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**Treatment of Water Contaminated with Heavy Metals
Cadmium and Lead Using Aquatic Plants *Eichhornia
crassipes* and *Azolla pinnata***

A Thesis

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of Al-Qadisiyah, in partial fulfillment of the requirements for
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BY

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

"وَلَسَوْفَ يُعْطِيكَ رَبُّكَ فَتَرْضَىٰ"

صدق الله العلي العظيم

الضحى: (٥)

Dedication

At the completion of this academic journey, I stand grateful for every challenge I encountered and for every blessing from Allah that enabled me to overcome obstacles and successfully complete this research.

To the symbol of sacrifice, dignity, and steadfastness, whose path inspires the values of patience, truth, and the pursuit of righteousness—my Master, Imam Hussein (Peace Be Upon Him).

To the light that illuminated my path and to the sincere prayers that continually strengthened my determination and hope—to my beloved mother, whose endless love, patience, support, and encouragement made this achievement possible.

To my dear father, who paved the way for my education, supported me with unwavering dedication, and has always been a source of guidance and inspiration.

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And to all those who seek knowledge, contribute to its advancement, and work sincerely toward the betterment of society and the preservation of our environment, I dedicate this humble work.

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Finally, I pray that this work will be beneficial and accepted with Allah's blessings, and that He rewards all those who contributed to its completion.

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List of Abbreviations

Abbreviation	Full Name
(ROS)	Reactive Oxygen Species
(ATP)	reducing energy production
(CAT)	Catalase
(CRD)	randomized design
(DOM)	dissolved organic matter
(Eh)	redox potential
(EPS)	Exopolysaccharides
(GSH)	Glutathione
(HDPE)	high-density polyethylene
(HRT)	hydraulic retention periods
(MTs)	Metallothionein
(OH)	hydroxyl radicals
(SOD)	superoxide dismutase
(PCs)	Phytochelatin
(POD)	Peroxidase
(RE %)	efficiency indices
(-SH)	sulfhydryl groups
(PGPB)	Plant Growth-Promoting Bacteria
(NRAMPs)	Natural Resistance-Associated Macrophage Protein
(HMA)	Heavy Metal ATPases

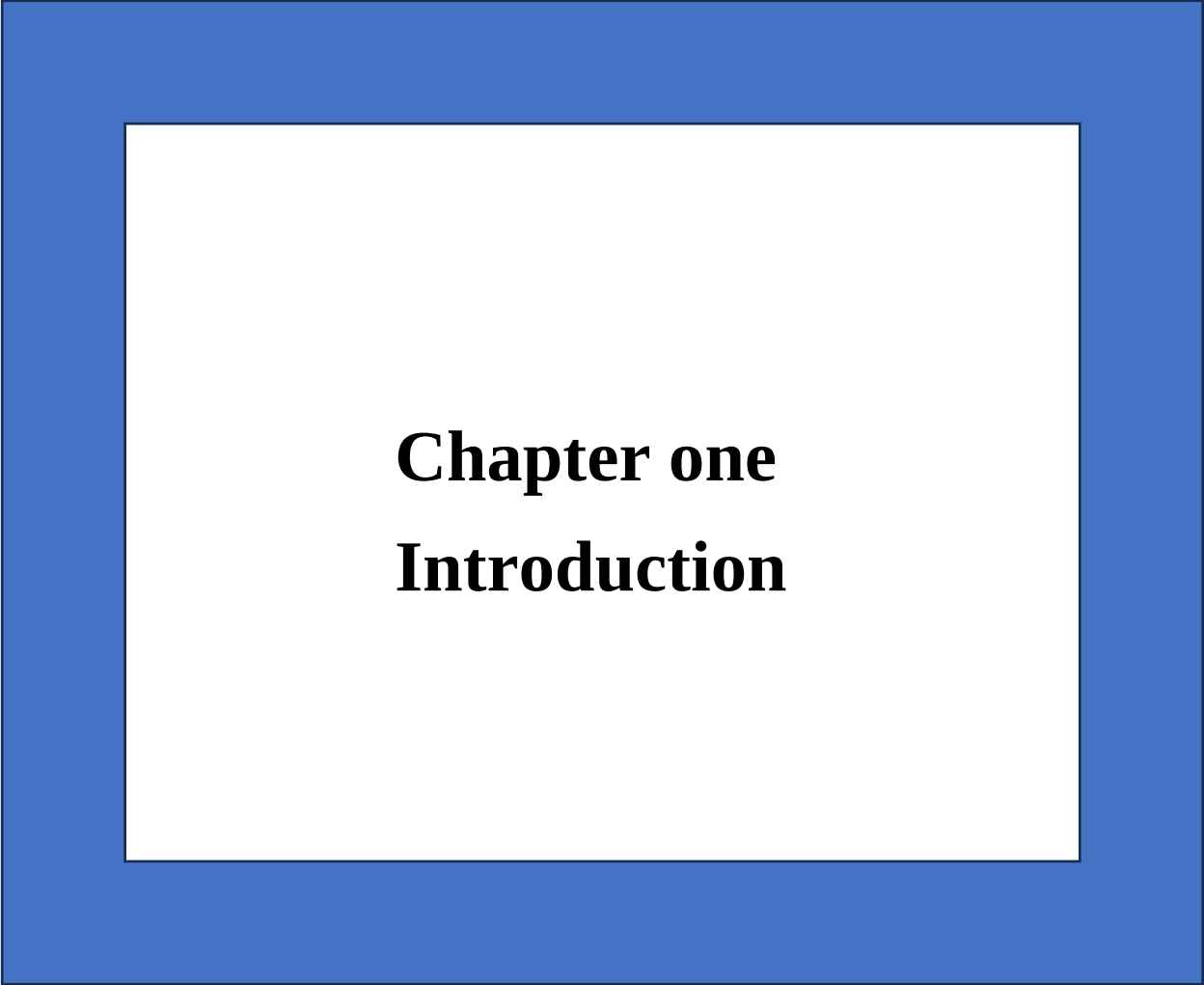
Summary

This study was conducted to evaluate the efficiency of *Eichhornia crassipes* and *Azolla pinnata* in removing and immobilizing lead and cadmium from contaminated water under open-field conditions in Al-Muthanna Governorate, southern Iraq, from January 16 to February 15, 2026. It also investigated the behavior of these elements in water, plant tissues, and sediments and analyzed their relationships with selected physicochemical water variables. A Completely Randomized Design (CRD) was employed to assess the ability of the plants investigated to remove lead and cadmium from water at concentrations of 0, 5, 10, and 25 mg/L for each element over a 30-day period. The results showed that water temperature ranged from 14 to 17°C before treatment, with a mean of 15.4°C, and from 18 to 21°C after treatment, with a mean of 19.3°C. The pH values in the *Eichhornia crassipes* basins under lead treatment were 7.200, 7.340, and 7.380 before treatment and 7.220, 7.270, and 7.197 after treatment at concentrations of 5, 10, and 25 mg/L, respectively. Under cadmium treatment, the corresponding pH values were 7.423, 7.460, and 7.510 before treatment and 7.177, 7.207, and 7.217 after treatment, respectively. *Azolla pinnata* exhibited pH values of 7.120, 7.240, and 7.280 before treatment and 6.417, 6.377, and 6.300 after treatment under lead treatment at concentrations of 5, 10, and 25 mg/L, respectively. Under cadmium treatment, the pH values were 7.430, 7.470, and 7.537 before treatment and 7.097, 7.060, and 7.000 after treatment, respectively. Electrical conductivity (EC) in the *Eichhornia crassipes* basins under lead treatment decreased from 1.929, 2.079, and 2.335 dS/m before treatment to 1.679, 1.739, and 1.889 dS/m after treatment at concentrations of 5, 10, and 25 mg/L, respectively. Under cadmium treatment, EC decreased from 1.969, 2.146, and 2.415 dS/m to 1.759, 1.879, and 2.059 dS/m, respectively. In the *Azolla pinnata* basins, EC under lead treatment decreased from 1.979, 2.139, and 2.405 dS/m before treatment to

1.779, 1.859, and 2.015 dS/m after treatment at concentrations of 5, 10, and 25 mg/L, respectively. Under cadmium treatment, EC decreased from 2.029, 2.215, and 2.505 dS/m to 1.849, 1.979, and 2.205 dS/m, respectively. This decrease may be attributed to the uptake of dissolved ions and minerals by the plants from the aqueous medium. *Eichhornia crassipes* achieved lead removal efficiencies of 63.577%, 65.509%, and 71.064% at concentrations of 5, 10, and 25 mg/L, respectively. The lead concentration in water decreased from 4.810, 9.820, and 24.320 mg/L before treatment to 1.750, 3.390, and 7.040 mg/L after treatment at the corresponding concentrations. Meanwhile, lead concentrations in plant tissues increased from 0.0010, 0.0011, and 0.0011 mg/kg before treatment to 1.340, 2.840, and 7.630 mg/kg after treatment, respectively. Lead accumulation in the sediments reached 1.730, 3.080, and 9.630 mg/kg at concentrations of 5, 10, and 25 mg/L, respectively. The highest cadmium removal efficiency achieved by *Eichhornia crassipes* was 65.443% at a concentration of 25 mg/L, whereas removal efficiencies at 5 and 10 mg/L were 56.197% and 58.025%, respectively. The cadmium concentration in water decreased from 4.833, 9.713, and 24.270 mg/L before treatment to 2.117, 4.077, and 8.387 mg/L after treatment at concentrations of 5, 10, and 25 mg/L, respectively. Cadmium concentrations in plant tissues increased from 0.0009, 0.0013, and 0.0010 mg/kg before treatment to 1.573, 3.277, and 9.483 mg/kg after treatment, respectively. Cadmium accumulation in the sediments reached 1.087, 2.257, and 6.287 mg/kg at the corresponding treatment concentrations. *Azolla pinnata* achieved lead removal efficiencies of 45.472%, 47.812%, and 50.975% at concentrations of 5, 10, and 25 mg/L, respectively. The lead concentration in water decreased from 4.823, 9.613, and 24.157 mg/L before treatment to 2.630, 5.017, and 11.843 mg/L after treatment at the corresponding concentrations.

Lead concentrations in plant tissues increased from 0.0007, 0.0006, and 0.0008

mg/kg before treatment to 1.133, 2.467, and 5.183 mg/kg after treatment, respectively. Lead concentrations in the sediments reached 0.990, 2.090, and 6.483 mg/kg at concentrations of 5, 10, and 25 mg/L, respectively. For cadmium, *Azolla pinnata* achieved removal efficiencies of 37.411%, 42.799%, and 47.671% at concentrations of 5, 10, and 25 mg/L, respectively. The cadmium concentration in water decreased from 4.750, 9.533, and 24.157 mg/L before treatment to 2.973, 5.453, and 12.503 mg/L after treatment at the corresponding concentrations. Cadmium concentrations in plant tissues increased from 0.0005, 0.0009, and 0.0007 mg/kg before treatment to 1.657, 2.820, and 6.760 mg/kg after treatment, respectively. Cadmium concentrations in the sediments after treatment were 1.055 and 4.385 mg/kg at concentrations of 10 and 25 mg/L, respectively.

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Chapter one

Introduction

Chapter one

1.Introduction

Water sustainability is a vital pillar for maintaining ecological balance and ensuring the continuity of human and developmental activities. However, aquatic systems are currently facing critical pressures and rapid deterioration as a result of population growth and uncontrolled industrial and agricultural expansion. This problem is more pronounced in the Iraqi environment, where the effects of severe climate change and successive droughts combine with declining freshwater flows. These conditions have contributed to the concentration of pollutants and the deterioration of surface water quality to a level that threatens both water and environmental security (Al-Khafaji et al., 2021). In Iraq, the direct discharge of industrial waste, agricultural runoff, and untreated municipal wastewater into rivers and drainage canals has further contributed to increasing concentrations of hazardous pollutants and heavy metals within vital aquatic systems (Ali *et al.*, 2019).

Among the wide range of environmental pollutants, heavy metals are classified as one of the most dangerous and toxic groups due to their chemical stability, persistence, and non-biodegradability. Unlike many organic compounds, which may undergo degradation or biodegradation over time, heavy metals can persist in aquatic environments for extended periods and can transform between different phases within ecosystems. They can migrate through different trophic levels and exhibit a high tendency for bioaccumulation in the tissues of living organisms. As these metals move through aquatic food chains from aquatic plants and algae to higher consumers such as fish and humans, they may undergo biomagnification, posing a direct threat to public health and aquatic ecosystems (Tchounwou *et al.*, 2012; Liu *et al.*, 2021).

Cadmium (Cd) and lead (Pb) are among the most problematic heavy metals because of their high toxicity and bioaccumulation capacity. Chronic exposure to cadmium has been associated with impaired kidney function and kidney tissue damage, osteoporosis, oxidative stress, and reproductive disorders. Lead, on the other hand, can cause severe structural and functional damage to the central nervous system, as well as impaired liver and kidney function (Ali *et al.*, 2019; Chun-Wu *et al.*, 2012). Therefore, the presence of these metals in aquatic environments represents a significant environmental and public-health concern, particularly in regions experiencing water scarcity and increasing anthropogenic pressures.

Despite the relative reliability of traditional physical and chemical treatment techniques used in water treatment plants, such as chemical precipitation, ion exchange, and membrane filtration, these methods exhibit clear technical and economic limitations when dealing with heavy metals, particularly at low concentrations. They can also be costly in terms of operation and maintenance and may generate secondary chemical sludge that requires additional treatment and appropriate disposal (Ray, 2009). Given these limitations, scientific research has increasingly focused on developing alternative, sustainable, and environmentally friendly strategies for the treatment of contaminated water.

Within this framework, phytoremediation has emerged as a promising green technology and a low-cost nature-based approach. It relies on the physiological and biochemical mechanisms of plants to absorb, sequester, transport, immobilize, or fix pollutants within plant biomass or the surrounding rhizosphere. This approach provides an environmentally friendly alternative for reducing the concentration and mobility of contaminants in polluted environments (Rai, 2009; Rezanian *et al.*, 2020). Floating aquatic plants have demonstrated considerable potential for this purpose because of their rapid growth rates, high biomass productivity, physiological

flexibility, and ability to tolerate environmental and chemical stresses in contaminated aquatic environments.

Among aquatic macrophytes, Water Hyacinth (*Eichhornia crassipes*) is one of the species that has received considerable attention in phytoremediation research. It possesses a dense and fibrous root system that provides a large surface area for the retention and accumulation of metal ions, thereby enhancing processes such as biosorption and ion exchange. Its application in phytoremediation can also provide an environmental management strategy for this species, which is classified as an invasive plant and can cause ecological problems in Iraqi waterways (Malik, 2007). The high biomass production and extensive root system of *Eichhornia crassipes* contribute to its potential for removing and retaining heavy metals from contaminated water.

In contrast, the aquatic fern *Azolla pinnata* exhibits notable biological and physiological characteristics that support its application in water remediation. Its symbiotic relationship with nitrogen-fixing cyanobacteria represents an important biological adaptation that contributes to rapid growth, nutrient acquisition, and adaptability under different environmental conditions. These characteristics, together with its capacity to absorb and accumulate toxic metals within its biomass, have increased scientific interest in its potential for phytoremediation and water purification (Sood *et al.*, 2012; Kumar *et al.*, 2023).

The use of aquatic plants for the remediation of contaminated water is therefore based on several interacting processes, including the absorption and accumulation of heavy metals within plant tissues, their retention on plant surfaces and roots, and their transfer or fixation within sediments. The efficiency of these processes may vary according to plant characteristics, metal type and concentration, exposure conditions, and physicochemical properties of the water. Understanding these

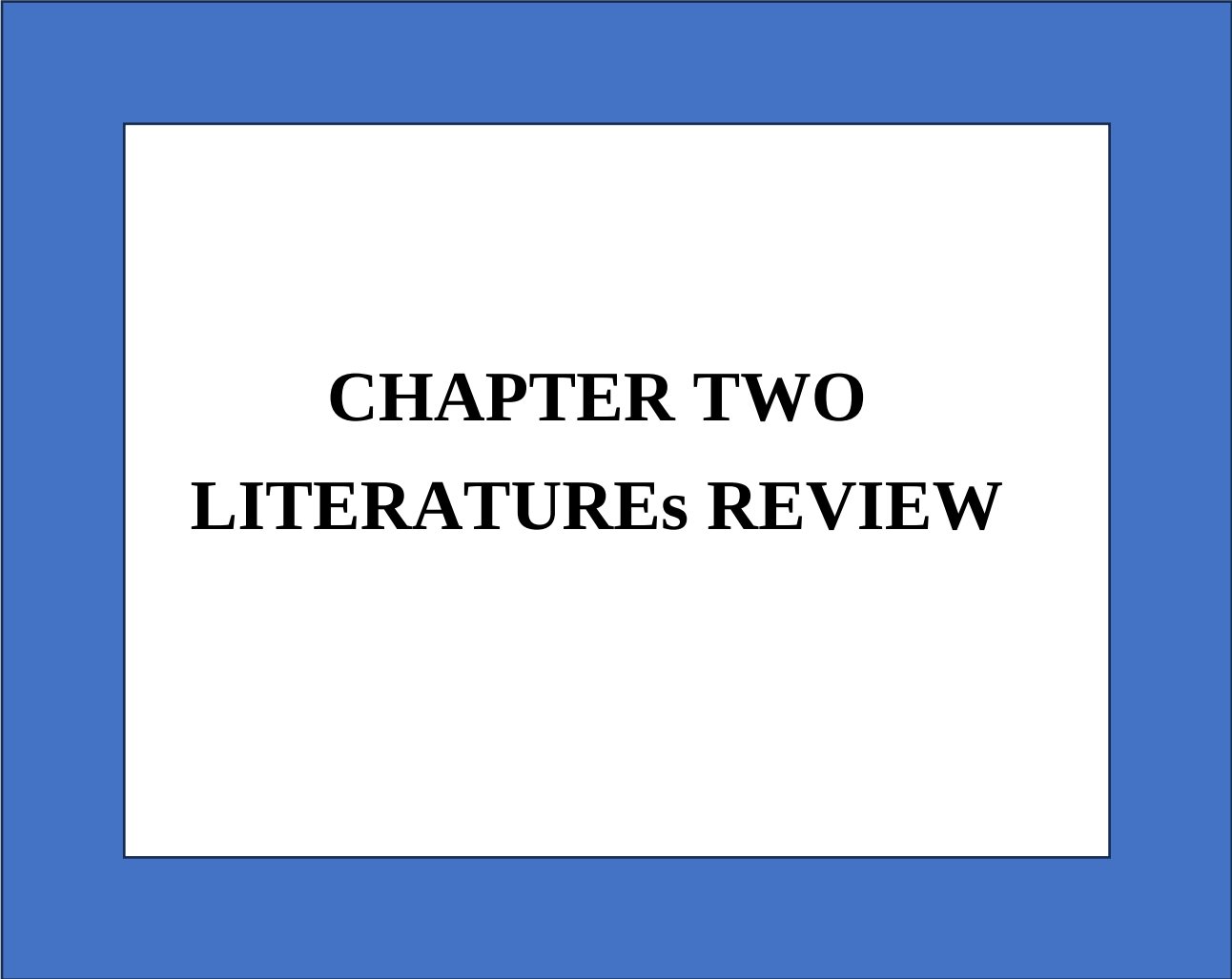
processes is particularly important under open-field conditions, where environmental factors may influence plant performance and contaminant behavior.

Accordingly, the present study investigates the efficiency of *Eichhornia crassipes* and *Azolla pinnata* in removing and fixing lead and cadmium from contaminated water under open-field conditions in Al-Muthanna Governorate. The study also examines the behavior and distribution of these heavy metals in the water, plant tissues, and sediments and investigates their relationship with selected physicochemical water variables. The findings are intended to contribute to the evaluation of aquatic plants as sustainable, environmentally friendly, and low-cost approaches for the treatment of heavy-metal-contaminated water.

1.2 Aim of study

This study aims to conduct a comparative evaluation of the efficiency of *Eichhornia crassipes* and *Azolla pinnata* in removing heavy metals from water by achieving the following objectives:

1. To measure and evaluate the removal efficiency of cadmium (Cd) and lead (Pb) from experimental aqueous media at different pollutant concentration levels.
2. To evaluate and compare the bioaccumulation capacity in the plant tissues of both plants and track the change in element concentrations before and after treatment.
3. To estimate and track the concentrations of cadmium and lead deposited in the sediments collected at the bottom of the experimental units after the plant incubation period.

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CHAPTER TWO

LITERATUREs REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy Metals in Aquatic Environments

The pollution of water bodies with heavy metals is a pressing environmental problem, primarily due to their chemical stability and inherent resistance to chemical or biological degradation. Heavy metals are naturally occurring with metallic chemical elements characterized by a high relative density (typically exceeding g/cm and a high atomic mass, while certain heavy metals serve as essential micronutrients required for specific biochemical and physiological functions at trace levels (e.g., copper, zinc, and iron), non-essential heavy metals such as lead Pb, cadmium Cd, mercury Hg, and arsenic as exert significant cytotoxic, mutagenic, and bioaccumulative toxicity even at low concentration thresholds (WHO, 2019). Unlike organic pollutants, which can be broken down and converted into non-toxic compounds through microbial metabolic pathways, heavy metals remain persistent in aquatic systems (rivers, lakes, and groundwater), these pollutants move through a dynamic cycle that includes dissolution in the water column, precipitation and binding to suspended particles, or settling in bottom sediments, which represent their final repository, thus exerting continuous toxic pressure on aquatic organisms (Ali *et al.*, 2019).

The danger of these metal lies in their ability to bioaccumulate in the living tissues of aquatic organisms at concentrations exceeding those found in the surrounding aquatic environment, this phenomenon is exacerbated when these metals are transferred through different trophic levels in a process known as biomagnification, they concentrate in the muscle tissues and vital organs of fish, posing a direct transmission risk to human health when contaminated aquatic products are consumed (Briffa *et al.*, 2020).

Although some heavy metals remain dissolved in the water column as free ions, the majority are attracted to colloidal surfaces and fine particles, eventually settling in benthic sediments. These sediments play a dual role here; While aquatic plants normally act as sites for collecting pollutants and limiting their spread in water, they can become secondary sources of pollution when any change occurs in the physicochemical balance of the aquatic environment such as a decrease in pH, a change in temperature, or a shift in redox potential which stimulates desorption processes and the re-release of metals into the water column, thereby increasing their bioavailability to aquatic organisms (Rezania *et al.*, 2020).

Studying the behavior of heavy metals in aquatic environments is particularly important when evaluating the efficiency of phytoremediation techniques, the success of aquatic plants in removing these metal depends not only on their total concentration but also on their bioavailability and distribution among water, sediments, and plant biomass, therefore, understanding the fate of cadmium and lead in these environmental components is a crucial step in evaluating the efficiency of phytoremediation systems and identifying mechanisms for removing pollutants from contaminated water.

2.1.1 Sources of Heavy Metal Pollution in Aquatic Environments

Heavy metals flow into aquatic ecosystems through pathways that can be divided into natural (geogenic) and anthropogenic sources. Natural sources include the physical and chemical weathering of rocks, natural soil erosion, and volcanic emissions, which release limited quantities within natural limits. In contrast, the significant increases in heavy metal concentrations over recent decades are attributed to uncontrolled human activities, primarily the direct discharge of untreated industrial wastewater, municipal runoff, and agricultural runoff laden with phosphate fertilizer and pesticide residues, mining activities and manufacturing

industries such as battery production, electroplating, dye manufacturing, and alloying also contribute to the release of cadmium and lead into waterways, leading to a continuous deterioration in water quality and aquatic ecosystem functions (Carvalho & Poté, 2022; Aziz *et al.*, 2023).

2.1.2 Fate and Transport of Heavy Metals in Aquatic Systems

Once they enter water bodies, heavy metals undergo complex physical and chemical processes that determine their environmental fate and distribution among the different phases of the aquatic environment, these processes begin with ionic dissolution in the water column, followed by adsorption and precipitation on suspended particles and colloids, over time, the majority of these pollutants are transported to benthic sediments, which become their primary reservoir, however, this stability is dynamic; Physicochemical changes in the aquatic environment, such as a decrease in pH or disturbance of water currents, can stimulate the ionic release of metals from sediments back into the water, at this stage, the free ions become more bioavailable for uptake by the root systems of aquatic plants such as *Eichhornia crassipes* and *Azolla*, these plants contribute to the removal of heavy metals from the water column through uptake and accumulation within their biomass, thus preventing their continued toxic movement within the ecosystem (Ali *et al.*, 2019; Rezania *et al.*, 2020).

2.1.3 Cadmium (Cd): Its Properties and Physiological Hazards

Cadmium is classified as a highly toxic heavy metal with no known physiological or biological role in plants or animals, in aquatic environments, cadmium is primarily found as the soluble divalent ion (Cd^{2+}), which is the most readily available form for uptake by the roots and cell surfaces of aquatic plants, the primary human sources of cadmium release include battery manufacturing waste, plastics production, and

mining operations, in addition to the significant role of excessive phosphate fertilizer use in agricultural lands adjacent to water bodies (Liu *et al.*, 2021).

When cadmium is absorbed by aquatic plants, it induces a cascade of physiological and biochemical responses associated with oxidative stress at the cellular level, its accumulation stimulates the excessive production of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide, causing oxidative stress that culminates in lipid peroxidation of cell membranes and the destruction of their structural integrity (Wang *et al.*, 2023). This mechanism is clearly reflected in the plant's morphology through toxicity symptoms, including chlorosis (yellowing of leaves due to the destruction of chlorophyll molecules), inhibition of root elongation, and a decrease in biomass production (Liu *et al.*, 2025). As for human health, the transfer of cadmium through the food chain causes chronic risks, as it concentrates in the kidneys, causing kidney failure, and replaces calcium in the bones, leading to their weakening and fragility, a clinical condition known as Itai-Itai disease (Tchounwou *et al.*, 2012; Amari *et al.*, 2017).

2.1.4 Lead (Pb): Accumulation and Environmental and Cellular Effects

Lead is a globally widespread metallic pollutant characterized by its high affinity for organic matter and fine particulate matter in water, due to its relatively low solubility at neutral pH levels, lead tends to precipitate rapidly in bottom sediments, which over time become a long-term reservoir of water pollution, anthropogenic sources of lead include industrial discharges, paint manufacturing waste, accumulated emissions from the combustion of formerly leaded fuels, and corrosion of aging water pipelines and infrastructure (Ali *et al.*, 2019).

From a biochemical perspective, the danger of lead ions (Pb^{2+}) lies in their ability to molecularly mimic the divalent basic metal ions, particularly calcium (Ca^{2+}) and

magnesium (Mg^{2+}), this mimicry allows lead ions to penetrate cellular barriers via calcium channels and bind to enzyme active sites, in aquatic plants, lead affects several enzymes involved in chlorophyll synthesis and photosynthesis, inhibiting photosynthetic efficiency and restricting vegetative growth (Tchounwou *et al.*, 2012; Sytar *et al.*, 2022). In aquatic organisms such as fish and invertebrates, lead accumulation causes neurotoxicity, embryonic developmental disorders, and damage to hematopoietic tissues, leading to reduced survival and growth rates (Tchounwou *et al.*, 2012; Lamhamdi *et al.*, 2011).

2.1.5 Physicochemical Factors Affecting the Bioavailability of Heavy Metals

The toxic effects of heavy metals in aquatic environments depend not only on their total concentration but also on their bioavailability, which is the fraction that can be absorbed and pass through the biological membranes of organisms, this dynamic is controlled by a of interconnected physicochemical factors, the primary factor is pH, A decrease in pH (increased acidity) leads to increased competition among hydrogen ions (H^+) for binding sites, causing heavy metals to be released and dissolve in the water column as free ions (Rezania *et al.*, 2020).

Soluble organic compounds (DOMs), such as humic acids, also play a pivotal role, they can form stable chelating complexes with metal ions, reducing the concentration of free ions and thus decreasing their bioavailability for plant uptake (Abduljaleel & Al-Hejuje, 2021). Temperature is another driving environmental factor, increased temperature leads to higher rates of chemical reactions and increased solubility of metal, while simultaneously activating metabolism, transpiration, and uptake rates in aquatic plants, this accelerates the extraction of pollutants up to a certain point, which then stops at the plant's physiological tolerance threshold, furthermore, the increase in mineral concentrations in water is directly

proportional to the amount of pollutant absorbed and retained within plant tissues until the point of cellular saturation is reached (Ali *et al.*, 2019; Briffa *et al.*, 2020).

2.1.6 Local and International Water Quality Standards

To ensure public health protection, environmental and health organizations have established stringent permissible limits for heavy metals as showing in table (2.1)

Table (2.1): Permissible limits for Cadmium and Lead (mg/L) Metal, WHO Standard, Iraq (Drinking Water Standards)

Heavy Metal	WHO Standard	Iraq (Drinking Water Standards)
Cadmium (Cd)	0.003 mg/L	0.005 mg/L
Lead (Pb)	0.01 mg/L	0.05 mg/L

(WHO (2017); COSQC (2009). Cadmium and lead are both highly toxic heavy metals that pose significant risks to human health. However, their toxic effects differ depending on the level and duration of exposure. Cadmium has a long biological half-life in the human body and can accumulate primarily in the kidneys, potentially causing renal damage and adverse effects on bone health. In contrast, lead is particularly harmful to the nervous system and brain development, especially in children (WHO, 2022). Although the WHO guideline value for cadmium is lower than that for lead, these values alone do not establish that cadmium is universally more toxic than lead.

2.1.7 Biochemistry and Toxicology of Heavy Metals: Lead and Cadmium

Heavy metals, particularly lead and cadmium, are among the most dangerous persistent inorganic pollutants in aquatic ecosystems, this is due to their thermodynamic stability, near-total resistance to biodegradation, and high capacity for bioaccumulation and biomagnification, which allows them to bounce back

through the food chain and reach humans (Ali *et al.*, 2019; Kumar *et al.*, 2023). These metals pose a dual physiological threat to macro aquatic plants, as their toxic effects extend beyond the surface to cause profound structural and molecular disturbances that undermine cellular metabolism and the overall stability of the aquatic ecosystem.

2.1.8 Chemical Behavior and Bioavailability in Aquatic Environments

The kinetics and bioavailability of lead and cadmium in aquatic environments are subject to complex physical and chemical constraints, most notably pH, salinity, and redox potential (Eh). In slightly alkaline aqueous environments, lead (Pb^{2+}) tends to react rapidly with carbonate, sulfate, and hydroxide ions, leading to its chemical precipitation as insoluble complexes in benthic sediments. This relatively limits its bioavailability to planktonic plants (Malik, 2007).

In contrast, cadmium (Cd^{2+}) exhibits high mobility and greater physiological stability in aqueous solution across a wide pH range, this facilitates its rapid and free translocation through the protein channels of root cell membranes, making it a more toxic, yet still, systemic pollutant compared to lead (Rezania *et al.*, 2020).

Morphological studies indicate that plants resist lead toxicity by stimulating the growth of shorter, thicker roots and calcifying cell walls as a localized retention mechanism to prevent its ascent to the leaves, cadmium, on the other hand, penetrates directly through epidermal and cortical cells, causing widespread tissue damage (Siddiqui *et al.*, 2022).

2.1.9 Physiological and Molecular Effects of Lead

Lead is classified as a non-essential element for plants, and its toxicity interferes with biochemical pathways along three main axes:

1. Induction of acute oxidative stress: Lead indirectly inhibits cellular defense enzymes, leading to the explosive production of reactive oxygen species (ROS) such

as hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH_2), these radicals attack unsaturated fatty acids in cell membranes, causing lipid peroxidation and selective permeability disruption (Sood *et al.*, 2012).

2. Inhibition of photosynthetic efficiency: Lead chemically interferes with the chlorophyll molecule by displacing and replacing the central magnesium ion (Mg^{2+}), leading to the degeneration of chloroplast structures and inhibition of the enzyme delta-levulinic acid dehydratase (-ALAD), which is responsible for chlorophyll biosynthesis. This manifests as yellowing and stunting of leaves (Vesk *et al.*, 1999).

3. Metabolic and Respiratory Disruptions: Lead disrupts oxidative phosphorylation pathways in mitochondria, reducing energy production (ATP) and forcing the plant to modify the activity of superoxide dismutase (SOD) and catalase (CAT) enzymes as a critical chemosensory defense against programmed cell death (Ali *et al.*, 2019).

2.1.10 Physiological Effects of Cadmium and Ionic Mimicry

The extreme molecular hazard of cadmium lies in its ability to mimic ions. It possesses an ionic radius and electrical charge similar to that of the primary nutrient cations, enabling it to penetrate membrane channels and specific transporters designated for the uptake of calcium (Ca^{2+}), zinc (Zn^{2+}), and iron (Fe^{2+}), and enter the cytoplasm (Choudhury *et al.*, 2017). Its molecular effects include:

1. Enzymatic interference and direct binding: Cadmium has a high affinity for binding to free sulfhydryl groups (-SH) located in the active sites of proteins and metabolic enzymes, altering their three-dimensional spatial structure and leading to complete inhibition of their vital functions (Kumar *et al.*, 2023).

2. Genotoxicity: Chronic intranuclear cadmium accumulation causes DNA fragmentation in meristematic cells of growing tips, inhibiting cell division and abruptly halting cell elongation and root system growth (Ali *et al.*, 2019).

3. Chromosomal and chemical defense responses: Cadmium stimulates cell physiology to synthesize

secondary metabolites and non-enzymatic antioxidants such as anthocyanins and phenolic compounds, which act as chemical barrier agents that isolate the metal and neutralize photo-oxidation resulting from the destruction of the electron transport system in chloroplasts (Sood *et al.*, 2012).

2.2 Phytoremediation: Conceptual Framework and Applications

Phytoremediation is a sustainable bioengineering approach that has garnered significant research attention in modern environmental studies for the rehabilitation of aquatic systems contaminated with heavy metals, this technology is defined as a solar-powered biosystem that utilizes plants (terrestrial or aquatic) to remove, transport, stabilize, or convert environmental pollutants into harmless forms in soil, water, and benthic sediments (Rai, 2009).

This method leverages the natural physiological capabilities of plants to absorb or adsorb inorganic pollutants onto their cellular surfaces, subsequently sequestering or metabolizing them via biochemical pathways within the tissues and the root microenvironment, the strategic value of this green technology lies in its low cost and its ability to purify water bodies without disrupting the natural ecological balance or causing secondary chemical or physical disturbances, this makes it an ideal option for application in resource-limited areas (Ali *et al.*, 2019; Rezanian *et al.*, 2020).

2.2.1 Phytoremediation Mechanisms in Aquatic Environments

The efficiency of aquatic plants in water purification is linked to a network of integrated physiological and physical mechanisms, classified into the following main pathways:

1. Phytoextraction: This involves the ability of the root system to absorb dissolved heavy metal ions and transport them via the xylem to the aerial parts (stems and leaves), where they are retained within the cells to reduce their free toxicity, this mechanism is considered the most sustainable method for permanently removing pollution by harvesting plant biomass and separating it from the aquatic system (Rai, 2009; Kumar *et al.*, 2023).
2. Rhizo filtration relies on the ability of dense roots to attract and retain pollutants on their cell surfaces(adsorption) due to the negative charges of the cell walls, or to deposit them within root tissues, this mechanism plays a crucial role in restricting the movement of high atomic weight heavy metals such as lead (Pb) in the first contact zone (Suman *et al.*, 2018).
3. Phyto stabilization aims to limit the movement and translocation of heavy metals in water or bottom sediments by secreting root exudates that chelate free ions and convert them into insoluble complexes. This reduces their bioavailability and prevents their transfer into the aquatic food chain (Rezania *et al.*, 2020).
4. Phyto transformation encompasses internal metabolic processes where plants modify the molecular structure of pollutants interacting with their tissues through specialized enzymes such as oxidation and reduction, converting them into less toxic and less mobile chemical forms (Ali *et al.*, 2019).
5. Rhizosphere Effect: Roots secrete sugars and amino acids that stimulate the activity of symbiotic microbial communities within their microenvironment, this biological interaction contributes to modifying geochemical conditions, which helps either improve the solubility of minerals to facilitate their absorption or accelerate their direct precipitation, depending on the nature of the medium (Kumar *et al.*, 2023).

2.2.2 Factors Affecting Phytoremediation Efficiency

The success and cumulative capacity of a phytoremediation system are influenced by a of interrelated environmental and physiological variables:

1. pH: Directly controls the chemical state and solubility of heavy metals, acidic environments increase the breakdown of complexes and the availability of ions for root absorption, while alkaline environments promote the precipitation of metals as hydroxides and carbonates, thus reducing the efficiency of plant accumulation (Liu *et al.*, 2021).
2. Temperature: Directly affects transpiration rates, photosynthesis, and cell membrane permeability. Moderately high temperatures activate enzymes and accelerate absorption, while extreme temperatures inhibit vital processes and growth (Rezania *et al.*, 2020).
3. Initial Metal Concentration: The relationship between concentration and absorption is directly proportional within certain limits. However, when a plant is exposed to critical concentrations exceeding its physiological tolerance threshold, its defense mechanisms collapse, leading to acute cytotoxicity due to increased chemical pressure and stress within the tissues (Kumar *et al.*, 2023).
4. Biomass and Species Plants: vary biologically in their accumulation capacity, Species with rapidly multiplying biomass and a dense fibrous root system provide a larger surface area for ion exchange and physical adsorption (Rai, 2009).
5. Exposure Time: Removal efficiency is related to the hydrological time of the plant's survival in the contaminated medium, plants exhibit the highest absorption rates in the first few days, then these rates gradually decrease as cell-binding sites fill up and tissues reach kinetic saturation.

2.2.3 Advantages and Limitations of Phytoremediation and Biomass Management

Phytoremediation is a non-invasive technique that preserves the natural appearance of the site and relies on solar energy, thus reducing operational costs and preventing the formation of hazardous chemical sludge, it is also aesthetically and socially acceptable (Rezania *et al.*, 2020). On the other hand, this process is slow, requiring several growing seasons compared to immediate physicochemical methods, and remains subject to climatic fluctuations and high levels of pollution (Ali *et al.*, 2019). The overall environmental sustainability of phytoremediation depends on the sound and safe management of the contaminated biomass after harvesting (biomass disposal), common technological options for reducing the volume of these plant residues and preventing the re-release of metallic pollutants into the ecosystem include: solar drying and compaction to reduce logistical volume, controlled landfilling in designated isolation sites, controlled incineration with the capture of toxic ash, or oxygen-free pyrolysis techniques to produce biochar, in addition to the possibility of utilizing the mineral-containing biomass through mineral recovery and recycling processes, subject to economic feasibility (Kumar *et al.*, 2023; Evangelou *et al.*, 2007).

2.2.4 The Importance of Aquatic Plants in the Bioremediation of Heavy Metals Aquatic

Macrophytes are among the most widely used plant organisms in phytoremediation applications for polluted water, this is due to their unique morphological and physiological characteristics, which enable them to efficiently handle various pollutants, particularly heavy metals, these plants are characterized by their ability to grow directly in aquatic environments, allowing for continuous contact with

dissolved pollutants and increasing the likelihood of their absorption or retention within plant tissues(Valipour *et al.*, 2018).

The sophisticated root systems of many aquatic plants provide a large surface area for the adsorption and accumulation of metal ions, Furthermore, these systems support the microbial communities within the root zone, which participate in numerous biological processes responsible for stabilizing pollutants or converting them into less toxic forms, these plants are also characterized by their rapid growth and high biomass production compared to many terrestrial plants, which enhances their ability to remove larger quantities of pollutants in relatively short periods (Rezania *et al.*, 2020).

Furthermore, many aquatic macrophytes possess the ability to tolerate high levels of heavy metals through specialized physiological mechanisms, including chelation of metals and activation of anti-stress systems. This has led to the adoption of several species, such as *Eichhornia crassipes* and *Azolla* , in numerous studies and practical applications for the treatment of industrial and agricultural wastewater and water bodies contaminated with lead, cadmium, and other heavy metals (Kumar *et al.*, 2023).

The environmental importance of these plants stems from their combination of high pollutant removal efficiency, low operational costs, and ease of management and harvesting, as well as their contribution to improving the physical and chemical properties of water and reducing the transfer of pollutants through the aquatic food chain, Therefore, aquatic plants have become a cornerstone of sustainable environmental management programs and the rehabilitation of water systems damaged by metal pollution.

2.2.5 Applications of Phytoremediation in Polluted Water Treatment

The engineering and environmental applications of phytoremediation systems in water purification are numerous, as they are used as practical solutions for treating various types of wastewaters, foremost among these applications is the treatment of industrial wastewater from electroplating, leather tanning, and battery manufacturing, which is often laden with critical concentrations of toxic metal, this technology is also successfully applied in the treatment of agricultural drainage water rich in fertilizer and pesticide residues containing metallic impurities, as well as in the purification of municipal wastewater, the efficiency of aquatic macrophytes is particularly evident when integrated into the design of constructed wetlands or artificial ponds designed to rehabilitate polluted lakes and rivers, where they function as low-cost, highly efficient biological filtration systems for reducing the chemical and biological loads of the water (Ali *et al.*, 2019; Rezania *et al.*, 2020).

2.2.6 Schematic representation of the accumulation and transport of heavy metals

The following schematic (Figure 2.1) shows the complex dynamic and physiological pathway of cadmium Cd^{2+} and lead Pb^{2+} ions as they move from polluted aquatic environments and penetrate through the cell walls of large aquatic plants (*Eichhornia crassipes* and *Azolla*), and then the mechanisms of their transport and internal bio segregation, reaching levels of biomagnification in fish tissues and the human body through successive trophic levels of the food chain (Ali *et al.*, 2019; Kumar *et al.*, 2023).



Figure (2-1): Diagram illustrating the process of heavy metal bioaccumulation and biomagnification from polluted water to humans.

The flowchart depicts the step-by-step movement of pollutants from polluted water into aquatic plants (*Azolla* and Water Hyacinth) through their roots. These contaminants then progress through various trophic levels, beginning with aquatic invertebrates, moving to fish, and ultimately affecting humans. This process can result in chronic toxicity and possible organ damage due to biomagnification.

2.2.7 Aquatic Plant Efficiency in Removing Lead and Cadmium

The efficiency of aquatic plants in removing heavy metals varies depending on the chemical properties of the pollutants and the bioaccumulation mechanisms possessed by different plant species. Lead (Pb) and cadmium (Cd) are among the most commonly studied heavy metals in environmental research due to their high toxicity and bioaccumulation potential within aquatic systems. Numerous studies indicate that floating aquatic plants have a high capacity to absorb this metal through their roots or water-contact vegetative surfaces and subsequently sequester them

within plant tissues via mechanisms including surface adsorption, chelation, and cellular kinetic sequestration (Rezania *et al.*, 2020).

Several types of aquatic macrophytes, including Water Hyacinth and *Azolla*, have demonstrated a remarkable ability to reduce lead and cadmium concentrations in polluted water, making them important bio models for phytoremediation applications of heavy metals (Malik, 2007).

2.3 Physical, Chemical, and Biological Factors Affecting

Phytoremediation Efficiency

The efficiency of macrophytes, such as *Eichhornia crassipes* and *Azolla pinnata*, in absorbing and sequestering heavy metals like lead (Pb^{2+}) and cadmium (Cd^{2+}) depends on a complex dynamic interaction between the plant's physiological and structural characteristics and the physicochemical conditions of the aquatic environment, the scientific literature indicates that the combined effect of these environmental and biological determinants is the decisive factor in determining the overall efficiency and kinetic rate of phytoremediation processes and their final outputs in open ecosystems (Malik, 2007; Rezania *et al.*, 2019).

