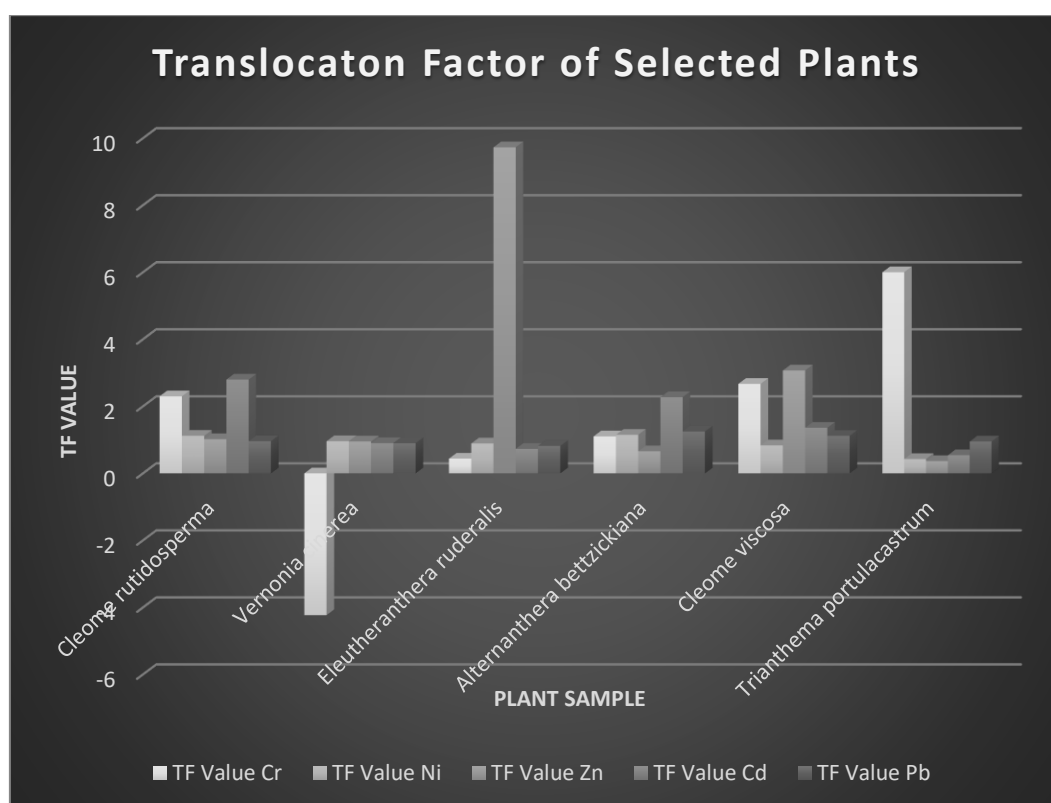
DISCUSSION

The important results of the experiments carried out to investigate the status and impact of heavy metals in soils and plants in the polluted areas presented in the preceding chapter are discussed in the

light of fundamental principles and evidences from published literature, keeping in view the objectives proposed in the study.

Status of heavy metals in soils and plants. The concentrations of heavy metals (Chromium, Nickel, Zinc, Cadmium, and Lead) in different parts (roots and shoots) of *Cleome rutidosperma* indicate how these metals are distributed within the plant. Chromium concentrations are higher in the shoots compared to the roots. This suggests that *Cleome rutidosperma* has the ability to accumulate chromium from the soil and transport it to the above-ground parts of the plant.

Nickel concentrations are slightly higher in the shoots than in the roots. Similar to chromium, *Cleome rutidosperma* shows a moderate accumulation of nickel in the above-ground parts.

Zinc concentrations are also higher in the shoots compared to the roots, indicating effective translocation of zinc within the plant.

Cadmium concentrations show a significant increase from roots to shoots. This suggests that *Cleome rutidosperma* not only absorbs cadmium from the soil but also accumulates it in higher concentrations in its above-ground parts.

Lead concentrations show a dramatic increase from roots to shoots, indicating a strong ability of *Cleome rutidosperma* to accumulate lead in its shoots.

Overall, these data indicate that *Cleome rutidosperma* is capable of accumulating significant amounts of heavy metals from the soil, with varying degrees of translocation to its shoots. This characteristic makes it potentially useful for phytoextraction purposes, where plants are used to remove heavy metals from contaminated soils. However, the high accumulation of metals like cadmium and lead in the shoots also raises environmental and health concerns, as these metals can be toxic to organisms including humans if they enter the food chain.

The negative value for chromium in the roots (-1.3 ppm) might indicate either an extremely low concentration near the detection limit or possibly an analytical anomaly. In contrast, chromium is detected in the shoots at 5.5 ppm. This suggests that *Vernonia cinerea* can accumulate chromium in its above-ground parts, indicating some level of phytoaccumulation capability. Chromium is not typically accumulated in roots in negative concentrations in natural contexts, so this negative value might warrant further investigation or confirmation.

Nickel concentrations are slightly higher in the roots compared to the shoots, but the difference is minimal. This indicates that *Vernonia cinerea* has a balanced distribution of nickel between its roots and shoots, with moderate translocation of nickel within the plant.