2.3.1 pH and Chemical Bioavailability

The pH of the aqueous medium is the most influential and critical physicochemical parameter in determining the chemical specificity of heavy metals and their bioavailability for plant adsorption, chemically, lower pH values (acidic environments) lead to increased solubility and mobility of heavy metal ions due to the dissociation of free carbonate and hydroxyl complexes. However, this increased bioavailability is counteracted by intense chemical competition between free positive hydrogen protons (H^+) and metal ions (Pb^{2+} and Cd^{2+}) for negatively charged cation exchange sites on root cell surfaces, this competition can inhibit the

plant's actual uptake rate despite the abundance of the metal in the water column (Rai, 2009).

In contrast, in slightly alkaline aqueous environments, lead tends to react rapidly and directly with the carbonate and sulfate ions present, leading to its mechanical precipitation as insoluble lead carbonate, this limits its cellular permeability via free radicals and stabilizes it in the bottom sediment, Cadmium, on the other hand, maintains greater stability and ionic mobility in solution under moderately alkaline conditions without rapid precipitation (Rezania *et al.*, 2019). Physiological studies confirm that the optimal pH range for efficient plant cellular metabolism and stable cell wall adsorption sites without damaging the cell membrane is precisely between 5.5 and 7.5 (Rai, 2009).

2.3.2 Temperature and Metabolic Thermodynamics

Metabolic and enzymatic reaction rates are linked to increasing temperature within the plant's physiological limits, moderate temperature increases (between 20°C and 30°C) directly regulate the rates of biochemical reactions, the kinetics of transport enzymes, the rate of transpiration, and photosynthetic processes that drive the movement of metal within plant tissues, this, in turn, enhances the efficiency of nutrient and mineral uptake, accompanied by a rapid increase in total plant biomass production (Sood *et al.*, 2012).

Conversely, lower temperatures during cold periods lead to a physiological inhibition and a decline in metabolic energy and intracellular ATP production, this negatively impacts the mechanisms of active transport across cell membranes, particularly in the small aquatic fern (*Azolla*), which exhibits greater sensitivity to chilling stresses, this slows its cell division kinetics and vegetative growth rates, in contrast, the *Eichhornia crassipes* demonstrates greater structural and thermal flexibility and a greater capacity to maintain its root system area for mechanical

retention and precipitation processes, despite the lower kinetic temperatures in open aquatic environments (Sood *et al.*, 2012; Newete & Byrne, 2016).

2.3.3 Initial Pollutant Concentration and Loading Capacity Kinetics

The relationship between the initial concentration of heavy metals in the aquatic medium and their rate of bioaccumulation within plant tissues is governed by a kinetic system similar to classical kinetic adsorption models (such as the Langmuir and Freundlich equations for surface adsorption), at low and prolonged concentrations, a clear direct relationship exists, where the rate of cellular uptake increases with increasing external concentration due to the availability of large areas and vacant binding sites on cell walls and root receptors (Malik, 2007).

However, this kinetic activity necessarily reaches a critical stage known as the structural saturation point and the plant's maximum uptake capacity, at this point, all binding and exchange sites and membrane protein receptors are fully occupied and can no longer accommodate additional ions. Scientific reports indicate that exceeding the critical limits of plant tolerance mechanisms, particularly for the highly systemic and kinetic element cadmium, leads to acute cytotoxicity. Its physiological symptoms appear rapidly as tissue necrosis, inhibition of anabolic enzymes, damage to chloroplasts, and stunting of the root system. Therefore, sustaining absorption efficiency requires activating a regular periodic harvesting strategy to remove saturated tissues and stimulate the plant to produce young vegetative and root growths that have new chemical loading and absorption capacity to ensure the continuity of biological cleansing (Malik, 2007; Mishra & Tripathi, 2008).

2.3.4 Ionic Interference and Osmotic Pressure (Salinity)

Heavy metals are present in polluted waters along with a wide spectrum of ions and dissolved salinity, presenting plants with the challenge of cross-linked ion

interference and competition. Due to the chemical similarity in charge and chemical conformation of the hydrated ionic radii, lead and cadmium ions compete directly with the essential cations and macronutrients required for plant growth. Cadmium (Cd^{2+}) competes with calcium (Ca^{2+}) and zinc (Zn^{2+}) for the same membrane and protein carriers, while lead (Pb^{2+}) interferes with the uptake kinetics of magnesium (Mg^{2+}) and iron (Fe^{2+}) in membrane channels (Vesk *et al.*, 1999). This intense competition significantly reduces the overall accumulation coefficient of the targeted pollutants.

On the other hand, high salinity levels increase the osmotic pressure in the aqueous solution surrounding the roots, hindering the roots' ability to draw water and causing severe physiological drought stress.

This physiological condition forces plant cells to direct their metabolic energy and biochemical resources toward the synthesis of internal osmotic pressure regulators (such as the amino acid proline and soluble sugars) to maintain cellular osmotic balance, at the expense of essential biological processes dedicated to biomass production and the synthesis of chelating proteins and peptides for sequestering and neutralizing toxic heavy metals (Liu *et al.*, 2020).

2.3.5 Specific Morphological and Biological Characteristics of Plants

The structural and morphological features of aquatic plants represent the primary biological and physical drivers determining the efficiency and mechanism of environmental remediation in biological environments, in the water hyacinth, the dense, branching, fibrous root system provides an enormous surface area, geometrically increasing the opportunities for direct contact with the water column, this makes it an ideal system for rhizo filtration, trapping suspended particles and mechanically sedimenting associated pollutants (Malik, 2007).

In contrast, the *Azolla* plant possesses a unique physiological structure that compensates for its small external morphological size: its specialized and close symbiotic relationship with nitrogen-fixing cyanobacteria (*Azolla anabaena*) residing in the leaf cavities, this unique biological feature gives the *Azolla* a physiological advantage in tolerating and resisting acute mineral stress, biologically fixed nitrogen contributes to supporting and building biomass without consuming the energy of traditional nutrient absorption, and directs the whole cell to synthesize phytokelatin complexes (PCs) and antioxidants necessary to restrict and neutralize the chemical toxicity of free ions within the cytoplasm (Sood *et al.*, 2012; Choudhury & Panda, 2019).

2.3.6 Dynamic Physiological Response and Ecosystem Sustainability

Reference data indicate that sustainable phytoremediation depends on the integrated physiological responses of aquatic plants to metallic, thermal, and environmental stress, including cellular protection, respiration regulation, and membrane permeability modification to mitigate metal toxicity (Lal *et al.*, 2015). Seasonal biomass dynamics highlight the need for effective environmental management, including periodic adjustment of plant growth and harvesting to maintain tissue vitality and prevent senescence and cell death. During these stages, membrane permeability may deteriorate, causing previously accumulated heavy metals to be released back into the water, thereby threatening the long-term sustainability and efficiency of phytoremediation (Lal *et al.*, 2015; Lu *et al.*, 2004).

2.4 Environmental and Physiological Characteristics of the Study Plants

2.4.1 (*Eichhornia crassipes*)

2.4.1.1 Morphological Characteristics and Scientific Classification of Water Hyacinth

Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Commelinales

Family: Pontederiaceae

Genus: *Eichhornia*

Species: *Eichhornia crassipes*

(Royal Botanic Gardens, Kew, 2026).

Water hyacinth is a free-floating aquatic plant characterized by glossy, oval-shaped green leaves, swollen spongy petioles, and an extensive fibrous root system. Its roots provide a large surface area for mineral absorption, ion exchange, and suspended particle retention, contributing to its phytoremediation efficiency (Malik, 2007; Rezaei et al., 2020).

2.4.1.2 Water Hyacinth Rhizo filtration Mechanisms

The effectiveness of Water Hyacinth removing metal pollutants such as cadmium (Cd) and lead (Pb) is attributed to the interplay of multiple biological reactions, these processes begin with the electrostatic passive adsorption of metals onto the negatively charged cell walls of the roots, followed by active uptake across cell membranes and vascular transport to the aerial parts, the plant's dense root system acts as a mechanical and physical "rhizo filtration" system, stimulating the clumping

and sedimentation of suspended matter at the bottom, This process is driven by root exudates that alter the biochemistry of the surrounding water, scientifically explaining the formation of the characteristic dark deposits around the roots after exposure to pollutants (Liu *et al.*, 2021; Kumar *et al.*, 2023).

2.4.1.3 Water Hyacinth Physiological Response to Metal Stress and Cellular Isolation

The Water Hyacinth exhibits remarkable adaptive resilience when exposed to critical levels and high concentrations of cadmium and lead in aquatic environments, this adaptation manifests as root elongation and increased surface density to compensate for the chemical stress, once heavy metal ions (Cd^{2+} and Pb^{2+}) enter the cells, isolation and inhibition mechanisms are activated to reduce the toxicity of the active cytoplasm, this allows the plant to maintain its physiological balance without acute biomass mortality, even under less-than-ideal environmental conditions or high concentrations of metal pollutants (Ali *et al.*, 2019; Malik, 2007).

2.4.1.4 Environmental Constraints and water hyacinth Harvesting Strategy

The processing efficiency of water hyacinth influenced by the physicochemical properties of the medium, a low pH increases the bioavailability of cadmium and lead ions by facilitating their dissociation, while light and nutrients stimulate overall plant metabolism, however, the literature indicates a physiological saturation threshold for tissues; prolonged overloading leads to cellular tissue damage and death. This environmental constraint necessitates a periodic and regular harvesting approach for mineral-saturated biomass to avoid tissue degradation, which inevitably releases trapped pollutants back into the aquatic ecosystem (Rezania *et al.*, 2020; Kumar *et al.*, 2023).

2.4.1.5 Cellular Fixation and Biochemical Resistance Mechanisms in Water Hyacinth

To counter cadmium and lead toxicity, water hyacinth employs cell-wall and molecular mechanisms. Negatively charged cell-wall components, such as pectin and cellulose, bind metal ions and limit their entry into the cytoplasm. If Cd^{2+} and Pb^{2+} cross the plasma membrane, cysteine-rich metallothioneins bind these ions and reduce their toxicity (Zhou et al., 2024).

Meanwhile, increased reactive oxygen species (ROS) stimulate antioxidant enzymes, including superoxide dismutase (SOD), which converts superoxide radicals into hydrogen peroxide, subsequently broken down by catalase (CAT) and peroxidase (POD). These enzymes help protect root cell membranes and maintain tissue integrity (Malik, 2007; Kumar et al., 2023).

2.4.2 (*Azolla pinnata*)

2.4.2.1 Morphological Characteristics, Scientific Classification, and Symbiotic Relationship of *Azolla*

Scientific Classification

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Polypodiinae

Order: Salviniiales

Family: Salviniaceae

Genus: *Azolla*

Species: *Azolla pinnata*

(Royal Botanic Gardens, Kew, 2026).

Azolla pinnata is a small, free-floating aquatic fern characterized by overlapping leaves and rapid growth. Its symbiotic relationship with nitrogen-fixing cyanobacteria, *Anabaena azollae*, within leaf cavities supports nitrogen nutrition and adaptation to environmental stress, including heavy metal toxicity (Sood et al., 2012; Kumar et al., 2023).

2.4.2.2 Mechanisms of Cadmium and Lead Absorption and Cellular Sequestration in *Azolla*

Azolla pinnata exhibits excellent selectivity for ensnaring lead (Pb^{2+}) and cadmium (Cd^{2+}) ions. Its tightly packed leaves form a biological shield that increases the contact area with dissolved pollutants, the metal is kinetically transported across the cell membrane into the cytoplasm, to maintain cell viability, immediate vacuolar sequestration is activated. The toxic ions bind to low molecular weight chelating proteins within the vacuole, preventing their interaction with cellular organelles and allowing the *Azolla* to continue its metabolic and growth processes despite the surrounding chemical stress (Rezania et al., 2020; Kumar et al., 2023).

2.4.2.3 Environmental Constraints and Biomass Management Strategy for *Azolla*

Despite *Azolla* 's high physiological resilience compared to conventional chemical techniques; its effectiveness remains limited by its biological tolerance limits. Since the treatment mechanism here relies entirely on internal biochemical retention within the cellular tissues without external physical precipitation, the fern reaching its saturation point represents a critical stage. Environmental studies emphasize the necessity of implementing a periodic and rigorous harvesting strategy for the resulting biomass before senescence and death, to prevent tissue decomposition that causes backflow of pollutants and the re-release of minerals into the water column (Lal et al., 2015; Kumar et al., 2023).

2.4.2.4 Biochemical Response Mechanisms and Resistance to Oxidative Stress in *Azolla*

The accumulation of cadmium and lead in *Azolla* ferns triggers a rapid biochemical response primarily dependent on the mutual metabolic support arising from its symbiotic relationship with the bacterium (*Anabaena azollae*), which enhances the overall metabolic capacity of the host. To overcome oxidative stress and reduce the destruction of biomolecules by reactive oxygen species (ROS), the production of specialized glutathione-derived peptide chelates known as phytochelatins is accelerated within *Azolla* cells, these phytochelatins neutralize the activity of free ions in the cytoplasm by forming stable complexes that are immediately pumped into the vacuoles (Sood *et al.*, 2008).

Simultaneously, the plant registers an intensive accumulation of the free amino acid “proline” (proline accumulation), which acts as an osmolality-protective compound and a non-enzymatic antioxidant that contributes to charge stabilization and protection of cell membranes, supported by the synergistic activity of catalase, peroxidase, and superoxide dismutase enzymes to reduce the severity of cytotoxicity and ensure the continuation of the rapid multiplication rate of the fern (Sood *et al.*, 2012; Lal *et al.*, 2015).

2.4.3 Mechanisms of Tolerance to Metal Stress and Toxicity in Aquatic Plants

Hyper accumulative aquatic plants rely on complex, multi-level biochemical and molecular strategies to neutralize the toxicity of cadmium (Cd^{2+}) and lead (Pb^{2+}) ions after they penetrate cell membranes. These integrated mechanisms aim to prevent free ions from reacting with vital organelles in the cytoplasm and can be summarized in the following physiological axes:

1. **Cell Wall Exclusion and Trapping:** Root cell walls represent the first line of chemical defense; a large proportion of lead and cadmium ions are trapped through electrostatic binding to the cell wall components of pectin, cellulose, and glycoproteins rich in carboxyl and phosphate groups. This localized trapping prevents the movement of metals through the apoplast pathway and limits their toxic influx into permanent cells and vascular tissues (Kumar *et al.*, 2023).
2. **Biosynthesis of chelating agents:** When metals cross the cell wall and enter the cytoplasm, metal stress stimulates the immediate gene expression of specialized sulfur-rich chelating compounds, most notably phytochelatins (PCs) and metallothionein's (MTs). These peptides bind to free metal ions via strong covalent bonds targeting thiol groups (-SH), forming stable complexes that prevent the metal from toxically reacting with cellular enzymes (Ali *et al.*, 2019; Sood *et al.*, 2012).
3. **Vacuole Segregation and Active Transport System:** Segregation within the vacuole is the most important molecular mechanism for achieving long-term endurance, chelated complexes or free ions are actively transported across the vacuole membrane (tonoplast) by specialized protein transporters that rely on ATP energy (such as ABC transporters and H^{+} -ATPase pumps). Once inside the vacuole, minerals are sequestered away from the cytoplasm and vital metabolic processes, allowing the cell to maintain its division and growth integrity (Rezania *et al.*, 2020).
4. **Activation of Enzymatic and Non-Enzymatic Defense Systems:** To counteract the oxidative explosion caused by the production of reactive oxygen species (ROS), the plant increases the activation rates of an integrated enzyme system that includes superoxide dismutase (SOD) to break down superoxide radicals, and catalase (CAT) and peroxidase (POD) to convert hydrogen peroxide into water and oxygen. This coincides with the intensive production of non-enzymatic antioxidants such as

glutathione (GSH) and the amino acid proline, which acts as an osmotic regulator and protects cell membranes from degradation (Lal *et al.*, 2015; Sood *et al.*, 2012).

2.4.4 Applied Conclusions for Aquatic Environmental Treatment

Understanding the precise physicochemical and biological behavior of both lead and cadmium forms the philosophical and engineering basis for designing and developing sustainable and highly efficient plant treatment systems, successful field application requires a thorough understanding of the dynamics of uneven pollutant distribution between root and shoot systems, and the plant's tolerance to intracellular oxidative stress (Kumar *et al.*, 2023).

Reference literature confirms that employing super accumulatory species such as Water Hyacinth and *Azolla* offers significant technological flexibility for combating mineral pollution, provided that the plant organism is engineered and selected based on the type and concentration of metal prevalent at the site, the anatomical structure of its root system, and the unique hydrobiological characteristics of the aquatic ecosystem to be rehabilitated (Malik, 2007; Rezanian *et al.*, 2020).

2.5 Interaction Between Aquatic Plants and Microorganisms

The Rhizosphere and Its Dynamic Role in Phyto remediation the rhizosphere—the narrow aquatic zone directly surrounding the roots of aquatic plants—is the most important dynamic element and the primary determinant of the efficiency and speed of phytoremediation processes, it represents the point of direct physical, chemical, and biological interaction between the root system and the surrounding aquatic environment. Hydrobiological literature reveals that the rhizosphere does not merely function as a mechanical substrate or a passive medium for water absorption but rather constitutes a complex micro-ecosystem teeming with continuous biochemical interactions between active root exudates and diverse microbial communities. This

directly impacts the kinetics and fixation of heavy metals such as lead (Pb^{2+}) and cadmium (Cd^{2+}) (Pilon-Smits, 2005; Rezanian *et al.*, 2019).

2.5.1 Root Exudates and Modification of Aquatic Chemistry:

The roots of floating aquatic plants continuously secrete a wide range of diverse organic compounds resulting from carbon metabolism, known as root exudates. These exudates include low molecular weight organic acids (such as citric, malic, and oxalic acids), as well as monosaccharides, amino acids, and exogenous enzymes (Liu *et al.*, 2020). These compounds play a dual role in the rhizosphere; firstly, they act as natural chelating agents (Phyto siderophores) that chemically bind to dissolved heavy metal ions, altering the ion's surface charge and hydrophobicity. These organic acids contribute to crucial local modifications of pH and redox potential (Eh) values in the rhizosphere microenvironment, this leads either to increased solubility of minerals for easier absorption or to their chemical precipitation in the form of complexes with phosphates or carbonates, depending on the nature of the metal and the prevailing chemical conditions (Lu *et al.*, 2004). Secondly, these organic products represent a rich source of energy and readily available carbon for absorption by microorganisms, contributing to the "rhizosphere effect." This effect attracts and activates specialized bacterial and fungal communities that associate with the roots and support environmental purification functions (Liu *et al.*, 2020).

2.5.2 The Microbial Role and Biofilms in Pollutant Retention

The microorganism's endemic to the rhizosphere of aquatic plants (such as PGPB) constitute a crucial complementary physiological arm for enhancing the efficiency of phytoremediation. When activated by chemically attracted substances in root exudates, these bacteria secrete exopolysaccharides (EPS) that form complex

networks known as microbial biofilms, which coat the root surfaces (Mishra & Tripathi, 2008).

These biofilms contain a high density of functional groups capable of trapping and containing cadmium and lead ions, thus limiting their free movement in water, furthermore, these microorganisms possess advanced metabolic mechanisms that contribute to the conversion of heavy metals through biomineralization or enzymatic reduction processes, transforming highly soluble and toxic ions into insoluble sulfide or carbonate salts that precipitate to the bottom of the ecosystem (Dowling & Doty, 2009; Mishra & Tripathi, 2008).

2.5.3 Root Density and Microbial Colonization Capacity

The size and activity of microbial interactions in the rhizosphere are directly related to the available root surface area for microbial colonization, The Water Hyacinth (*E. crassipes*), with its branching fibrous root system, allows for higher microbial colonization capacity and the formation of wider biofilms across the water column, thus increasing root filtration rates and the capture of dissolved free ions (Malik, 2007). In contrast, the *Azolla* (*A. pinnata*) has a shorter rhizosphere and limited surface area, making it primarily dependent on the direct biochemical efficiency of the cell walls of its vegetative tissues and metabolic integration with symbiotic bacteria residing in its leaves to compensate for the physical limitations of its roots (Sood *et al.*, 2012). This structural difference leads to a kinetic difference in the rate and pattern of mineral retention and sequestration between the two plant systems (Malik, 2007).

2.5.4 Integrated Systemic Perspective and Treatment System Design

Scientific data derived from rhizosphere dynamics studies confirm that phytoremediation efficiency cannot be reduced to the plant's independent capacity for absorption and cellular accumulation, rather, it is the inevitable outcome of an

integrated, collaborative ecosystem that combines the plant, the physicochemical environment of the water, and the associated microbiome (Rhizosphere microbiome) (Pilon-Smits, 2005).

This evolving understanding compels designers of bioremediation systems to view the plant not as a single, isolated metal, but as a holistic, interconnected biological system, deciphering these rhizosphere interactions contributes to improving the efficiency of designed systems (such as constructed wetlands) by selecting diverse root systems, stimulating specific bacterial inoculation, and adjusting environmental parameters to ensure the highest rates of disinfection and removal of stubborn mineral pollutants from water in natural environments (Rezania *et al.*, 2019).

2.6 Extraction Mechanisms and Kinetic Modeling of Heavy Metals in Plant Tissues

Studying the physiological, molecular, and mathematical mechanisms controlling the extraction, transport, and accumulation of heavy metals in plant tissues is fundamental to deciphering the key to evaluating and improving the efficiency of phytoremediation systems, this dynamic cannot be reduced to simple physical processes of surface adsorption; rather, it is a complex cellular kinetic process comprised of several sequential physiological stages, governed by the properties of plasma membranes and the gene expression of specific transporters. Target metal ions, such as lead (Pb^{2+}) and cadmium (Cd^{2+}), are transported from the external aqueous solution into the cytoplasm, followed by internal tissue transport and sequestration pathways that are rigorously evaluated kinetically and temporally (Pilon-Smits, 2005; Dal Corso *et al.*, 2019).

2.6.1 Biphase Kinetics: Surface Adsorption and Active Cellular Uptake

Heavy metal ions move from the aqueous medium into the biological tissues of aquatic plants via a biphase kinetic mechanism, this process begins with a first, rapid phase (passive adsorption) that is not dependent on cellular or metabolic energy, in this phase, positively charged ions bind to negatively charged functional groups on the natural polymers that make up the outer cell walls of roots (such as carboxyl, hydroxyl, phosphate, and amine groups, which are abundant in pectin and cellulose), this chemical phase acts as a primary protective barrier and a surface reservoir with a high capacity for trapping large quantities of free radicals (Rezania *et al.*, 2019).

This is immediately followed by a second, slower phase: active absorption and cellular entry through the kinetic plasma membrane, this transport occurs via two main hydrostatic pathways: the apoplastic pathway, which continues across cell walls and intercellular spaces without membrane permeability, and the deeper symplastic pathway, which necessarily requires crossing the semipermeable cell membrane via specialized transport proteins and ion channels under the direct consumption of metabolic energy in the form of adenosine triphosphate (ATP), since the movement of ions here often occurs against the electrochemical concentration gradient within the target cells (Lux *et al.*, 2011).

2.6.2 Ionic Mimicry and Molecular Protein Carrier Families

Toxic heavy metal ions exploit cellular transport channels physiologically and practically dedicated to the uptake of essential micronutrients through a biological and molecular phenomenon known as ionic mimicry. This behavior is clearly demonstrated in the case of the cadmium ion (Cd^{2+}), which shares a critically similar ionic radius and electrical charge with essential metal such as calcium (Ca^{2+}) and

zinc (Zn^{2+}). This allows it to penetrate living plasma membranes by utilizing zinc-iron permeases (ZIPs) and NRAMPs (Non-Iron Permeases) (Clemens, 2006).

The lead ion (Pb^{2+}), on the other hand, follows transport pathways via voltage-gated calcium channels and other non-specialized transport channels. However, lead exhibits significantly slower transport kinetics within plant cymbals compared to cadmium, this is primarily due to its high chemoattractant affinity for cellulose cell products and cell walls, leading to its trapping and dense, localized accumulation in the root tissues of Water Hyacinth and *Azolla*, this serves as a physiological defense mechanism that prevents or limits its free ascent to the aerial parts (Pourrut *et al.*, 2011).

2.6.3 Cellular Defense: Organic Complexes and Vacuole Trapping

Once toxic heavy metal ions penetrate the plasma membrane and enter the cytoplasm, the plant's physiological system responds by activating robust cellular protection and purification mechanisms. These mechanisms primarily aim to neutralize the chemical activity of free ions and prevent the destructive oxidative stress that can damage organelles, this mechanism involves the stimulation and synthesis of bioactive chelating complexes via immediate gene expression to produce thiol-rich peptides and proteins, primarily phytochelatins (PCs) synthesized enzymatically from glutathione (GSH), and metallothioneins (MTs) genetically encoded within the nucleus (Clemens, 2006; Dal Corso *et al.*, 2019).

These specialized compounds attract and bind Cd^{2+} and Pb^{2+} ions to form highly stable organometallic complexes with low cytotoxicity. The internal purification process is completed by the transfer of these stable complexes via the primary active transport pumps dependent on the energy (ATP) of the (ABC) transporter family across the vacuolar membrane or what is known as the tonoplast, to be permanently isolated and stored within the vacuolar sequestration, thus protecting the efficient

sensitive organelles such as chloroplasts and mitochondria from enzymatic inhibition and programmed cell death (Dowling & Doty, 2009).

2.6.4 Vascular Tissue Transport and Distribution Dynamics

The long-distance tissue transport of minerals from the stored root system to the shoot system (leaves and stems) via the xylem vessels is a crucial stage that determines the final and standard pattern of plant processing, specialized protein families, such as heavy metal transporters of the ATPase type (specifically the HMA family), control the pumping and packaging of minerals within the xylem (Lux *et al.*, 2011).

In this context, the tissue distribution dynamics within the growth stages are linked to two highly important physiological concepts in the ecological literature; The first is bioaccumulation potential, which reflects the efficiency of plant tissues in drawing in and retaining ions from the surrounding aqueous environment, the second is translocation capability, which expresses the flexibility of ion movement through vascular tissues and their ascent to the aerial parts above water (Malik, 2007).

Aquatic plants vary widely in these dynamics, some floating plants with large biomass (such as the water hyacinth) tend physiologically to exhibit superior nutrient accumulation within their dense root network, with limited tissue translocation to the leaves as a defense mechanism to protect their photosynthesis from toxicity.

Other plants (such as *Azolla*) exhibit more balanced distribution dynamics thanks to their rapid ion exchange, ultimately enabling the complete removal of metals from the aquatic ecosystem through periodic and systematic harvesting of the growing biomass (DalCorso *et al.*, 2019; Rezanian *et al.*, 2019).

2.6.5 Multilevel Functional Integration

These physical, physiological, and molecular mechanisms operate within a sequential, multilevel integrated system, no biological mechanism can be isolated

from the others; rather, they all dynamically interact, starting with external surface adsorption on the cell wall and ending with vacuole deposition and long-range vascular transport (Pilon-Smits, 2019 ; 2005; DalCorso *et al.*, 2019) The overall and organizational performance of remediation plants such as Water Hyacinth and *Azolla* depends on a delicate kinetic balance between the velocity of influx and entry of toxic metal ions (Pb^{2+} and Cd^{2+}) across the membranes and the time efficiency of the internal defense systems of bio chelation and antioxidants (Clemens, 2006).

This network-based functional integration explains the variation in the efficiency and adaptive capacity of aquatic plants under different pollution stresses the success of the system depends not only on the surface adsorption capacity of the roots but also on the speed with which cells summon bio chelates and actively transport the resulting complexes to the vacuoles to avoid cytoplasmic toxicity (Ali *et al.*, 2019). This mechanistic interrelationship confirms to the academic community that phytoremediation represents a complex, integrated molecular biological response, and not merely a simple filtration or physical absorption process of pollutants from the environment. Surrounding aquatic environment (Kumar *et al.*, 2023).

2.6.6 Determinants of Physiological Stress and Biological Breakpoint

The kinetics and efficiency of these cellular defense pathways are directly influenced by the physicochemical determinants of the aquatic environment, excessive concentrations of external heavy metals lead to chemical saturation of adsorption sites on cell walls and inhibition of energy-consuming active transport channels due to ATP depletion (Rezania *et al.*, 2019).

When concentrations exceed the cell's toxic threshold, endogenous chelation and vacuole sequestration mechanisms fail to absorb the influx of free ions, leading the plant to reach its "biological breakpoint" at this stage, reactive oxygen species (ROS) are released explosively, attacking the chloroplast membrane and causing

chlorophyll degradation and inhibition of carbon-fixing enzymes, this translates phenotypically and visually into visible signs of physiological stress, such as leaf yellowing, chlorosis or necrosis, accompanied by a sharp slowdown in growth and biomass production, can lead to physiological variability, this physiological variation necessitates understanding the critical limits for ensuring the continuity of vital plant functions as an integrated biological system capable of productivity under conditions of acute mineral stress (Pilon-Smits, 2005; Lux *et al.*, 2011).

2.6.7 Kinetic Modeling and Mathematical Interpretation of Removal Processes Over Time

Recent and rigorous studies in the field of bioremediation increasingly employ temporal kinetic mathematical modeling as an advanced theoretical tool for understanding the dynamics of heavy metal removal from aquatic systems, rather than relying solely on surface descriptive analysis or fixed instantaneous measurements (Tang *et al.*, 2023; Zhou *et al.*, 2024). Well-known kinetic models, such as the pseudo-first-order and pseudo-second-order kinetics, are extensively utilized to estimate time-dependent removal rate constants and theoretical maximum adsorption capacities, linking dynamic concentration changes with the physiological saturation stages and defensive responses of plant cells under continuous toxic stress (Priya *et al.*, 2022; El-Sharkawy *et al.*, 2024; Segneanu *et al.*, 2024). Applying such mathematical models strictly requires empirical data collected across multiple time intervals to ensure reliable parameter estimation and theoretical fit (Al-Musawi *et al.*, 2023). However, kinetic modeling was not applied in the present study because the experimental design focused primarily on evaluating overall phytoremediation capacity, incorporating measurements solely at the Pre-treatment and Post-treatment stages after a 30-day exposure period without intermediate temporal sampling. Consequently, the study evaluated heavy metal removal performance based on the

net removal efficiency (%) and concentration differentials between the initial and final stages (Saba *et al.*, 2022).

2.7 Previous Studies

Previous studies were categorized using a chronological approach and within two structural axes to link local indicators with global data:

2.7.1 Local and Regional Studies (Iraq)

Al-Khafaji's study (2016): The researcher evaluated the efficiency of a group of locally grown aquatic macrophytes, including water hyacinth, in removing heavy metals from drainage canals in central Iraq, the results demonstrated that *Eichhornia crassipes* has a high capacity to retain and concentrate lead and cadmium in its root system compared to the vegetative parts, with a decrease in the pH of the aquatic environment due to the plant's acidic root exudates.

Khalid and Ganjo (2021) investigated the phytoremediation potential of native aquatic plants collected from Soran City, Erbil, Iraq, under outdoor experimental conditions. The study examined the removal and accumulation of several heavy metals, including lead, using different aquatic plant species. The results demonstrated that the selected plants were capable of adapting to contaminated conditions and accumulating metals through different phytoremediation mechanisms. The free-floating plant *Nasturtium officinale* showed a particularly notable capacity for lead uptake and removal, while other species demonstrated root accumulation and Phyto stabilization behavior.

Najem (2023) evaluated the biosorption capacity of water hyacinth (*Eichhornia crassipes*) root for several heavy metals, including lead and cadmium, using aqueous solutions under different initial metal concentrations and pH conditions. The study demonstrated a high biosorption capacity of the water hyacinth root, with lead showing the highest removal among the investigated metals. The results also showed

that metal removal was influenced by the initial concentration and pH of the solution, supporting the potential use of water hyacinth in wastewater treatment applications. Hassan et al., (2024) conducted a local study that tested the efficiency of the water fern (*Azolla pinnata*), isolated from Iraqi environments, in purifying industrially polluted water contaminated with cadmium and lead under continuous laboratory conditions. The study recorded a removal efficiency exceeding 78% for cadmium at low concentrations, while it showed signs of phytotoxicity, represented by yellowing of leaves and stunted growth, when the cadmium concentration exceeded 5 mg/L due to oxidative stress.

Huda and Ebrahim (2024) evaluated the performance of *Azolla pinnata* in a free-water-surface constructed wetland for removing lead and cadmium from simulated industrial wastewater under laboratory conditions in Iraq. The experiment included Pb and Cd concentrations of 5, 10, and 20 mg/L. The results showed that *Azolla pinnata* achieved higher removal efficiencies for lead, reaching 90.1%, 86.67%, and 81.3%, compared with 79.3%, 78.4%, and 69.7% for cadmium at the respective concentrations after five days of treatment. The study also reported changes in pH and dissolved oxygen during treatment, supporting the potential application of *Azolla* in phytoremediation systems.

Al-Ankaz et al., (2025) investigated the role of *Azolla* in the phytoremediation of heavy metals in sediments of the Tigris River in Kut City, Iraq. The study assessed several heavy metals, including lead, in sediment and *Azolla* samples and found that *Azolla* was capable of accumulating several metals and reducing their pollution effects in the sediments. Lead showed the highest accumulation potential among the investigated metals, indicating the suitability of *Azolla* for the remediation of contaminated sediments under Iraqi environmental conditions.

2.7.2 Global Studies

Jain et al., (2010) conducted a comparative analysis to evaluate the efficiency of Water Hyacinth and other aquatic plants in treating wastewater contaminated with varying concentrations of cadmium and lead under hydraulic retention periods (HRT) ranging from 7 to 21 days, the results showed the superior kinetic activity of Water Hyacinth in capturing and fixing lead ions in its root tissues during the first seven days via root filtration.

Sood et al., (2012) focused on the physiological potential of the genus *Azolla* as a biological tool for environmental remediation, experimental data demonstrated that symbiotic interaction with cyanobacteria partially protects the fern against the toxic effects of metals, enabling *Azolla* leaves to continue growing and accumulating cadmium at concentrations hundreds of times higher than those of the surrounding environment without cellular collapse.

The study by Rezanian et al., (2016) evaluated the biochemical efficiency of Water Hyacinth in extracting heavy metals from industrial wastewater by calculating biodegradation coefficients, the results demonstrated that the plant acts as an excellent Phyto stabilizer of lead due to its ability to restrict the metal's movement and retention through phosphorylation and retention mechanisms within its dense root system, preventing its translocation to the leaves.

The study by Ghosh et al., (2019) investigated the cytotoxicity pathways resulting from exposure of *Azolla filiculoides* to divalent cadmium ions (Cd^{2+}). The results showed that high concentrations induce a sharp increase in lipid peroxidation within cell membranes and inhibit the activity of antioxidant enzymes, leading to pronounced yellowing and a decrease in the plant's dry weight.

Kumar et al., (2021) evaluated the cadmium and lead removal efficiency of a co-planting system based on the alternation and integration of Water Hyacinth and

Azolla , the results showed that this bioengineering achieved 92% filtration efficiency for lead and 84% for cadmium, due to the synergy between the large surface area of Water Hyacinth roots and the rapid ion exchange facilitated by the cell walls of (*Azolla*).

Al-Baldawi et al., (2022) investigated the phytoremediation potential of *Azolla filiculoides* and *Lemna minor* for removing copper from aqueous solutions under different metal concentrations and exposure periods. The study demonstrated that *A. filiculoides* achieved high copper removal efficiency, reaching 100% at an initial concentration of 1.00 mg/L by the fifth day. The study also showed that removal kinetics could be described using a pseudo-second-order model, highlighting the importance of exposure time and plant biomass in determining phytoremediation performance.

Tang et al., (2023) investigated the role of physicochemical factors (pH, temperature, and dissolved organic matter (DOM) in controlling the uptake kinetics of heavy metals by floating macrophytes. The results indicated that lowering the pH to 5.5 doubles the rate of cadmium uptake due to increased solubility, while very high temperatures (above 35°C) impair the efficiency of active cellular uptake due to the effect on biofilm carriers.

Thomas et al., (2023) evaluated the phytoremediation potential of *Azolla filiculoides* and *Lemna minor* for removing multiple heavy metals, including cadmium and lead, from industrial wastewater in Ethiopia. The experiment demonstrated that both macrophytes were capable of accumulating heavy metals and reducing their concentrations in the wastewater, with measurable differences in removal efficiency and bioconcentration factors between the two plant species.

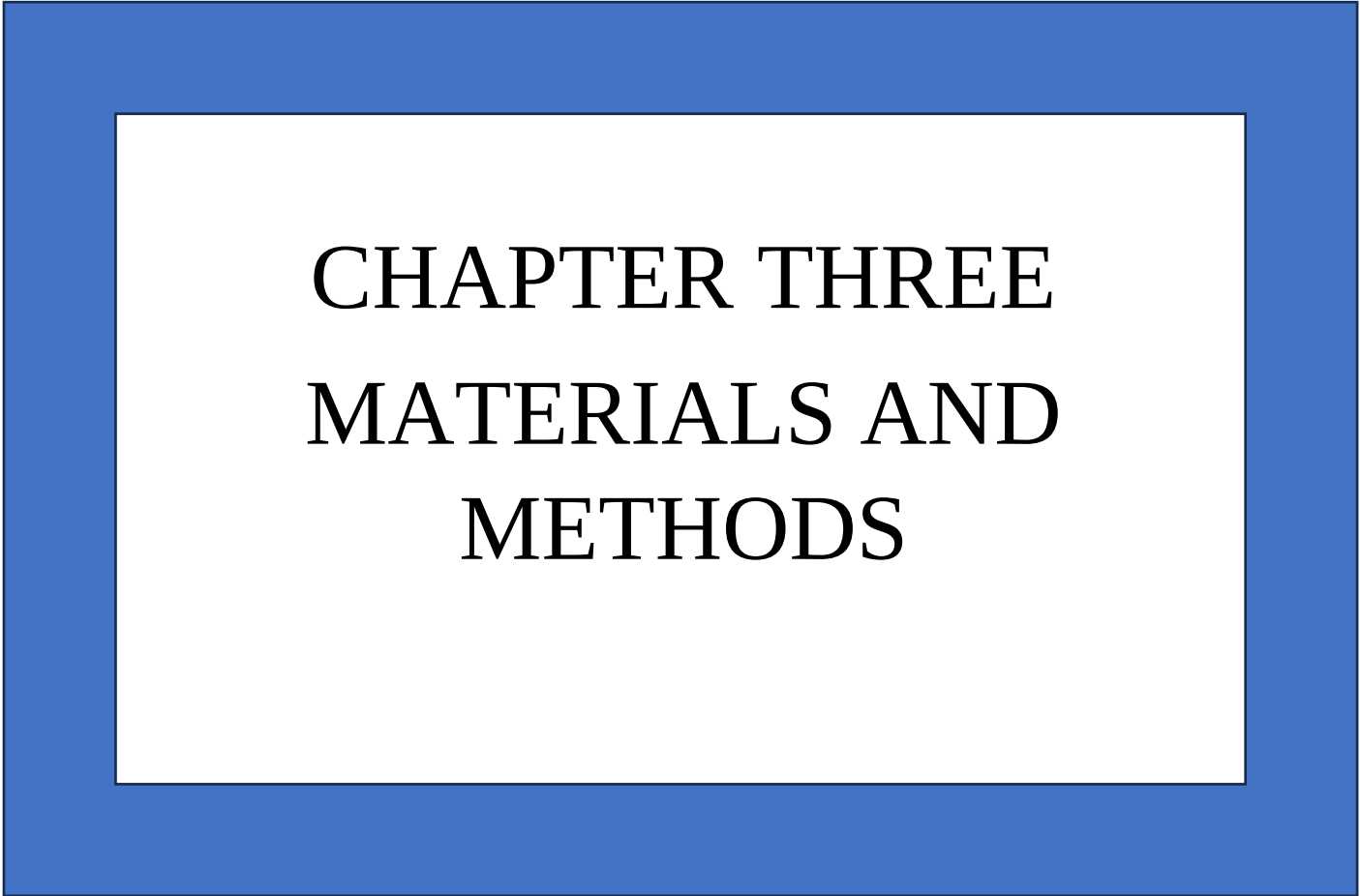
Kumari and Bauddh (2023) reviewed the application of *Eichhornia crassipes* for removing inorganic contaminants from aquatic ecosystems.

The study highlighted the ability of water hyacinth to remove several toxic metals, including cadmium and lead, through phytoremediation and adsorption-related processes, supporting its potential as an environmentally friendly and cost-effective treatment option for contaminated aquatic systems.

Al-Baldawi et al., (2023) evaluated the phytoremediation potential of *Eichhornia crassipes* in freshwater, industrial wastewater, and urban wastewater environments. The results demonstrated that the plant was capable of accumulating several heavy metals, including cadmium and lead, with higher bioconcentration factors generally observed in roots than in shoots. The study further showed that metal accumulation and translocation varied according to the characteristics of the contaminated water source, supporting the importance of physicochemical conditions in determining phytoremediation performance.

Noman et al., (2023) investigated the biochemical responses and phytoremediation potential of *Azolla imbricata* exposed to waste metal cutting fluid under different temperature and humidity conditions. The study reported substantial accumulation of Cd and Pb and demonstrated that the phytoremediation performance of *Azolla* was influenced by the environmental conditions and the composition of the contaminated medium. These findings emphasize the importance of environmental factors when evaluating the performance of floating aquatic plants for heavy-metal remediation.

Monroy-Licht and Carranza-Lopez (2024) reviewed the potential of *Eichhornia crassipes* for wastewater treatment and reported that water hyacinth has considerable capacity for the phytoremediation of heavy metals, including lead and cadmium. The review also highlighted improvements in several water-quality parameters and emphasized the potential of *E. crassipes* as a low-cost and sustainable option for contaminated aquatic environments.

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CHAPTER THREE MATERIALS AND METHODS

CHAPTER THREE

3.MATERIALS AND METHODS

3.1 chemical and devices

The chemical and devices used in the study showing in table (3.1) and (3.2) respectively

Table [3.1] chemicals used in the study.

<i>Mater</i>	<i>Manufacturer</i>
Lead nitrate ($\text{Pb}(\text{NO}_3)_2$)	Sigma-Aldrich (Merck Group)
Cadmium nitrate tetrahydrate ($\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$)	Sigma-Aldrich (Merck Group)
Nitric acid (HNO_3)	Merck
Perchloric acid (HClO_4)	Merck
Hydrochloric acid (HCl)	Spectrum Chemical
Calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$]	Sigma-Aldrich (Merck Group)

Table [3.2] equipment used in the study.

<i>Equipment</i>	<i>Model</i>	<i>Origin</i>
Atomic Absorption Spectrophotometer (AAS)	AAAnalyst 800	USA
pH meter	Orion Star A111	USA
EC meter	Lovibond Sensodirect 150	Germany
Digital thermometer	LaMotte	USA
Drying oven	Memmert UN55	Germany
Analytical balance	Sartorius	Germany

Hot plate	GER Retsch	Germany
Centrifuge	Sigma 3-16KL	China
Fume hood	LFH-13S / LFH-16S / LFH-18S	Korea
Micropipette	Eppendorf Research Plus	Germany
Volumetric flask	---	Germany
Beaker	---	Germany
Graduated cylinder	---	Germany
Glass funnel	---	Germany
Whatman No.42 filter paper	Whatman	England
Ceramic crucible	---	Germany
Ceramic mortar and pestle	---	Germany
HDPE plastic tanks	---	Local market
HDPE plastic containers	---	Local market

3.2 Experimental Timeline and Site Characterization

This study was conducted as an open-field experiment in a field containing a private nursery located approximately 5 km from the center of Samawah, Al-Muthanna Governorate, southern Iraq, at geographical coordinates of 31.403650° N latitude and 45.1985947° E longitude. The experiment aimed to evaluate the efficiency of water hyacinth (*Eichhornia crassipes*) and *Azolla pinnata* in removing lead (Pb) and cadmium (Cd) from water under natural environmental conditions.

The total study period lasted six weeks, including two weeks for acclimatization and physiological stabilization, followed by a 30-day period of actual exposure to heavy metal pollutants, from January 16, 2026, to February 15, 2026. This duration is

sufficient and appropriate for studying the uptake and bioaccumulation efficiency of heavy metals in these aquatic plants (Stepniewska *et al.*, 2012).

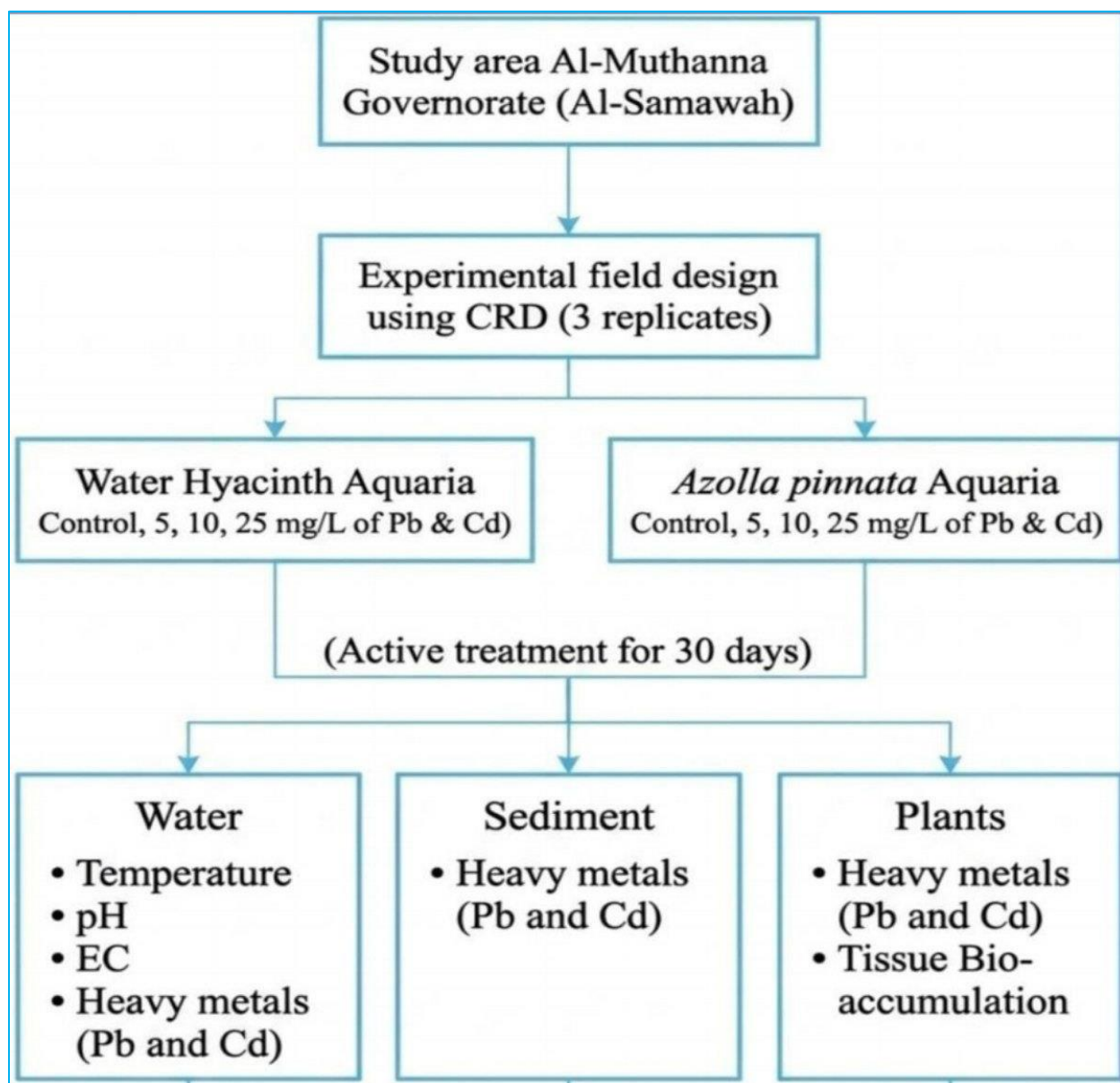


Figure (3.1) experimental design

3.2.1 Climatic Characteristics of the Study Site

Al-Muthanna Governorate is located within the semi-arid to arid climate zone, characterized by high levels of solar radiation and temperature, and low annual rainfall, these environmental factors directly affect open aquatic systems by

increasing evaporation and transpiration rates, making it an ideal and suitable environment for studying the performance of aquatic plants in treating polluted water under realistic field conditions similar to those prevailing in southern Iraq (Al-Anbari *et al.*, 2018).

3.3 Plant Source and Acclimatization Protocol

The acclimatization and preparation phase is a fundamental pillar for ensuring the accuracy of field results and was implemented according to the following methodological steps:

3.3.1 Plant Procurement and Cleaning

Live and healthy Water Hyacinth and *Azolla* plants were obtained from their natural sources in Baghdad Governorate, due to the potential presence of suspended particles or impurities on the foliage or roots, the plants were thoroughly and carefully washed with clean tap water to remove dirt and suspended materials before being introduced into the acclimatization environment.

3.3.2 Acclimatization Protocol

The plants underwent a two-week acclimatization period at the study site in Al-Muthanna Governorate, during this period, the plants were placed in basins containing clean water with very low and diluted concentrations of nutrient solutions to provide a stable growth environment, ensure their physiological stability, and minimize the impact of translocation shock before actual exposure to heavy metals (Upadhyay & Tripathi, 2007).

3.4 Preparation of Stock Solutions

To ensure the accuracy of the required experimental exposure concentrations, stock solutions were prepared at a concentration of 1000 mg/L for each of the heavy metals under study, following the following laboratory steps (Naghipour *et al.*, 2018).

3.4.1 Cadmium Stock Solution

A 1000 mg/L cadmium stock solution was prepared using 308.48 g/mol cadmium nitrate tetrahydrate $[\text{Cd}(\text{NO}_3)_2\text{O}]$, following these steps:

2.7473 g of the salt was accurately weighed using a sensitive four-tiered analytical balance.

The weighed amount was dissolved in 30 mL of a dilute acidic solution (consisting of 20 mL of distilled water with 10 mL of concentrated hydrochloric acid), this was done to ensure complete dissolution of the salt and prevent the precipitation of free cadmium ions by hydrolysis.

After complete dissolution, the solution was transferred to a 1-liter volumetric flask and diluted with distilled water up to the mark, resulting in a solution where each 1 mL contains 1 mg of active cadmium.

3.4.2 Lead Stock Solution

A 1000 mg/L lead stock solution was prepared using lead nitrate $[\text{Pb}(\text{NO}_3)_2]$ with a molecular weight of 331.2 g/mol, following these steps:

1.5985 g of the pure salt was accurately weighed.

The substance was dissolved in 30 mL of dilute acidic solution (20 mL distilled water + 10 mL hydrochloric acid) to keep the ions dissolved and prevent hydrolysis.

The resulting solution was transferred to a 1-liter volumetric flask, and the volume was topped up with distilled water to the mark. The solution was then ready for use, with each 1 mL containing 1 mg of active lead.

3.4.3 Preparation of Experimental Concentrations

The general dilution equation was adopted to determine the volumes of concentrated solutions to be drawn and prepared to obtain the target concentrations of (5, 10, 25) mg/L, according to the following equation:

$$\boxed{N_1 V_1 = N_2 V_2} \dots\dots\dots (1)$$

Where:

N 1: Concentration of the standard concentrated solution extracted with distilled water (1000 mg/L).

V 1: The volume to be withdrawn from the concentrated solution (mL).

N 2: The target experimental concentration in the tank (5, 10, or 25 mg/L).

V 2: The final total volume of water in the field experimental unit.

3.5 Experimental Design and Setup

In this study, a static batch system was implemented within a completely randomized design (CRD) to ensure homogeneity of environmental conditions and minimize the impact of uncontrolled external factors (Sood *et al.*, 2012). Accordingly, two main phases were adopted for measurement and analysis:

1.Pre-treatment Phase: This represents the baseline, where samples were taken and the physicochemical properties of the water and background metal in the plants were measured immediately after the acclimatization period and before the addition of the contaminating mineral concentrations.

2.Post-treatment phase: This represents the final evaluation phase, where water samples, complete plant biomass, and bottom sediments that appeared in the basins after the 30-day exposure period were collected to calculate removal efficiency, chemical sedimentation, and bioaccumulation.

The choice of a completely randomized experimental design (CRD) was based on the high environmental homogeneity among the experimental units and the absence of significant spatial gradients within the open field study site (such as sharp variations in light intensity, wind direction, or temperature), which reduces the likelihood of experimental bias.

This design is one of the most efficient and widely used statistical designs in environmental and field studies with homogeneous units, it provides high degrees of

freedom, which contribute to highly accurate estimation of experimental error, it also allows for direct statistical comparisons between different treatments and their interactions, efficiently isolating the influence of uncontrolled external factors (Gomez & Gomez, 1984).

The field experiment was divided into two completely independent systems based on plant type:

3.5.1 Water Hyacinth (*Eichhornia crassipes*) System

In this system, homogeneous plastic tanks made of high-density polyethylene (HDPE) were used, due to its chemical inertness which prevents the adsorption of metal ions on the walls (Vesk *et al.*, 1999).

Water Volume and Biomass: The volume of clean water in each tank was fixed at 10 liters to provide a water medium that allows for the normal growth of the dense root system (Lu *et al.*, 2004). An initial biomass of 100 grams of healthy fresh plants was added to each tank (at a density of 10 g/L), which is suitable for reducing internal competition and maintaining metabolic rates (Malar *et al.*, 2016).

Treatment and Replication Distribution: Three concentrations of heavy metals (5, 10, and 25 mg/L) were applied independently for both lead (Pb) and cadmium (Cd). The experiment was designed with three replications for each concentration, in addition to two separate control tanks (one for the lead experiment and one for the cadmium experiment) to ensure accurate statistical comparison and isolate the effect of each element. The total number of experimental units for the *Eichhornia* Water Hyacinth was 19.



Figure (3.2) *Eichhornia crassipes*

3.5.2 *Azolla* (*Azolla pinnata*) System

Due to the small morphological size and floating nature of the surface-dwelling *Azolla* plant, this system was designed using homogeneous plastic containers made of high-density polyethylene (HDPE), HDPE is chemically inert, preventing the adsorption of metal ions onto the walls (Vesk *et al.*, 1999), and its properties are consistent with standard.

Water and Biomass Volume: The volume of clean water in each container was fixed at 1 liter, and an initial biomass of 8 grams of fresh *Azolla* was added to each container.

Distribution of Treatments and Replications: The same exposure concentrations mentioned above (5, 10, and 25 mg/L) were applied for lead and cadmium, with 3 replications for each concentration. In addition, two separate control tanks (one for the lead test and one for the cadmium test) were used to ensure accurate statistical comparison and to isolate the effect of each element. The total number of experimental units for the *Azolla* system was 19 tanks.



(Figure 3.3) *Azolla pinnata*

3.6 Water Management and Nutrient Solution

The open-field nature of the experiment necessitated strict monitoring to maintain a stable aquatic environment throughout the 30-day experiment period. This was achieved through the following procedures:

3.6.1 Daily Water Replenishment

Due to the open climatic conditions at the study site, which lead to water evaporation and transpiration through the plants, water levels in the ponds were monitored daily, the ponds were periodically replenished with distilled water to restore them to their target volume (10 liters for Water Hyacinth and 1 liter for *Azolla*), this ensured that the concentration of pollutants and nutrients remained stable, preventing fluctuations caused by water drying and isolating the effect of volumetric changes.

3.6.2 Preparation of the Standard Nutrient Solution

To ensure the continued provision of nutrients to the plants after the acclimatization phase and to prevent the appearance of nutrient deficiency symptoms that could impair treatment efficiency, a complete and stable nutrient solution was used in all 40 experimental units throughout the trial period. The nutrient solution was prepared based on the standard formulation proposed by Issa (2006).

Due to the ongoing field need to replenish lost nutrients, the total quantities of chemicals required to prepare a total volume of 260 liters of nutrient solution were calculated to ensure sufficiency throughout the study period, as shown in Table (3.3).

Table 3.3: Standard formulation used to prepare the 260-liter standard nutrient solution (adapted from Issa, 2006)

Chemical Material	Chemical Formula	Addition Rate (g/L)	Total Amount Required for 260L (g)
Calcium Nitrate	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1.423	369.98
Potassium Nitrate	KNO_3	0.802	208.52
Monopotassium Phosphate	KH_2PO_4	0.175	45.50
Magnesium Sulfate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.307	79.82
Chelated Iron	Fe-EDTA	0.050	13.00
Manganese Sulfate	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.025	6.50
Boric Acid	H_3BO_3	0.003	0.78
Copper Sulfate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.008	2.08
Zinc Sulfate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.004	1.04
Ammonium Molybdate	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	0.006	1.56

3.7 Field Monitoring and Physicochemical and Chemical Analysis of the Aqueous Medium

3.7.1 Daily Temperature Measurement and Documentation (Winter Experiment)

Daily temperature variations in the aqueous medium were monitored and recorded for all 40 experimental units throughout the 30-day winter experiment to assess plant

tolerance to low temperatures. Measurements were taken daily at 12:00 PM to standardize ambient solar radiation and minimize temporal variability, a digital thermometer with a submerged probe was used, immersed in the mid-depth of each experimental unit to ensure accurate and stable readings, while avoiding the influence of surface and bottom layers, in accordance with standard guidelines (APHA, 2017).

3.7.2 pH and EC Measurement Protocol

Physicochemical measurements of both pH and EC were performed at two specific time points: the first, the pre-treatment phase, immediately after the addition and homogenization of the designated heavy-metal concentration in the experimental basins; the nutrient solution had been added to the distilled water prior to the addition of the heavy metals. Thus, this measurement represented the initial physicochemical conditions of the experimental water medium at the beginning of the phytoremediation treatment. The second, the post-treatment phase, was conducted at the end of the 30-day exposure period (February 15, 2026), as follows:

pH Measurement: A digital laboratory pH meter was used. Before measurements began, the probe underwent three-point calibration using standard buffer solutions at values of 4.01, 7.00, and 10.01 to ensure a stable response. The electrode was washed with distilled water and dried between samples to prevent cross-contamination (APHA, 2017).

Electrical Conductivity (EC) Measurement: A digital EC meter was used after calibration with a standard potassium chloride (KCl) solution of a known concentration, yielding a conductivity equivalent to 1.413 dS/m at 25°C. Measurements were performed by directly immersing the conductivity cell in the aqueous medium with automatic temperature compensation (ATC) enabled to mitigate the effects of temperature variations on the reading (APHA, 2017).

3.7.3 Measurement of Heavy Metal (Pb and Cd) Concentrations and Mass Balance Protocol

Due to the open field environment and the interaction of nutrient solution components with element ions, chemical precipitation was observed at the bottom of the tanks and experimental vessels, to ensure accurate mass balance calculations that illustrate the actual chemical pathway of heavy metal removal from the aqueous medium and guarantee the highest scientific reliability of the results, a comprehensive chemical analysis protocol was adopted to measure lead (Pb) and cadmium (Cd) concentrations in three baseline environments before the start of the experiment (as a baseline) and after the 30-day exposure period:

1.In the aqueous medium (Water Phytoremediation): to assess the efficiency of effective removal and the percentage reduction of pollutants from the aqueous solution throughout the experiment.

2.In the whole plant biomass: to determine the levels of bioaccumulation and the chemical distribution of metal within the total biomass of Water Hyacinth and *Azolla* plants without tissue separation.

3.In bottom sediments: to measure and determine the amount of heavy metals removed from the water column through natural chemical precipitation mechanisms and bottom stabilization at the bottom of the experimental units, and to compare them with the control parameters.

3.7.4 Quality Control/Quality Assurance

All field and laboratory measurements underwent a rigorous quality control system to ensure data reliability and statistical comparability. This included daily periodic calibration of instruments before use, verification of the stability of the electrodes, and remeasurement of randomly selected duplicate samples to confirm the

consistency of results and minimize experimental error, in accordance with internationally recognized standard procedures (APHA, 2017).

3.8 Contamination of the Water Hyacinth (*Eichhornia crassipes*) System

Each experimental basin containing Water Hyacinth (*Eichhornia crassipes*) was prepared with a water volume of 10 L. The required concentrations of lead (Pb) and cadmium (Cd) were prepared from a 1000 mg/L standard stock solution. The appropriate volume of the stock solution was measured using a graduated pipette and added directly to the designated experimental basins containing clean water and nutrient solution. The required volumes were calculated according to the target concentration and the water volume in each basin, as follows:

Concentration 5 mg/L: 50 mL of the 1000 mg/L stock solution was added to the experimental basin.

Concentration 10 mg/L: 100 mL of the 1000 mg/L stock solution was added to the experimental basin.

Concentration 25 mg/L: 250 mL of the 1000 mg/L stock solution was added to the experimental basin.

The same preparation procedure was applied separately to the Pb and Cd experimental setups. The preparation of the aqueous metal concentrations was conducted according to established principles for the preparation and analysis of aqueous samples (APHA, AWWA, and WEF, 2017).

3.9 Contamination of the *Azolla pinnata* System

For the *Azolla pinnata* experimental system, each experimental basin was prepared with a water volume of 1 L. The required Pb and Cd concentrations were prepared from the 1000 mg/L standard stock solution. The appropriate volume of the stock solution was measured using a graduated pipette and added directly to the designated

experimental basins containing clean water and nutrient solution, as follows:
Concentration 5 mg/L: 5 mL of the 1000 mg/L stock solution was added to the experimental basin.

Concentration 10 mg/L: 10 mL of the 1000 mg/L stock solution was added to the experimental basin.

Concentration 25 mg/L: 25 mL of the 1000 mg/L stock solution was added to the experimental basin.

The same procedure was applied separately to the Pb and Cd treatments.

3.10 Water Sample Collection and Determination of Dissolved Pb and Cd

Water samples were collected from each experimental basin at two sampling stages. The initial water samples were collected immediately after the addition and homogenization of the designated Pb or Cd concentration and before the beginning of the 30-day treatment period. Therefore, the initial concentration represented the initial concentration (C_i) of the metal in the experimental water at the beginning of the phytoremediation period. The final water samples were collected after completion of the 30-day treatment period and represented the residual concentration (C_f) remaining in the water after treatment. Before collecting each water sample, the upper part of the water column was mixed to obtain a representative water sample, while care was taken to avoid disturbing or resuspending the sediment at the bottom of the experimental basin. A 50 mL representative water sample was then collected from each experimental basin using clean sampling containers for the determination of Pb and Cd concentrations. Thus, the collected sample represented the water phase without intentionally incorporating the sediment layer. For the determination of Pb and Cd concentrations. Thus, the collected sample represented the water phase without intentionally incorporating the sediment layer. For the determination of

dissolved Pb and Cd, the collected water samples were filtered through a 0.45 µm membrane filter. The filtrate was then collected in clean sample containers and acidified with nitric acid to pH < 2 prior to instrumental analysis. Filtration through a 0.45 µm membrane before acidification is the operational definition used for dissolved elements in aqueous samples, and acidification of the filtrate to pH < 2 with nitric acid is a standard preservation procedure for dissolved metals (APHA, AWWA, and WEF, 2017; U.S. EPA Method 200.8). The concentrations of Pb and Cd in the filtered water samples were determined using Atomic Absorption Spectrophotometry (AAS). The analytical procedure was conducted according to established procedures for the determination of metals in water, including preliminary sample treatment and atomic absorption spectrometric determination (APHA, AWWA, and WEF, 2017). The removal efficiency (RE%) of Pb and Cd from the water was calculated using the initial and final concentrations according to the following equation:

$$R(\%) = \frac{\{C_i - C_f\}}{C_i} * 100 \dots\dots\dots(2)$$

where C_i represents the initial dissolved metal concentration in the water immediately after addition and homogenization of the metal solution, and C_f represents the final dissolved metal concentration remaining in the water after the 30-day treatment period (Lazo et al., 2022).

3.11 Plant Biomass Collection, Processing, and Acid Digestion. (Water Hyacinth and *Azolla*)

Representative portions of the plant biomass of Water Hyacinth (*Eichhornia crassipes*) and *Azolla pinnata* were collected from their respective experimental basins at two sampling stages to determine the concentrations of Pb and Cd

accumulated in the plant biomass. For each plant species, separate experimental basins were used for the Pb and Cd treatments, with each metal maintained in its corresponding experimental setup. Accordingly, plant samples exposed to Pb were collected from the Pb treatment basins, whereas plant samples exposed to Cd were collected from the Cd treatment basins. The first plant samples (pre-treatment) were collected before the addition of the respective heavy-metal solution to the experimental basins. These samples represented the initial metal status of the plant biomass prior to exposure to the experimental Pb or Cd concentrations. The second plant samples (post-treatment) were collected at the end of the 30-day exposure period from the corresponding Pb or Cd treatment basins. A representative portion of the plant biomass was carefully collected from each respective experimental basin. The entire biomass plant present in the basin was not collected for analysis; rather, a representative portion was taken for laboratory processing. The collected plant material was initially washed with running tap water to remove adhering sediment, silt, and visible impurities, followed by successive rinsing with deionized distilled water to remove residual surface-associated materials. The samples were then surface-dried using clean laboratory filter paper and transferred to clean ceramic crucibles. The plant material was dried in a convection oven at 70°C for 48 h until a constant dry weight was obtained (Allen, 1989). The dried plant material was ground and sieved to obtain homogeneous material suitable for chemical analysis. Accurately weighed portions of the dried and homogenized plant material were then transferred into clean conical flasks for acid digestion. A sample weight of 1.0 g was used for *Eichhornia crassipes*, whereas 0.5 g was used for *Azolla pinnata*. Acid digestion was performed using a mixture of concentrated hydrochloric acid (HCl) and nitric acid (HNO₃) at a ratio of 3:1 (v/v). The use of HCl: HNO₃ (3:1, v/v), commonly referred to as aqua regia, is an established acid-digestion approach for the extraction of trace and heavy metals from plant materials prior to instrumental

analysis (Assessment of Extraction Methods of Trace Metallic Elements in Plants, 2022; Comparison of Digestion Methods Using Atomic Absorption Spectrometry for the Determination of Metal Levels in Plants, 2023). The acid mixture was applied at a standardized ratio of 10 mL per gram of dry sample. Accordingly, 10 mL of the acid mixture was used for the 1.0 g *Eichhornia crassipes* sample, consisting of 7.5 mL HCl and 2.5 mL HNO₃. For the 0.5 g *Azolla pinnata* sample, 5 mL of the acid mixture was used, consisting of 3.75 mL HCl and 1.25 mL HNO₃. The samples were digested on a hot plate inside a fume hood, with the temperature gradually increasing to approximately 120°C until sufficient decomposition of the organic matter and a clear digest were obtained. After cooling, the digested solutions were filtered through ash-free Whatman No. 42 filter paper. The filtrates were quantitatively transferred and diluted with deionized distilled water to a final volume of 50 mL for both plant species. The prepared solutions were subsequently analyzed for Pb and Cd concentrations using Atomic Absorption Spectrophotometry (AAS). Acid digestion followed by filtration and instrumental determination is consistent with established procedures for the preparation of plant samples for heavy-metal analysis (Assessment of Extraction Methods of Trace Metallic Elements in Plants, 2022; Comparison of Digestion Methods Using Atomic Absorption Spectrometry for the Determination of Metal Levels in Plants, 2023). The same acid composition, acid-to-sample ratio, digestion conditions, filtration procedure, final extraction volume, and analytical technique were applied to the representative plant samples collected from the corresponding Pb and Cd treatment basins of both *Eichhornia crassipes* and *Azolla pinnata*.

3.12 Acid Digestion of Sediments (Water Hyacinth and *Azolla pinnata*)

At the end of the 30-day experimental period, following collection of the final water samples and separation of the plant biomass, the sediment accumulated at the bottom of each experimental basin was collected. The experiment consisted of separate experimental basins for each plant species and each heavy metal treatment. Water Hyacinth (*Eichhornia crassipes*) and *Azolla pinnata* were maintained in separate basins. For each plant species, the Pb and Cd treatments were also established in separate basins, with a corresponding control basin for each metal treatment setup. Thus, the sediment from the *Eichhornia crassipes*–Pb, *Eichhornia crassipes*–Cd, *Azolla pinnata*–Pb, and *Azolla pinnata*–Cd experimental setups was collected and processed separately. The entire amount of sediment accumulated at the bottom of each experimental basin was collected because the quantity of sediment was limited. The collected sediment was transferred to clean glass dishes and air-dried at room temperature in a clean environment until completely dry. The dried sediment was gently ground using an inert ceramic mortar and sieved to obtain homogeneous material suitable for chemical analysis. Sample homogenization and controlled acid digestion are important steps in the preparation of sediment samples for the determination of heavy metals by atomic spectrometric techniques (U.S. EPA Method 3050B). After homogenization, accurately weighed portions of the dried sediment were transferred into clean conical flasks and subjected to acid digestion. Acid digestion was performed using a mixture of concentrated hydrochloric acid (HCl) and nitric acid (HNO₃) at a ratio of 3:1 (v/v). The acid mixture was applied at a standardized ratio of 10 mL per gram of dry sample. Accordingly, 10 mL of the acid mixture was used for the 1.0 g sediment sample from the *Eichhornia crassipes* system, whereas 2 mL was used for the 0.2 g sediment sample from the *Azolla*

pinnata system. The corresponding acid volumes were 7.5 mL HCl + 2.5 mL HNO₃ for the 1.0 g sample and 1.5 mL HCl + 0.5 mL HNO₃ for the 0.2 g sample. The HCl: HNO₃ ratio of 3:1 is an established aqua regia digestion composition used for heavy-metal extraction from solid environmental matrices before instrumental determination. The digestion was carried out on a hot plate inside a fume hood, with the temperature gradually increased from approximately 80°C to 120°C until sufficient decomposition of the organic matter and a clear digest were obtained. After cooling, the digested extracts were filtered through ash-free Whatman No. 42 filter paper. The filtrates were then quantitatively transferred and diluted with deionized distilled water to the predetermined final volumes of 50 mL for *Eichhornia crassipes* sediment and 25 mL for *Azolla pinnata* sediment. The prepared extracts were subsequently analyzed for Pb and Cd concentrations using Atomic Absorption Spectrophotometry (AAS). Acid digestion, filtration, and dilution to a known volume are consistent with established sample-preparation principles for sediment samples intended for heavy-metal analysis by atomic spectrometry (U.S. EPA Method 3050B). The same acid composition, acid-to-sample ratio, digestion temperature, filtration procedure, dilution principle, and analytical technique were applied to the collected sediments from the four experimental setups to ensure consistency and comparability of the analytical procedure.

3.13 Spectrophotometric Analysis and Mathematical Calculations of Metal Removal Concentrations and Efficiencies

3.13.1 Measurement of Heavy Metal Concentrations (Pb and Cd)

The concentrations of lead (Pb²⁺) and cadmium (Cd²⁺) ions were measured in filtrate, aqueous extracts, sedimentary deposits, and final plant extracts using an atomic absorption spectrophotometer atomic. Calibration curves were prepared for each

element using standard solutions of known concentrations to ensure the accuracy of volumetric readings according to international standards (APHA, 2017).

3.13.2 Equation for Calculating Metal Concentration in Plant and Sediment

To calculate the actual concentration of heavy metals accumulated in the whole plant biomass and bottom sediments, measured in micrograms per gram ($\mu\text{g/g}$ dry weight), equivalent to mg/kg , the standard mathematical equation applied in digesters was adopted as follows:

$$E_{\text{Con.}} = \frac{A \cdot B \cdot df}{D} \dots\dots\dots(3)$$

Where:

E: Con: Actual concentration of the heavy metal in the plant sample or sediment ($\mu\text{g/g}$ or mg/kg).

A: Atomic absorption spectrometry (AAS) reading of the digester sample, measured in mg/L .

B: Final volume of the extract solution after filtration and dilution, measured in milliliters (mL), (50 mL for water hyacinth, *Azolla*, and Water Hyacinth sediment, and 25 mL for *Azolla* sediment).

df: Dilution Factor, which is equal to (1) if no further dilution of the extract is performed.

D: Total dry weight of the plant powder or sediment sample used in the digestion process, measured in grams (g), (1.0 g for Water Hyacinth and its sediment, 0.5 g for *Azolla*, and 0.2 g for the *Azolla* sediment).

3.13.3 Calculation of Heavy Metal Removal Efficiency from Water

The percentage removal efficiency (R/%) of lead and cadmium ions from the aqueous medium was calculated exclusively by experimental plant systems based on the change in water concentrations between the beginning and end of the experiment, according to the following equation (Kadlec & Wallace, 2008):

$$R(\%) = \frac{\{C_i - C_f\}}{C_i} * 100 \dots\dots\dots(4)$$

Where:

R%: Percentage or percentage removal efficiency from water (%).

C_i: Initial concentration of the heavy metal in the aqueous medium at the beginning of the experiment (mg/L).

C_f: Final concentration of the heavy metal remaining in the aqueous medium at the end of the experimental period (mg/L).

3.13.4 Calculating the Mass Lost (Removed) of Metal from the Aqueous Phase

To track the actual mass loss of heavy metal ions from the aqueous medium and determine the total mass loss of the metal in milligrams (mg) for mass balance calculations, the following volumetric equation was applied:

$$M_{\{Removed\}} = (C_i - C_f) * V_{Water} \dots\dots\dots(5)$$

Where:

M: Removed: Total mass of metal lost (removed) from the water, expressed in milligrams (mg).

C_i: Initial concentration of the metal in the water (mg/L).

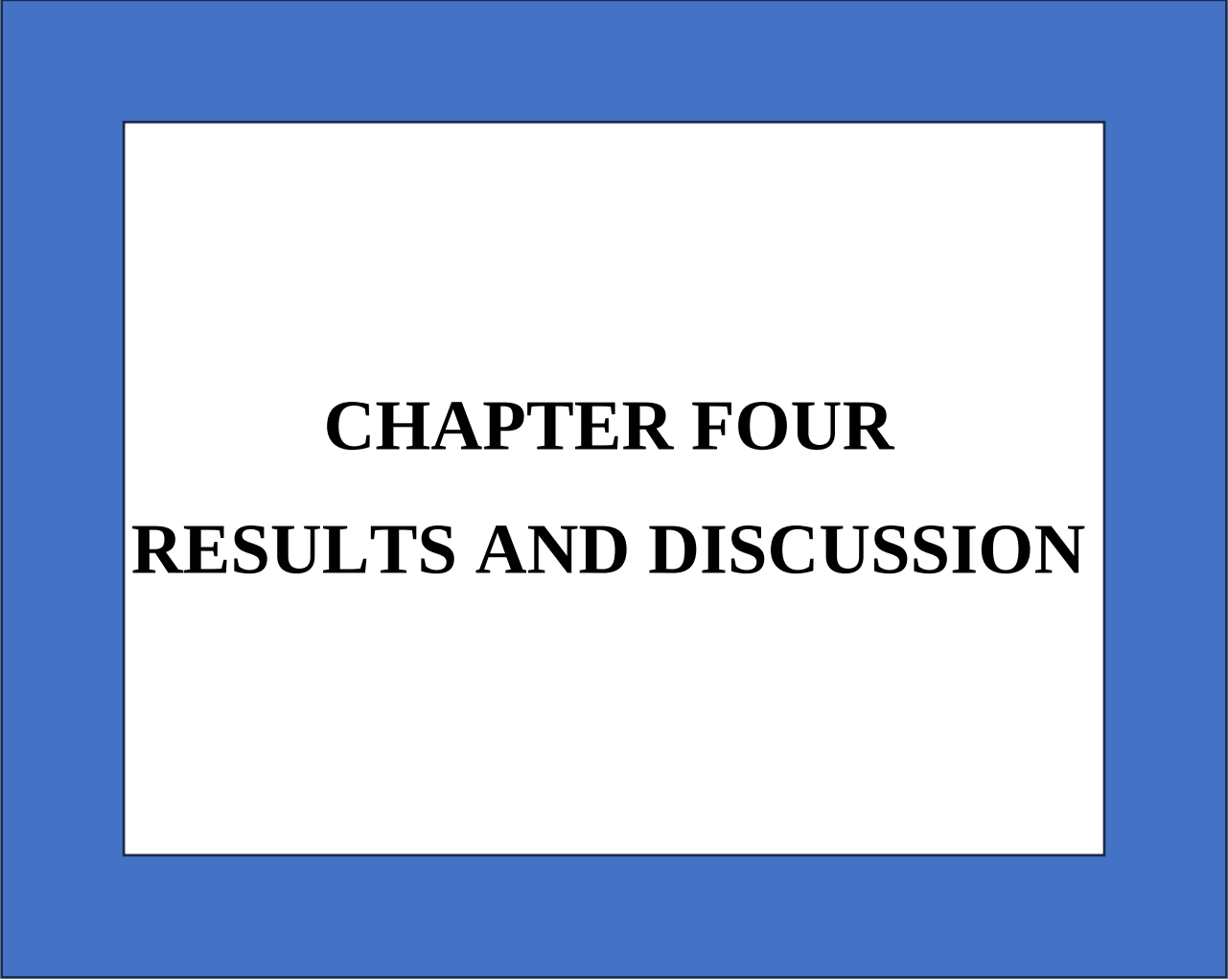
C_f: Final concentration of the metal in the water (mg/L).

V: Water: The total constant volume of water in the experimental basin, measured in liters (L), (10 L for Water Hyacinth basins and 1 L for *Azolla* vessels).

3.14 Statistical Analysis

The experimental data were statistically analyzed to evaluate the effect of different treatments (heavy metal concentrations), time intervals, and their combined effects on the studied classes, the statistical analysis was performed as a factorial experiment using a completely randomized design (CRD) with three replicates per treatment (Steel & Torrie, 1980).

The GenStat statistical software (Twelfth Edition) was used to perform a two-way ANOVA and to test the significance of differences between means (Payne *et al.*, 2009). To compare the mean values of treatments and pairwise interactions when significant differences were found, the Least Significant Difference (LSD) test was used at a significance level of 0.05, following the statistical methodology adopted in environmental and agricultural experiments (Gomez & Gomez, 1984). Furthermore, to understand the nature and strength of the reciprocal directional relationships between the physicochemical variables (temperature, pH and EC) and heavy metal concentrations in different phases (water, whole plant, and bottom sediment), Pearson's correlation coefficient (R) was calculated to determine the mathematical correlation between these treatments and track the overall efficiency of the biological system (Schober *et al.*, 2018).

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CHAPTER FOUR

RESULTS AND DISCUSSION

CHAPTER FOUR

Results and Discussion

4.1 Efficiency of (*Eichhornia crassipes*) in Treating Lead (Pb)-Contaminated Water

4.1.1 Effect of Physicochemical Factors on Lead (Pb) Removal

4.1.1.1 Dynamics and Influence of Daytime Temperature on the Phytoremediation System

The results of the field study showed that the temperature readings during the Pre-treatment and Post-treatment periods in the lead and cadmium treatment system basins using *Eichhornia crassipes* exhibited clear variations. The temperature values across the treatments ranged from a low of (15°C) to a high of (20°C), as shown in Figure (4.1).

The statistical analysis revealed significant differences between the measurement periods. The Pearson correlation results showed a positive correlation in the lead (Pb) treatment system between temperature and the rate of metal accumulation in the plant ($r = +0.70$), and a positive correlation with its accumulation in the bottom sediment ($r = +0.64$), compared to an inverse correlation with the residue in the water column ($r = -0.42$). In the cadmium (Cd) treatment system, temperature showed a positive correlation with the metal accumulation in plant tissues ($r = +0.78$), a positive correlation with its accumulation in the bottom sediment ($r = +0.73$), and an inverse correlation with the residue in the water column ($r = -0.30$). Temperature also showed a negative correlation with electrical conductivity ($r = -0.51$) and an inverse correlation with pH ($r = -0.29$), as shown in Table (4.1) and Table (4.2).

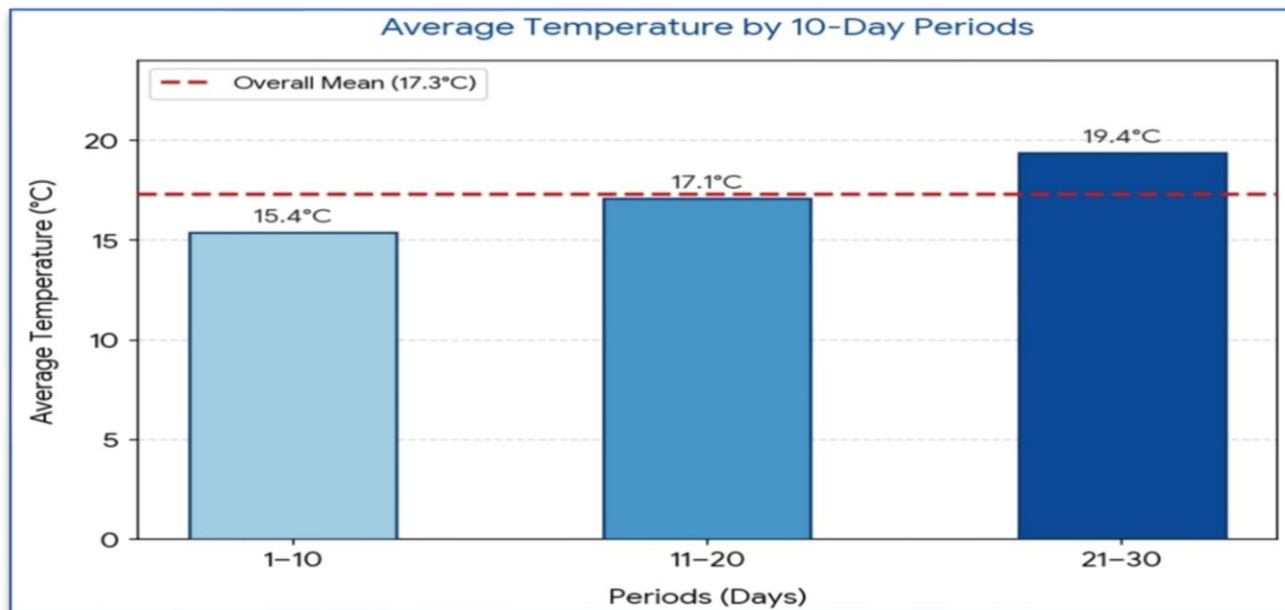


Figure (4.1): Thermal changes observed during the two study periods: Pre-treatment and Post-treatment by (*Eichhornia crassipes*)

The aforementioned statistical indicators indicate that the gradual increase in temperature during the post-treatment period was associated with changes in the distribution of lead and cadmium between the aqueous phase, plant tissues, and bottom sediment in the study environment. This is consistent with the observed relationships between temperature and metal concentrations in the different components of the phytoremediation system. However, these correlations indicate an association between temperature and metal distribution and do not by themselves confirm that the increase in temperature directly caused the uptake or accumulation of lead and cadmium. From a physiological perspective, the temperature fluctuations and increase during the post-treatment period may have contributed to changes in the activity of both the shoot and root systems of *Eichhornia crassipes*. The increase in temperature during the experimental period may have supported plant metabolic activity, water movement, and ion exchange processes at the root–water interface. These processes may affect the interaction between the plant and dissolved ions,

including lead and cadmium, during the treatment period (Kumar *et al.*, 2022). The observed changes in pH and electrical conductivity may also be related to nutrient uptake, ion exchange, and metabolic activity in the root environment (Singh & Balomajumder, 2024). The observed metal distribution during the lower temperatures in the pre-treatment period can also be related to the physicochemical interaction between the metal ions and the plant surfaces. Surface adsorption and binding of metal ions to functional groups on the root and plant surfaces may contribute to metal retention and can occur in addition to biological uptake processes (Valenzuela *et al.*, 2023). Therefore, the changes in temperature during the study period may have influenced the overall phytoremediation environment, while the observed metal reduction in water was associated with the combined contribution of plant interaction and retention within the bottom sediment (Nguyen *et al.*, 2021).

4.1.1.2 Effect of pH on the Bioavailability and Removal of Lead (Pb)

The results of the field experiment showed changes in the pH of the water medium between the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*) exposed to different concentrations of lead (Pb). During the pre-treatment stage, the pH values were (7.220), (7.340), and (7.380) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the pH values were (7.200), (7.270), and (7.197) at (5, 10, and 25 mg/L), respectively. In the control treatment, the pH increased slightly from (7.110) during the pre-treatment stage to (7.140) during the post-treatment stage. as shown in Table (4.1). and Figure (4.2)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed relationships between pH and the measured Pb-related parameters. Specifically, pH was associated with residual Pb concentration in water ($r = -0.59$), Pb accumulation

in plant tissues ($r = -0.55$), Pb accumulation in sediments ($r = -0.56$), and EC ($r = -0.90$), as shown in Table (4.2).