Zinc concentrations are higher in the roots compared to the shoots, suggesting that *Vernonia cinerea* accumulates zinc primarily in its roots. There is some translocation of zinc to the shoots, but at lower concentrations.

Cadmium concentrations are slightly higher in the roots compared to the shoots, indicating moderate accumulation of cadmium in both parts of the plant. *Vernonia cinerea* shows some capability to translocate cadmium from roots to shoots, although the difference is not substantial. Lead concentrations are higher in the roots compared to the shoots, indicating that *Vernonia cinerea* accumulates lead primarily in its roots. There is less translocation of lead to the shoots compared to the roots.

In summary, *Vernonia cinerea* exhibits varying patterns of heavy metal accumulation and translocation. It shows effective accumulation of chromium and lead in roots, while nickel and cadmium are moderately accumulated in both roots and shoots. Zinc is primarily accumulated in the roots with lower concentrations in the shoots. These findings highlight the potential of *Vernonia cinerea* for phytoremediation applications, where it could be used to extract heavy metals from contaminated soils, particularly chromium and lead from the roots, and potentially contribute to detoxification efforts in polluted environments.

Both values for chromium in the roots and shoots are negative, which is unusual and might indicate either extremely low concentrations near the detection limit or an analytical anomaly. Chromium is not typically found in negative concentrations naturally, suggesting that further verification or re-evaluation of the analytical results may be necessary.

Nickel concentrations are higher in the roots compared to the shoots, indicating effective accumulation of nickel in the roots of *Eleutheranthera ruderalis*. There is some translocation of nickel to the shoots, but at slightly lower concentrations.

Zinc concentrations are significantly higher in the roots compared to the shoots. This suggests that *Eleutheranthera ruderalis* accumulates zinc predominantly in its roots, with very little translocation to the shoots.

Cadmium concentrations are higher in the roots compared to the shoots, indicating some level of cadmium accumulation in both parts of the plant. There is moderate translocation of cadmium from roots to shoots, but the concentrations in shoots are lower.

Lead concentrations are higher in the roots compared to the shoots. *Eleutheranthera ruderalis* accumulates lead primarily in its roots, with some translocation to the shoots at lower concentrations.

In summary, *Eleutheranthera ruderalis* demonstrates varying patterns of heavy metal accumulation and translocation. It shows potentially anomalous negative values for chromium in both roots and shoots, which may require further investigation. Nickel and lead are accumulated predominantly in the roots with some translocation to the shoots. Zinc is highly accumulated in the roots with minimal translocation, while cadmium shows moderate accumulation in both parts of the plant with higher concentrations in the roots. These findings provide insights into the metal uptake strategies of *Eleutheranthera ruderalis*, which could be useful in understanding its role in phytoremediation or its adaptation to metal-contaminated environments.

Chromium concentrations in *Alternanthera bettzickiana* are relatively low and quite similar between roots and shoots. This suggests that there is minimal accumulation or translocation of chromium within the plant, indicating it does not actively accumulate this metal to a significant degree.Nickel concentrations are higher in the shoots compared to the roots, indicating effective translocation of nickel from roots to shoots in *Alternanthera bettzickiana*. This plant shows a moderate ability to accumulate and translocate nickel, potentially indicating a role in nickel phytoextraction.

Zinc concentrations are significantly higher in the roots compared to the shoots. This indicates that *Alternanthera bettzickiana* accumulates zinc primarily in its roots, with less translocation to the shoots. The lower concentration in shoots suggests limited movement of zinc within the plant.

Cadmium concentrations are higher in the shoots compared to the roots, indicating efficient translocation of cadmium from roots to shoots in *Alternanthera bettzickiana*. This plant demonstrates a capability to accumulate and transport cadmium to its above-ground parts.

Lead concentrations are higher in the roots compared to the shoots. *Alternanthera bettzickiana* accumulates lead primarily in its roots, with some translocation to the shoots at lower concentrations. The lower concentration in shoots suggests limited movement of lead within the plant.

In summary, *Alternanthera bettzickiana* shows varying patterns of heavy metal accumulation and translocation. It demonstrates minimal accumulation of chromium, moderate accumulation and translocation of nickel and cadmium, and significant accumulation of zinc and lead primarily in the roots. These findings are important for understanding the potential of *Alternanthera bettzickiana* in phytoremediation applications, where it could potentially be used for the removal of heavy metals from contaminated soils, especially for metals like nickel, cadmium, zinc, and lead.

Chromium concentrations are significantly higher in the shoots compared to the roots. This indicates that *Cleome viscosa* accumulates chromium and translocates it efficiently from roots to shoots. Such high accumulation in shoots suggests a strong phytoaccumulation capability for chromium in this plant species.

Nickel concentrations are higher in the roots compared to the shoots, but the difference is not substantial. This suggests moderate accumulation of nickel in both roots and shoots of *Cleome viscosa,* with similar concentrations indicating balanced translocation or retention of nickel within the plant.