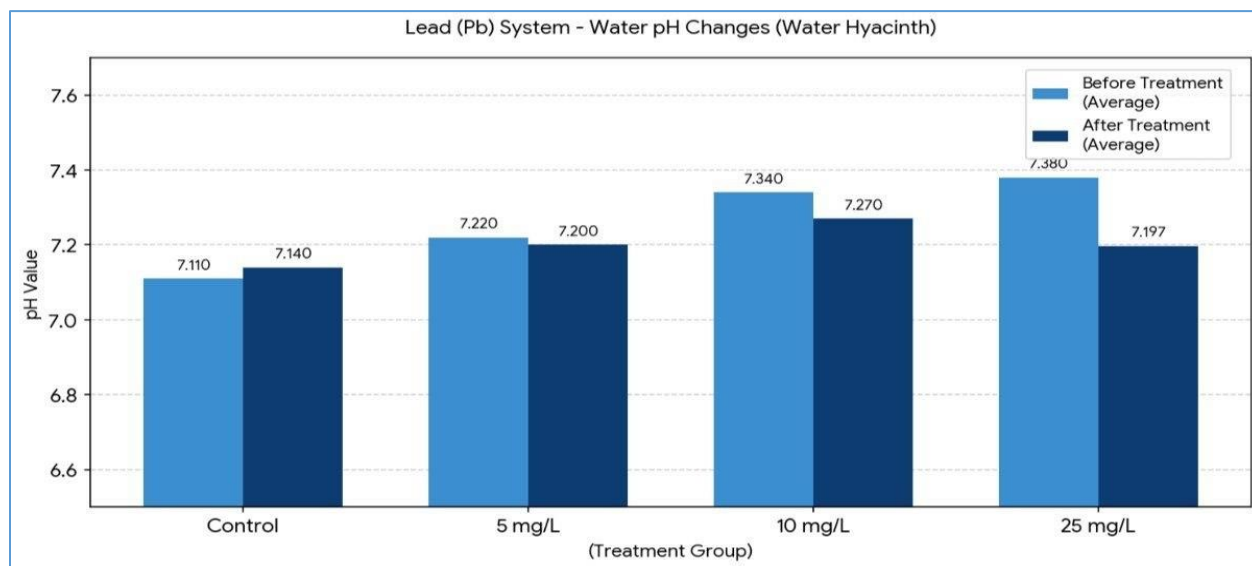


Figure (4.2): pH measurement in water with Pb by (*Eichhornia crassipes*)

The changes in pH between the pre- and post-treatment stages can be explained by the interaction between the presence of lead in the aqueous medium and the continued physiological activity of the water hyacinth, along with changes in dissolved ions resulting from the nutrient solution. Since all treatments received the same nutrient medium, the varying pH responses across different lead concentrations suggest that the increased metal load may have affected the chemical balance of the medium and the interaction between the roots and water, rather than the nutrient content alone. Furthermore, the fact that the pH values remained within the near-neutral range indicates that the plant maintained its physiological activity during the lead exposure period, which is consistent with the water hyacinth's known tolerance to heavy metals and their accumulation in its biomass (Newete & Byrne, 2016). From a physiological perspective, this change may be related to root activity in regulating the ionic balance between the plant and the aqueous medium. Nutrient

uptake from the environment can be accompanied by hydrogen ion (H^+) exchange at the root zone. Heavy metal stress can also affect the regulation of proton movement through the activity of cell membrane enzymes such as H^+ -ATPase (Jan *et al.*, 2017). Therefore, the presence of lead in the environment, along with the availability of nutrients, can lead to a change in the balance between ion uptake and root secretions, which is reflected in the pH of the water surrounding the roots (Vargas *et al.*, 2021). Thus, the observed change in pH can be interpreted as a combined response of the plant to lead and to the ongoing change in the ionic environment surrounding the roots. Furthermore, root secretions can contribute to modifying the chemical environment near the roots. Plants may release organic acids and other compounds into the root environment. These compounds can react with metal ions, affecting their mobility and availability, as well as influencing the pH balance (Montiel-Rozas *et al.*, 2016; Xia *et al.*, 2024).

This mechanism becomes important in explaining the differences between treatments, as increased lead concentration can increase chemical stress on the plant, potentially altering root activity and the nature of the root-water interaction. Furthermore, the respiration activity of roots and microorganisms associated with the root zone can contribute to pH modification. Carbon dioxide production during respiration can increase dissolved carbon dioxide in the water, potentially leading to carbonic acid formation and varying degrees of pH decrease (Kuzuyakov & Razavi, 2019). Therefore, changes in pH should not be explained solely by lead, but rather by the interaction between metal stress, plant and root activity, and changes in the aqueous environment and available nutrients (Kumar *et al.*, 2024).

The correlations between pH and lead in water, biomass, and sediments support a relationship between the pH state and the distribution of lead among the different components of the experimental system. This may be related to the effect of pH on the behavior of lead in the aqueous environment, including its movement, its

adhesion to solid surfaces, and its distribution among the aqueous phase, sediments, and biomass. Thus, a decrease in pH in some treatments may be associated with an increase in the presence of lead in these components. However, the correlation coefficients do not prove that pH change alone is the direct cause of lead uptake or accumulation (Tariq *et al.*, 2019; Gupta *et al.*, 2021).

The strong relationship between pH and EC can be explained by the fact that both variables reflect changes in the ionic environment of the water during the experimental period. Electrical conductivity is affected by the total number of dissolved ions, not just lead. Therefore, its value can be influenced by changes resulting from nutrient uptake by the plant, ion exchange at the roots, and the interactions of lead with the medium's components.

Thus, the relationship between pH and EC reflects a shared change in the aqueous chemistry, not necessarily a direct causal relationship between the two variables. The difference between the treatments is more pronounced at the highest lead concentration, where the change in pH was more significant, while the change at the lower concentration was limited. This could indicate that the increased lead load led to greater chemical and biological changes in the root zone, while the effect of the lower concentration treatment remained more limited (Srivastava *et al.*, 2017). In contrast, the small change in the control group suggests that some of the pH change can occur naturally as a result of the plant's interaction with water and nutrients, even in the absence of direct lead addition.

In general, the results indicate that the pH response in the water hyacinth system under lead treatment resulted from the simultaneous interaction of several factors, most importantly the presence of lead, nutrient availability, root activity, ion exchange, and chemical changes in the aqueous medium. Therefore, maintaining a pH within the near-neutral range, with variations between treatments, suggests that the system underwent a significant change in the chemical environment surrounding

the roots, while the plant continued to perform its vital functions during the treatment period.

4.1.1.3 Effect of Electrical Conductivity (EC) and Ionic Strength on Lead (Pb) Removal

The results of the field experiment showed a clear decrease in electrical conductivity (EC) of the water medium between the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*) exposed to different concentrations of lead (Pb). During the pre-treatment stage, the EC values were (1.929), (2.079), and (2.335 dS/m) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the EC values decreased to (1.679), (1.739), and (1.889 dS/m) at (5, 10, and 25 mg/L), respectively. The decrease was observed at all Pb concentrations. In the control treatment, EC decreased from (1.819 dS/m) during the pre-treatment stage to (1.710 dS/m) during the post-treatment stage. as shown in Table (4.1).and Figure (4.3)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed associations between EC and the measured Pb-related parameters. EC was associated with residual Pb concentration in water ($r = +0.82$), Pb accumulation in plant tissues ($r = +0.85$), and Pb accumulation in sediments ($r = +0.84$). In contrast, EC showed a strong association with pH ($r = -0.90$) and an association with temperature ($r = -0.51$), as shown in Table (4.2).

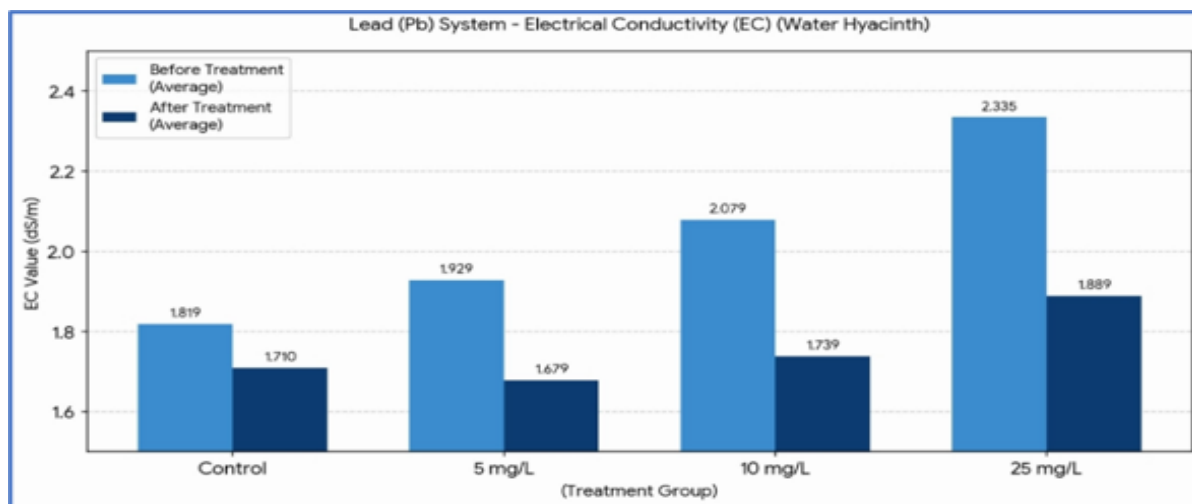


Figure (4.3) EC measurement in water with Pb by (*Eichhornia crassipes*)

Electrical conductivity (EC) reflects the overall ionic state of the aqueous medium. Therefore, the change observed between the pre- and post-treatment phases can be explained by the change in the total dissolved ions during the experimental period. Since the aqueous medium contained the nutrients necessary for plant growth, the initial EC value was not due to lead alone, but rather to the total ions present in the nutrient medium plus those resulting from the addition of the lead compound. Thus, the increase in EC with increasing lead concentration before treatment corresponds to an increased ionic load in treatments with higher metal concentrations. The significant decrease in EC after treatment can be primarily explained by the continued activity of the water hyacinth plant and its absorption of dissolved ions from the medium. The plant requires a range of mineral elements present in the nutrient solution for continued growth and metabolic processes. Consequently, the removal of these ions from the water during the exposure period can lead to a decrease in the ionic state of the medium and a corresponding decrease in EC (Lu *et al.*, 2018). Therefore, a decrease in EC does not necessarily represent a decrease in lead alone but rather reflects a broader change in the composition of the aqueous solution resulting from continuous plant activity.

The presence of lead may also have contributed to this change through its interaction with the root system of the water hyacinth. The plant's roots represent a large contact area with water, and the root surfaces and functional groups on them can act as binding sites for some metal ions, in addition to adsorption and ion exchange processes (Mishra *et al.*, 2020). Studies have shown the ability of water hyacinth to retain and accumulate metals from the aqueous medium, particularly through the interaction of metals with the roots and the translocation of some of them to the plant biomass (Newete & Byrne, 2016). Therefore, the translocation of some ionic substances from the aqueous phase to the plant or to root-associated surfaces can contribute to a decrease in EC during the treatment period.

On the other hand, the appearance of sediment in the basins provides an additional pathway for changes in the ionic state of the medium. Some lead ions and dissolved components may react with organic matter and solid surfaces in the system, then gradually migrate from the aqueous to the solid phase (Vazquez *et al.*, 2020). This migration can reduce the amount of mobile ions or dissolved substances in the water, thus contributing to a decrease in EC. However, EC represents the total dissolved ions, so its decrease cannot be considered direct evidence of lead precipitation alone but rather reflects the overall change in the water's chemical environment. The control group is particularly important in interpreting this result, as the decrease in EC in this group occurred in the absence of direct lead addition. This suggests that part of the decrease was related to natural processes occurring in the plant, water, and nutrient system, particularly nutrient uptake, plant growth, and the continuous change in the water's composition. Therefore, the observed decrease in lead coefficients represents an interaction between these fundamental processes, also present in the control group, and the additional effects associated with the presence of lead.

The relationship between EC and residual lead concentration in water is consistent with this interpretation, as the correlation value indicates that treatments that maintained higher levels of water ionicity were also associated with higher levels of residual lead. Furthermore, the correlation between EC and lead accumulation in plants and sediments suggests that changes in the medium's ionic environment coincided with lead redistribution between water, biomass, and sediments (Rezania *et al.*, 2019).

These relationships could reflect the interaction of uptake, root retention, and phase-transport processes; however, the statistical correlation does not prove that changes in EC were the direct cause of lead removal or accumulation.

The strong relationship between EC and pH indicates that changes in the medium's ionic state occurred concurrently with changes in the water's pH balance. This is consistent with the nature of plant systems, as ion uptake from the medium, root exudates, and chemical interactions between medium components can affect both ion composition and pH simultaneously. Thus, the relationship between the two variables reflects the combined change in the aqueous chemistry during the treatment period.

As for the relationship with temperature, it could be related to the effect of thermal conditions on plant biotic activity, ion mobility, and chemical reactions in the medium. Furthermore, the EC reading itself is affected by water temperature (Hayat *et al.*, 2021), which makes the interpretation of this correlation dependent on the environmental conditions accompanying the measurements. Therefore, the recorded relationship with temperature should be interpreted as a relationship between concurrent experimental variables, and not as evidence of a direct effect of temperature on lead removal.

In general, the decrease in EC after treatment indicates a significant change in the ionic environment of the aqueous medium during the exposure period. This change

appears to have resulted from the interaction of nutrient uptake, root activity, the interaction of lead with root surfaces, and the transfer of some substances between water, biomass, and sediment. The occurrence of a decrease in control also confirms that the normal activity of the plant in the nutrient medium was a key factor in the change in EC, while the presence of lead added other effects related to its behavior and interaction with the plant and water system.

4.1.2 Dynamics and Removal Efficiency of Lead (Pb) in the Water Medium

The results of the field experiment showed a clear decrease in lead (Pb) concentration in the water medium between the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*). During the pre-treatment stage, the Pb concentrations were (4.810), (9.820), and (24.320 mg/L) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the Pb concentrations decreased to (1.750), (3.390), and (7.040 mg/L) at (5, 10, and 25 mg/L), respectively. The removal efficiency (RE%) was (63.577%), (65.509%), and (71.064%) at (5, 10, and 25 mg/L), respectively. as shown in Table (4.1). and Figure (4.4)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed associations between residual Pb concentration in water and EC ($r = +0.82$), Pb accumulation in plant tissues ($r = +1.00$), and sediment Pb concentration ($r = +1.00$). Associations were also observed between residual Pb concentration and pH ($r = -0.59$) and temperature ($r = -0.42$), as shown in Table (4.2).

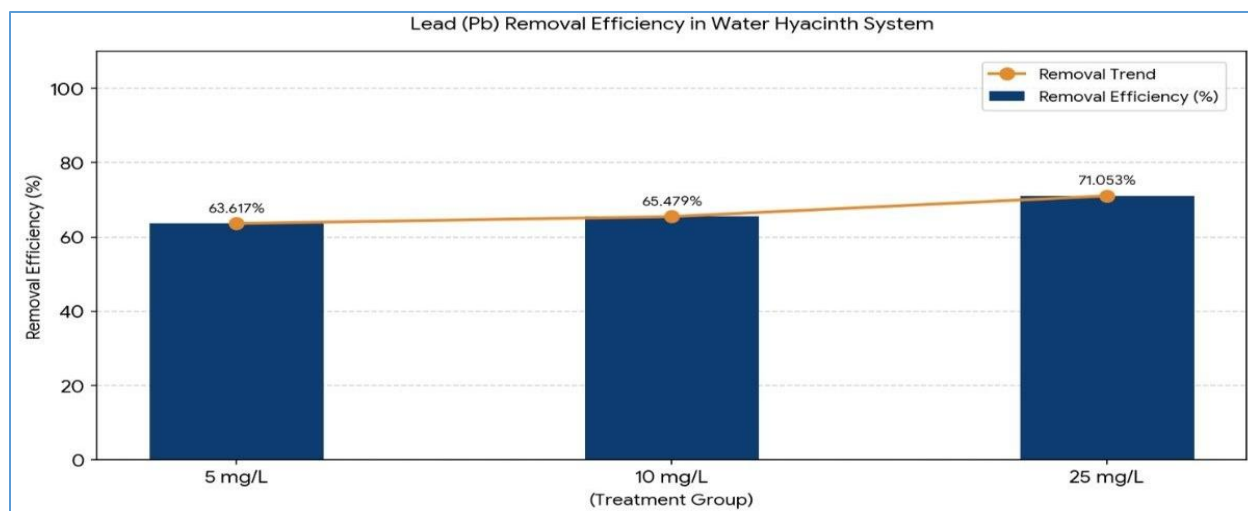


Figure (4.4): Pb Removal Efficiency (%) in Water-by (*Eichhornia crassipes*)

The decrease in lead concentration in the water during the treatment period can be explained by the gradual transfer of lead from the aqueous phase to other components of the experimental system, particularly to the plant biomass and benthic sediments. This change is consistent with the ability of water hyacinth to sequester heavy metals from the aqueous medium, as its roots possess a large surface area that allows for continuous interaction with metal ions present in the water. Studies have demonstrated the ability of *Eichhornia crassipes* to remove heavy metals from water and accumulate them in biomass, supporting the possibility that the plant contributed to the observed lead reduction (Newete & Byrne, 2016; Yan *et al.*, 2020).

The roots likely represented the initial site of lead interaction with the plant due to the direct and continuous contact between the roots and the aqueous medium. Lead can bind to root surfaces through adsorption, ion exchange, and binding to functional groups within the plant material, and some of it can then be translocated into plant tissues during the exposure period. Therefore, the increase in lead accumulation in biomass after treatment, coupled with a decrease in its concentration in the water,

supports the existence of lead transfer from the aqueous medium to the plant phase, although other pathways may still contribute to lead removal from the water (Newete & Byrne, 2016; Saleh *et al.*, 2021).

This uptake should also be considered in the presence of nutrients in the medium. The plant continued to absorb ions necessary for its growth while simultaneously being exposed to lead ions. Thus, the root zone represented an environment of continuous interaction between nutrients, lead, and root surfaces (Lyu *et al.*, 2022). This interaction may affect the binding sites and ion distribution between the water and the roots, potentially contributing to the varying behavior of lead at different concentrations. Consequently, the decrease in lead should not be attributed solely to plant uptake, but rather to the interaction between the plant's physiological activity and the accompanying ionic and chemical processes.

The results show that the amount of lead removed from the water increased with increasing initial lead concentration, which is expected because a higher concentration provides a greater quantity of lead available to interact with roots and associated surfaces. However, this does not necessarily mean that the plant became more efficient by the same percentage; the amount of metal removed represents the amount of lead that migrated or disappeared from the aqueous phase, while removal efficiency expresses the ratio of this reduction to the initial concentration (Kaur *et al.*, 2021). Therefore, the increased amount of lead removed at higher concentrations can be explained by an increase in the available metal load for the system, rather than necessarily by a proportional increase in the plant's relative removal capacity. In addition to the plant pathway, some of the reduction in water lead may be due to its migration to the solid phase. Lead in the medium may react with organic and inorganic materials, root-associated solid surfaces, and suspended particles. Adsorption, ion exchange, complexation, and precipitation processes can all contribute to reducing the dissolved or mobile fraction of the metal. Some lead-

associated particles or materials may then settle at the bottom of the ponds, which explains the presence of lead in the sediments after the treatment period. This finding supports the importance of viewing lead removal as a redistribution of the metal between water, plants, and sediments, rather than its disappearance from the system (Tariq *et al.*, 2019; Gupta *et al.*, 2021; Shahid *et al.*, 2021).

The correlations between lead in water and both biomass and sediments support this interpretation. The simultaneous change in these components indicates that the decrease in lead in the aqueous phase occurred concurrently with the presence of lead in the other phases. Furthermore, the relationship with EC suggests that lead behavior is linked to the overall ionic state of the medium. Since EC is influenced by the total dissolved ions, including nutrients and ions resulting from lead salts, this relationship could reflect the combined change in the ionic environment and the distribution of lead during the treatment period. However, the statistical correlations do not prove that changes in EC or increased lead accumulation in the plant or sediments were the direct cause of the lead reduction in the water. The relationship between residual lead and temperature may reflect the concurrent change in environmental conditions during the winter experimental period. Temperature can affect plant physiological activity, the rate of chemical reactions, and ion mobility, and thus the nature of the interaction between roots and lead may change with varying thermal conditions (Al-Jobori & Ahmed, 2020). However, the correlation value alone does not allow for determining the effect of temperature in isolation, as environmental and biological changes occurred simultaneously during the experimental period.

The control response is important in interpreting removal efficiency. The decrease in lead in the control, despite the absence of experimental lead application, indicates that the observed change in lead concentration was not solely related to added lead exposure. This change may be related to low background concentrations, changes in

the nutrient and plant medium, or analytical variability at very low concentrations (Gautam *et al.*, 2020). Therefore, the high removal efficiency in the control system should not be interpreted as the plant's processing efficiency for added lead but rather used to understand the extent of the underlying change that occurred in the system without the intended exposure to lead.

In general, the results indicate that the reduction in lead in the water during the treatment period was most likely due to the interaction of several pathways, including lead retention on the roots, accumulation of some lead in the biomass, translocation of some lead to sediment, and chemical and ionic reactions in the aqueous medium. The presence of nutrients in the medium also provided an environment conducive to continued plant activity, allowing for ongoing interaction between the roots, water, and lead during the exposure period. Therefore, the contribution of water hyacinth to lead reduction is best understood within a comprehensive system integrating phytochemical and physicochemical processes, rather than through a single mechanism.

4.1.3 Bioaccumulation of Lead (Pb) in Whole Plant Tissues

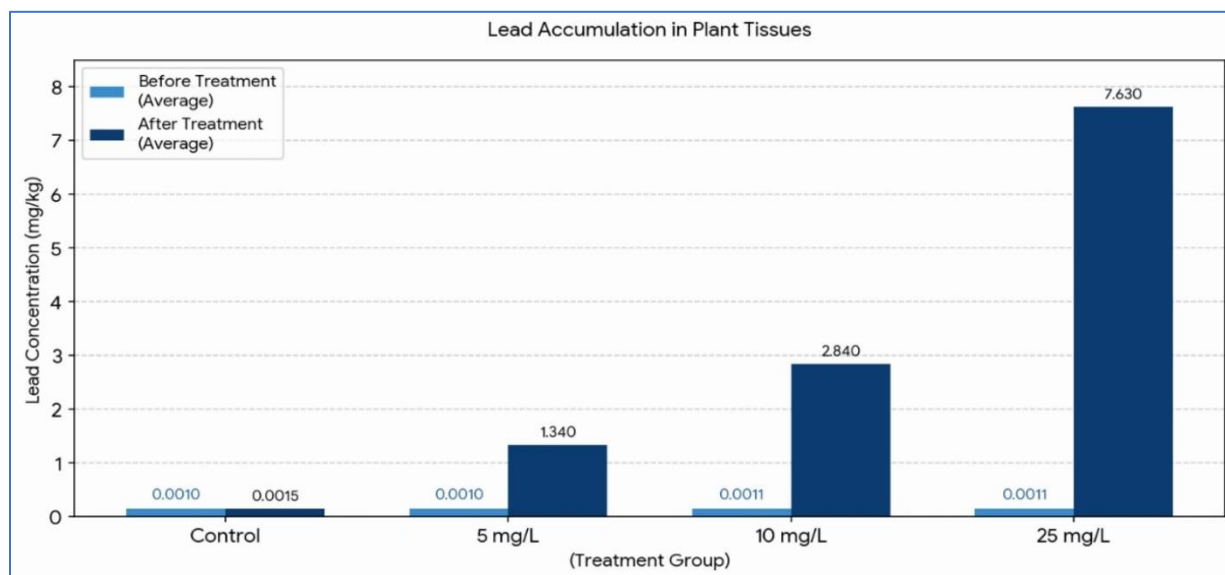
The results of the field experiment showed a significant increase in lead (Pb) accumulation in the whole plant tissues of water hyacinth (*Eichhornia crassipes*) between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, Pb concentrations in plant tissues were (0.0010), (0.0011), and (0.0011 mg/kg) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, tissue Pb concentrations increased to (1.340), (2.840), and (7.630 mg/kg) at (5, 10, and 25 mg/L), respectively. The increase was observed across all tested Pb concentrations, with the highest accumulation recorded at (25 mg/L). In contrast, the control treatment showed a small increase in tissue Pb concentration from (0.0010 mg/kg)

during the pre-treatment stage to (0.0015 mg/kg) during the post-treatment stage. as shown in Table (4.1). and Figure (4.5)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed relationships between Pb accumulation in plant tissues and the measured experimental parameters. The relationships with residual Pb concentration in water ($r = +1.00$), sediment Pb concentration ($r = +1.00$), EC ($r = +0.85$), and water temperature ($r = +0.70$) were observed, while the relationship with water pH was ($r = -0.55$), as shown in Table (4.2).

Figure (4.5): Pb concentration in Plant Tissues (mg/kg) by (*Eichhornia crassipes*)

The increase in lead (Pb) concentration within plant tissues after treatment indicates



that some of the lead present in the aqueous medium was translocated into the plant tissue and retained within the tissues of the water hyacinth (*Eichhornia crassipes*). The very low values before treatment suggest that the plant tissues contained limited background amounts of lead prior to exposure. The accumulation increased with increasing lead concentration, indicating that the increased availability of lead in the

medium provided a greater opportunity for it to react with the roots and subsequently translocate into the plant tissues (Eid *et al.*, 2021).

The nature of the water hyacinth's root system also contributed to the increased potential for lead retention. The plant's roots possess a large surface area that allows them to interact with metal ions present in the water. Lead retention may initially occur on the root surface through adsorption and ion exchange, and then some of it may translocate into the plant tissues, explaining the increased concentration observed after exposure (Newete & Byrne, 2016). The presence of nutrients in the medium can also influence this process, as plant nutrient uptake and the maintenance of intracellular ionic balance are linked to root activity and physiological processes (Al-Saeed *et al.*, 2023). Therefore, the lead accumulation observed in this experiment can be interpreted as a result of an interaction between lead availability, root activity, and the ionic conditions of the nutrient medium, rather than a single factor. The increased lead accumulation with rising external lead concentration suggests a pollutant-concentration-dependent pattern. As the lead concentration in the water increases, the amount of lead available to interact with the root surface also increases, thus increasing the potential for lead retention within the plant. This aligns with the ability of aquatic plants to accumulate metals when exposed to high concentrations of pollutants (Newete *et al.*, 2016). However, the high correlation coefficient between water lead and plant tissue lead reflects a simultaneous change in both variables and cannot be considered conclusive evidence that water lead concentration alone is the direct cause of increased accumulation (Mahmood *et al.*, 2022).

Furthermore, the high correlation between lead in plant tissues and lead in sediments indicates that lead was distributed throughout the experimental period between the water, plant tissues, and sediments (Hassan *et al.*, 2022). This is important in

explaining the low lead concentration in the water, as a decrease does not necessarily mean that all the lost water was transferred to the plant. Some of it could have been retained in the sediments as a result of physical and chemical processes such as adsorption, ion exchange, and precipitation. Therefore, lead accumulation in the plant represents one pathway for lead retention within the aqueous system, in addition to the sediment's contribution to retaining some of the metal.

As for the correlation between lead accumulation in tissues and electrical conductivity (EC) values, this can be explained in light of changes in the ionic state of the medium. Electrical conductivity reflects the total ions dissolved in water and not just lead; therefore, this correlation may reflect the simultaneous change in the concentration of lead and other ionic elements present in the nutrient medium (Mustafa *et al.*, 2023). Furthermore, plant nutrient uptake and root interaction with the environment can lead to changes in dissolved ion concentrations. Therefore, a relationship may emerge between EC and lead accumulation, without implying that EC itself is a direct cause of lead uptake. The correlation between lead accumulation and water temperature indicates that temperature changes coincided with changes in lead accumulation during the experiment. This may be related to changes in plant physiological activity, as temperature can affect metabolic activity, root activity, and nutrient uptake and transport processes. However, this correlation does not prove that higher temperature directly led to increased lead uptake but rather suggests that thermal changes and processes associated with lead accumulation occurred simultaneously during the experiment (Gupta *et al.*, 2021).

The relationship with pH indicates that changes in the chemical environment coincided with changes in lead accumulation in plant tissues. pH can influence the behavior of lead in aqueous environments, including its mobility and interaction with solid surfaces and roots. Consequently, the amount of lead available to react with

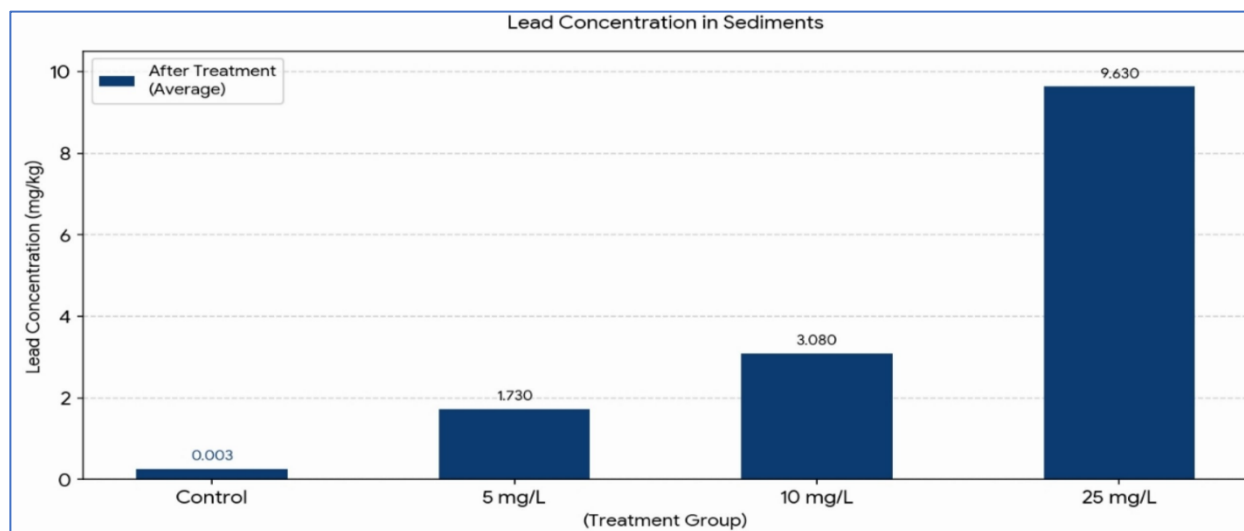
plants may change with pH variations (Tariq *et al.*, 2019; Gupta *et al.*, 2021). Therefore, the observed relationship between pH and lead accumulation may reflect the impact of changes in the water's chemical environment on lead distribution and its interaction with roots. In general, the accumulation of lead in plant tissues after treatment indicates that water hyacinth was able to sequester some of the lead during the exposure period. This accumulation appears to be related to increased lead availability, the efficiency of the root system in reacting with the metal, and the ionic and chemical conditions of the nutrient medium. Furthermore, the presence of lead in the sediments suggests that lead removal from the water resulted from its distribution among multiple components of the system, rather than its transfer to the plant alone. Therefore, lead accumulation in plant tissues represents an important pathway for metal sequestration, while the correlation coefficients reflect the relationship between the measured variables and do not establish a direct causal relationship between them.

4.1.4 Deposition and Accumulation Dynamics of Lead (Pb) in Bottom Sediments

The results of the field experiment showed a clear accumulation of lead (Pb) in the bottom sediments after the treatment period in the basins containing water hyacinth (*Eichhornia crassipes*). During the post-treatment stage, sediment Pb concentrations were (1.730), (3.080), and (9.630 mg/kg) at (5, 10, and 25 mg/L), respectively. Sediment Pb concentration increased with increasing applied Pb concentration, with the highest accumulation recorded at (25 mg/L). In the control treatment, the sediment Pb concentration was (0.003 mg/kg). as shown in Table (4.1). and Figure (4.6). Statistical analysis indicated a significant difference ($P \leq 0.05$) among the treatments. The Pearson correlation analysis showed associations between sediment Pb concentration and the measured experimental parameters. The relationship with

total plant tissue Pb accumulation was ($r = +0.99$), with residual Pb concentration in water was ($r = +0.99$), with EC was ($r = +0.84$), and with water temperature was ($r = +0.64$), while the relationship with water pH was ($r = -0.56$), as shown in Table (4.2).

Figure (4.6): Pb concentration in Sediments (mg/kg) by (*Eichhornia crassipes*)



The increased accumulation of lead in bottom sediments with increasing lead concentration can be attributed to the increased availability of lead in the aquatic medium. This leads to a greater opportunity for lead to bind to particles and materials present in the medium, with some of it then migrating to the bottom of the basin. As the treatment period continues, lead-bearing particles can settle to the bottom, further increasing lead accumulation in the sediments. This explanation is consistent with the nature of lead, which can bind to suspended matter, solid surfaces, and organic matter, facilitating its transfer from water to sediments (Newete & Byrne, 2016; Vargas *et al.*, 2021; Zhao *et al.*, 2022).

The dense root system of water hyacinth may also play a role in this distribution. The roots provide a large surface area for lead to interact with the plant surface, and their density can also help trap some of the particles and organic matter nearby. As

the plant continues to be exposed to lead, some of the metal can bind to these materials and then settle to the bottom of the basin. Therefore, the presence of lead in sediments, along with its accumulation in plant tissues, indicates that lead was not solely retained within the plant, but rather distributed among multiple components within the system (Newete & Byrne, 2016; Han *et al.*, 2023).

Furthermore, the presence of nutrients in the medium may be indirectly related to this process. Plant nutrient uptake and ion exchange at the root zone can alter the ionic and chemical conditions surrounding the roots. This can influence the interaction of lead with particles and surfaces in the medium, and consequently, its distribution among water, root-associated materials, and sediments (Li *et al.*, 2023). Thus, lead accumulation in sediments could be a result of the interaction between lead availability, root activity, and the chemical and ionic conditions of the medium, rather than a single precipitation process.

The very low lead concentration in the sediments of the control treatment can be explained by the absence of direct lead addition to the medium. Consequently, a sufficient quantity of the metal was not available to allow for significant accumulation at the bottom. This indicates that sediment formation alone does not explain the elevated lead levels in the treatments; rather, the accumulation is linked to the presence of a contaminating load in the medium (Nasser *et al.*, 2022). Changes in pH during the treatment period contributed to variations in the medium's chemical conditions. These changes can influence lead mobility and its interaction with particles and solid surfaces. When pH values change, lead's solubility or binding capacity to substances in water and sediment may also change, potentially affecting its migration from the aqueous medium to the bottom (Newete & Byrne, 2016; Gupta *et al.*, 2021; El-Khaldy *et al.*, 2023). This helps explain the correlation between lead distribution and changes in the medium's chemical conditions. Furthermore, changes in electrical conductivity reflect alterations in the medium's

ionic state resulting from plant interaction with water, nutrient uptake, and changes in the distribution of dissolved ions during the treatment period. Since EC does not represent lead alone, its relationship to lead accumulation in sediments can reflect combined changes in the ionic environment and the metal's distribution among system components. Therefore, the higher lead accumulation in sediments at higher treatments can be understood within the overall change in the aqueous medium, rather than as a result of lead alone (Xia *et al.*, 2024; Kumar *et al.*, 2024).

Furthermore, changes in water temperature during the experimental period may be related to changes in plant and root activity and certain physicochemical processes in the medium. These changes can indirectly affect particle movement and the interaction of metals with different surfaces (Raza *et al.*, 2023), but this does not imply that temperature was the direct cause of lead accumulation in the sediments. Overall, the lead accumulation in the sediments indicates that the decrease in water lead concentration during the treatment period was not solely due to its accumulation in plant tissues but was also associated with its retention in bottom sediments. This suggests that the treatment system had multiple pathways for lead distribution, involving water, plants, and sediments. Therefore, sediments represent an important component in the fate of lead within the system and have contributed to its retention along with the ability of the Nile grass to accumulate some of it in its tissues.

4.2 Efficiency of Water Hyacinth (*Eichhornia crassipes*) in Treating Cadmium (Cd)-Contaminated Water

4.2.1 Effect of Physicochemical Factors on Cadmium (Cd) Removal

4.2.1.1 Effect of pH on the Bioavailability and Removal of Cadmium (Cd)

The results of the field experiment showed that pH values in the water medium remained relatively stable within the near-neutral range during the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*) exposed to different Cd concentrations. During the pre-treatment stage, the pH values were (7.423), (7.460), and (7.510) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the pH values decreased to (7.177), (7.207), and (7.217) at (5, 10, and 25 mg/L), respectively. The decrease was observed at all tested Cd concentrations. shown in Table (4.1). and Figure (4.7)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed relationships between pH and the measured Cd-related parameters. The relationship with residual Cd concentration in water was ($r = +0.29$), while the relationships with Cd accumulation in plant tissues and sediment Cd concentration were ($r = +0.25$) and ($r = +0.26$), respectively, shown in Table (4.2)

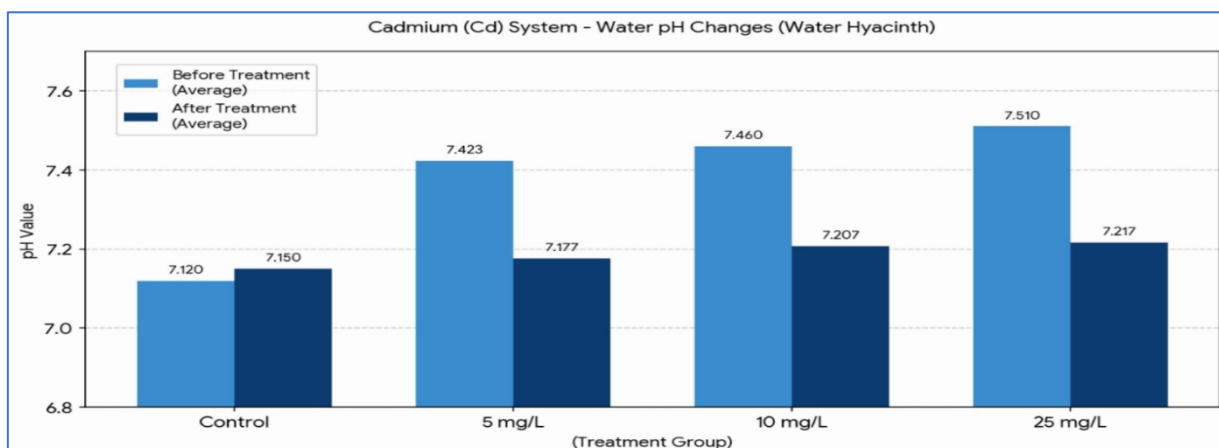


Figure (4.7): pH measurement in water under Cadmium (Cd) treatment by (*Eichhornia crassipes*).

The fact that the pH remained within the near-neutral range during the treatment period indicates that the aqueous medium maintained a relatively stable chemical state despite the plant's exposure to cadmium (Cd). The slight decrease in pH after treatment can be explained by the continued biological activity of the plant and the

interaction of its roots with the aqueous medium (Ahmad *et al.*, 2022). This change is expected in systems containing aquatic plants, as root activity and ion uptake processes can affect the ionic balance of the root-bound medium. The nutrient solution in the medium may also play a role in maintaining suitable ionic conditions for water hyacinth growth during the experiment, since nutrient uptake by the roots is accompanied by ion exchange with the medium. When the plant is exposed to cadmium, nutrient uptake can interfere with the root response to the metal, potentially leading to limited changes in proton mobility and the ionic state around the roots (Salama *et al.*, 2023). Therefore, limited pH changes can reflect the combined interaction of plant activity, nutrient uptake, and cadmium exposure, rather than the effect of cadmium alone.

pH changes can also be linked to root activity in the plant-water contact zone. Roots can alter hydrogen ion mobility through transmembrane transport, and root respiration and carbon dioxide production can contribute to limited changes in the surrounding environment. Furthermore, the secretion of certain organic compounds from roots can modify the nearby chemical environment, influencing cadmium behavior and its interaction with root surfaces (Vargas *et al.*, 2021; Xia *et al.*, 2024; Kumar *et al.*, 2024).

Chemically speaking, maintaining a pH close to neutral may be important in determining cadmium mobility and interaction with the surrounding environment. pH changes can affect cadmium solubility, mobility, and interaction with surfaces and roots, and thus the amount of the metal available for interaction with the plant. The binding of cadmium to functional groups on the root surface, such as carboxyl and hydroxyl groups, can also be affected by changes in the chemical conditions of the medium (Lopez *et al.*, 2021; Ibrahim *et al.*, 2022). Therefore, the relatively stable pH helped maintain the chemical conditions of the medium within a range that allowed cadmium to continue reacting with the plant and sediments.

The limited changes in pH and their relationship to the distribution of cadmium between water, plants, and sediments can be explained by the fact that, during the treatment period, cadmium did not remain in a single form within the system, but rather was distributed among the different components. A portion could react with the roots and enter plant tissues, while another portion could bind to particles and substances in the medium and reach the sediments (Zhao *et al.*, 2023). Therefore, pH changes may be one of the factors that contributed to determining the direction of cadmium distribution, along with plant activity and the ionic conditions of the medium (Newete & Byrne, 2016; Tariq *et al.*, 2019; Gupta *et al.*, 2021).

In general, the relative stability of pH indicates that the water hyacinth maintained its biological activity during the cadmium exposure period, with limited changes in the medium's chemical environment (Mahmoud *et al.*, 2024). This change could be attributed to the interaction between root activity, nutrient uptake, and the plant's response to cadmium, in addition to natural chemical changes in the medium. Furthermore, the role of pH in this experiment is related to its ability to influence cadmium behavior and distribution among water, plants, and sediments, and thus it can be considered one of the important environmental factors associated with cadmium removal in the water hyacinth system.

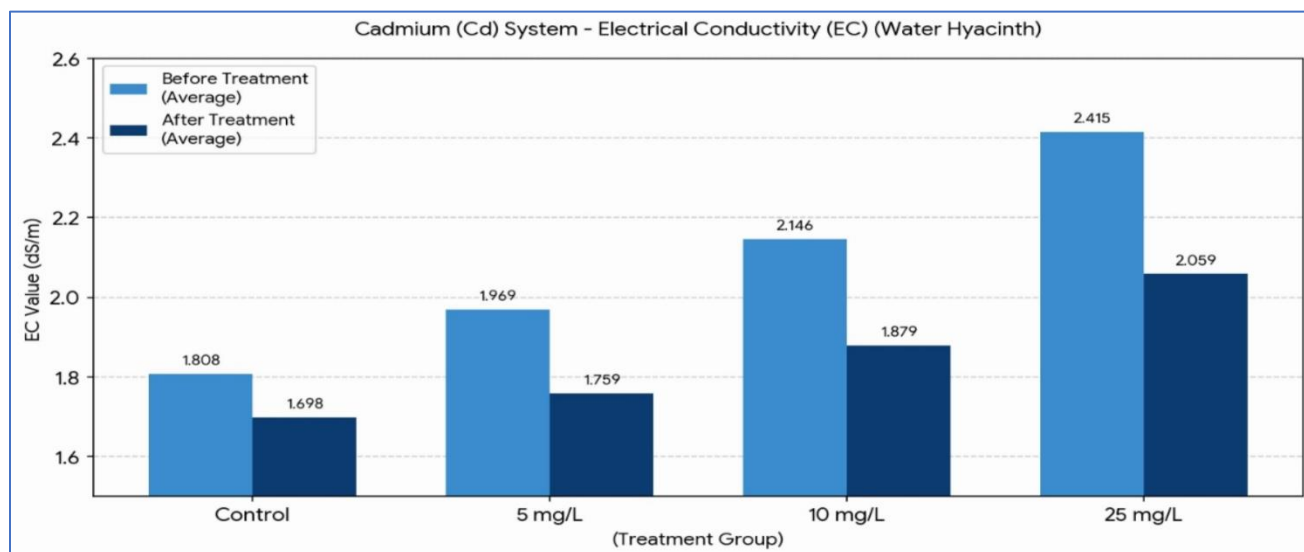
4.2.1.2 Effect of Electrical Conductivity (EC) and Ionic Strength on Cadmium (Cd) Removal

The results of the field experiment showed a clear decrease in electrical conductivity (EC) of the water medium between the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*) exposed to different Cd concentrations. During the pre-treatment stage, the EC values were (1.969), (2.146), and (2.415 dS/m) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the EC values decreased to (1.759), (1.879), and (2.059 dS/m) at (5, 10, and

25 mg/L), respectively. The decrease was observed at all tested Cd concentrations. In the control treatment, EC decreased from (1.808 dS/m) during the pre-treatment stage to (1.698 dS/m) during the post-treatment stage. These variations are presented in Table (4.1) and Figure (4.8).

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed relationships between EC and the measured Cd-related parameters. The relationships with Cd concentration in water, Cd accumulation in plant tissues, and sediment Cd concentration were ($r = +0.99$), ($r = +0.98$), and ($r = +0.98$), respectively. The relationship with water pH was ($r = +0.41$), while the relationship with temperature was ($r = -0.51$), as presented in Table (4.2).

Figure (4.8): EC measurement in water with Cd by (*Eichhornia crassipes*)



The high electrical conductivity values observed in treatments exposed to cadmium concentrations of 5 to 25 mg/L before treatment can be explained by the increased ionic load in the medium with the added cadmium concentration, in addition to the ions already present in the nutrient medium. Since EC reflects the total ions dissolved in water, the increased cadmium concentration led to a greater contribution

of treatment-related ions to the ionic state of the medium, resulting in higher values at higher concentrations (Adamu *et al.*, 2022; Vargas *et al.*, 2021; Usman *et al.*, 2023).

The significant decrease in EC after the treatment period can be explained by the continued activity of water hyacinth and its absorption of nutrients and dissolved ions from the medium. The plant requires these elements to maintain its growth and physiological activity, and continued exposure during the experiment can reduce the amount of some dissolved ions due to their absorption by the roots, thus affecting the EC. Therefore, a decrease in EC does not represent cadmium removal alone but rather reflects a general change in the ionic composition of the medium resulting from plant activity (Newete & Byrne, 2016; Al-Farraj *et al.*, 2022).

Furthermore, an increase in cadmium concentration in the medium (from 5 to 25 mg/L) signifies an increased pollutant load to which the roots are exposed. Water hyacinth roots act as the primary contact point between the plant and water. Some cadmium can bind to the root surface through adsorption and ion exchange, while other cadmium can be translocated into plant tissues. As these reactions continue, the ion distribution between water and root-bound portion can change, contributing, along with nutrient uptake, to a decrease in EC after treatment (Gupta *et al.*, 2021; Kumar *et al.*, 2024; Tariq *et al.*, 2023).

Moreover, an increase in cadmium concentration does not mean that its effect on EC is isolated from the other components of the medium; Since cadmium was present alongside other nutrients and ions, nutrient uptake, ion exchange, and interactions between the roots and the medium occurred during the treatment period. Therefore, the change in EC at different treatments reflects the overall change in the ionic environment with increasing pollutant load, rather than the effect of cadmium alone (Farid *et al.*, 2023).

These processes also contributed to the distribution of cadmium among water, plant tissues, and sediments. When plants are exposed to higher concentrations, a greater amount of cadmium is available to interact with roots and particles, increasing the likelihood of its retention in plant tissues or its binding to substances and particles in the medium, with some of it being transported to the sediments. Thus, the change in EC coincides with the change in cadmium distribution among the system components (Xia *et al.*, 2024; Shanker *et al.*, 2023). However, this coincidence does not imply that a decrease in EC is the direct cause of cadmium removal. The relationship between EC and pH can be explained by the combined change in the chemical and ionic conditions of the medium. Nutrient uptake and root interaction with cadmium can influence ion mobility and the chemical equilibrium near the roots, potentially leading to simultaneous changes in both EC and pH. Temperature during the experiment can also affect plant activity and ion mobility, in addition to its impact on electrical conductivity. Therefore, changes in EC may be related to a combination of environmental and physiological conditions rather than a single factor (El-Nagar *et al.*, 2022).

The decrease in EC in the control treatment is significant in interpreting this result, as the control was not exposed to cadmium. Therefore, the observed decrease suggests that part of the change in EC was related to natural processes within the system, particularly nutrient uptake by the water hyacinth and changes in the concentration of dissolved ions during the treatment period.

Consequently, the decrease in cadmium coefficients can be understood as a result of the interaction between plant activity and nutrient uptake with root interaction with cadmium and changes in ion distribution within the medium (Ibrahim *et al.*, 2023). In general, the change in EC across cadmium treatments reflects the change in the ionic state of the medium with increasing contaminant load, followed by a decrease

in the fraction of dissolved ions during the treatment period due to continued plant activity and reactions in the root zone. These processes interfere with the distribution of cadmium between water, plants, and sediments, making EC an indicator of the overall change in the medium during treatment, rather than a direct measure of the amount of cadmium removed by the plant.

4.2.2 Dynamics and Removal Efficiency of Cadmium (Cd) in the Water Medium

The results of the field experiment showed a clear decrease in cadmium (Cd) concentrations in the water medium between the Pre-treatment and Post-treatment stages in basins containing water hyacinth (*Eichhornia crassipes*). During the pre-treatment stage, the Cd concentrations were (4.833), (9.713), and (24.270 mg/L) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the residual Cd concentrations decreased to (2.117), (4.077), and (8.387 mg/L) at (5, 10, and 25 mg/L), respectively. The removal efficiency (RE%) was (56.174%), (58.045%), and (65.443%) at (5, 10, and 25 mg/L), as shown in Table (4.1). and Figure (4.9).

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages. The Pearson correlation analysis showed strong associations between residual Cd concentration in water and Cd accumulation in plant tissues ($r = +0.99$), sediment Cd concentration ($r = +0.99$), and electrical conductivity ($r = +0.99$). The relationship between residual water Cd and pH was ($r = +0.29$), while the relationship with water temperature was ($r = -0.30$), shown in Table (4.2)

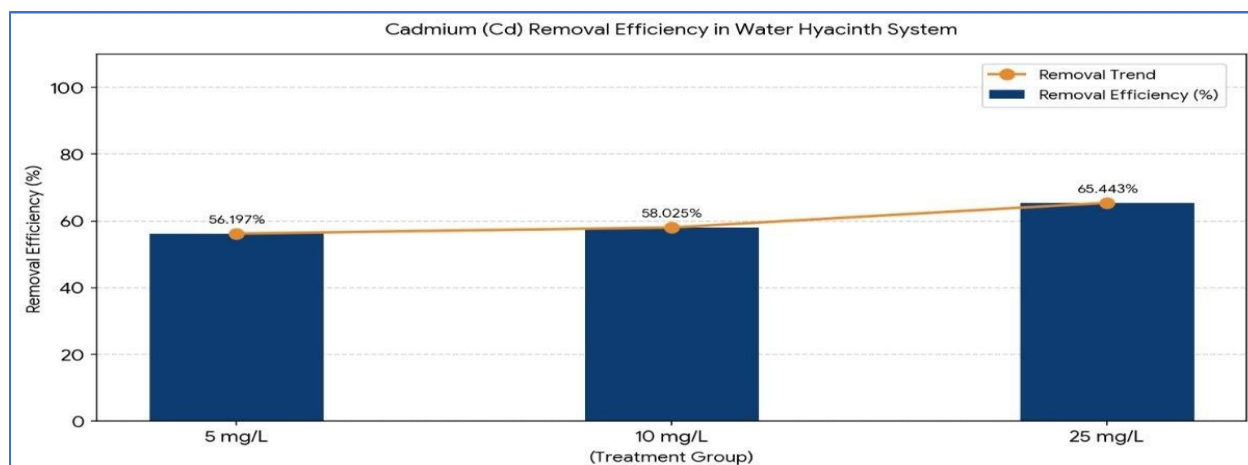


Figure (4.9): Cd Removal Efficiency (%) in Water-by (*Eichhornia crassipes*)

The decrease in cadmium (Cd) concentration in the aqueous medium after treatment can be explained by the translocation of some cadmium from the water to other components within the system, particularly plant tissues and sediments (Ibrahim *et al.*, 2023). This change was more pronounced in the cadmium-treated treatments compared to the control group, suggesting that the presence of water hyacinth and its interaction with the medium contributed to reducing the residual cadmium in the water. Furthermore, increasing the added cadmium concentration (from 5 to 25 mg/L) means increasing the amount of the metal available to interact with the roots and particles in the medium, thus explaining the continued presence of cadmium in the water even after the treatment period.

The dense root system of water hyacinth is another important factor in explaining the reduction of cadmium in the water, as the roots provide a large surface area for interaction with metal ions. When a plant is exposed to cadmium, some of it can bind to the root surface, some can be transferred to the plant tissues, and some can remain bound to other substances and particles in the medium (Al-Soliman *et al.*, 2023). These interactions facilitate the transfer of cadmium from the aqueous phase to the

plant-bound phase, thus contributing to a reduction in its residual concentration in the water (Vargas *et al.*, 2021; Xia *et al.*, 2024).

Furthermore, the presence of nutrients in the medium was crucial for the plant's continued activity during the treatment period, as nutrient uptake and the maintenance of ionic balance are linked to root activity and plant physiological processes. As the water hyacinth continued to grow, its roots continued to interact with the medium and absorb ions while simultaneously being exposed to cadmium (Habib *et al.*, 2024). Therefore, the decrease in Cd in the water can be explained by the interaction between the mineral's availability, root activity, and nutrient uptake, rather than solely by cadmium absorption.

The increased cadmium concentration in the medium led to a greater amount of the metal available for interaction with the plant and other materials in the medium. Therefore, at higher treatment levels, a greater volume of cadmium was available for distribution among the water, plant tissues, and sediments. This suggests that the amount of cadmium remaining in the water after treatment represents the portion that did not migrate to other components during the experiment, while the decrease in water concentration reflects the migration and retention of some of the metal outside the aqueous phase (Qasim *et al.*, 2023).

The decrease in water cadmium cannot be attributed solely to plant activity, as sediment formation during the experiment provides another pathway for metal retention. Cadmium can bind to suspended particles or to the surfaces of sediments and organic matter and then settle at the bottom of the tank. Therefore, the decrease in water cadmium better reflects a distribution process involving the metal between the plant, sediments, and the aqueous medium, rather than pure plant removal (Mahmood *et al.*, 2022; Xia *et al.*, 2024).

Furthermore, the ionic conditions of the medium can influence cadmium behavior and distribution. The presence of nutrients and dissolved ions alongside cadmium

can affect reactions in the root zone and the metal's binding to various surfaces. Therefore, the change in electrical conductivity that accompanied the decrease in cadmium reflects a general change in the medium's ionic environment resulting from the interaction of plant nutrient uptake with cadmium distribution between water, plants, and sediments (Adamu *et al.*, 2022).

Meanwhile, limited variations in pH and temperature may have contributed to changes in the chemical and physiological conditions of the medium during the experiment. pH can influence cadmium mobility and its interaction with roots and particles, while temperature may affect plant activity and processes associated with metal uptake (Saleh *et al.*, 2022).

However, these factors cannot be considered as single, direct causes responsible for the cadmium reduction, but rather as part of the conditions under which the metal's distribution occurred (Gupta *et al.*, 2021; Mahmood *et al.*, 2022).

The reduction observed in the control treatment is significant in interpreting removal efficiency, as the control treatment was not exposed to cadmium addition. Therefore, the change in the control treatment does not represent the removal of added cadmium but could be related to natural changes in the medium, nutrient uptake, plant activity, or metabolic changes at low background concentrations. In contrast, the reduction in cadmium treatments is associated with the presence of a genuine contaminant load and the interaction of this metal with the plant, the medium, and sediments. In general, the decrease in cadmium concentration in water after treatment indicates that some of the metal has migrated from the aqueous phase and become trapped within various system components. This can be explained by the interaction of cadmium with the root system of water hyacinth, with some being absorbed into plant tissues and some binding to particles and sediments. Plant activity, nutrient uptake, and the chemical and ionic conditions of the medium also contributed to determining the behavior of cadmium during the treatment period. Therefore, the

decrease in Cd in the water reflects a multi-path distribution and retention process within the system, with water hyacinth contributing to the removal of some cadmium from the aqueous medium.

4.2.3 Bioaccumulation of Cadmium (Cd) in Whole Plant Tissues

The results of the field experiment showed a clear increase in cadmium (Cd) accumulation in the whole-plant tissues of water hyacinth (*Eichhornia crassipes*) between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, Cd concentrations in plant tissues were (0.0009), (0.0013), and (0.0010 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, Cd concentrations increased to (1.573), (3.277), and (9.483 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. The increase in Cd accumulation was greater at the higher applied Cd concentrations, with the highest accumulation recorded at (25 mg/L). In the control treatment, Cd concentration increased from (0.0012 mg/kg) during the pre-treatment stage to (0.0016 mg/kg) during the post-treatment stage. shown in Table (4.1) and Figure (4.10).

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages, The Pearson correlation analysis showed strong associations between Cd accumulation in plant tissues and Cd concentration in water ($r = +0.99$), sediment Cd concentration ($r = +0.99$), and EC ($r = +0.98$). The relationship with temperature was ($r = +0.78$), while the relationship with pH was ($r = +0.25$), as shown in Table (4.2)

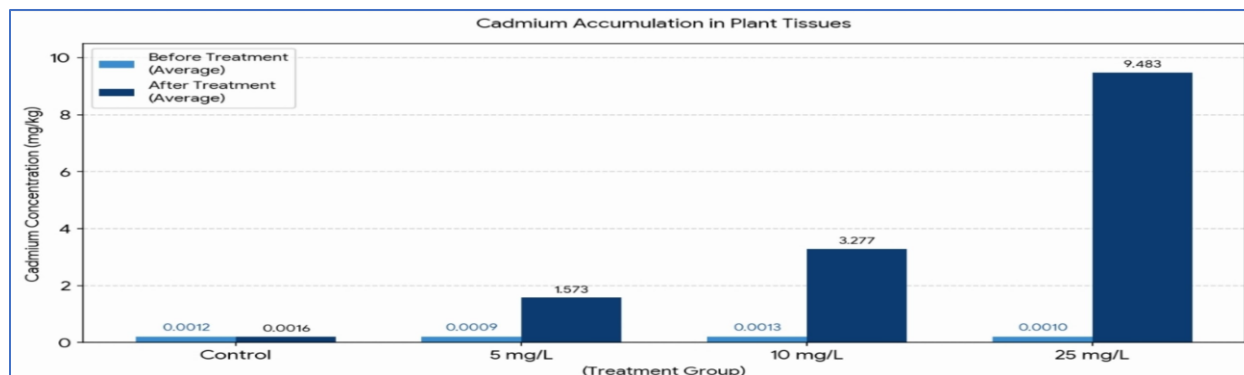


Figure (4.10): Cd concentration in Plant Tissues (mg/kg) by *Eichhornia crassipes*)

The increase in cadmium (Cd) concentration in plant tissues after treatment indicates that the cadmium present in the aqueous medium did not remain entirely in the water, but rather some of it was translocated into the water hyacinth biomass and retained within its tissues (Al-Soliman *et al.*, 2024). This increase was more pronounced when the added cadmium concentration was raised from 5 to 25 mg/L, as a higher contaminant load provides a greater quantity of Cd for interaction with the plant's root system, thus increasing the amount of the metal available for adsorption, absorption, and accumulation within the tissues.

This is particularly relevant to the dense root system of water hyacinth, which provides a large surface area for direct interaction with the contaminated water. At concentrations of 5, 10, and 25 mg/L, the metal can first react with the root surfaces, and then some of it can be translocated into the plant tissues. Therefore, the increased Cd concentration in the tissues with increasing applied concentration reflects increased root exposure to the metal and an increased amount of Cd available for uptake and plant retention (Siddiqui *et al.*, 2021; Qasim *et al.*, 2024).

Furthermore, the continued presence of nutrients in the medium throughout the experimental period was crucial for maintaining plant physiological activity. Nutrient uptake is linked to root activity and ion movement between water and plant

tissues, while simultaneously, the roots were exposed to cadmium. Therefore, Cd accumulation in the tissues could be attributed to the interaction of metal exposure with normal root activity and ion uptake processes, rather than solely to the presence of Cd (Habib *et al.*, 2025). This explains why cadmium accumulation became more pronounced with increasing concentration in the medium while plant activity continued throughout the treatment period.

After Cd enters the plant, its retention in the tissues helps explain the significant increase in its concentration following treatment. Plants can retain some of the absorbed cadmium by binding to various cellular components or sequestering it within their tissues, potentially reducing its toxicity and allowing for continued accumulation. However, this study measured Cd concentration in total plant tissues and did not identify the specific cellular components or the precise intracellular location of the metal. Therefore, these mechanisms are potential explanations rather than directly established mechanisms in this experiment (Siddiqui *et al.*, 2021; Saleh *et al.*, 2023).

Furthermore, the elevated Cd levels in plant tissues coincided with the presence of Cd in the water and sediment, indicating that the metal was distributed among different system components during the treatment period. Some of the Cd available in the water could interact with the roots and enter plant tissues, while another portion could bind to particles or settle in the sediment. Therefore, the accumulation of Cd in the plant does not necessarily mean that all the cadmium lost from the water was transferred to the plant but rather represents one of the distribution pathways of the metal within the experimental system.

The relationship between Cd accumulation and electrical conductivity can be explained by the overall change in the ionic environment of the medium. Electrical conductivity does not reflect Cd concentration alone, but is influenced by the total

dissolved ions, including nutrients and ions resulting from the addition of the metal compound. As Cd concentration increases, the amount of ions in the medium also increases, while plant activity and nutrient uptake alter this ionic environment during the treatment period. Therefore, the correlation between EC and Cd accumulation reflects the synchronization of changes in ion load with cadmium exposure and is not evidence that EC itself causes Cd accumulation in the plant (Adamu *et al.*, 2022). Temperature may also have influenced the level of plant physiological activity during the experiment. As temperature conditions change, the intensity of physiological processes, root activity, and ion mobility may alter, potentially affecting Cd translocation to plant tissues. However, temperature was not an independent experimental factor in this study; therefore, its relationship to Cd accumulation is interpreted as part of the environmental conditions accompanying the experiment, rather than as a direct cause of accumulation (Mishra *et al.*, 2023; Kumar *et al.*, 2024).

Changes in pH can influence the chemical behavior of cadmium and its availability for interaction with root surfaces. However, the limited change in pH and its weak correlation with Cd accumulation suggest that pH was not the primary factor explaining the variation in tissue cadmium accumulation during this experiment. Therefore, it appears that Cd availability in the medium and root interaction with it were more closely associated with increased plant accumulation than the limited changes in pH (Usman *et al.*, 2024).

It is noteworthy that the control treatment showed only a slight increase in tissue Cd, which is expected given its lack of exposure to added Cd concentrations (5, 10, and 25 mg/L). This limited increase could be attributed to background levels in the medium, natural variations in ionic and nutrient conditions, or analytical variability at low concentrations. Conversely, a large increase in contaminated treatments is

more consistent with direct plant exposure to added cadmium and its increased availability to the roots. In general, the increased accumulation of Cd within water hyacinth tissues reflects the plant's ability to take up a portion of the available cadmium from the medium and retain it within its biomass (Farid *et al.*, 2024). This accumulation increased with the applied Cd concentration, resulting from a higher pollutant load and the greater availability of the metal for interaction with the root system. Root activity, nutrient uptake, and changes in the ionic and chemical environment of the medium also contributed to determining Cd behavior during the treatment period. Thus, tissue accumulation of cadmium represents one of the main pathways for Cd transfer from the aqueous medium to system components, with another pathway being its retention within the sediments.

4.2.4 Deposition and Accumulation Dynamics of Cadmium (Cd) in Bottom Sediments

The results of the field experiment showed that cadmium (Cd) accumulated in the bottom sediments during the post-treatment stage in all added Cd treatments compared with the control treatment. During the post-treatment stage, Cd concentrations in the sediments were (1.087), (2.257), and (6.287 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. The highest Cd accumulation in the sediments was recorded at the highest applied concentration of (25 mg/L). In the control treatment, the Cd concentration in the sediment was (0.001 mg/kg) dry weight. shown in Table (4.1) and Figure (4.11).

Statistical analysis revealed a significant difference ($P \leq 0.05$), The Pearson correlation analysis showed a very strong association between Cd accumulation in plant tissues and sediment Cd concentration ($r = +0.99$). The relationships with pH, temperature, and EC were ($r = +0.26$), ($r = +0.73$), and ($r = +0.98$), respectively, as

shown in Table (4.2)

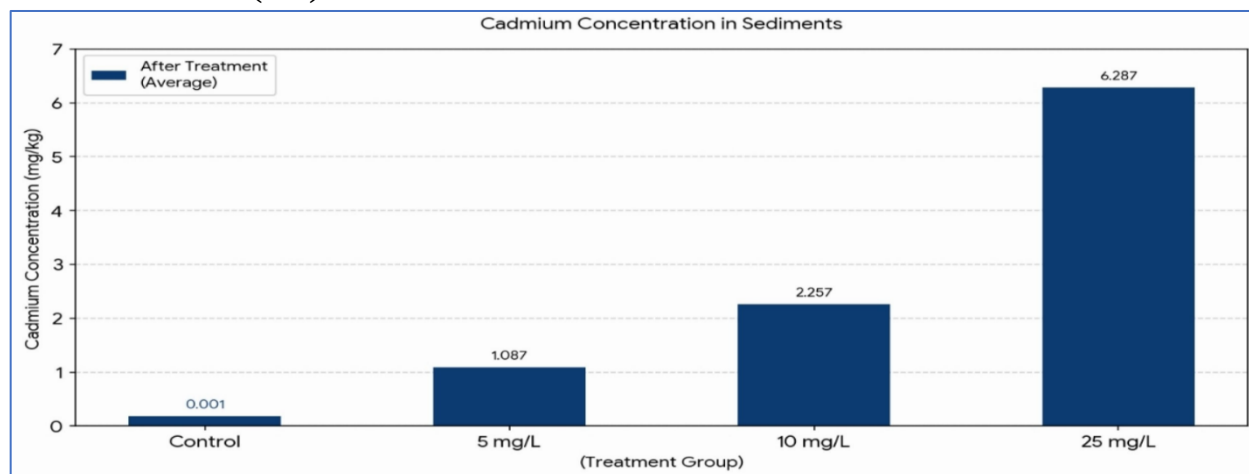


Figure (4.11): Cd concentration in Sediments (mg/kg) by Water Hyacinth

The presence of cadmium (Cd) in the treated benthic sediments indicates that some of the cadmium in the aqueous medium was transferred from the water phase to the sediments and retained there during the experiment (Ozturk *et al.*, 2023). Cd accumulation was highest with increasing added concentrations (5 to 25 mg/L), which is associated with an increased amount of cadmium entering the aqueous medium and, consequently, an increased amount available for interaction with roots, particles, and substrates at the bottom of the ponds. Therefore, the high Cd accumulation in the sediments at 25 mg/L primarily reflects an increased pollutant load available for distribution within the system.

The nature of the water hyacinth ecosystem contributed to creating conditions conducive to the transfer of some cadmium to the sediments. The plant's dense root system interacts continuously with water and its particles, and can trap some particles near the root zone, which are then transported to the pond bottom. Cd can also bind to suspended matter or organic components in the medium before settling in the sediments (El-Din *et al.*, 2023). Therefore, the presence of roots not only facilitates the transfer of cadmium (Cd) to the plant but can also influence the

distribution of the metal between the water, roots, and sedimentary materials. Furthermore, the continued plant activity and nutrient uptake during the treatment period contribute to changes in the ionic and chemical environment around the roots. These changes affect the interaction of ions with root surfaces and particles, potentially contributing to the transfer of some cadmium from the water to sediment-associated materials. Thus, the accumulation of cadmium at the bottom can be explained by the interaction between the pollutant load, root activity, nutrient uptake, and particle movement within the medium, rather than by a single precipitation process (Kassim *et al.*, 2024).

From a physical and chemical perspective, some cadmium can be transferred to the sediment through the stabilization of metal-bearing particles, the binding of cadmium to particle surfaces, and interaction with organic matter or other sediment components. These processes may lead to the retention of some of the metal at the bottom and reduce its permanence in the water. However, this study did not identify the chemical forms of cadmium within the sediments or directly measure each mechanism. Therefore, these processes are considered possible explanations for the observed Cd accumulation, rather than independently established mechanisms (Tariq *et al.*, 2019; Al-Shammari *et al.*, 2023).

Furthermore, the increased Cd levels in the sediments coincided with increased accumulation in plant tissues, reflecting the presence of multiple pathways for cadmium distribution within the system. Cadmium available in the water can interact with roots and enter plant tissues, while another portion can bind to particles and become deposited in the sediments. Therefore, the simultaneous accumulation of Cd in plants and sediments does not imply that one directly caused the other, but rather suggests that both represent pathways for cadmium transport and retention within the experimental system.

Changes in pH may affect cadmium mobility and its interaction with particle and sediment surfaces. However, the limited relationship between pH and Cd accumulation in the sediments suggests that pH change was not the most significant factor determining the amount of Cd accumulated at the bottom during this experiment. With pH remaining within the near-neutral range, the chemical conditions of the medium can be relatively stable, and this study provides no direct evidence that chemical precipitation was the primary mechanism for Cd retention (Srivastava *et al.*, 2024).

Changes in electrical conductivity are related to the overall change in the ionic environment of the medium, as EC is affected by nutrients, dissolved ions, and cadmium. As the concentrations of added Cd varied and the plant continued to absorb nutrients, the ionic environment of the water changed during the treatment period. These changes can influence the interactions between Cd and the particles and materials present in the sediments. Therefore, the relationship between EC and Cd accumulation reflects the synchronization of changes in the ionic environment with cadmium distribution, rather than a direct effect of electrical conductivity on the metal's accumulation (Badr *et al.*, 2023).

Temperature may also have contributed to variations in plant and root activity during the experiment. As thermal conditions change, the intensity of physiological processes, ion mobility, and root-intermediate interactions may alter, potentially indirectly affecting Cd distribution among water, plants, and sediments. However, temperature was not an independent experimental factor and therefore cannot be considered a direct cause of Cd accumulation in the sediments. Instead, it is an environmental factor that accompanied the distribution process during the experiment (Rai *et al.*, 2022; Mwangi & Zhou, 2024).

The low cadmium value in the control sediments suggests the absence of an added load of Cd compared to the contaminated treatments. This limited amount could be

attributed to background levels, natural variations in the medium, or analytical variability at low concentrations. In contrast, the increase in Cd in the treated sediments more likely reflects the effect of adding cadmium at concentrations of 5, 10, and 25 mg/L and its distribution within the system during the treatment period. In general, Cd accumulation in benthic sediments is a significant pathway for cadmium retention within the water hyacinth system. This accumulation increased with increasing Cd concentration due to the increased pollutant load and the amount available for transfer from water to particles and sediments (Shehata *et al.*, 2024). Root interaction with the medium, nutrient uptake, and changes in the ionic environment and physicochemical conditions also contributed to determining cadmium distribution. Therefore, the decrease in Cd from the aqueous medium should not be interpreted solely as a result of plant accumulation, but rather reflects the distribution of cadmium among water, plant tissues, and sediments, with sediments being an important component in the retention of the metal within the system.

4.2.5 Comprehensive Evaluation and Comparative Assessment of Lead (Pb) and Cadmium (Cd) Removal by (*Eichhornia crassipes*)

The overall numerical outputs of the statistical comparison of the kinetics and behavior of lead (Pb) and cadmium (Cd) within the open field treatment system using *Eichhornia crassipes* (with an initial weight of 100 g) under winter weather conditions at an average daytime temperature of (17.3°C) showed a clear statistical superiority for lead. The efficiency of removing lead from the pond waters reached (63.577%) and (65.509%) at low and medium initial concentrations of (5 and 10 mg/L), respectively, while the removal of cadmium for the same treatments recorded lower values of (56.174%) and (58.045%). At the highest treatment concentration of (25 mg/L), lead removal efficiency increased to (71.064%), with Pb accumulation

in plant tissues reaching (7.630 mg/kg) dry weight. Meanwhile, cadmium removal efficiency from the water increased to (65.443%), accompanied by the highest Cd accumulation in plant tissues, reaching (9.483 mg/kg) dry weight. These results indicate that the response of *Eichhornia crassipes* differed between the two metals. Lead showed a higher percentage of removal from the water, whereas cadmium showed a higher level of accumulation in plant tissues at the highest applied concentration. This difference may be related to differences in the interaction of each metal with the plant surface, water chemistry, and plant physiological responses during the treatment period.

The Pearson correlation supported the observed differences in metal distribution among the water, plant, and sediment compartments. The relationships between metal concentration in water and accumulation in plant tissues, as well as between plant tissues and bottom sediments, were very strong for both metals ($r = +0.99$). This indicates that changes in metal concentration in the water occurred together with changes in metal accumulation in plant tissues and sediments. The results therefore suggest that plant accumulation and sediment retention were important compartments associated with the decrease in dissolved metal concentrations. However, these relationships represent associations and do not establish a direct transfer pathway between the different compartments.

The temperature relationships also provide an indication of the influence of the winter field conditions on metal distribution. Temperature was associated with plant accumulation of both Pb and Cd, while its association with sediment accumulation was also observed. In contrast, temperature showed an inverse association with metal concentration in the water. These patterns may be related to changes in plant physiological activity and physicochemical processes as temperature changed during the experimental period. Increasing temperature within the observed winter range may have supported greater biological activity and interaction between the

plant and the contaminated water. However, temperature was not experimentally controlled, so these relationships should be considered as associations with the environmental conditions rather than direct temperature effects.

Electrical conductivity (EC) also showed an important relationship with the behavior of both metals. The association between EC and metal concentration in the water was stronger for Cd than for Pb. This may reflect the greater contribution of changes in the overall dissolved ionic condition of the water at increasing Cd concentrations. Because EC represents the total dissolved ionic content rather than a specific metal, the observed relationship should be interpreted as an indication that changes in Cd concentration occurred together with changes in the ionic condition of the water. Similar relationships were observed between EC and metal accumulation in plant tissues and sediments, suggesting that changes in water chemistry occurred simultaneously with changes in metal distribution. From a biochemical and physiological perspective, the variation in removal efficiency and accumulation between the two metals may be related to differences in their chemical properties and their interactions with functional groups on the plant surface. The root system of *Eichhornia crassipes* contains surfaces and functional groups that can interact with dissolved metal ions through adsorption and ion-exchange processes. Such interactions may contribute to the initial retention of Pb and Cd at the plant surface before or during uptake into plant tissues. Lead may show strong interaction with negatively charged sites containing functional groups such as carboxyl and hydroxyl groups, which can contribute to its retention by plant biomass (Oladipo *et al.*, 2021; Fessel *et al.*, 2022). However, the present study did not directly measure the specific binding sites or chemical forms of Pb and Cd, so these mechanisms should be considered possible explanations rather than directly confirmed processes. The lower Cd removal efficiency at the low and medium concentrations, followed by the increase at (25 mg/L), may be related to the greater Cd load available for

interaction with the plant biomass. The higher Cd accumulation measured in plant tissues at the highest concentration indicates that the plant retained a considerable proportion of the metal within its biomass. Cadmium is known to interact with cellular transport and detoxification mechanisms in plants, including chelation and compartmentalization processes. Phytochelatins and other metal-binding compounds can contribute to Cd detoxification, while vacuolar sequestration can reduce the concentration of Cd in sensitive cellular compartments (Siddiqui *et al.*, 2021; Tan *et al.*, 2024). Nevertheless, because the present study did not measure phytochelatin concentration, transporter activity, or intracellular Cd localization, these mechanisms cannot be considered directly demonstrated in *Eichhornia crassipes* under the present experimental conditions. Similar studies have reported that Cd stress can activate chelation and compartmentalization mechanisms in plants, supporting the interpretation that these processes may contribute to Cd tolerance and accumulation (He *et al.*, 2024).

The difference between water removal efficiency and tissue accumulation is also important when comparing Pb and Cd. Removal efficiency describes the percentage reduction in the aqueous concentration, whereas tissue accumulation represents the amount of metal detected within the plant biomass. Therefore, a higher tissue concentration does not necessarily mean a higher percentage of removal from the water. In the present study, Pb showed higher removal efficiency at all tested concentrations, while Cd showed greater accumulation in plant tissues at the highest concentration. This indicates that the fate of the metals was not controlled by plant uptake alone and that other processes, including sediment retention and interactions within the water medium, may have contributed to the overall reduction in dissolved metal concentrations.

The accumulation of metals in bottom sediments provides further evidence that the decrease in water concentration cannot be attributed only to plant uptake. The strong

association between water metal concentration and sediment accumulation indicates that part of the metals was retained in the sedimentary compartment during the treatment period. Settling of particulate materials, adsorption onto sediment surfaces, and interactions with organic and mineral components may contribute to this retention. Therefore, the phytoremediation process in the present field system should be considered as a combined biological and physicochemical process involving the water, plant biomass, and bottom sediment.

From a design and methodological standpoint, maintaining the experimental water volume and providing clean water and essential nutrients when required helped reduce changes associated with water loss during the experimental period. Evaporation can alter water volume and dissolved ion concentrations, and therefore maintaining the water conditions helps reduce the possibility that changes in concentration were caused mainly by changes in water volume. This is important when interpreting EC and metal concentrations because water loss through evaporation can increase dissolved solute concentrations even when the total amount of solute remains unchanged. Therefore, maintaining relatively stable water conditions supported a more consistent interpretation of the changes observed during the treatment period (Zheng *et al.*, 2022).

The results also indicate that the behavior of Pb and Cd was influenced by the interaction between biological activity and the physicochemical conditions of the treatment system. The strong association of Cd with EC suggests that changes in Cd concentration occurred together with changes in the overall ionic condition of the water. However, EC should not be interpreted as a direct measure of Cd concentration because it represents the total dissolved ionic condition of the water. Similarly, the relationships between metal concentration, pH, temperature, plant accumulation, and sediment accumulation indicate that several factors changed simultaneously during the treatment period. These relationships provide evidence of

coordinated changes in the experimental system but do not establish a single controlling mechanism (Andrade *et al.*, 2022; Xia *et al.*, 2024).

Overall, the study results show that *Eichhornia crassipes* exhibited a clear ability to reduce both Pb and Cd concentrations in contaminated water under the prevailing winter field conditions. The plant showed a relative advantage in Pb removal at low, medium, and high concentrations, while Cd showed a higher accumulation level in plant tissues at the highest applied concentration. The results also indicate that metal removal was associated with both plant accumulation and sediment retention, together with changes in pH, EC, and temperature. Therefore, *Eichhornia crassipes* can be considered a promising aquatic plant for phytoremediation of Pb- and Cd-contaminated water, particularly under field conditions, while the differences in metal behavior should be considered when evaluating its removal performance.

Table 4.1: Physicochemical parameters and heavy metal concentrations (Range, Mean $\pm$ SD, LSD, RE%) in the water, plant tissues, and sediments of the Water Hyacinth (*Eichhornia crassipes*) system under Pb and Cd treatments. Water Hyacinth (*Eichhornia crassipes*) system under Pb and Cd treatments.

Parameters	Treatment	Before Treatment (Range Mean $\pm$ SD)	After Treatment (Range Mean $\pm$ SD)	LSD	(RE%)
Temperature ($^{\circ}$ C)	General	14–17 15.4 $\pm$ 1.07	18–21 19.3 $\pm$ 1.07	1.14	
pH measure ment in water with Pb	Control	7.104– 7.113 7.110 $\pm$ 0.005	7.132– 7.148 7.140 $\pm$ 0.008	0.0370	
	5 mg/L	7.190 – 7.240 7.220 $\pm$ 0.026	7.170 – 7.220 7.200 $\pm$ 0.026		

	10 mg/L	7.310 – 7.360 7.340 ± 0.026	7.240 – 7.290 7.270 ± 0.026		
	25 mg/L	7.350 – 7.400 7.380 ± 0.026	7.160 – 7.220 7.197 ± 0.032		
EC measurement in water with Pb dS/m	Control	1.803 – 1.834 1.819 ± 0.016	1.693 – 1.724 1.710 ± 0.016	0.0283	
	5 mg/L	1.914 – 1.941 1.929 ± 0.014	1.662 – 1.693 1.679 ± 0.016		
	10 mg/L	2.061 – 2.092 2.079 ± 0.016	1.721 – 1.753 1.739 ± 0.016		
	25 mg/L	2.312 – 2.353 2.335 ± 0.021	1.873 – 1.902 1.889 ± 0.015		
Pb concentration in water (mg/L)	Control	0.0017 – 0.0017 0.0017 ± 0.000	0.0002 – 0.0002 0.0002 ± 0.000	0.0565	
	5 mg/L	4.780 – 4.830 4.810 ± 0.025	1.720 – 1.780 1.750 ± 0.031		63.577%
	10 mg/L	9.780 – 9.850 9.820 ± 0.036	3.350 – 3.430 3.390 ± 0.040		65.509%
	25 mg/L	24.260 – 24.370 24.320 ± 0.056	7.010 – 7.070 7.040 ± 0.031		71.064%
Pb concentration in Plant Tissues (mg/kg)	Control	0.0008 – 0.0012 0.0010 ± 0.0002	0.0012 – 0.0018 0.0015 ± 0.0003	0.0977	
	5 mg/L	0.0009 – 0.0011 0.0010 ± 0.0001	1.290 – 1.410 1.340 ± 0.061		
	10 mg/L	0.0008 – 0.0013 0.0011 ± 0.0003	2.790 – 2.910 2.840 ± 0.061		
	25 mg/L	0.0010 – 0.0012 0.0011 ± 0.0001	7.530 – 7.750 7.630 ± 0.111		
Pb concentration ratio	Control	—	0.003 – 0.003 0.003 ± 0.000	0.0891	

	5 mg/L	—	1.650 – 1.810 1.730 ± 0.080		
	10 mg/L	—	3.010 – 3.170 3.080 ± 0.081		
	25 mg/L	—	9.530 – 9.700 9.630 ± 0.091		
pH measurement in water with Cd	Control	7.116 – 7.124 7.120 ± 0.004	7.142 – 7.157 7.150 ± 0.008	0.0356	
	5 mg/L	7.410 – 7.440 7.423 ± 0.015	7.150 – 7.200 7.177 ± 0.025		
	10 mg/L	7.430 – 7.480 7.460 ± 0.026	7.190 – 7.220 7.207 ± 0.015		
	25 mg/L	7.480 – 7.530 7.510 ± 0.026	7.200 – 7.230 7.217 ± 0.015		
EC measurement in water (dS/m) with Cd	Control	1.791 – 1.826 1.808 ± 0.0018	1.682 – 1.715 1.698 ± 0.0017	0.0671	
	5 mg/L	1.951 – 1.983 1.969 ± 0.016	1.741 – 1.772 1.759 ± 0.016		
	10 mg/L	2.124 – 2.162 2.146 ± 0.020	1.861 – 1.894 1.879 ± 0.017		
	25 mg/L	2.391 – 2.432 2.415 ± 0.022	2.042 – 2.073 2.059 ± 0.016		
Cd Concentration in water (mg/L)	Control	0.0012 – 0.0012 0.0012 ± 0.0000	0.0001 – 0.0001 0.0001 ± 0.0000	0.0420	
	5 mg/L	4.790 – 4.870 4.833 ± 0.040	2.080 – 2.150 2.117 ± 0.035		56.197%
	10 mg/L	9.670 – 9.750 9.713 ± 0.040	4.020 – 4.130 4.077 ± 0.055		58.025%

	25 mg/L	24.230 – 24.310 24.270 ± 0.040	8.310 – 8.470 8.387 ± 0.080		65.443%
Cd concentration in Plant Tissues (mg/kg)	Control	0.0009 – 0.0015 0.0012 ± 0.0003	0.0014 – 0.0017 0.0016 ± 0.0002	0.0541	
	5 mg/L	0.0008 – 0.0010 0.0009 ± 0.0001	1.530 – 1.610 1.573 ± 0.040		
	10 mg/L	0.0010 – 0.0016 0.0013 ± 0.0003	3.210 – 3.330 3.277 ± 0.061		
	25 mg/L	0.0008 – 0.0012 0.0010 ± 0.0002	9.430 – 9.530 9.483 ± 0.050		
Sed Cd concentration in iments (mg/kg)	Control	—	0.001 – 0.001 0.001 ± 0.000	0.0543	
	5 mg/L	—	1.040 – 1.130 1.087 ± 0.045		
	10 mg/L	—	2.190 – 2.350 2.257 ± 0.083		
	25 mg/L	—	6.190 – 6.390 6.287 ± 0.100		

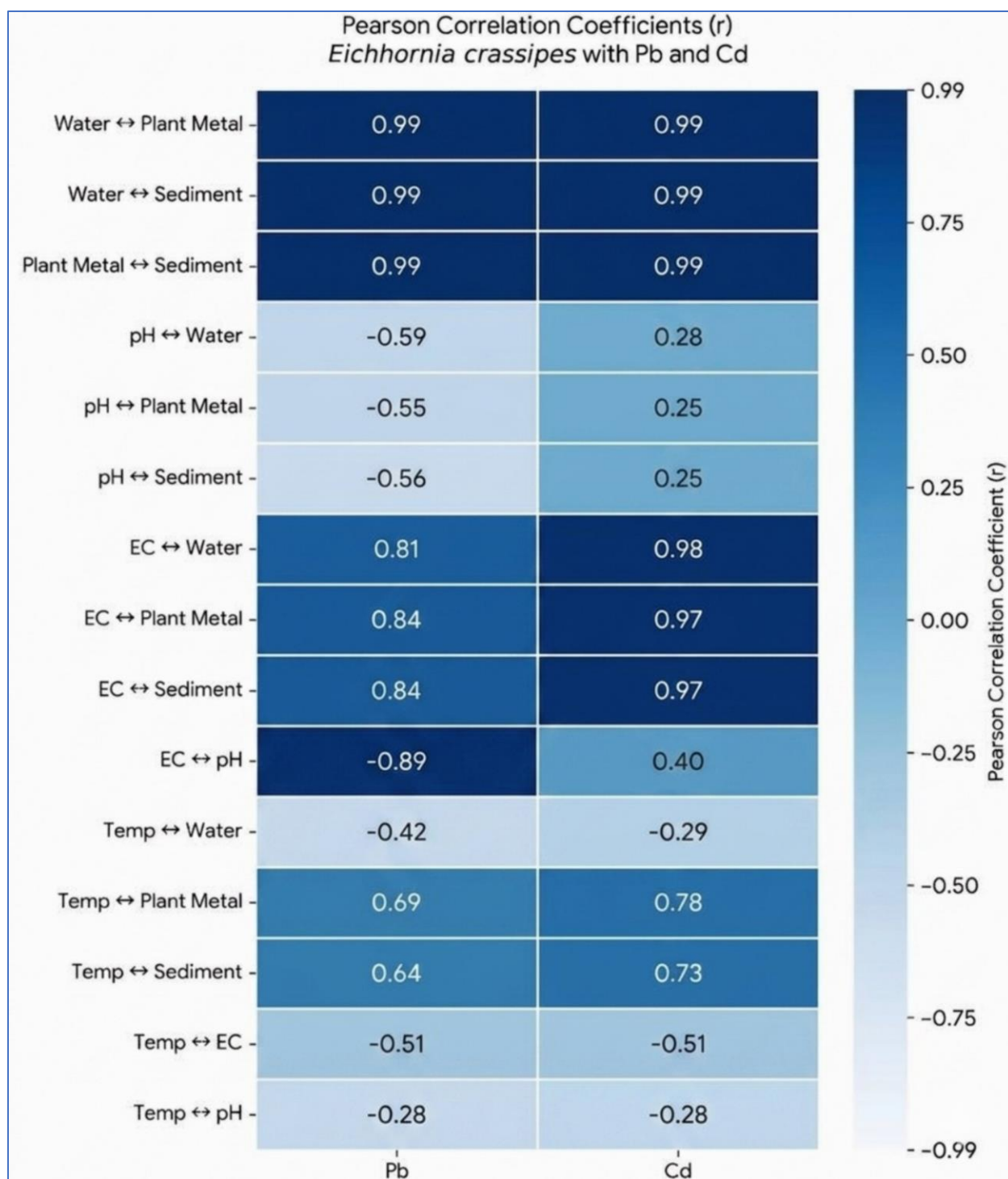


Table. (4.2): Statistical correlation analysis (r) showing relationships among physicochemical parameters and heavy metal concentration in the Water Hyacinth system.