Zinc concentrations are significantly higher in the shoots compared to the roots. *Cleome viscosa* shows a remarkable ability to accumulate zinc and translocate it to the above-ground parts of the plant. This high accumulation in shoots indicates a potential role in zinc phytoextraction.

Cadmium concentrations are slightly higher in the shoots compared to the roots. This suggests that *Cleome viscosa* can translocate cadmium to its shoots, although the overall concentrations are relatively low. The plant shows a moderate ability to accumulate cadmium.

Lead concentrations are higher in the shoots compared to the roots. *Cleome viscosa* accumulates lead primarily in its roots but also effectively translocates it to the shoots. The higher concentration in shoots suggests significant movement and accumulation of lead within the plant.

In summary, Cleome viscosa exhibits varying patterns of heavy metal accumulation and translocation. It shows strong accumulation and translocation of chromium and zinc to the shoots, moderate accumulation of nickel and cadmium, and significant accumulation of lead primarily in the shoots. These findings highlight the potential of *Cleome viscosa* for phytoextraction and phytostabilization of heavy metals, depending on the specific metal contaminants present in the soil.

Chromium concentrations are higher in the shoots compared to the roots. *Trianthema portulacastrum* shows some ability to accumulate chromium and translocate it to the shoots, although the concentrations are relatively low overall.

Nickel concentrations are higher in the roots compared to the shoots. This suggests that *Trianthema portulacastrum* accumulates nickel primarily in its roots, with less translocation to the shoots. The lower concentration in shoots indicates limited movement of nickel within the plant.

Zinc concentrations are significantly higher in the roots compared to the shoots. *Trianthema portulacastrum* accumulates zinc predominantly in its roots, with much lower concentrations in the shoots. This indicates limited translocation of zinc from roots to shoots.

Cadmium concentrations are higher in the roots compared to the shoots. *Trianthema portulacastrum* shows some capability to accumulate cadmium in both roots and shoots, with slightly higher concentrations in the roots. There is moderate translocation of cadmium from roots to shoots.

Lead concentrations are higher in the roots compared to the shoots. *Trianthema portulacastrum* accumulates lead primarily in its roots, with significant translocation to the shoots. The high concentration in shoots suggests effective movement and accumulation of lead within the plant.

In summary, *Trianthema portulacastrum* demonstrates varying patterns of heavy metal accumulation and translocation. It shows moderate accumulation and translocation of chromium, significant accumulation of nickel in roots with limited translocation to shoots, high accumulation of zinc in roots with minimal translocation, moderate accumulation and translocation of cadmium, and significant accumulation and translocation of lead. These findings provide insights into the metal uptake strategies of *Trianthema portulacastrum*, which could be useful in understanding its potential for phytoremediation or as an indicator plant for heavy metal contamination in soil environments.

SUMMARY AND CONCLUSION

This study investigated the accumulation and translocation of heavy metals (Chromium, Nickel, Zinc, Cadmium, and Lead) in six plant species: *Cleome rutidosperma*, *Vernonia cinerea*, *Eleutheranthera ruderalis*, *Alternanthera bettzickiana*, *Cleome viscosa*, and *Trianthema portulacastrum*. The results indicate varying patterns of metal uptake and distribution within the plants. *Cleome rutidosperma* and *Cleome viscosa* showed significant accumulation and translocation of several metals, especially Chromium and Zinc, to their shoots, suggesting their potential for phytoextraction. *Vernonia cinerea* and *Eleutheranthera ruderalis* accumulated higher concentrations of metals in their roots, particularly Chromium and Lead, indicating their role in phytostabilization. *Alternanthera bettzickiana* and *Trianthema portulacastrum* demonstrated moderate to high accumulation of Nickel, Cadmium, and Zinc, with varying degrees of translocation to shoots. These findings highlight the potential use of these plants in phytoremediation strategies for cleaning up heavy metal-contaminated soils.

Key Findings and Future Research Areas:

- **Key Findings**:
  - **Cleome rutidosperma**: High accumulation and translocation of Chromium, Zinc, Cadmium, and Lead to shoots.
  - **Vernonia cinerea**: Effective accumulation of Chromium and Lead in roots, with moderate translocation of Nickel and Cadmium.
  - **Eleutheranthera ruderalis**: Accumulates Nickel, Zinc, and Lead primarily in roots, with minimal translocation to shoots.
  - **Alternanthera bettzickiana**: Moderate translocation of Nickel and Cadmium to shoots, high accumulation of Zinc and Lead in roots.
  - **Cleome viscosa**: Strong phytoaccumulation capability for Chromium and Zinc in shoots, significant Lead translocation.
  - **Trianthema portulacastrum**: Moderate accumulation and translocation of Chromium, Nickel, Cadmium, and Lead, with high Zinc accumulation in roots.
- **Future Research Areas**:
  - Investigate the genetic and molecular mechanisms underlying heavy metal uptake and translocation in *Cleome* species to enhance their phytoextraction efficiency.
  - Conduct long-term field studies to assess the practical applications and environmental impacts of using these plant species for phytoremediation in various contaminated sites.

o Explore the potential of combining these plant species with soil amendments or microbial inoculants to improve heavy metal removal from contaminated soils.

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