4.3 Efficiency of *Azolla pinnata* in Treating Lead (Pb)-Contaminated Water

4.3.1 Effect of Physicochemical Factors on Lead (Pb) Removal

4.3.1.1 Effect of Temperature on the Availability and Removal of Lead (Pb) and Cadmium (Cd) by *Azolla*

Field study results showed that temperatures in the *Azolla pinnata* treatment basins gradually increased between the Pre-treatment and Post-treatment phases. The temperature values across the treatments ranged from a low of (15°C) to a high of (20°C), as shown in Table (4.3). and Figure (4.12) Furthermore, Pearson's correlation for the lead (Pb) treatment system revealed an inverse correlation between temperature and lead concentration in the water ($r = -0.41$), a positive correlation with lead accumulation in plant tissues ($r = +0.69$), and a positive correlation with lead accumulation in bottom sediments ($r = +0.59$). In the cadmium (Cd) treatment system, the results showed an inverse correlation between temperature and cadmium concentration in the water ($r = -0.28$), a positive correlation between temperature and cadmium accumulation in plant tissues ($r = +0.76$), and a positive correlation with its accumulation in bottom sediments ($r = +0.71$). Temperature also showed an inverse correlation with electrical conductivity (EC) in both systems ($r = -0.50$) and an inverse correlation with pH ($r = -0.26$), as shown in Table (4.4).

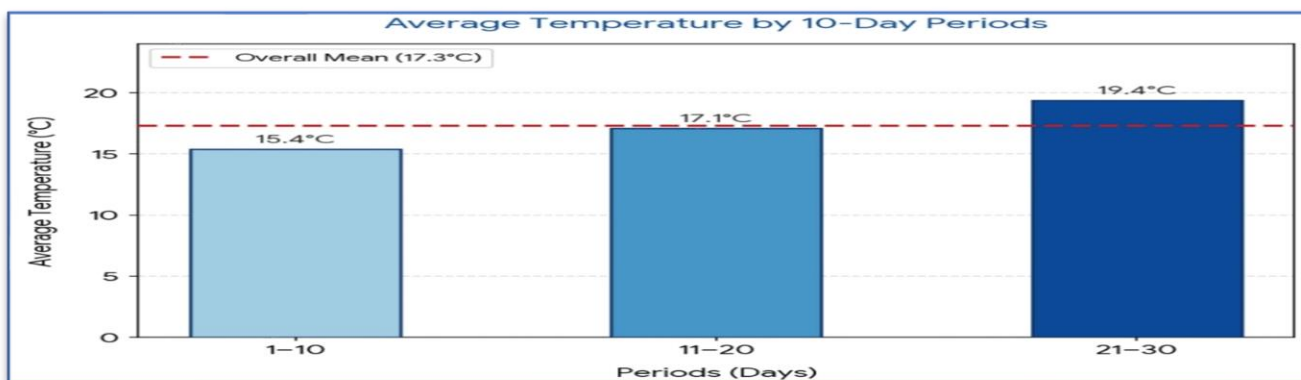


Figure (4.12): Thermal changes observed during the two study periods: Pre-treatment and Post treatment by *Azolla pinnata*

These results indicate that the gradual increase in temperature during the experiment was associated with changes in the distribution of lead and cadmium between the aqueous medium, plant tissues, and bottom sediments. The decrease in the concentrations of these two metals in the water occurred together with their increased accumulation in plant tissues and bottom sediments. This may be related to the fact that the temperature range recorded during the study provided suitable conditions for maintaining the physiological activity of the *Azolla* plant, which may have contributed to plant–water interactions and metal retention during the treatment period (Sattar *et al.*, 2021).

The inverse relationship between temperature and electrical conductivity and pH can also be explained by the reflection of plant biological activity on the aqueous medium. Increased metabolic activity and ion uptake may lead to gradual changes in the properties of the water surrounding the plant, including changes in the concentration of dissolved ions and pH values within the surrounding medium (Thangaraj *et al.*, 2022).

The continued efficiency of lead and cadmium removal despite winter conditions may be related to the contribution of passive biosorption during the initial interaction between heavy metal ions and the plant surfaces. This physicochemical process involves the binding of metal ions to functional groups on plant cell walls and does not directly depend on metabolic energy (ATP). Therefore, the temperature conditions recorded during the study may have allowed *Azolla* to maintain its interaction with lead and cadmium during the treatment period (Sadeghi *et al.*, 2013; Mishra *et al.*, 2023).

4.3.1.2 Effect of pH on the Bioavailability and Removal of Lead (Pb) by *Azolla*

The results of the field experiment showed clear changes in the pH values in the lead (Pb) treatment basins containing *Azolla pinnata* between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, the pH values were (7.120), (7.240), and (7.280) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the pH values decreased to (6.417), (6.377), and (6.300) at (5, 10, and 25 mg/L), respectively. The decrease was observed at all tested Pb concentrations, with the lowest pH value recorded at (25 mg/L) during the Post-treatment stage. In the control treatment, pH increased from (7.068) during the pre-treatment stage to (7.108) during the post-treatment stage as shown in Table (4.3) and Figure (4.13). Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages, The Pearson correlation analysis showed strong associations between pH and the measured Pb-related parameters. Specifically, pH was associated with residual Pb concentration in water ($r = -0.96$), Pb accumulation in plant tissues ($r = -0.96$), and Pb accumulation in bottom sediments ($r = -0.96$), as shown in Table (4.4)

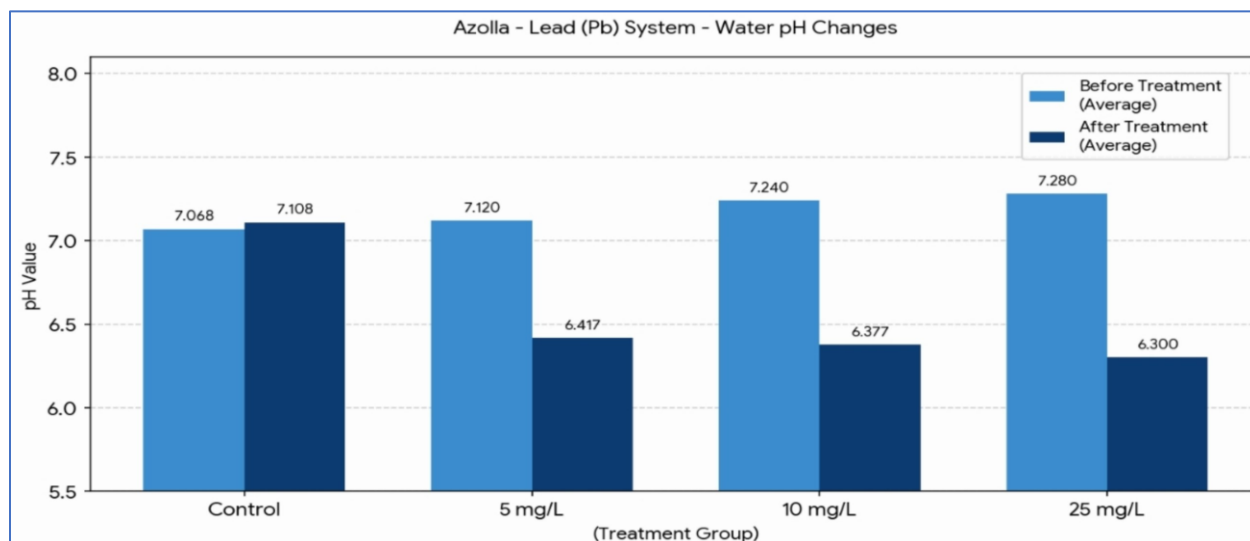


Figure (4.13): pH measurement in water with Pb by *Azolla pinnata*

The decrease in pH values after treatment in all lead treatments (5, 10, and 25 mg/L) can be explained by the continued physiological activity of *Azolla pinnata* and its interaction with the aqueous medium during lead exposure (Sood *et al.*, 2022). The decrease was more pronounced with increasing lead concentration, suggesting that a higher pollutant load was associated with a greater change in the chemical conditions of the medium. Meanwhile, the higher pH in control treatment indicates that the change in lead treatments was not solely due to the passage of time but was also influenced by the presence of lead and the plant's interaction with the medium.

The decrease in pH may be related to *Azolla* activity and ion exchange processes between the plant and the aqueous medium. During treatment, the plant continues to absorb nutrients and dissolved ions necessary for growth, and changes in hydrogen ion (H^+) flux may occur at the plant-water contact zones (Barkat *et al.*, 2023). Therefore, continued root activity and ion exchange can alter the chemical equilibrium of the medium and lead to a decrease in pH. Plant and microbial respiration processes can also contribute to an increase in dissolved carbon dioxide and create relatively more acidic conditions in the aqueous medium (Malar *et al.*, 2016; Zeraatkar *et al.*, 2016).

This change becomes more significant at higher lead treatments because increasing the added concentration (from 5 to 25 mg/L) means increasing the amount of lead available to react with the plant and other components of the medium (Nassir *et al.*, 2024). The plant surface and contact areas with the medium can provide binding sites for lead ions, and these sites can change depending on the surrounding chemical conditions. Thus, the decrease in pH is not solely attributable to lead, but rather to the interaction between the contaminating load, *Azolla* activity, and the ionic and chemical changes that occurred during the treatment period.

Furthermore, a decrease in pH is important in explaining the behavior of lead within the experimental system, as changes in acidity can affect lead solubility, mobility, and interaction with plant surfaces and particles in the medium. Changes in pH alter the nature of binding and ion exchange sites on various surfaces, potentially impacting lead transfer between water, plants, and sediments. Therefore, a decrease in pH during the treatment period may be one factor contributing to changes in lead distribution within the system, though not the sole factor responsible (Akhoondi *et al.*, 2020; Al-Jaberi *et al.*, 2023).

The relationship between pH and residual lead in water demonstrates that changes in acidity coincided with changes in the amount of lead present in the aqueous medium. This can be explained by the fact that pH changes affect the chemical state of lead and its persistence in a dissolved form or its binding to other components in the medium (Chandra & Kumar, 2022). As the treatment continued, lead migrated from the water to other components of the system, including plant tissues and sediments. Therefore, the relationship between pH and lead in the water reflects a combined change in chemical conditions and lead distribution and does not prove that the decrease in pH alone caused the decrease in lead in the water. Similarly, the association between a decrease in pH and increased lead accumulation in *Azolla pinnata* tissues can be explained by changes in the availability of lead around the plant. When the lead concentration in the water increases, the amount of lead available to react with the plant surface also increases, and some of the lead may be retained on the plant surfaces before some is transferred to the tissues (Ayyappan *et al.*, 2023). Furthermore, changes in pH can affect the properties of binding sites on the plant surface, and thus may alter the interaction of lead with the plant.

However, this result does not imply that a decrease in pH is the direct cause of lead

accumulation, as nutrient uptake, plant activity, changes in EC, and other environmental conditions occurred simultaneously during the experiment. The correlation between decreased pH and increased lead accumulation in sediments can be explained by the fact that changes in the medium's chemical conditions may affect the interaction of lead with particles, organic matter, and other components present in the sedimentary portion. As the concentration of added lead increases, the amount of lead available to bind to particles becomes greater, increasing the likelihood of some of it being transferred from the water to the sediments through adsorption, ion exchange, precipitation, or gradual stabilization of the particles (Roy *et al.*, 2024). The presence and activity of *Azolla* at the water surface may also indirectly influence the distribution of particles and suspended matter; however, these mechanisms were not measured independently in the experiment and are therefore considered potential explanations rather than directly established mechanisms.

It is also important to interpret the results in light of the control treatment, in which the pH increased during the experiment despite the absence of lead. This suggests that the change in pH within the lead-treated conditions was more strongly associated with the presence of the pollutant load and the interaction between the lead, the plant, and the medium, while the change in the control treatment reflects natural system processes such as nutrient uptake, ion exchange, and plant biological activity (Mishra & Sharma, 2022). Therefore, comparing lead parameters with the control helps to separate changes associated with the presence of lead from natural changes that can occur during the treatment period.

4.3.1.3 Effect of Electrical Conductivity (EC) and Ionic Strength on Lead (Pb) Removal by *Azolla*

The results of the field experiment showed that the electrical conductivity (EC) values in the lead (Pb) treatment basins containing *Azolla pinnata* increased with increasing applied Pb concentrations during the pre-treatment stage. During the pre-treatment stage, the EC values were (1.979), (2.139), and (2.405 dS/m) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the EC values decreased to (1.779), (1.859), and (2.015 dS/m) at (5, 10, and 25 mg/L), respectively. A decrease was also observed in the control treatment, where EC decreased from (1.758 dS/m) during the pre-treatment stage to (1.637 dS/m) during the post-treatment stage. as shown in Table (4.3) and Figure (4.14)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages, The Pearson correlation analysis showed strong associations between EC and the measured Pb-related parameters. Specifically, EC was associated with residual Pb concentration in water ($r = +0.99$), Pb accumulation in plant tissues ($r = +0.99$), and Pb accumulation in bottom sediments ($r = +0.99$). The relationship between EC and pH was ($r = -0.98$), as shown in Table (4.4)

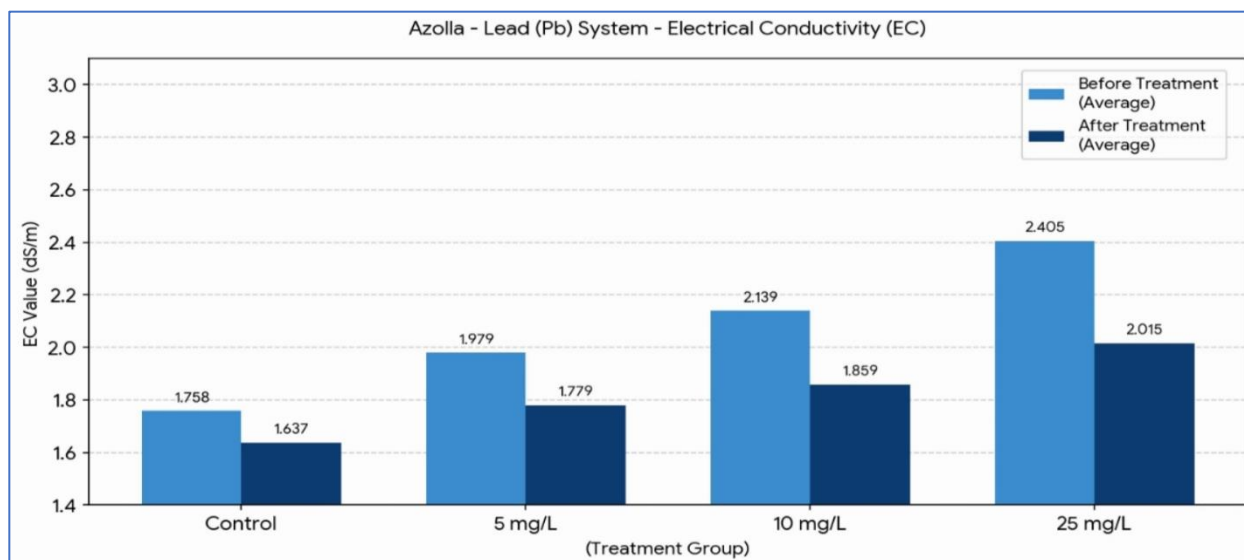


Figure (4.14): EC measurement in water with Pb by *Azolla pinnata*

The increase in electrical conductivity (EC) values before treatment with increasing lead concentrations (5 to 25 mg/L) can be explained by the increased ionic load in the aqueous medium with the added lead, in addition to the ions already present in the nutrient medium (Al-Farji *et al.*, 2023). Since EC reflects the overall ionic state of the solution and does not measure lead in isolation, its increase at higher concentrations reflects the increase in the total dissolved ions associated with the treatment and the nutrient medium. Therefore, the difference in EC between the 5, 10, and 25 mg/L treatments is related to the increased contaminant load along with the other ionic components present in the medium.

The decrease in EC after the treatment period in all lead treatments can be explained by the continued activity of *Azolla pinnata* throughout the experiment and its absorption of nutrients and dissolved ions from the medium. *Azolla* requires these elements to maintain its growth and activity. As the interaction between the plant and water continues, the amount of some ions present in the dissolved form can decrease due to their absorption or a change in their distribution among the system components (Ebrahim *et al.*, 2024). Therefore, the decrease in EC after treatment does not solely represent lead removal but rather reflects a general change in the ionic composition of the medium resulting from *Azolla* activity and the changes that occurred during the treatment period.

Furthermore, increasing the lead concentration from 5 to 25 mg/L means increasing the load to which the *Azolla* was subjected, and consequently increasing the amount of lead available to react with the plant surfaces and the surrounding environment (Saleh *et al.*, 2023). Plant surfaces and water contact areas can retain some of the lead ions through adsorption and ion exchange, while others can be transferred to plant tissues. As these reactions continue, the distribution of ions between the water, the plant, and the sediment changes, which may contribute to the decrease in EC

after treatment. However, these changes do not necessarily mean that lead is the sole factor responsible for the decrease in EC, as nutrient uptake and changes in other ions occur simultaneously.

This interpretation becomes even more significant when comparing different treatments. The higher EC before treatment (at 25 mg/L) compared to lower concentrations reflects the increased ionic load associated with higher lead concentrations. After the treatment period, the EC decreased in all treatments, but the values remained related to the initial ionic load level of the treatment (Kumar *et al.*, 2023). This suggests that the system experienced not only a change in lead but also a change in the ionic environment of the water due to the ongoing interaction between the *Azolla*, the nutrient medium, lead, and other components of the system. The interactions of lead with the plant and sediment also contributed to the change in the ionic state of the medium. As the lead concentration increases, the amount of lead available to interact with particles and materials in the medium increases, thus increasing the likelihood of some of it being transferred from the water to plant tissues or binding to materials and particles that settle at the bottom of the container (Hasan *et al.*, 2024). Thus, the change in EC coincides with a change in lead distribution between water, plants, and sediments, and a decrease in EC cannot be considered a direct indicator of the amount of lead removed by the *Azolla*. The relationship between EC and pH can be explained by the combined change in the ionic and chemical state of the medium during the treatment period. Nutrient uptake, ion exchange at the plant surface, and interaction with lead can alter the chemical environment surrounding the *Azolla*, leading to simultaneous changes in EC and pH (Mahmood *et al.*, 2023).

Therefore, the relationship between the two variables reflects a combined change in the medium conditions and does not imply that EC directly caused the pH decrease.

The decrease in EC in the control treatment is significant in interpreting the result because the control was not exposed to lead. Therefore, the decrease observed in the control suggests that part of the change in EC resulted from natural processes within the *Azolla* system, particularly nutrient uptake, changes in dissolved ion concentrations, and the ongoing interaction between the plant and water (Prasad *et al.*, 2023). Therefore, the decrease in lead coefficients represents an interaction between these natural processes and the effects of the pollutant load and the interactions of lead with the plant and the medium.

Furthermore, the presence of lead in the system does not necessarily mean that all of the lead remained in its water-soluble form throughout the treatment period. As the experiment continues, its distribution between water, plant tissues, and sediments can change depending on its interaction with plant surfaces and the particles and materials present in the medium. Therefore, the change in EC should be understood as a reflection of the overall change in the ionic environment of the system as these processes occur, rather than as a direct indicator of lead removal (Rahman *et al.*, 2024). In general, a rise in EC with increasing lead concentration before treatment reflects an increase in the ionic load in the medium, while a decrease after treatment reflects continued *Azolla pinnata* activity, nutrient uptake, and a change in the distribution of ions and lead between the water, the plant, and the sediment. Therefore, in this experiment, EC represents an indicator of the overall change in the ionic state of the medium during water treatment with *Azolla*, and not a direct measure of the amount of lead removed by the plant.

4.3.2 Dynamics and Removal Efficiency of Lead (Pb) in the Water by *Azolla*

The results of the field experiment showed a clear decrease in lead (Pb) concentration in the water medium between the Pre-treatment and Post-treatment

stages in the basins containing *Azolla pinnata*. During the pre-treatment stage, the Pb concentrations were (4.823), (9.613), and (24.157 mg/L) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the Pb concentrations decreased to (2.630), (5.017), and (11.843 mg/L) at (5, 10, and 25 mg/L), respectively. The removal efficiency (RE%) was (45.472%), (47.812%), and (50.975%) at (5, 10, and 25 mg/L), respectively. as shown in Table (4.3) and Figure (4.15)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages, The Pearson correlation analysis showed a very strong association between residual Pb concentration in water and Pb accumulation in plant tissues ($r = +0.99$), and between residual Pb concentration in water and Pb accumulation in bottom sediments ($r = +0.99$), as shown in Table (4.4)

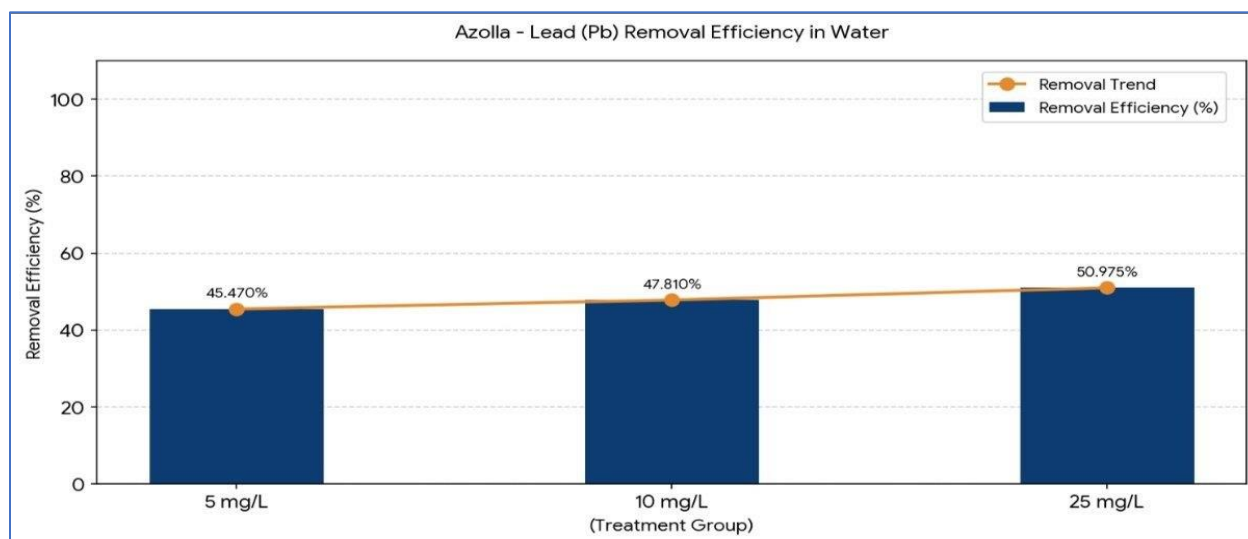


Figure (4.15): Pb Removal Efficiency (%) in Water by *Azolla pinnata*

The decrease in lead (Pb) concentration in the water following the treatment period across all *Azolla pinnata* treatments can be attributed to the transfer of a portion of the lead from the aqueous phase to other components within the experimental system specifically, plant tissues and sediments (Al-Heety *et al.*, 2023). This explanation becomes more evident as the initial lead concentration increases (from 5 to 25 mg/L);

a higher pollutant load implies a greater quantity of lead available to interact with the *Azolla* as well as with particles and components present in the medium. Consequently, the reduction in residual lead in the water reflects a shift in lead distribution during the treatment period, rather than necessarily indicating lead removal solely by the plant.

Azolla pinnata contributed to this redistribution through the interaction of its surfaces with the lead present in the water. *Azolla* possesses plant surfaces that provide binding sites for metal ions; a fraction of the lead may be sequestered on the plant surface, while another portion is translocated into the tissues. As the lead concentration in the medium rises, the amount of lead available for interaction with the plant increases, helping to explain the greater lead accumulation observed in *Azolla* tissues at higher treatment levels. Previous studies have highlighted the capacity of aquatic plants to sequester heavy metals through adsorption, absorption, and accumulation within plant biomass (Soni *et al.*, 2018; Mahmud *et al.*, 2023). At the same time, the decrease in lead concentration in the water cannot be attributed solely to plant uptake, as a portion of the lead may also migrate to the sediments at the bottom of the treatment tanks. As the concentration of added lead increases, the amount of lead available to bind with particles, organic matter, and mineral components rises, thereby increasing the likelihood of its sequestration in the sedimentary fraction of the system (Tariq & Ahmed, 2024). Furthermore, the settling of suspended particles and their subsequent deposition on the bottom contribute to increased lead accumulation in the sediments. Consequently, the presence of lead in the sediments constitutes a significant pathway influencing the concentration of residual lead in the water.

The nutrient medium present in all treatments also plays a crucial role in explaining this result, as *Azolla* continued to absorb nutrients and dissolved ions throughout the

treatment period. Sustained plant activity induced changes in the ionic and chemical environment surrounding the plant, coinciding with the interaction of lead with plant surfaces and particles within the medium (Al-Khafaji *et al.*, 2023). Thus, the reduction of lead from the water occurred within a system comprising plants, nutrients, water, and sediments, rather than in a medium containing lead in isolation. The increase in relative removal efficiency from 45.472% at 5 mg/L to 50.975% at 25 mg/L demonstrates that a larger proportion of the added lead was removed from the aqueous phase at the higher concentration. This can be attributed to the increased quantity of lead available to interact with *Azolla* and the sediments as the applied concentration rose (Mustafa & Al-Zwainy, 2024). However, a higher relative removal efficiency does not imply a lower final lead concentration at 25 mg/L, given that the initial lead quantity was significantly higher in this treatment. Therefore, RE% should be interpreted as the proportion of the initial lead removed from the water, rather than as the absolute concentration of remaining lead. The simultaneous increase in lead accumulation within *Azolla* tissues and the sediment supports the interpretation that lead redistribution occurred within the system. The decrease of lead in the water alongside its increased presence in the plant and sediment can be understood as a transfer or sequestration of lead among the various components of the experimental system (Siddiqui *et al.*, 2023). This implies that *Azolla* served as one pathway for lead sequestration, while the sediment constituted another significant pathway. However, these results alone do not allow for determining the precise contribution of the plant versus the sediment to the overall reduction of lead in the water.

The strong relationship between residual lead in the water and its accumulation in plant tissues and sediment indicates that changes in water lead concentration coincided with changes in accumulation within these two system components. This

can be explained by the fact that increased lead exposure simultaneously raised the quantity available for interaction with the plant and sediment (Hussein *et al.*, 2024). Thus, while these relationships support the concept of lead redistribution among the water, plant, and sediment, they do not prove that lead accumulation in the plant or sediment was the sole cause of the reduction in water lead levels. The interpretation of the decrease in lead concentration in the control treatment differs from that of the treatments with added lead. Since no lead was added to the control, the slight decrease observed there does not represent the removal of added lead by *Azolla*. This variation may be linked to background lead levels, changes in ionic composition within the medium, the binding of trace amounts of lead to surfaces or materials present in the system, or analytical fluctuations at very low concentrations (Saleem & Aziz, 2023). Consequently, the decline observed at the 5, 10, and 25 mg/L levels is particularly significant for assessing lead behavior under conditions of cumulative exposure.

4.3.3 Bioaccumulation of Lead (Pb) in Whole Plant Tissues by *Azolla*

The results of the field experiment showed a clear increase in lead (Pb) accumulation in the whole-plant tissues of *Azolla pinnata* between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, Pb concentrations in plant tissues were (0.0007), (0.0006), and (0.0008 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, Pb concentrations increased to (1.133), (2.467), and (5.183 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. The highest Pb accumulation was recorded at the highest applied concentration of (25 mg/L). In the control treatment, Pb concentration increased from (0.0005 mg/kg) during the pre-treatment stage to (0.0010 mg/kg) during the post-treatment stage. as shown in Table (4.3) and Figure (4.16)

Statistical analysis indicated a significant difference ($P \leq 0.05$) between the Pre-treatment and Post-treatment stages, The Pearson correlation analysis showed very strong associations between Pb accumulation in plant tissues and Pb concentration in water ($r = +0.99$), Pb accumulation in bottom sediments ($r = +0.99$), and EC ($r = +0.99$). The relationship with temperature was ($r = +0.68$), while the relationship with pH was ($r = -0.96$), as shown in Table (4.4)

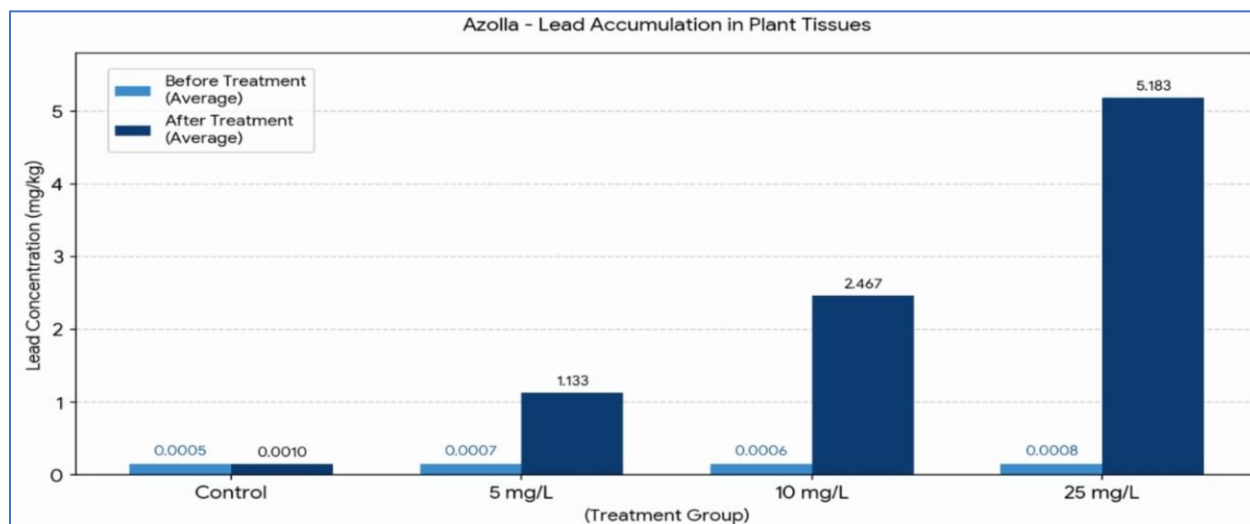


Figure (4.16): Pb concentration in Plant Tissues (mg/kg) by *Azolla pinnata*

The increase in lead (Pb) concentration in *Azolla* plant samples following the treatment period can be attributed to the increased exposure of *Azolla pinnata* to the lead present in the aqueous medium and the plant's continued interaction with the medium throughout the experiment (Al-Tamimi *et al.*, 2023). As the applied lead concentration increased (from 5 to 25 mg/L), the amount of lead available for interaction with the plant increased, resulting in greater accumulation within the plant samples at higher concentrations. This pattern indicates that a higher pollutant load in the water led to an increase in the amount of lead sequestered by the plant sample during the treatment period.

Lead sequestration likely begins with interactions at the plant surfaces and the contact zones between the *Azolla* and the water. Components on the plant surfaces and within cell walls can provide binding sites for metal ions, facilitating the sequestration of a portion of the lead, while another portion may be translocated into the plant tissues. Consequently, the rise in lead concentration within the plant sample observed when the applied concentration was increased can be linked to the greater availability of Pb for binding and sequestration sites (Rai, 2012; Ahmad *et al.*, 2024). However, these processes represent potential mechanisms, as the current experiment did not separately measure the amount of lead adsorbed onto the surface versus the amount present within the tissues.

Furthermore, the increased lead accumulation observed at 25 mg/L, compared to lower concentrations, can be explained by the higher quantity of lead available in the plant's immediate environment at this concentration (Raza *et al.*, 2023). Higher lead concentrations in the medium increase the likelihood of interaction with the *Azolla* surface during the treatment period, potentially leading to a greater amount of lead being sequestered by the plant sample. Consequently, the gradient in Pb accumulation across the treatments (5, 10, and 25 mg/L) corresponds to the varying levels of lead exposure in the aqueous medium.

It is important to interpret this result in light of the study's sampling method. Analysis was performed on a sample taken from the *Azolla* plants within the experimental units, rather than on the entire plant biomass present in the unit. Therefore, the measured values represent the lead concentration in the analyzed plant sample and cannot be used to determine the total quantity of lead in the entire biomass. Furthermore, since the sample was not separated into distinct plant parts, the data do not allow for the quantification of lead in specific plant sections or for

directly distinguishing between surface-adsorbed lead and lead internalized within the tissues (Obaid *et al.*, 2023).

The presence of the nutrient medium during the treatment period must also be considered when interpreting lead accumulation, as *Azolla* continued to absorb nutrients and ions essential for its activity and growth while simultaneously being exposed to lead (Mahdi *et al.*, 2024). Nutrient uptake and ion exchange at the plant surface can alter the ionic environment surrounding the *Azolla*, while lead continues to interact with both plant surfaces and the surrounding medium. Thus, lead accumulation in the plant sample reflects the interplay between lead exposure, plant activity, and ionic and chemical changes in the medium, rather than the isolated effect of lead concentration alone. Concurrently, the increase in lead content within the plant samples coincided with changes in lead concentration in the water and increased accumulation in the sediments. This can be attributed to the redistribution of lead among the system's components during the treatment period (Hussain *et al.*, 2023). A portion of the lead present in the water can interact with the plant and become sequestered within the plant sample, while another portion may bind to particles and materials in the medium and settle into the sediments.

Therefore, a high accumulation of Pb in the plant sample does not imply that the entire amount lost from the water was transferred to the plant, as the sediments represented an alternative pathway for lead sequestration.

The relationship between lead levels in the water and its accumulation in plant samples supports this interpretation, as increased exposure to lead in the medium coincided with increased accumulation in the plant (Karim *et al.*, 2024). This can be explained by the fact that treatments with higher concentrations provided a greater quantity of Pb to interact with the *Azolla*. However, this relationship does not prove that plant uptake alone was responsible for the reduction of lead in the water, as the

transfer of lead to sediments and other physicochemical interactions may have also contributed to the change in concentration.

Furthermore, the simultaneous accumulation of lead in plant samples and sediments indicates that both components played a role in the distribution of lead within the experimental system (Ibrahim *et al.*, 2023). As the applied concentration increased, the amount of Pb available to both the plant and the particles in the medium increased, leading to greater accumulation in both components. This relationship does not imply that lead accumulation in the plant directly caused its accumulation in the sediments or vice versa—but rather reflects a concurrent shift in lead distribution as the pollutant load increased.

As for the relationship between lead accumulation in plant samples and electrical conductivity (EC), it can be explained by the fact that EC reflects the overall ionic state of the medium rather than the Pb concentration alone. EC values rose at the beginning of the experiment alongside increasing lead concentrations, driven by the heightened ionic load in addition to the ions and nutrients already present in the medium. As treatment progressed, the ionic status shifted due to *Azolla* 's uptake of nutrients and changes in the distribution of lead among the water, plant, and sediment (Yousif *et al.*, 2024). Therefore, the relationship between electrical conductivity (EC) and lead (Pb) accumulation reflects a concurrent change in the pollutant load and the ionic environment of the medium; it does not imply that EC directly caused the increased lead accumulation in the plant. The relationship with temperature can be explained by the environmental conditions during the experiment. Temperature changes may alter plant physiological activity, ion mobility, and reactions at the plant surface, potentially coinciding with changes in lead accumulation. However, temperature was not an independent experimental factor in this study and therefore cannot be considered a direct cause of Pb

accumulation. Rather, it is an environmental factor that coincided with changes occurring during the treatment period (Stepniewska *et al.*, 2005; Zheng *et al.*, 2023). Regarding pH, its decrease during the treatment period coincided with increased lead accumulation in the plant samples. pH changes can influence lead mobility, availability, and interaction with plant surfaces and particles in the medium (Jassim *et al.*, 2023). Therefore, lower pH conditions may have contributed to changes in Pb interaction with *Azolla*. However, lead accumulation cannot be explained by pH alone, as EC, plant activity, the nutrient medium, and the distribution of lead between water and sediment also changed during the treatment period. The slight increase in lead concentration in the control sample can be explained differently than in the treatments with added lead. The absence of Pb in the control means that the slight increase does not represent accumulation due to exposure to experimental concentrations. It could be related to background lead levels, natural variations in the medium, or analytical variability at very low concentrations (Hamid *et al.*, 2024).

In contrast, the significant increase in lead concentration in plant samples from treatments exposed to 5, 10, and 25 mg/L is more consistent with increased lead exposure and retention in the *Azolla* during the treatment period.

In general, the increase in lead concentration in *Azolla* plant samples with increasing applied concentrations (5 to 25 mg/L) reflects increased lead exposure and retention capacity during the treatment period. This accumulation occurred concurrently with a decrease in water lead and an increase in sediment accumulation, suggesting redistribution of lead among the components of the experimental system (Sadiq *et al.*, 2024). Therefore, *Azolla pinnata* represented one of the important pathways for lead retention under experimental conditions, while other physical and chemical precipitation and reactions contributed to determining the final distribution of lead within the system.

4.3.4 Deposition and Accumulation Dynamics of Lead (Pb) in Bottom Sediments by *Azolla*

Laboratory analysis of bottom sediment samples obtained from the basins of the *Azolla pinnata* plant system showed a clear accumulation of lead (Pb) during the Post-treatment stage. During the post-treatment stage, Pb concentrations in the bottom sediments were (0.990), (2.090), and (6.483 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. The concentration of Pb in the bottom sediments increased with increasing initial Pb concentrations added to the experimental water. The highest Pb accumulation in the sediment was observed at the highest applied concentration of (25 mg/L), while the control treatment recorded a very low concentration of (0.0002 mg/kg) dry weight. as shown in Table (4.3) and Figure (4.17)

The statistical analysis indicated significant differences at the probability level ($P \leq 0.05$), The Pearson's correlation showed a very strong association between Pb accumulation in bottom sediments and Pb concentration in water ($r = +0.99$), as well as Pb accumulation in whole-plant tissues ($r = +0.99$). The relationship between sediment Pb and temperature was ($r = +0.59$), while the relationship with pH was ($r = -0.96$). A strong association was also observed between sediment Pb and EC ($r = +0.98$), as shown in Table (4.4)

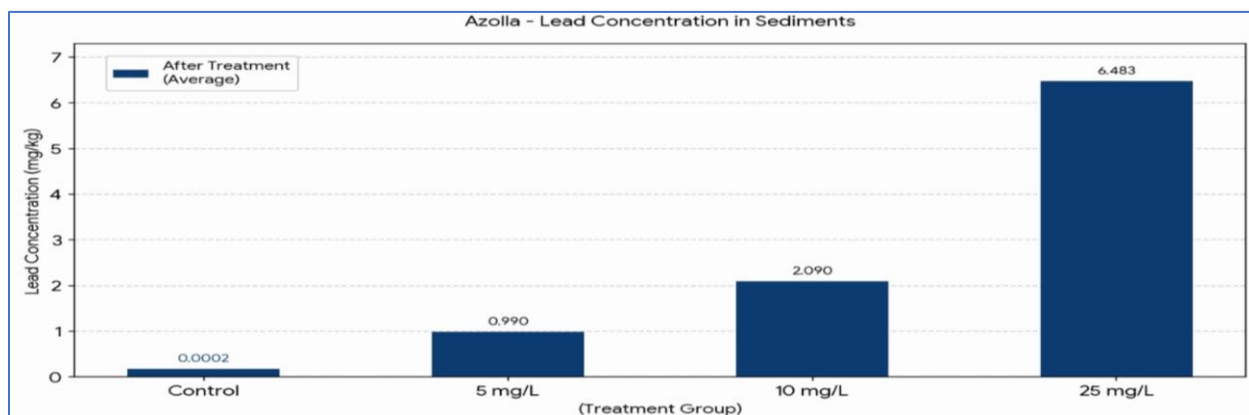


Figure (4.17): Pb concentration in Sediments (mg/kg) by *Azolla pinnata*

The increase in lead (Pb) accumulation in the sediments as the applied concentration increased from 5 to 25 mg/L can be attributed to the higher pollutant load in the aqueous medium. This increased the amount of Pb available to interact with the *Azolla pinnata* root zone, as well as with suspended particles and other materials present in the experimental medium (Al-Khafaji & Al-Zubaidi, 2024). Consequently, the greater Pb accumulation in the sediments at 25 mg/L reflects the higher quantity of lead available for distribution among the different components of the experimental system during the treatment period rather than the isolated effect of the sediments.

The nature of *Azolla pinnata* may have contributed to this distribution. Its root system extends into the aqueous medium and provides surfaces for interaction with dissolved substances and suspended particles. As the Pb concentration increased, the amount of lead available to interact with the root zone and surrounding particles also increased. The roots may retain or intercept a portion of suspended particles, which can subsequently settle gradually toward the bottom of the basin. This process may contribute to the transfer of part of the Pb from the water column to the sediments. Therefore, the presence of the plant roots may influence not only Pb retention within the plant but also the distribution of lead among the water, plant tissues, and sediments (Ozturk *et al.*, 2023; El-Din *et al.*, 2023).

Furthermore, the continued activity of *Azolla pinnata* throughout the treatment period involved the ongoing uptake of nutrients and ions from the medium while the roots remained exposed to Pb. Nutrient uptake and ion exchange in the root zone can modify the surrounding ionic and chemical conditions, potentially affecting the interactions of Pb with roots, suspended particles, and other substances in the medium (Zhang *et al.*, 2023). Consequently, the increased accumulation of Pb in the sediments at higher concentrations may be related to the combined influence of

increased pollutant loading, root activity, particle dynamics, and changes in the rhizosphere environment rather than a single process (Kassim *et al.*, 2024).

From a physicochemical perspective, a portion of the Pb present in the water may be transferred to the sediments through interactions with suspended particles, organic matter, and mineral components, which subsequently settle at the bottom of the basins. As the applied Pb concentration increases, the amount of lead available for these interactions also increases, potentially enhancing its retention within the sediment. However, the current study did not determine the chemical speciation of Pb within the sediments or measure each of these mechanisms individually; therefore, these processes represent plausible explanations for Pb accumulation rather than independently proven mechanisms (Tariq *et al.*, 2019; Al-Shammari *et al.*, 2023).

4.4 Efficiency of *Azolla pinnata* in Treating Cadmium (Cd)-Contaminated Water

4.4.1 Effect of Physicochemical Factors on Cadmium (Cd) Removal

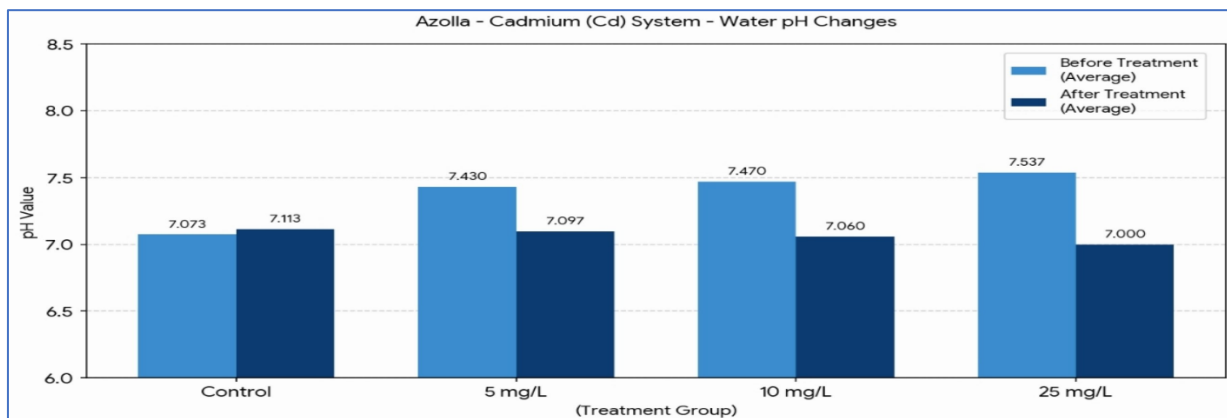
4.4.1.1 Effect of pH on the Bioavailability and Removal of Cadmium (Cd) by *Azolla*

The results of the field study showed a clear decrease in pH values in the *Azolla pinnata*-treated cadmium (Cd) treatment basins between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, the pH values were (7.430), (7.470), and (7.537) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the pH values decreased to (7.097), (7.060), and (7.000) at (5, 10, and 25 mg/L), respectively. The decrease in pH was observed across the Cd treatments at the applied concentrations of (5, 10, and 25 mg/L). In the control treatment, pH increased from (7.073) during the pre-treatment stage to (7.113) during the post-treatment stage. as shown in Table (4.3) and Figure (4.18)

Statistical analysis indicated significant differences at the probability level ($P \leq 0.05$), Pearson's correlation showed a strong association between pH and Cd concentration in water ($r = -0.97$), Cd accumulation in whole-plant tissues ($r = -0.96$), and Cd accumulation in bottom sediments ($r = -0.97$), as shown in Table (4.4)

Figure (4.18): pH measurement in water with (Cd) by *Azolla pinnata*

The decrease in pH values in the cadmium treatments (5 to 25 mg/L) after treatment can be explained by the continued physiological activity of the *Azolla plant* and its interaction with the aqueous medium throughout the experimental period (Al-Farji & Al-Husseini, 2024). The decrease in pH coincided with an increase in the applied



cadmium concentration, indicating that a higher contaminant load was associated with a greater change in the chemical conditions of the medium. In contrast, the higher pH in the control treatment suggests that the change in cadmium values was not solely due to the passage of time or plant activity but was also related to the presence of cadmium in the medium.

The decrease in pH could be related to *Azolla* activity in the water contact zone, as the uptake of nutrients and ions from the medium, along with ion exchange processes at plant surfaces, can alter the ionic balance of the aqueous medium (Zhao *et al.*, 2023). Plant physiological activity and processes related to respiration and ion exchange can contribute to changes in hydrogen ion concentration, and

consequently, pH. Therefore, the observed decrease in pH should not be interpreted solely as a result of cadmium exposure, but rather as a consequence of the interaction between cadmium exposure, plant biological activity, and the chemical conditions of the medium (Barkat *et al.*, 2024; Xia *et al.*, 2024).

Furthermore, increasing the cadmium concentration from 5 to 25 mg/L means increasing the amount of cadmium available to react with the *Azolla* surface and the surrounding medium. Changes in pH can affect the state of cadmium in water and its interaction with plant surfaces and particles in the medium (Mustafa & Abbas, 2024). Changes in the chemical conditions of the medium can alter the cadmium's ability to bind or remain in a dissolved form, thus affecting its distribution between water, plants, and sediments. Therefore, the decrease in pH in cadmium treatments may be related to changes in cadmium behavior and distribution during the treatment period, rather than simply a discrete change in pH value (Lopez *et al.*, 2021; Xia *et al.*, 2024).

The relationship between pH and residual cadmium concentration in water suggests that the decrease in pH coincided with an increase in cadmium concentration in water across the treatments (Patel *et al.*, 2023). This could be explained by the fact that a decrease in pH might increase the retention of a larger fraction of cadmium in the aqueous medium and limit its binding to certain surfaces or components within the system, thus preserving its mobility and reactivity with plants and sediments. However, this explanation represents a potential mechanism, as the current study did not directly identify the chemical species of cadmium, and the correlation coefficient only demonstrates the synchronicity between variables and does not prove that the decrease in pH was the direct cause of the increase in residual cadmium in water. Regarding cadmium accumulation in *Azolla* tissue samples, the correlation between low pH and high cadmium concentration in plant samples may be related to changes

in cadmium availability for interaction with plant surfaces and tissues during the exposure period (Salih *et al.*, 2024). The high applied cadmium concentration increased the pollutant load available for interaction with the plant, and this process can be influenced by the chemical conditions of the medium, including pH. Plant surfaces and components within plant tissues can bind to cadmium ions to varying degrees. However, the current study did not differentiate between surface-bound and tissue-bound cadmium; therefore, the contribution of each mechanism cannot be directly determined (Mishra *et al.*, 2023; Kumar *et al.*, 2024).

Furthermore, the relationship between low pH and high cadmium in sediments suggests that changes in the chemical conditions of the medium coincided with the translocation of some cadmium from the water to the sediment (Guo *et al.*, 2023). Cadmium retention in sediments can be influenced by its interaction with organic particles, materials, and mineral surfaces in the medium. The presence and activity of *Azolla* can also affect the movement of particles and materials near the water's surface, potentially contributing to the transfer of some cadmium to the sediments. However, the specific chemical reaction responsible for cadmium retention was not directly identified in this study; therefore, the correlation between pH and cadmium cannot be considered indicative of a specific chemical reaction. It is also important to interpret the change in pH in conjunction with the nutrient medium used in the experiment, because *Azolla* did not react with cadmium in isolation. Rather, cadmium was present with a range of ions and nutrients in the medium (Al-Mansoor *et al.*, 2024). As the plant continued to absorb nutrients and interact with ions, changes occurred in the surrounding ionic and chemical environment. Therefore, the change in pH reflects the overall chemical state of the medium during treatment, resulting from the interaction of plant activity with cadmium exposure and the altered ion distribution among the system components.

The control treatment, on the other hand, showed an increase in pH despite not being exposed to added cadmium (Verma *et al.*, 2023). This result is significant because it demonstrates that the normal activity of *Azolla* and the nutrient medium can lead to pH changes even in the absence of cadmium. Consequently, the greater decrease in cadmium concentrations can be attributed to the interaction of cadmium with plant activity and the chemical changes in the medium, rather than to plant activity alone. In general, a decrease in pH in cadmium treatments indicates a change in the chemical conditions of the medium during the treatment period, coinciding with the distribution of cadmium between water, plant tissue samples, and sediments (Naji *et al.*, 2024). While pH may be a factor influencing cadmium mobility and its interaction with system components, the behavior of cadmium in this experiment reflects the interaction of *Azolla*'s biological activity with the ionic and chemical conditions of the medium. Therefore, pH alone cannot be considered the sole factor responsible for changes in cadmium concentration or its accumulation in the various components.

4.4.1.2 Effect of Electrical Conductivity (EC) and Ionic Strength on Cadmium (Cd) Removal

The results of the field study showed a clear decrease in electrical conductivity (EC) values in the *Azolla pinnata*-treated cadmium (Cd) treatment basins between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, the EC values were (2.029), (2.215), and (2.505 dS/m) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the EC values decreased to (1.849), (1.979), and (2.205 dS/m) at (5, 10, and 25 mg/L), respectively. The decrease was observed across all tested Cd concentrations. In the control treatment, EC decreased from (1.761 dS/m) during the pre-treatment stage to (1.640 dS/m) during the post-treatment stage. as shown in Table (4.3) and Figure (4.19)

Statistical analysis indicated significant differences at the probability level ($P \leq 0.05$), The Pearson's correlation showed a very strong association between EC and Cd concentration in water ($r = +0.99$), Cd accumulation in whole-plant tissues ($r = +0.99$), and Cd accumulation in bottom sediments ($r = +0.99$). The relationship between EC and pH was ($r = -0.98$), as shown in Table (4.4)

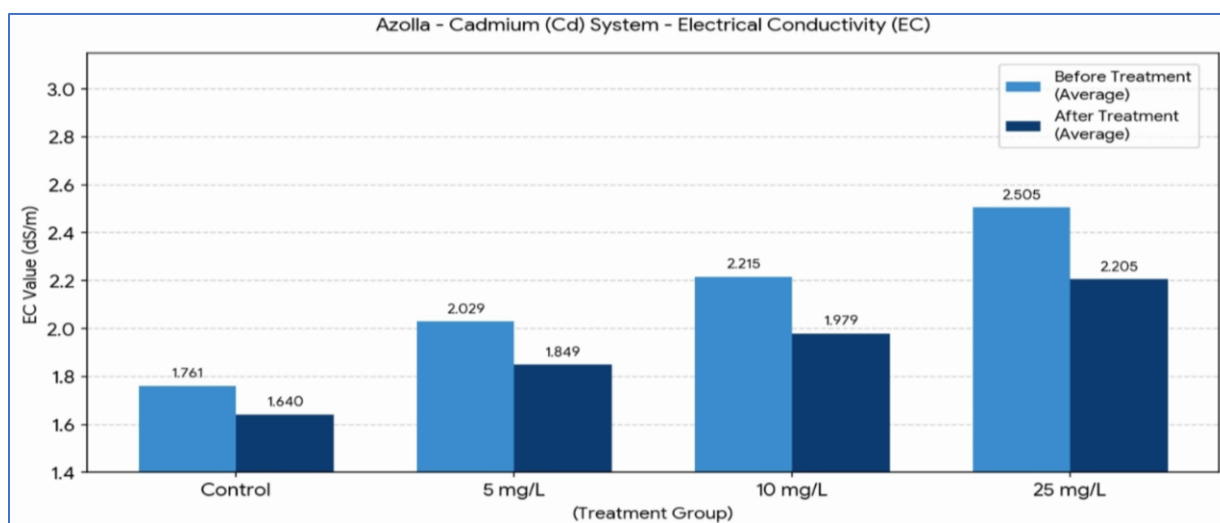


Figure (4.19): EC measurement in water with (Cd) by *Azolla pinnata*

The rise in electrical conductivity (EC) values across cadmium treatments (ranging from 5 to 25 mg/L) prior to treatment can be attributed to the increased ionic load in the medium—resulting from both the added cadmium and the ions originally present in the nutrient solution (Al-Rubaye & Al-Hussainy, 2024). Since EC reflects the total dissolved ions in the water, higher cadmium concentrations led to a greater treatment-associated ionic load, resulting in higher EC values at these elevated concentrations. Consequently, the variation in EC among the cadmium treatments prior to exposure reflects changes in the medium's overall ionic status rather than the isolated effect of cadmium alone.

The decrease in EC following the treatment period can be explained by the ongoing physiological activity of the *Azolla* plant and its interaction with the aqueous

medium (Sun *et al.*, 2023). The plant requires nutrients and dissolved ions to sustain its activity and growth; as exposure continues, the concentration of certain ions declines due to absorption and exchange processes at the plant surface, thereby lowering the EC. Thus, the post-treatment drop in EC does not signify cadmium removal in isolation but rather reflects a general shift in the medium's ionic composition driven by *Azolla* activity and the interactions occurring during the treatment period (Kumar *et al.*, 2024).

Furthermore, increasing the cadmium concentration from 5 to 25 mg/L implies a higher pollutant load to which the *Azolla* plant was exposed (Jassim & Al-Niemi, 2024). As this load increases, the amount of cadmium available to interact with plant surfaces and the surrounding medium also rises; a portion may bind to the plant surface or translocate into plant tissues, while another portion may migrate to the sediments. Consequently, the decrease in EC coincided with a shift in cadmium distribution among the water, plant tissue samples, and sediments; however, this does not imply that the drop in EC was the direct cause of cadmium removal. The strong correlation between EC and cadmium concentration in the water can be explained by the fact that treatments with higher initial cadmium concentrations carried a greater ionic load, resulting in higher initial EC values (Zhou *et al.*, 2024). As the treatment progressed, both EC and aqueous cadmium levels declined, indicating that changes in the medium's ionic status and cadmium concentration occurred simultaneously. This phenomenon may be attributed to the uptake of nutrients and ions by *Azolla*, as well as interactions involving cadmium, the plant, and particles or substances present in the medium. Nevertheless, EC is not a direct measure of cadmium concentration, as it is influenced by all dissolved ions present in the water. The correlation between EC and cadmium accumulation in plant tissue samples can be explained by the fact that treatments with higher ionic loads were also those in which the plants were exposed to higher concentrations of cadmium

(Al-Baidhani *et al.*, 2024). As the available cadmium in the medium increased, so did its potential to react with the *Azolla* surface and be retained or translocated into plant tissues. Therefore, the increase in EC and cadmium accumulation in plant samples reflects the combined effect of the change in ionic load and the level of cadmium exposure. This does not imply that EC alone caused cadmium uptake, as the relationship between the two variables may reflect the effect of the treatment concentration and the accompanying ionic conditions.

Furthermore, the correlation between EC and cadmium accumulation in sediments suggests that the increase in ionic load and cadmium in the medium coincided with increased cadmium retention in the sediment fraction (Wang *et al.*, 2023). The translocation of cadmium from water to particles and materials at the bottom of the medium, followed by precipitation, could contribute to its increased concentration in the sediments. Changes in the ionic environment of the medium can also affect cadmium's interactions with surfaces and particles. However, EC cannot be considered the direct cause of cadmium precipitation, as cadmium accumulation in sediments depends on a complex interplay of physical and chemical processes within the system.

The relationship between EC and pH indicates that changes in the ionic state of the medium coincide with changes in water pH (Ibrahim & Al-Sultani, 2024). This can be explained by the interaction of *Azolla* activity with the absorption and exchange of nutrients and ions with the medium, potentially leading to simultaneous changes in the ionic and chemical equilibrium of the water. Furthermore, changes in pH can influence cadmium's behavior and its interactions with plant surfaces and particles. Therefore, the relationship between EC and pH reflects a shared change in the chemical conditions of the medium and does not prove that one variable directly causes the other.

It is also important to interpret the decrease in EC in the control treatment, since the control was not exposed to cadmium (Chaudhary *et al.*, 2023). Therefore, the decrease in EC in this treatment suggests that part of the change in electrical conductivity was related to natural processes within the system, particularly *Azolla* activity, nutrient and dissolved ion uptake, and changes in the medium composition during the treatment period. This supports the interpretation of the decrease in EC in the cadmium treatments as a result of the interaction between plant activity and the change in ion load caused by the presence of cadmium, rather than solely due to cadmium removal.

In general, the decrease in EC after treatment reflects a reduction in a portion of the dissolved ion load resulting from continued *Azolla* activity and changes in the aqueous medium (Al-Saffar *et al.*, 2024). Simultaneously, the high concentration of applied cadmium led to an increase in the contaminant load and a change in its distribution between the water, plant tissue samples, and sediment. Therefore, EC is an important indicator of the overall change in the ionic state of the medium during treatment, but it is not a direct measure of the amount of cadmium removed by *Azolla*, because its change is influenced by a range of ions and biological, physical and chemical processes within the system (Barkat *et al.*, 2024; Xia *et al.*, 2024).

4.4.2 Dynamics and Removal Efficiency of Cadmium (Cd) in the Water

Field measurements of residual cadmium (Cd) concentration in the aqueous phase of the *Azolla pinnata* system showed a significant decrease in all Cd treatments during the post-treatment stage compared with the pre-treatment stage. During the pre-treatment stage, the Cd concentrations were (4.750), (9.533), and (24.157 mg/L) at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, the Cd concentrations decreased to (2.973), (5.453), and (12.503 mg/L) at (5, 10, and 25 mg/L), respectively. The removal efficiency (RE%) was (37.411%), (42.799%), and

(47.671%) at (5, 10, and 25 mg/L), respectively. as shown in Table (4.3) and Figure (4.20).

Statistical analysis indicated significant differences at the probability level ($P \leq 0.05$), The Pearson's correlation showed a very strong association between Cd concentration in water and EC ($r = +0.99$), Cd accumulation in whole-plant tissues ($r = +0.99$), and Cd accumulation in bottom sediments ($r = +0.99$). The relationship between Cd concentration in water and pH was ($r = -0.97$), as shown in Table (4.4)

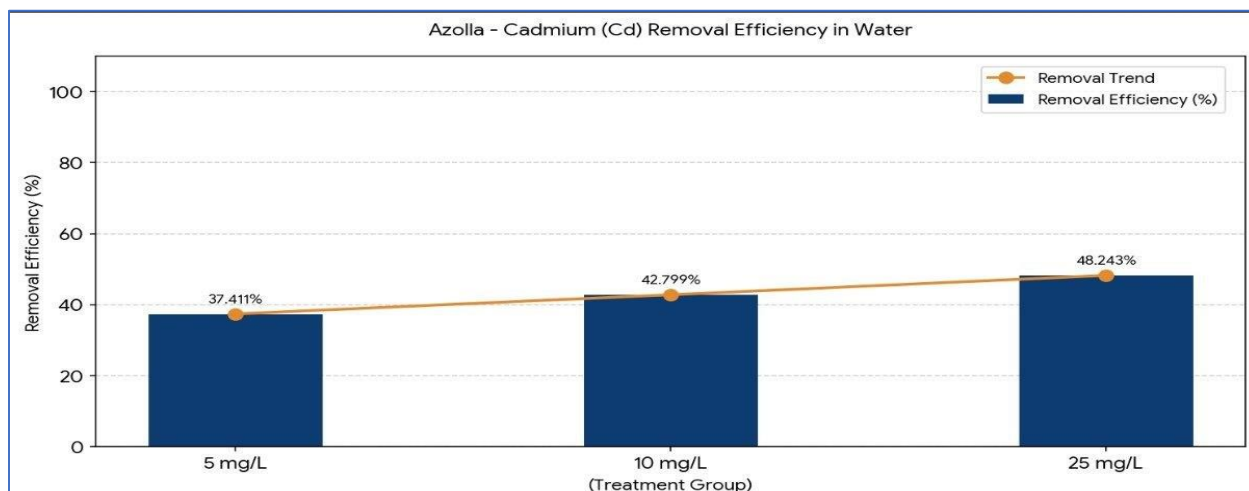


Figure (4.20): Cd Removal Efficiency (%) in Water by *Azolla pinnata*

The decrease in cadmium (Cd) concentration in the water between the pre- and post-treatment stages in all cadmium treatments (5 to 25 mg/L) indicates a change in cadmium distribution within the experimental system during the treatment period (Al-Hasnawi & Al-Hameedi, 2024). This decrease can be explained by the interaction of cadmium with *Azolla* plants, along with the transfer or retention of some of it in the sediments and other physical and chemical processes within the medium. Therefore, the decrease in cadmium in the water does not represent pure vegetation removal but rather reflects a redistribution of cadmium between the water, plants, and sediments.

Increasing the applied cadmium concentration (5 to 25 mg/L) means an increased pollutant load available for interaction with *Azolla* plants and other materials in the medium (Tang *et al.*, 2023). As the pollutant load increases, a greater quantity of cadmium becomes available to interact with plant surfaces and waterborne particles. This may lead to increased cadmium retention on the plant surface or translocation to plant tissues, with some cadmium being transferred to sediments. *Azolla* surfaces and their plant components can provide binding sites for cadmium ions. However, the current study did not directly identify the chemical species of cadmium or differentiate between cadmium adsorbed on the plant surface and cadmium within the tissues. Therefore, these mechanisms remain potential explanations rather than definitively proven processes (Kumar *et al.*, 2024).

The presence of the nutrient medium during the treatment period is also important in explaining the decrease in water cadmium levels, as the plant continued to absorb nutrients and ions and interact with the medium while simultaneously being exposed to cadmium (Al-Saeedi & Al-Janabi, 2024). Plant activity may alter the ionic and chemical environment near its surface, affecting cadmium movement and distribution among water, plant surfaces, and particles. Therefore, the change in cadmium concentration during the treatment period reflects the interaction of cadmium exposure with the physiological activity of *Azolla* and the chemical conditions of the medium, rather than the effect of cadmium alone.

The accumulation of cadmium in plant tissue samples supports the existence of transfer or retention of a portion of the cadmium bound to the plant system as its concentration in water decreases (Ren *et al.*, 2024). As the applied cadmium concentration increased, the amount of cadmium available to react with the plant also increased, resulting in higher concentrations in plant tissue samples at higher treatment levels. It is important to note that the plant measurements in this study represent a sample taken from the experimental units and analyzed; the entire plant

mass within the unit was not analyzed. Therefore, these results represent the cadmium concentration in the analyzed plant sample and cannot be used to determine the total amount of cadmium present in the entire *Azolla* mass. Meanwhile, the accumulation of cadmium in the sediments suggests that some of the cadmium that fell from the water was retained in the sedimentary layer (Al-Kenaany *et al.*, 2024). This could occur through the deposition of cadmium-bearing particles or the binding of cadmium to the surfaces of particles, organic matter, and other components within the sediments. The presence of *Azolla* and its interaction with the surrounding environment could also influence the movement of certain particles and materials in the water, potentially contributing to the transport of some cadmium to the bottom. However, the current study did not identify the contribution of each process individually, and therefore it cannot be determined that any one of these mechanisms was solely responsible for the accumulation of cadmium in the sediments. Regarding the relationship between cadmium in water and electrical conductivity (EC), the simultaneous increase in cadmium concentration and EC in higher treatments can be explained by the increase in the total ionic load of the medium with increasing treatment concentration (Li *et al.*, 2023). After treatment, both cadmium concentration and EC decreased, indicating a simultaneous change in cadmium concentration and the ionic state of the water. However, EC reflects the total ions dissolved in the medium, and therefore its decrease cannot be considered direct evidence of cadmium removal, as part of the decrease in EC may be related to nutrient and ion uptake by *Azolla* and changes in the ionic composition of the medium.

As for the relationship between cadmium and pH, the increase in cadmium concentration in water coincided with a decrease in pH across treatments. Changes in pH can affect cadmium mobility and its interaction with plant surfaces, particles, and sediments, and thus may influence its distribution among system components.

Azolla activity, nutrient uptake, and ion exchange can also contribute to pH changes during the treatment period. Therefore, the relationship between pH and cadmium reflects a combined change in the chemical conditions of the medium and does not imply that a decrease in pH alone caused a decrease in cadmium from the water (Barkat *et al.*, 2024; Xia *et al.*, 2024).

It is important to interpret the relationship between cadmium in water, plant tissue samples, and sediment together, because the results showed that a decrease in cadmium in water coincided with the presence of cadmium in both the plant sample and the sediment portion (Al-Zubaidy & Al-Azzawi, 2024). This indicates that cadmium was not only distributed in the aqueous phase but also distributed among several components of the system during the treatment period. However, the strength of the correlation between these components does not prove that cadmium transfer occurred directly from the water to the plant or directly from the water to the sediment but rather suggests that changes in these components occurred simultaneously.

The control treatment, on the other hand, showed a decrease in cadmium concentration despite the absence of cadmium addition (Singh *et al.*, 2023). Therefore, a decrease in control cannot be considered evidence of the plant's effectiveness in treating the added cadmium. This change may be related to very low background cadmium levels, natural variations in the aqueous and nutrient medium, plant activity, or analytical variability at low concentrations. Thus, the interpretation of the decrease in cadmium coefficients should primarily depend on the changes that occurred under the applied cadmium concentrations.

The increase in relative removal efficiency (RE%) with increasing applied concentration (from 5 to 25 mg/L) indicates that a greater proportion of the initial cadmium was removed from the aqueous phase at the higher concentration (Al-Bayati *et al.*, 2024). This could be related to an increased amount of cadmium

available to react with *Azolla*, particles, and sediments at higher contaminant loads. However, a distinction must be made between the removal percentage and the final residual concentration in the water; a higher RE% at the higher concentration does not mean that the water now contains the lowest final cadmium concentration, but rather that a greater proportion of the initial amount has been transferred or retained outside the aqueous phase.

In general, the decrease in cadmium concentration in the water during the treatment period reflects its redistribution between the aqueous phase, plant tissue samples, and sediments, with a possible contribution from the physicochemical reactions that occurred in the medium (Chen *et al.*, 2024). *Azolla* contributed to the retention and accumulation of some cadmium, while the sediments represented another important pathway for cadmium retention. Therefore, the aqueous removal efficiency recorded in this experiment should not be interpreted as solely due to plant uptake, but rather as a result of the interaction between *Azolla* activity and its interactions with cadmium, the aqueous medium, and the sediments during the treatment period.

4.4.3 Bioaccumulation of Cadmium (Cd) in Whole Plant Tissues

The results of the field study showed a clear increase in cadmium (Cd) concentrations in the whole-plant tissues of *Azolla pinnata* between the Pre-treatment and Post-treatment stages. During the pre-treatment stage, Cd concentrations in plant tissues were (0.0005), (0.0009), and (0.0007 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. During the post-treatment stage, Cd concentrations increased to (1.657), (2.820), and (6.760 mg/kg) dry weight at (5, 10, and 25 mg/L), respectively. The highest Cd accumulation was recorded at the highest applied concentration of (25 mg/L). In contrast, the Cd concentration in the control treatment increased from (0.0007 mg/kg) during the pre-treatment stage to (0.0011 mg/kg) during the post-treatment stage. as shown in Table (4.3) and Figure (4.21)

Statistical analysis indicated significant differences at the probability level ($P \leq 0.05$), The Pearson's correlation showed a very strong association between Cd accumulation in plant tissues and Cd concentration in water ($r = +0.99$), Cd accumulation in bottom sediments ($r = +0.99$), and EC ($r = +0.99$). The relationship between plant Cd accumulation and temperature was ($r = +0.76$), while the relationship with pH was ($r = -0.96$), as shown in Table (4.4)

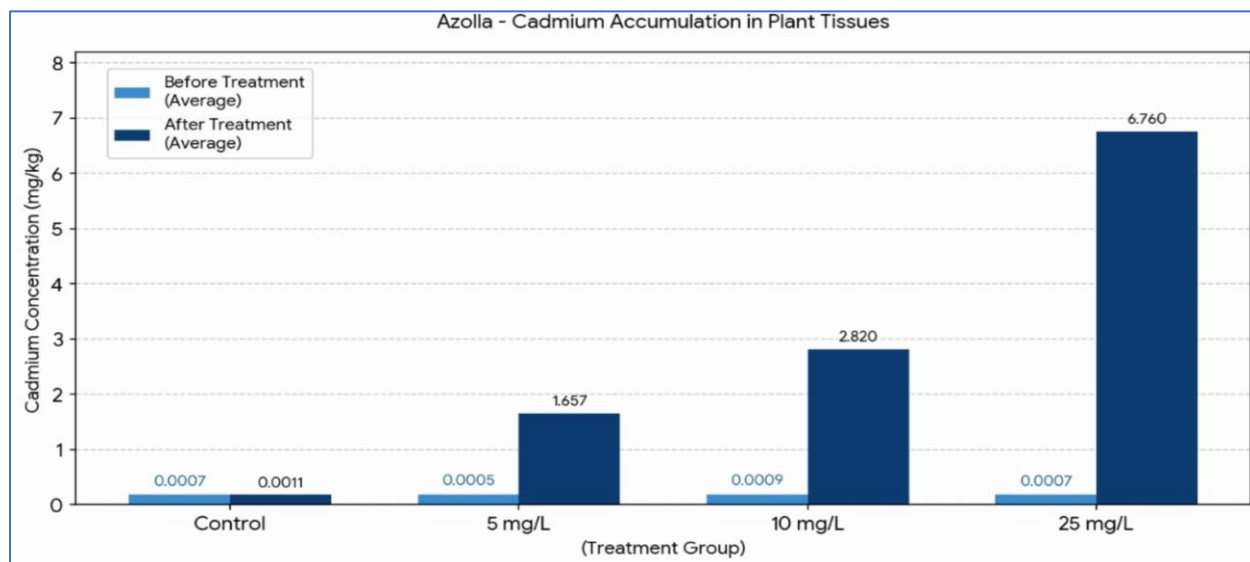


Figure (4.21): Cd concentration in Plant Tissues (mg/kg) by *Azolla pinnata*

The increase in cadmium (Cd) concentration in *Azolla* plant tissue samples between the pre- and post-treatment stages indicates that cadmium became bound to the plant system during the exposure period (Al-Gharibawi & Al-Rubaie, 2024). The increased cadmium concentration in plant samples with increasing applied concentration (5 to 25 mg/L) can be explained by the increased amount of cadmium available for interaction with the plant at higher contaminant loads. As the treatment period continued, the opportunity for cadmium to interact with the *Azolla* surface and plant tissues increased, reflected in the higher concentrations in the analyzed plant samples.

Cadmium binding to the plant can begin through its interaction with plant surfaces and functional groups within cell components, as some of these sites can bind to and retain cadmium ions (Wu *et al.*, 2023). Some of the surface-bound cadmium may then be translocated into plant tissues through the plant's metabolic processes. However, the current study did not differentiate between cadmium bound to the plant surface and cadmium within the tissues, nor did it directly identify the chemical species of cadmium or its mechanism of entry into cells. Therefore, these processes are considered possible explanations for the results, not directly established mechanisms (Siddiqui *et al.*, 2021; Kumar *et al.*, 2024).

The higher cadmium accumulation in the treatment with concentrations above 25 mg/L can be attributed to the increased amount of cadmium available to react with *Azolla* compared to treatments with lower concentrations (Al-Khafaji *et al.*, 2024). As the cadmium concentration in the water increases, the pollutant load to which the plants are exposed also increases, thus increasing the likelihood of cadmium binding to plant surfaces and some retention in the tissues. This indicates that the level of cadmium exposure was related to the degree of accumulation observed in the plant samples during the treatment period.

It is important to clarify here that the plant measurements represent a sample of *Azolla* taken from the experimental units and analyzed in the laboratory; the entire plant mass in each experimental unit was not analyzed (Xu *et al.*, 2024). Therefore, the recorded cadmium values represent the cadmium concentration in the analyzed plant sample and not the total amount of cadmium present in the entire *Azolla* mass. Furthermore, since the plant sample was not separated into different plant parts, it is not possible to determine whether cadmium accumulated to a greater extent in a specific plant part or to directly differentiate between surface and internal accumulation.

The relationship between cadmium accumulation in plant samples and its

concentration in water can be explained by the fact that a higher cadmium concentration in the medium provided a greater quantity of the metal available to react with the plant. Therefore, higher exposure levels coincided with greater accumulation in plant samples (Al-Musawi & Al-Hussain, 2024). This result indicates a correlation between the amount of cadmium present in the water and the amount of cadmium measured in the plant sample, but it does not prove that all the cadmium that dropped from the water was transferred directly to the plant, because some of the cadmium was also present in the sediment and could have been retained through various physical and chemical processes. Furthermore, the accumulation of cadmium in plant samples coincided with its accumulation in sediments (Yang *et al.*, 2023). This can be explained by the fact that cadmium was distributed among several components of the system during the treatment period, including water, plants, and sediments. As the contaminant load increased, the amount of cadmium available to react with plants, particles, and other materials in the medium also increased, potentially leading to some being retained in the plant sample and some in the sediments.

Therefore, the relationship between plant and sediment cadmium reflects the simultaneous distribution of cadmium among system components and does not imply that its accumulation in plants directly caused its accumulation in sediments, or vice versa.

The correlation between cadmium accumulation in plant samples and electrical conductivity (EC) can be explained by the fact that treatments with higher cadmium concentrations also had a higher ionic load in the medium (Al-Shammari & Al-Tae, 2024). Since EC reflects the total dissolved ions and not just cadmium, elevated EC and cadmium accumulation in plant samples can reflect a combined change in the concentration of cadmium, ions, and nutrients in the medium. Therefore, EC cannot be considered a direct cause of increased cadmium accumulation, but rather an

indicator of the overall change in the ionic state of the medium during the treatment period.

Furthermore, the relationship between cadmium accumulation and temperature can be explained by the environmental changes that occurred during the experiment (Zhang *et al.*, 2024). Temperature can affect the plant's physiological activity and the vital processes associated with growth and ion exchange; thus, changes in temperature may coincide with changes in the plant's ability to react with cadmium. However, temperature was not independent experimental control; therefore, its relationship to cadmium accumulation represents a correlation with the environmental conditions during the experiment and cannot be considered evidence that higher temperature directly caused increased cadmium accumulation (Siddiqui *et al.*, 2021; Kumar *et al.*, 2024).

The relationship between cadmium accumulation and pH can be explained by the fact that a decrease in pH may coincide with a change in cadmium's behavior and its interaction with plant surfaces and medium components (Al-Jebouri *et al.*, 2024). A change in pH can affect cadmium mobility and its availability to react with plants, and *Azolla* activity, nutrient uptake, and ion exchange can also contribute to pH changes. Therefore, increased cadmium accumulation with a decrease in pH may reflect the interaction of the medium's chemical conditions with plant exposure to cadmium, rather than the effect of pH alone (Barkat *et al.*, 2024; Xia *et al.*, 2024). The accumulation process may involve several stages, beginning with cadmium reacting with the plant surface, then being retained by plant components, and some of it may translocate into internal tissues (Zhao *et al.*, 2024). Certain cell wall components and functional groups may provide sites for metal ion binding, and internal mechanisms may contribute to cadmium tolerance and retention within cells. However, this study did not directly examine cell wall functional groups, Phyto lactins, vacuoles, or the internal location of cadmium. Therefore, these mechanisms

cannot be considered experimentally confirmed in this study, but they can be proposed as possible explanations for the increased cadmium concentration observed in the plant samples (Siddiqui *et al.*, 2021; Kumar *et al.*, 2024).

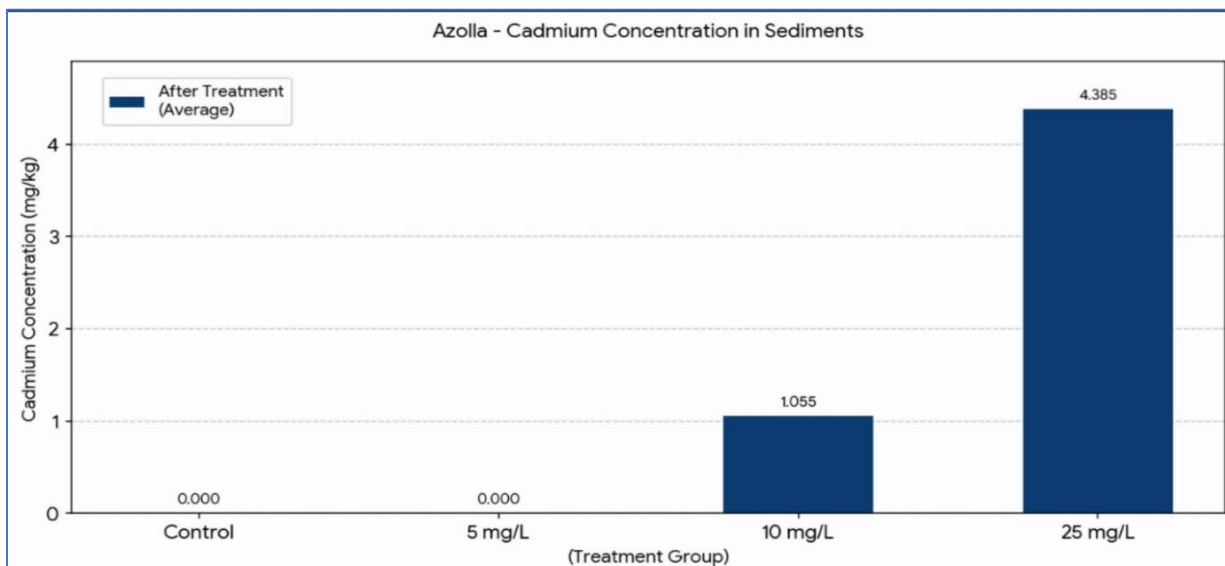
The slight increase in cadmium concentration in the control treatment should be interpreted with caution, as this treatment was not subjected to cadmium addition among the contaminated treatments (Tariq *et al.*, 2023). Therefore, this increase cannot be considered evidence of cadmium accumulation resulting from exposure to applied concentrations. It may be related to very low background cadmium levels, natural variations in the medium and nutrients, plant activity, or analytical variability at low concentrations. In contrast, the larger increase observed in cadmium coefficients is more clearly associated with exposure to applied cadmium during the treatment period.

In general, the increase in cadmium concentration in *Azolla* tissue samples after treatment indicates the plant's ability to retain and accumulate cadmium in the analyzed plant sample during the exposure period, with accumulation increasing at higher applied concentrations (Al-Sultani & Al-Maamori, 2024). At the same time, the presence of cadmium in the sediments and its decrease in the water suggests that cadmium is distributed among several components of the system, and therefore plant accumulation cannot be considered the sole pathway for cadmium reduction from the water. Taken together, the results reflect the interaction of *Azolla* with cadmium and the ionic, chemical, and sedimentary changes that occurred in the medium during the treatment period.

4.4.4 Deposition and Accumulation Dynamics of Cadmium (Cd) in Bottom Sediments

Laboratory analysis of settled bottom sediment samples from the basins of the *Azolla pinnata* system showed clear accumulation of cadmium (Cd) during the Post-

treatment stage. No detectable Cd accumulation was recorded in the (5 mg/L)



treatment. In the (10 mg/L) treatment, the mean Cd concentration in the bottom sediments was (1.055 mg/kg), while in the (25 mg/L) treatment it was (4.385 mg/kg). The control treatment recorded a very low concentration of (0.0001 mg/kg). as shown in Table (4.3) and Figure (4.22)

Statistical analysis indicated significant differences at the probability level ($P \leq 0.05$). The Pearson's correlation showed a very strong association between Cd accumulation in bottom sediments and Cd accumulation in *Azolla pinnata* tissues ($r = +0.99$), Cd concentration in water ($r = +0.99$), and EC ($r = +0.99$). The relationship between sediment Cd and temperature was ($r = +0.71$), while the relationship with pH was ($r = -0.97$), as shown in Table (4.4)

figure (4.22): Cd concentration in Sediments (mg/kg) by *Azolla pinnata*

The presence of cadmium in benthic sediment samples after treatment, with its concentration increasing as the applied concentration (10–25 mg/L) increased, indicates that some of the cadmium in the system became trapped in the sedimentary phase during the treatment period (Al-Fatlawi & Al-Sudani, 2024). This pattern can be explained by the increased availability of cadmium in the water at higher

concentrations, which enhances its potential to react with particles and other materials in the medium, leading to the subsequent migration of some of it to the bottom. While sedimentation and adhesion to the surfaces of particles, organic matter, and mineral components may contribute to cadmium retention (Liu *et al.*, 2023), the current study did not directly identify the chemical species of cadmium or the specific mechanism responsible for its retention in the sediments. Furthermore, the higher accumulation of cadmium in the sediments at the applied concentration of 25 mg/L compared to lower concentrations reflects an increased pollutant load in the medium (Al-Rubaie *et al.*, 2024). When the amount of available cadmium is greater, the likelihood increases that some of it will be present in a form capable of reacting with particles and materials in the water, and that these materials will then be transported to the bottom of the basin. Therefore, an increase in cadmium in the sediments with increasing applied concentration could reflect an increase in the amount of cadmium available for distribution between the water and the sediments, and not necessarily the occurrence of a specific chemical precipitation process, as this process was not directly measured in this study.

The absence of detectable cadmium in the sediments at an applied concentration of 5 mg/L, compared to its presence at 10 and 25 mg/L, could indicate that cadmium retention in the sediments becomes more pronounced with increasing contaminant load (Huang *et al.*, 2024). The amount of cadmium that reached the sediments at the lower concentration may be limited, or its concentration may be below the analytical detection limit used in the measurement. Therefore, the absence of detection at 5 mg/L should not be interpreted as evidence of the absence of any cadmium in the sediments, but rather as meaning that the cadmium was not detected at a concentration detectable by the analytical method used.

Azolla may play an indirect role in this process through its interaction with the aquatic environment and the particles present within it (Al-Khafaji & Al-Tamimi, 2024). The plant's presence and activity can influence the movement of certain particles and materials near its surface, potentially leading to the trapping of some cadmium-bearing substances, which then gradually migrate to the bottom. Simultaneously, cadmium can react directly with sediment components and particles in the water. Therefore, the accumulation of cadmium in sediments should not be attributed directly to the plant but rather considered a result of the interaction between plant-related processes and the physical and chemical processes within the environment. The relationship between sediment cadmium and water cadmium concentration indicates that treatments with a higher water cadmium load were also associated with higher sediment accumulation (Gao *et al.*, 2023). This can be explained by the increased availability of cadmium for interaction with particles and sedimentary materials at higher applied concentrations. As water cadmium decreased and sediment cadmium appeared during the treatment period, it appears that some cadmium was distributed outside the aqueous phase. However, this correlation does not prove that the transfer of cadmium from water to sediment occurred via a single pathway or specific direct process.

Furthermore, sediment cadmium accumulation coincided with elevated concentrations in *Azolla* tissue samples (Al-Mussawi *et al.*, 2024). This can be explained by the fact that cadmium was distributed among multiple components of the system during the treatment period, including water, plants, and sediment. As the applied concentration increased, the amount of cadmium available to react with the plant and the particles simultaneously increased, resulting in a simultaneous increase in the measured cadmium in both the plant sample and the sediment. However, this does not imply that the cadmium accumulated in the plant was subsequently

transferred to the sediment, nor that the plant directly caused the accumulation of cadmium at the bottom.

It is important to emphasize that the plant measurements in this study represent a sample of *Azolla* plants taken from the experimental units and analyzed in the laboratory, and do not represent the total amount of cadmium in the entire plant mass present in the unit (Song *et al.*, 2024). Therefore, the relationship between sediment cadmium and plant cadmium reflects the simultaneous change in cadmium concentration in the analyzed plant sample and the sediment and cannot be used to calculate the total amount of cadmium stored in the entire *Azolla* plant.

The correlation between precipitate cadmium and electrical conductivity (EC) can be explained by the fact that treatments with a higher cadmium load had a higher ionic state in the medium (Al-Sultani & Al-Husseini, 2024). Since EC reflects the total dissolved ions and not cadmium alone, the relationship between EC and precipitate cadmium may reflect the combined effect of cadmium, nutrients, and other ions in the medium. Therefore, EC cannot be considered a direct cause of cadmium precipitation but rather an indicator of the overall change in the ionic state of the water during treatment.

The relationship between precipitate cadmium and temperature can be explained within the context of environmental changes that occurred during the experiment (Sun *et al.*, 2024). Temperature can affect plant activity, particle movement, and certain physical and chemical processes in the medium; therefore, temperature changes may coincide with changes in the movement and retention of cadmium-bearing materials. However, temperature was not an independent experimentally controlled factor, and therefore the relationship cannot be considered evidence that increased temperature directly caused cadmium accumulation in the sediments. Regarding the relationship between sediment cadmium and pH, a decrease in pH

coincided with increased cadmium accumulation in the sediments (Al-Azzawi & Al-Zubaidi, 2024). Changes in pH can affect cadmium mobility and its interaction with particle surfaces, organic matter, and mineral components in the medium, thus potentially influencing its distribution between water and sediments. *Azolla* activity, nutrient uptake, and ion exchange can also contribute to pH changes during the treatment period. Therefore, the relationship between pH and sedimentary cadmium suggests a combined change in chemical conditions and cadmium distribution and does not prove specific chemical precipitation or that a decrease in pH was the direct cause of cadmium accumulation in the sediments (Barkat *et al.*, 2024; Xia *et al.*, 2024).

The control treatment showed very low cadmium concentrations in the sediments despite the absence of added cadmium (Wu *et al.*, 2024). Therefore, this value cannot be considered indicative of cadmium retention resulting from contaminating treatments. It may reflect the natural background level of cadmium, the presence of minor traces of contamination, or analytical variability at low concentrations. The higher concentrations observed in the 10 and 25 mg/L treatments are more closely associated with the presence of added cadmium and the experimental exposure conditions.

Overall, the accumulation of cadmium in the bottom sediments at the higher concentrations suggests that the sediments were an important component of cadmium retention in the *Azolla* system during the treatment period (Al-Gharibawi *et al.*, 2024). This retention coincided with a decrease in cadmium in the water and an increase in its concentration in the plant tissue samples, indicating that cadmium was distributed among several components of the system. Therefore, the decrease in cadmium in water should not be interpreted as being due to plant accumulation alone, but rather to the interference of cadmium retention in the plant sample and

sediments with the physical and chemical processes that occurred in the medium during the treatment period.

4.4.5 Comprehensive Evaluation and Comparative Assessment of Lead (Pb) and Cadmium (Cd) Removal by *Azolla pinnata*

Field measurements and analytical indicators revealed a clear variability in the efficiency and competitiveness of *Azolla pinnata* in removing lead (Pb) and cadmium (Cd) from polluted aquatic environments and their accumulation. The plant demonstrated a relatively superior ability to remove lead at high concentrations, while exhibiting unique and distinct cumulative behavior for cadmium across the measured environmental stages.

Removal efficiency from the aqueous phase: In lead (Pb) treatments, the plant recorded removal rates of (45.472%) for the first treatment (5 mg/L), (47.812%) for the second treatment (10 mg/L), and peaked at (50.975%) for the highest treatment (25 mg/L). The lead concentration in the water during the pre-treatment reached (24.157 mg/L) at the highest treatment and decreased during the post-treatment to (11.843 mg/L). This gradual increase in removal efficiency with increasing Pb concentration indicates that *Azolla pinnata* was able to reduce the dissolved Pb concentration under the experimental conditions. The reduction may be related to the interaction of Pb with the plant surface and tissues, together with the retention of part of the metal in the bottom sediment. Therefore, Pb removal from the water may be considered a combined process involving plant uptake, surface adsorption, and physicochemical retention in the aquatic system.

In contrast, cadmium (Cd) treatments achieved removal rates of (37.411%) at a concentration of (5 mg/L), (42.799%) at (10 mg/L), and (47.671%) at the highest concentration of (25 mg/L). Cadmium concentration in the water during the pre-treatment reached (24.157 mg/L) at the highest treatment and decreased during the

post-treatment to (12.503 mg/L). The increase in Cd removal efficiency with increasing applied concentration suggests that *Azolla pinnata* maintained the ability to remove Cd from the aqueous phase under the tested conditions. The lower removal efficiency of Cd compared with Pb may be related to differences in the chemical behavior of the two metals and their interactions with plant tissues and sediment surfaces. These differences can affect the distribution of metals between the dissolved, plant-associated, and sediment-associated phases.

Bioaccumulation in plant tissues and benthic deposition: Lead content in *Azolla pinnata* plant tissues increased with increasing Pb concentration, reaching a final post-treatment mean of (5.183 mg/kg) at (25 mg/L). At the same treatment, benthic lead deposits reached a mean concentration of (6.483 mg/kg). The increase in both plant and sediment Pb concentrations indicates that the metal removed from the water was distributed between the plant biomass and the bottom sediment. This pattern suggests that Pb removal was not dependent on plant accumulation alone. Part of the metal may have been retained through interaction with plant surfaces and tissues, while another part may have become associated with suspended particles and sediment surfaces. Such distribution is consistent with the ability of Pb to interact with mineral particles and organic matter in aquatic sediments (Ali *et al.*, 2022; Wang *et al.*, 2023).

In a cadmium-treated environment, plant tissues exhibited a clear internal retention capacity at the highest concentration (25 mg/L). Cadmium accumulation in the tissues increased to a final post-treatment mean of (6.760 mg/kg), while cadmium deposits at the bottom reached a mean concentration of (4.385 mg/kg), as shown in Table (4.2) and Figure (4.23). The higher Cd accumulation in plant tissues compared with the sediment at the highest treatment indicates that a considerable proportion of the retained Cd was associated with the plant biomass. This may be related to the ability of aquatic plants to bind metal ions to functional groups on the cell wall and

to retain absorbed metals within plant tissues. In addition, phytochelatins may contribute to the binding and intracellular compartmentalization of Cd, which can support the tolerance of plants exposed to elevated Cd concentrations (Rizwan *et al.*, 2021). Therefore, the accumulation observed in *Azolla pinnata* may reflect both adsorption at plant surfaces and uptake followed by internal retention.

The physiological and hadrochemical variation in the kinetics of lead and cadmium removal by *Azolla pinnata* is attributed to the overall differences in physical properties, ionic behavior, and chemical affinity of each metal towards exchange sites on plant cell walls and bottom soil colloids. Lead demonstrated a higher overall removal efficiency from the water column at the high-level treatment (50.975%). The distribution of Pb between plant tissues and sediments indicates that sediment retention may have contributed to the reduction of dissolved Pb in the water. Pearson's correlation showed very strong associations between Pb concentration in water, plant tissues, and sediments, indicating that changes in these compartments occurred in a closely related pattern. However, these relationships do not by themselves prove that precipitation was the only mechanism responsible for Pb removal. The affinity of Pb for mineral particles, organic matter, and sediment surfaces may contribute to its retention in the bottom sediment (Ali *et al.*, 2022; Wang *et al.*, 2023). This suggests that the removal of Pb in the *Azolla pinnata* system involved both biological retention by the plant and physicochemical retention within the sediment.

On the other hand, the system revealed a distinctive physiological behavior of *Azolla pinnata* towards cadmium. Although its removal rate from water was relatively lower compared to lead, its accumulation and retention within plant tissues reached a high value of (6.760 mg/kg) at the highest treatment. Pearson's correlation showed very strong associations among Cd concentrations in water, plant tissues, and sediments. This pattern indicates that the three compartments changed in a closely

related manner during the experiment and suggests that Cd was distributed between the aqueous phase, plant biomass, and sediment. The observed accumulation in plant tissues may be related to the ability of *Azolla pinnata* to bind Cd through cell-wall components and intracellular ligands, including phytochelatins, which can contribute to metal detoxification and compartmentalization within plant cells (Rizwan *et al.*, 2021). These processes may allow *Azolla pinnata* to retain Cd within its biomass and reduce the proportion of Cd remaining in the aqueous phase. This ionic behavior is further associated with the response of physicochemical variables. Pearson's correlation showed strong associations between Cd concentration in water and pH and EC, indicating that changes in the hydrochemical conditions of the experimental basins occurred together with changes in Cd concentration. pH can influence the availability and surface interactions of metal ions, while EC reflects changes in the concentration of dissolved ions in the water. Therefore, the observed changes in Cd concentration may have resulted from the combined effects of plant uptake, adsorption, ion exchange, and redistribution between the aqueous and sediment phases rather than from a single mechanism. Similar interactions between metal behavior and physicochemical conditions have been reported in phytoremediation systems (Singh & Prasad, 2023).

Overall, the results indicate that *Azolla pinnata* was able to remove both Pb and Cd from the aqueous phase under the tested field conditions, with a higher removal efficiency for Pb than Cd at the highest applied concentration. At the same time, the accumulation of both metals in plant tissues and sediments indicates that metal removal involved more than one pathway. Pb showed a clear association with sediment accumulation, whereas Cd showed a clear accumulation in plant tissues. This difference may reflect the different chemical behavior and distribution of the two metals within the water–plant–sediment system. Thus, the efficiency of *Azolla pinnata* in phytoremediation should be evaluated not only from the percentage

reduction in water concentration, but also from the distribution and retention of metals in plant tissues and bottom sediments.

Table (4.3): Physicochemical parameters and heavy metal concentrations (Range, Mean $\pm$ SD , LSD, RE%) in the water plant tissues and sediments of the *Azolla* system under Pb and Cd treatments.

<i>Parameters</i>	<i>Treatment</i>	<i>Before Treatment (Range Mean $\pm$ SD)</i>	<i>After Treatment (Range Mean $\pm$ SD)</i>	<i>LSD</i>	<i>(RE%)</i>
Temperature ($^{\circ}\text{C}$)	General	14–17 15.4 $\pm$ 1.07	18–21 19.3 $\pm$ 1.07	1.14	
pH measurement in water with Pb	Control	7.065– 7.071 7.068 $\pm$ 0.003	7.103 – 7.114 7.108 $\pm$ 0.006	0.0370	
	5 mg/L	7.090 – 7.140 7.120 $\pm$ 0.026	6.380 – 6.440 6.417 $\pm$ 0.032		
	10 mg/L	7.210 – 7.260 7.240 $\pm$ 0.026	6.340 – 6.400 6.377 $\pm$ 0.032		
	25 mg/L	7.250 – 7.300 7.280 $\pm$ 0.026	6.270 – 6.320 6.300 $\pm$ 0.026		
EC measurement (d S/m)	Control	1.742 – 1.771 1.758 $\pm$ 0.015	1.621 – 1.652 1.637 $\pm$ 0.016	0.0292	
	5 mg/L	1.961 – 1.993 1.979 $\pm$ 0.016	1.762 – 1.793 1.779 $\pm$ 0.016		
	10 mg/L	2.123 – 2.152 2.139 $\pm$ 0.015	1.841 – 1.873 1.859 $\pm$ 0.016		
	25 mg/L	2.383 – 2.421 2.405 $\pm$ 0.020	1.993– 2.032 2.015 $\pm$ 0.020		

Pb concentration in water (mg/L)	Control	0.0009 – 0.0009 0.0009 ± 0.0000	0.0002 – 0.0002 0.0002 ± 0.0000	0.0559	
	5 mg/L	4.790 – 4.870 4.823 ± 0.042	2.590 – 2.660 2.630 ± 0.036		45.472%
	10 mg/L	9.570 – 9.650 9.613 ± 0.040	4.980 – 5.050 5.017 ± 0.035		47.812%
	25 mg/L	24.120 – 24.190 24.157 ± 0.035	11.810 – 11.880 11.843 ± 0.035		50.975%
Pb concentration in Plant Tissues (mg/kg)	Control	0.0004 – 0.0006 0.0005 ± 0.0001	0.0008 – 0.0013 0.0010 ± 0.0003	0.0674	
	5 mg/L	0.0005 – 0.0010 0.0007 ± 0.0003	1.110 – 1.150 1.133 ± 0.021		
	10 mg/L	0.0005 – 0.0007 0.0006 ± 0.0001	2.410 – 2.530 2.467 ± 0.060		
	25 mg/L	0.0006 – 0.0010 0.0008 ± 0.0002	5.090 – 5.270 5.183 ± 0.090		
Pb concentration in Sediments (mg/kg)	Control	—	0.0002 – 0.0002 0.0002 ± 0.0000	0.3080	
	5 mg/L	—	0.970 – 1.010 0.990 ± 0.020		
	10 mg/L	—	2.030 – 2.130 2.090 ± 0.053		
	25 mg/L	—	5.910 – 6.830 6.483 ± 0.496		
pH measurement in water with Cd	Control	7.070 – 7.076 7.073 ± 0.003	7.108 – 7.120 7.113 ± 0.006	0.0383	
	5 mg/L	7.400 – 7.450 7.430 ± 0.026	7.060 – 7.120 7.097 ± 0.032		
	10 mg/L	7.440 – 7.490 7.470 ± 0.026	7.030 – 7.080 7.060 ± 0.026		

	25 mg/L	7.500 – 7.560 7.537 ± 0.032	6.970 – 7.020 7.000 ± 0.026		
EC measurement in water with Cd ds/m	Control	1.745 – 1.776 1.761 ± 0.016	1.625 – 1.655 1.640 ± 0.015	0.0308	
	5 mg/L	2.012 – 2.043 2.029 ± 0.016	1.831– 1.863 1.849 ± 0.016		
	10 mg/L	2.193 – 2.331 2.215 ± 0.020	1.962 – 1.994 1.979 ± 0.016		
	25 mg/L	2.481 – 2.522 2.505 ± 0.022	2.183 – 2.221 2.205± 0.020		
Cd concentration in water (mg/L)	Control	0.0007 – 0.0007 0.0007 ± 0.0000	0.0001 – 0.0001 0.0001 ± 0.0000	0.06320	
	5 mg/L	4.720 – 4.780 4.750 ± 0.030	2.930 – 3.010 2.973 ± 0.040		37.411%
	10 mg/L	9.470 – 9.580 9.533 ± 0.057	5.420 – 5.490 5.453 ± 0.035		42.799%
	25 mg/L	24.120 – 24.190 24.157 ± 0.035	12.460 – 12.550 12.503 ± 0.045		47.671%
Cd concentration in Plant Tissues (mg/kg)	Control	0.0005 – 0.0008 0.0007 ± 0.0002	0.0009 – 0.0012 0.0011 ± 0.0002	0.0592	
	5 mg/L	0.0004 – 0.0006 0.0005 ± 0.0001	1.590 – 1.710 1.657 ± 0.061		
	10 mg/L	0.0006 – 0.0012 0.0009 ± 0.0003	2.760 – 2.870 2.820 ± 0.056		
	25 mg/L	0.0005 – 0.0008 0.0007 ± 0.0002	6.710 – 6.810 6.760 ± 0.050		
Cd concentration in Sediments (mg/kg)	Control		0.0001 – 0.0001 0.0001 ± 0.0000	0.0463	
	5 mg/L	—	—		

	10 mg/L	—	1.090 – 1.020 1.055 ± 1.060		
	25 mg/L	—	4.450 – 4.320 4.385 ± 4.393		

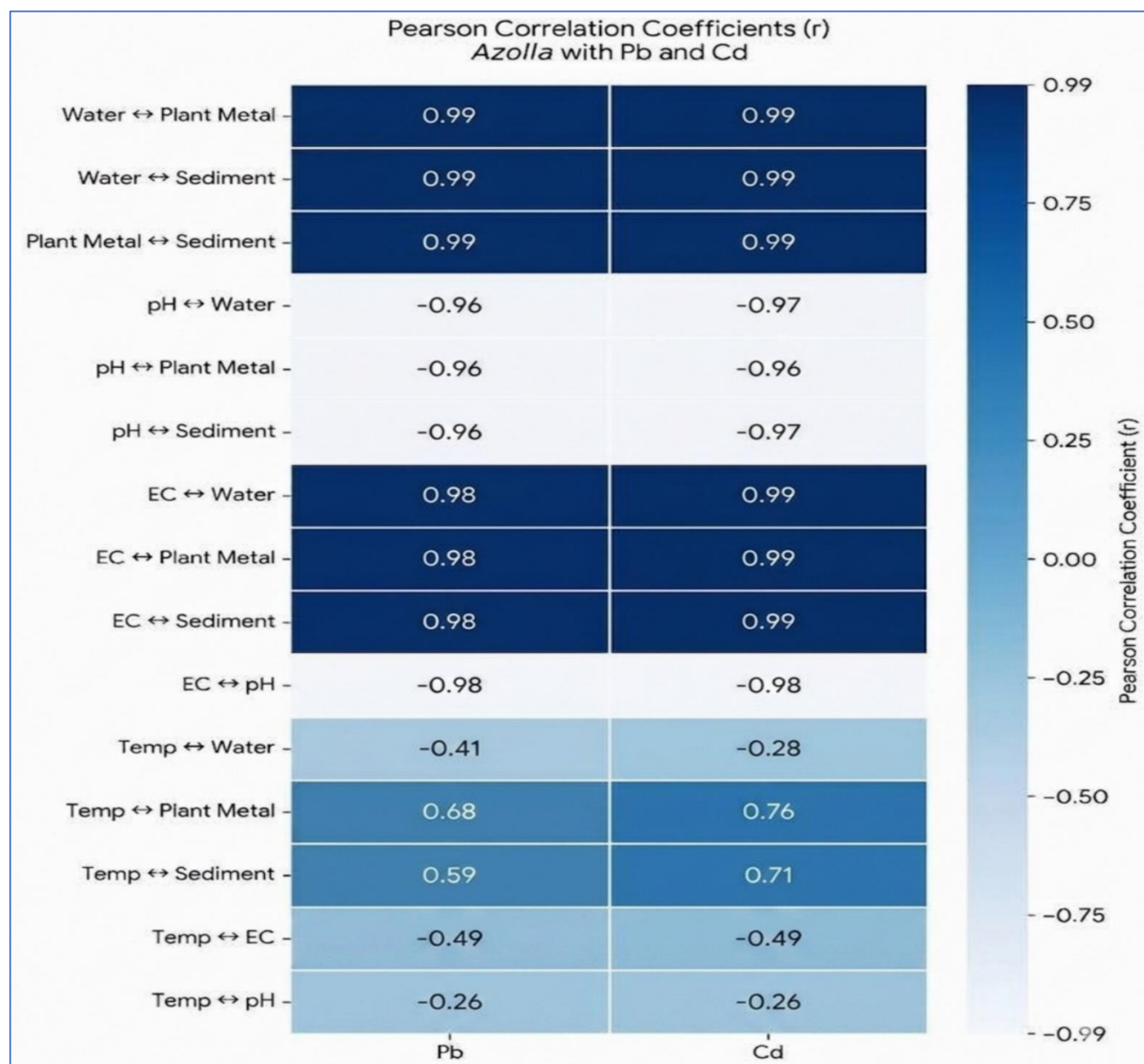


Table (4.4): Statistical correlation analysis (r) showing relationships among physicochemical parameters and heavy metal concentration in the *Azolla* system.

4.6 Comprehensive Comparison of the Efficiency of Lead and Cadmium Removal from Aqueous Media between Water Hyacinth and *Azolla*

The results of the comprehensive comparative evaluation showed that Water Hyacinth (*Eichhornia crassipes*) achieved higher removal efficiency than *Azolla pinnata* in reducing the aqueous concentrations of both heavy metals. At the highest treatment concentration (25 mg/L), Water Hyacinth recorded removal efficiencies of 71.064% for lead (Pb) and 65.443% for cadmium (Cd), whereas *Azolla* recorded lower removal efficiencies of 50.975% for Pb and 47.671% for Cd under the same treatment concentration. These results indicate a greater capacity of Water Hyacinth to reduce the dissolved metal concentrations under the experimental conditions of the present study. The ability of *E. crassipes* to accumulate and remove heavy metals from contaminated water has also been reported in previous studies, particularly for Pb and Cd (Mishra and Tripathi, 2008; Eid *et al.*, 2021).

The biomass loading used in the two experimental systems may also have contributed to the observed differences in removal efficiency. Water Hyacinth was introduced at 100 g fresh biomass per 10 L of water, corresponding to 10 g/L, whereas *Azolla* was introduced at 8 g fresh biomass per 1 L of water, corresponding to 8 g/L. The slightly higher biomass loading in the Water Hyacinth system may have provided a greater amount of plant material available for interaction with dissolved metal ions and may therefore have contributed to the higher removal observed. Previous studies have shown that biomass amount can influence metal removal by aquatic plants through the availability of additional sorption and uptake sites (Han *et al.*, 2010; Carreño Sayago *et al.*, 2020). However, biomass loading alone cannot explain the difference between the two species, because their

morphological characteristics, metal uptake behavior, and physiological responses to heavy-metal exposure are also different.

The higher performance of Water Hyacinth may therefore be related to its well-developed fibrous root system and its capacity to accumulate heavy metals within plant tissues. The roots of *E. crassipes* provide an extensive interface with the surrounding water and have been reported to accumulate higher concentrations of several heavy metals than other plant organs. In particular, Pb and Cd have been reported to accumulate strongly in the roots of Water Hyacinth, indicating an important role of the root system in metal retention and removal from the aqueous medium (Eid *et al.*, 2021; Zhang *et al.*, 2020).

In contrast, *Azolla pinnata* is also capable of removing and accumulating heavy metals from contaminated water, but its response may vary according to the metal type and exposure concentration. Previous research has demonstrated that exposure to Cd and other heavy metals can reduce growth, chlorophyll content, photosynthetic activity, and other physiological parameters in *Azolla*, with Cd showing a relatively strong toxic effect at increasing concentrations. Such physiological responses may affect the plant's growth and metabolic activity during prolonged exposure and may consequently influence its metal-removal performance (Rai, 2008).

Overall, the higher removal efficiencies obtained with Water Hyacinth in the present study can reasonably be associated with the combined effect of its biomass loading, extensive root system, and capacity for heavy-metal accumulation, together with the species-specific physiological responses of the two aquatic plants. Therefore, under the experimental conditions of the present study, *Eichhornia crassipes* demonstrated a higher efficiency than *Azolla pinnata* in reducing aqueous Pb and Cd concentrations.

4.5.1 Bioaccumulation capacity within plant tissues

In a related context, this water efficiency was reflected in the actual bioaccumulation levels. Water Hyacinth showed a higher bioaccumulation capacity within its tissues (when analyzing the whole plant) at a concentration of (25 mg/L). Accumulation values ranged from a low of (7.530 mg/kg) to a high of (7.750 mg/kg) for lead, while cadmium accumulation ranged from a low of (9.430 mg/kg) to a high of (9.530 mg/kg), registering an LSD value of 0.0977 for lead and 0.0541 for cadmium. It significantly outperformed *Azolla* fern, which recorded lower final tissue concentrations. Lead accumulation values ranged from a low of 5.090 mg/kg to a high of 5.270 mg/kg, while cadmium values ranged from a low of 6.710 mg/kg to a high of 6.810 mg/kg, with an LSD value of 0.0674 for lead and 0.0592 for cadmium. The biomass loading used in the two experimental systems may also have contributed to the differences observed in tissue metal accumulation. Water Hyacinth was introduced at 100 g fresh biomass per 10 L of water, corresponding to 10 g/L, whereas *Azolla pinnata* was introduced at 8 g fresh biomass per 1 L of water, corresponding to 8 g/L. The higher biomass loading of Water Hyacinth provided a greater amount of plant material relative to the treated water volume, which may have increased the available biomass for metal uptake and accumulation. However, biomass loading alone cannot explain the differences in tissue metal concentrations, as the two species also differ in their root structure, growth characteristics, metal uptake capacity, and physiological responses to heavy-metal exposure. The scientific discussion regarding the reasons for the higher tissue accumulation of Water Hyacinth may be related to its cellular defense mechanisms, which may contribute to its ability to tolerate and accumulate heavy metals. The heavy ions absorbed by Water Hyacinth can stimulate the production of thiol-rich peptides known as phytochelatins, which chelate toxic lead and cadmium ions within the

cytoplasm and form relatively stable complexes (Thakur *et al.*, 2021). These complexes may then be transported and retained through vacuolar sequestration, away from vital cellular components, allowing the plant to continue accumulating metals while reducing their immediate toxic effects. In contrast, the *Azolla* fern may rely more strongly on cell-wall binding and other metal-tolerance mechanisms, which can influence its capacity to accumulate high concentrations under increasing metal exposure (Gomes *et al.*, 2023).

It is also important to note that lead and cadmium did not necessarily show identical distribution patterns within the plant systems. Although both metals were accumulated in the plant tissues, their concentrations and distribution between plant biomass and the surrounding medium may differ because of differences in their chemical properties, mobility, and interactions with plant tissues and other components of the experimental system. Therefore, the higher accumulation of a particular metal in plant tissues does not necessarily indicate that the same metal will show a similar distribution in the sediment. This difference in the behavior and distribution of Pb and Cd is considered when interpreting the sediment accumulation results presented in the following section.

4.5.2 Dynamics of Sedimentation and Fixation in Basin Sediments

Regarding bottom sedimentation, the results showed a clear difference between the Water Hyacinth and *Azolla* systems in the accumulation of heavy metals in the basin sediments. Sediments in the Water Hyacinth basins under the highest treatment recorded lead accumulation values ranging from a low of (9.530 mg/kg) to a high of (9.700 mg/kg), while cadmium values ranged from a low of (6.190 mg/kg) to a high of (6.390 mg/kg), with LSD values of 0.0891 for lead and 0.0543 for cadmium. In contrast, *Azolla* fern basin sediments exhibited lower accumulation values for lead. Lead levels ranged from a low of 5.910 mg/kg to a high of 6.830 mg/kg, with

an LSD value of 0.3080. The cadmium sediment values for the highest treatment require verification from the original statistical data before being included in the final comparison.

The difference in sediment accumulation between the two systems may be related, in part, to the different characteristics of their root systems. The long and dense roots of Water Hyacinth can act as physical structures within the water column, potentially reducing localized water movement and facilitating the settling of suspended particles associated with metals (Verma *et al.*, 2022). On the other hand, the roots of Water Hyacinth release compounds and colloidal substances (exudates) into the surrounding rhizosphere, which may influence local chemical conditions and contribute to the retention and transformation of heavy metals in the sediment. Such processes may include changes in redox conditions and local pH that can affect metal precipitation and immobilization, while the shorter root system of *Azolla* may provide a different degree of hydraulic and rhizospheric interaction with the basin sediment (Valipour *et al.*, 2020).

The gradual increase in temperature from the pre-treatment phase to the post-treatment phase may also have influenced plant physiological activity during the experimental period. Higher temperatures can affect metabolic activity, enzyme processes, and transpiration in aquatic plants, which may influence metal uptake and accumulation during the treatment period (Kumar *et al.*, 2023; Su *et al.*, 2022). However, because temperature was not experimentally controlled as an independent treatment in the present study, its effect should be considered a possible environmental influence rather than a direct cause of the observed differences in metal accumulation.

Overall, the observed sediment data indicate that Water Hyacinth was associated with greater Pb and Cd accumulation in the basin sediments under the highest treatment compared with the corresponding *Azolla* treatments for which valid data

were available. This pattern may reflect the combined influence of plant root structure, sediment interaction, and metal-retention processes within the experimental basins. Therefore, the sediment results provide additional evidence of the role of Water Hyacinth in the overall removal and retention of heavy metals within the experimental system, while the response of *Azolla* varied according to the metal and treatment concentration.

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Conclusions and Recommendations

5. Conclusions

1. The results of this study demonstrated that phytoremediation using aquatic plants is an effective and environmentally friendly method for treating water contaminated with heavy metals. Both Water Hyacinth (*Eichhornia crassipes*) and Azolla fern (*Azolla pinnata*) showed a clear ability to remove lead (Pb) and cadmium (Cd) from the aquatic medium under open field conditions. However, there was a clear difference in removal efficiency and bioaccumulation between the two plants, depending on their physiological and structural characteristics.
2. The results showed that Water Hyacinth significantly outperformed *Azolla* in removing lead and cadmium from water, recording the highest removal rates at a concentration of 25 mg/L. This reflects its more developed root system, larger surface area, and higher biomass, which provided a greater number of adsorption and ion exchange sites and increased its ability to withstand the stress caused by heavy metal concentrations compared to *Azolla*.
3. The study also showed that removal efficiency increased gradually with increasing concentrations of added lead and cadmium, indicating a direct relationship between increased metal load and the plants' response to biosorption. Both plants maintained their biological activity throughout the experiment, confirming their potential use in treating water with moderate to high concentrations of heavy metals.
4. Laboratory analysis showed that the concentrations of lead and cadmium in the plant biomass increased after the treatment period. This indicates that metal accumulation in the plant biomass contributed to the reduction of Pb and Cd concentrations in the aqueous medium. However, the specific mechanisms responsible for metal retention, including biosorption and internal accumulation, were not directly determined in this study.

5. The results also showed that Water Hyacinth exhibited higher values than *Azolla* for lead and cadmium accumulation within plant tissues. This indicates a greater capacity for internal heavy metal sequestration, which is linked to the high efficiency of its cellular transport systems, the efficiency of its root system, and the effectiveness of compounds responsible for chelating and sequestering heavy metals within vacuoles. This, in turn, limits their toxic effects on plant cells.

6. The study further revealed that bottom sediments served as an important reservoir for the fixation of some heavy metals after treatment. Lead and cadmium accumulation in the sediments increased with increasing initial concentrations, demonstrating that the removal of heavy metals was not solely dependent on plant uptake. Chemical precipitation, ion exchange, and binding to fine organic matter at the bottom of the basins also played a role.

7. The results of the physicochemical variables showed that both phytoremediation processes contributed to improving water properties. Electrical conductivity (EC) values decreased gradually in all treatments, reflecting a reduction in the total ionic load resulting from the removal of metal ions from the aqueous medium. Furthermore, the pH values remained within the appropriate limits for the continuation of the biological processes of both plants. It was noted that *Azolla* caused a greater decrease in pH values compared to water hyacinth.

8. The statistical correlation results also showed that a decrease in electrical conductivity and pH values was associated with a decrease in heavy metal concentrations in the water. This indicates that these variables can be used as environmental indicators to monitor the efficiency of phytoremediation systems and evaluate their performance during different operating periods.

9. The study concludes that integrating bioaccumulation, internal accumulation, and chemical precipitation processes within the phytoremediation system resulted in a significant reduction in lead and cadmium concentrations. This confirms the potential of aquatic plants as a low-cost, environmentally friendly, and sustainable technology for treating water polluted with heavy metals. However, it is essential to manage the plant biomass safely after treatment to prevent the re-release of heavy metals into the environment.

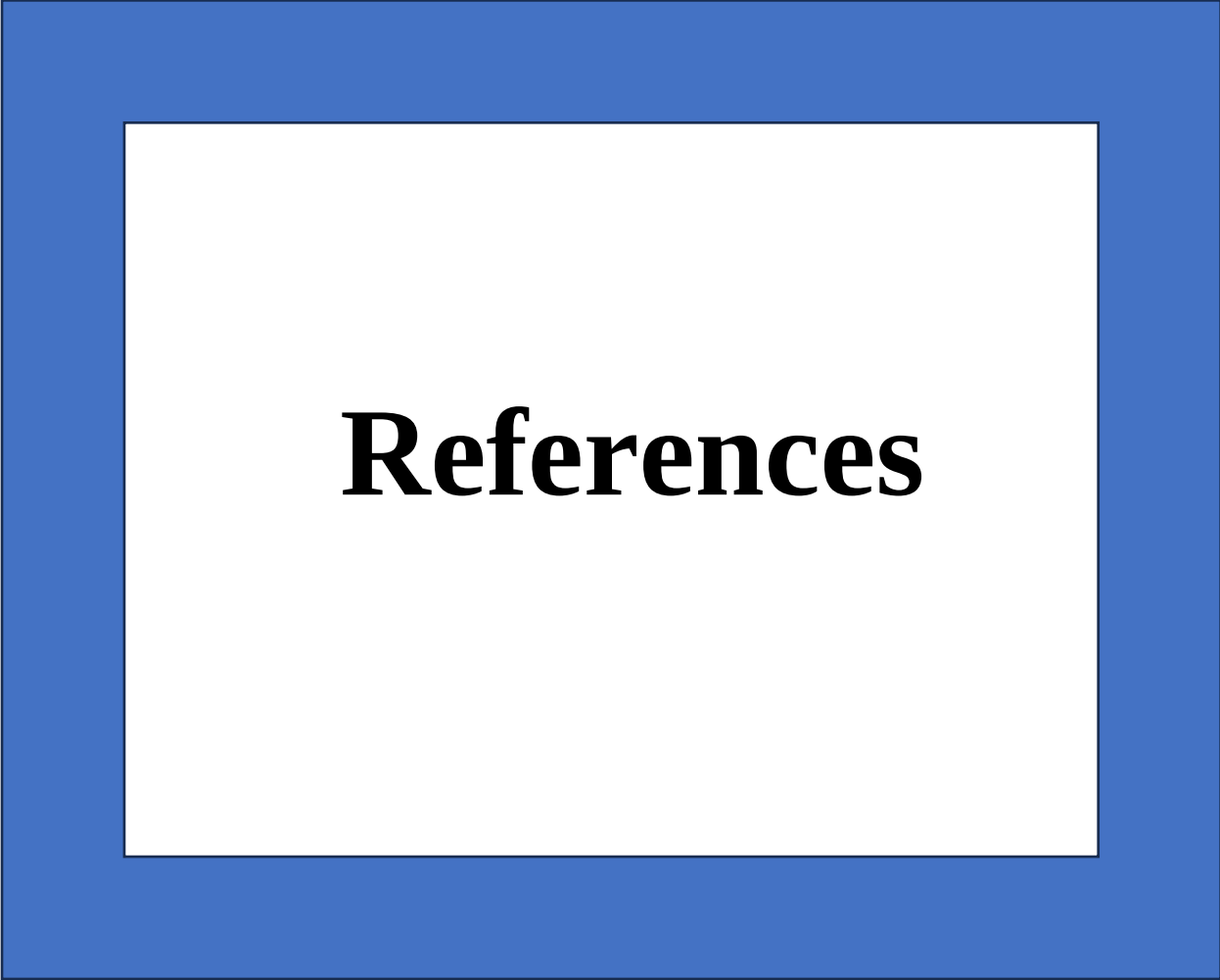
5.2 Recommendations

In light of the findings of the current study, which demonstrated the efficiency of aquatic plants in removing lead and cadmium from polluted water, the researcher recommends the following:

1. Expanding the application of phytoremediation techniques in rivers, streams, drains, marshes, and artificial ponds in Iraq is crucial due to these techniques' advantages: low operating costs, ease of application, reduced energy consumption, and environmental friendliness compared to traditional chemical or physical treatment methods
2. Adopting Water Hyacinth (*Eichhornia crassipes*) as a suitable option for phytoremediation of lead- and cadmium-contaminated water, particularly under conditions similar to those investigated in this study, due to its higher removal efficiency and greater metal accumulation in plant biomass under the experimental conditions. *Azolla* (*Azolla pinnata*) may also be considered for treating water with low to moderate heavy-metal concentrations or integrated with other aquatic plants in combined treatment systems to potentially improve pollutant removal efficiency and treatment stability. Further studies are recommended to evaluate these applications under different environmental conditions and at larger scales.

3. Conducting long-term studies to evaluate the efficiency of *Eichhornia crassipes* and *Azolla* over several-month operating periods is essential. This aims to determine the optimal operational lifespan of the plants and monitor physiological changes that may affect heavy metal removal efficiency over time.
4. Future studies should be expanded to include other heavy metals such as nickel (Ni), chromium (Cr), copper (Cu), zinc (Zn), mercury (Hg), and arsenic (As) to assess the efficiency of aquatic plants in treating mixed metallic pollutants under local environmental conditions.
5. Conduct advanced physiological and molecular studies to understand the mechanisms of biosorption, internal transport, accumulation, and detoxification within plants, focusing on the role of proteins, chelating compounds, and enzymes associated with resistance to metal stress.
6. Study the impact of various environmental factors, such as temperature, light intensity, pH, electrical conductivity (EC), and dissolved oxygen, on the efficiency of heavy metal removal, in order to determine the optimal operating conditions for phytoremediation systems.
7. Ensure the safe management of plant biomass after treatment, given its high concentrations of heavy metals, by implementing safe disposal or recycling methods that prevent the re-release of pollutants into the environment.
8. Conduct comparative studies between native and introduced aquatic plants to identify the most efficient and tolerant species to Iraqi environmental conditions, thus contributing to the selection of the most suitable plants for field applications.
9. Conduct semi-industrial and large-scale field trials to verify the applicability of the study results in water treatment plants, drainage networks, and polluted water bodies, and to evaluate plant efficiency under actual operating conditions.

- 10.** Utilize the study's findings to develop national programs for treating water contaminated with heavy metals and encourage environmental institutions and government agencies to adopt phytoremediation techniques within water resource protection plans and to achieve sustainable environmental development.
- 11.** Develop hybrid phytoremediation systems that combine multiple aquatic plant species or phytoremediation with physical or chemical methods, aiming to increase pollutant removal efficiency and reduce treatment time.
- 12.** Conduct economic and environmental studies to evaluate the cost of implementing phytoremediation techniques compared to traditional treatment methods, while calculating the long-term environmental and economic benefits resulting from their use.
- 13.** Encourage collaboration between universities, environmental institutions, and executive bodies to conduct applied projects based on phytoremediation, thereby contributing to the translation of scientific research findings into practical applications and leveraging them to reduce water resource pollution with heavy metals.

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الخلاصة

الخلاصة

أُجريت هذه الدراسة لتقييم كفاءة نباتي *Azolla pinnata* و *Eichhornia crassipes* في إزالة الرصاص والكاديوم وتشبيتهما من المياه الملوثة تحت ظروف الحقل المفتوح في محافظة المثنى، جنوب العراق، خلال المدة من 16 كانون الثاني إلى 15 شباط 2026. كما هدفت الدراسة إلى بحث سلوك هذين العنصرين في الماء والأنسجة النباتية والرواسب، وتحليل علاقتهما ببعض المتغيرات الفيزيوكيميائية المختارة للمياه. اعتمد التصميم العشوائي الكامل (Completely Randomized Design, CRD) لتقييم قدرة النباتات المدروسة على إزالة الرصاص والكاديوم من الماء عند تراكيز 0 و 5 و 10 و 25 mg/L لكل عنصر، على مدى 30 يومًا. أظهرت النتائج أن درجة حرارة الماء تراوحت بين 14 و 17 °C قبل المعالجة، بمتوسط بلغ 15.4 °C، وبين 18 و 21 °C بعد المعالجة، بمتوسط بلغ 19.3 °C. بلغت قيم الأس الهيدروجيني (pH) في أحواض نبات *Eichhornia crassipes* المعاملة بالرصاص 7.200 و 7.340 و 7.380 قبل المعالجة، و 7.220 و 7.270 و 7.197 بعدها، عند تراكيز 5 و 10 و 25 mg/L، على التوالي. أما في معاملة الكاديوم، فقد بلغت قيم الأس الهيدروجيني المقابلة 7.423 و 7.460 و 7.510 قبل المعالجة، و 7.177 و 7.207 و 7.217 بعدها، على التوالي. وسجل نبات *Azolla pinnata* قيم أس هيدروجيني بلغت 7.120 و 7.240 و 7.280 قبل المعالجة، و 6.417 و 6.377 و 6.300 بعدها، تحت معاملة الرصاص عند تراكيز 5 و 10 و 25 mg/L، على التوالي. أما تحت معاملة الكاديوم، فقد بلغت القيم 7.430 و 7.470 و 7.537 قبل المعالجة، و 7.097 و 7.060 و 7.000 بعدها، على التوالي.

انخفضت التوصيلية الكهربائية (EC) في أحواض نبات *Eichhornia crassipes* المعاملة بالرصاص من 1.929 و 2.079 و 2.335 dS/m قبل المعالجة إلى 1.679 و 1.739 و 1.889 dS/m بعدها، عند تراكيز 5 و 10 و 25 mg/L، على التوالي. وفي معاملة الكاديوم، انخفضت التوصيلية الكهربائية من 1.969 و 2.146 و 2.415 إلى 1.759 و 1.879 و 2.059 dS/m، على التوالي. وفي أحواض نبات *Azolla pinnata*، انخفضت التوصيلية الكهربائية تحت معاملة الرصاص من 1.979 و 2.139 و 2.405 dS/m قبل المعالجة إلى 1.779 و 1.859 و 2.015 dS/m بعدها، عند تراكيز 5 و 10 و 25 mg/L، على التوالي. أما تحت معاملة الكاديوم، فقد انخفضت من 2.029 و 2.215 و 2.505 إلى 1.849 و 1.979 و 2.205 dS/m، على التوالي. وقد يُعزى هذا الانخفاض إلى امتصاص النباتات للأيونات والمعادن الذائبة من الوسط المائي. حقق نبات *Eichhornia crassipes* كفاءات إزالة للرصاص بلغت 63.577% و 65.509% و 71.064% عند تراكيز 5 و 10 و 25 mg/L، على التوالي. وانخفض تركيز الرصاص في الماء من 4.810 و 9.820

و24.320 mg/L قبل المعالجة إلى 1.750 و3.390 و7.040 mg/L بعدها، عند التراكيز المقابلة. وفي الوقت نفسه، ارتفعت تراكيز الرصاص في الأنسجة النباتية من 0.0010 و0.0011 و0.0011 mg/kg قبل المعالجة إلى 1.340 و2.840 و7.630 mg/kg بعدها، على التوالي. وبلغ تراكم الرصاص في الرواسب 1.730 و3.080 و9.630 mg/kg عند تراكيز 5 و10 و25 mg/L ، على التوالي.

بلغت أعلى كفاءة لإزالة الكاديوم بواسطة نبات *Eichhornia crassipes* نسبة 65.443% عند تركيز 25 mg/L، في حين بلغت كفاءتا الإزالة عند تركيزي 5 و10 mg/L نسبة 56.197% و58.025%، على التوالي. وانخفض تركيز الكاديوم في الماء من 4.833 و9.713 و24.270 mg/L قبل المعالجة إلى 2.117 و4.077 و8.387 mg/L بعدها، عند تراكيز 5 و10 و25 mg/L ، على التوالي. وارتفعت تراكيز الكاديوم في الأنسجة النباتية من 0.0009 و0.0013 و0.0010 mg/kg قبل المعالجة إلى 1.573 و3.277 و9.483 mg/kg بعدها، على التوالي. وبلغ تراكم الكاديوم في الرواسب 1.087 و2.257 و6.287 mg/kg عند تراكيز المعالجة المقابلة.

حقق نبات *Azolla pinnata* كفاءات إزالة للرصاص بلغت 45.472% و47.812% و50.975% عند تراكيز 5 و10 و25 mg/L ، على التوالي. وانخفض تركيز الرصاص في الماء من 4.823 و9.613 و24.157 mg/L قبل المعالجة إلى 2.630 و5.017 و11.843 mg/L بعدها، عند التراكيز المقابلة. وارتفعت تراكيز الرصاص في الأنسجة النباتية من 0.0007 و0.0006 و0.0008 mg/kg قبل المعالجة إلى 1.133 و2.467 و5.183 mg/kg بعدها، على التوالي. وبلغت تراكيز الرصاص في الرواسب 0.990 و2.090 و6.483 mg/kg عند تراكيز 5 و10 و25 mg/L ، على التوالي.

أما بالنسبة إلى الكاديوم، فقد حقق نبات *Azolla pinnata* كفاءات إزالة بلغت 37.411% و42.799% و47.671% عند تراكيز 5 و10 و25 mg/L ، على التوالي. وانخفض تركيز الكاديوم في الماء من 4.750 و9.533 و24.157 mg/L قبل المعالجة إلى 2.973 و5.453 و12.503 mg/L بعدها، عند التراكيز المقابلة. وارتفعت تراكيز الكاديوم في الأنسجة النباتية من 0.0005 و0.0009 و0.0007 mg/kg قبل المعالجة إلى 1.657 و2.820 و6.760 mg/kg بعدها، على التوالي. أما تراكيز الكاديوم في الرواسب بعد المعالجة، فقد بلغت 1.055 و4.385 mg/kg عند تركيزي 10 و25 mg/L ، على التوالي.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
كلية العلوم/ جامعة القادسية
قسم البيئة

معالجة المياه الملوثة بالمعادن الثقيلة الكاديوم والرصاص باستخدام النباتات المائية عشبة النيل والازولا

رسالة

مقدمة إلى مجلس كلية العلوم في جامعة القادسية، وهي جزء من متطلبات
نيل درجة الماجستير في علوم البيئة

بواسطة

مريام طالب نابعي

بكالوريوس علوم البيئة

كلية العلوم 2023/جامعة القادسية

تحت إشراف

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