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# Comparative Analysis of Water Hyacinth Efficiency as Biosorbent and Phyto remediating Plant for Removal of Lead (Pb) Water Contaminants

**Nwagbara, Victor Uzoma**

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University of the Witwatersrand, Johannesburg

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# Comparative Analysis of Water Hyacinth Efficiency as Biosorbent and Phyto-remediating Plant for Removal of Lead (Pb) Water Contaminants

**By**

**Victor Uzoma Nwagbara**

**A research report submitted to the Faculty of Science in partial fulfilment of the requirement for the degree of Master of Animal, Plant and Environmental Sciences**

**School of Animal, Plant and Environmental Sciences (AP&ES)**

**Supervisor: Prof Solomon Newete**

**Co-supervisor: Prof Francesca Parrini**

**August 2023, Johannesburg**

**Declaration**

I, Victor Uzoma Nwagbara, hereby declare that the work contained in the dissertation, entitled "Comparative Analysis of Water Hyacinth Efficiency as Biosorbent and Phyto-remediating Plant for Removal of Lead Water Contaminants" is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.



30/08/2023

Signature: ..... Date: .....

**Abstract**

One of the major problems facing the modern society is the issues of water contamination. The ability of aquatic plants to serve as both heavy metal biomarkers and phytoremediators has been advocated for many years. The purpose of this study was therefore to compare the efficacy of water hyacith as a biosorbent and phytoremediating plant for removal of lead from contaminated waters. Dry and fresh water hyacinth biomass were exposed to Pb-contaminated water at different time intervals of 1, 7, 14 and 21 days. The results showed that with the increase of the exposure time, the Pb removal capacity of the plant biomass also increased. The Pb water concentration in day 1 and day 7 were significantly different from those in day 14 and day 21 which were not significantly different from each other. The average Pb removal from the Pb-treated waters for the different time exposure of 1, 7, 14 and 21 days in the fresh water hyacinth biomass were 40%, 56%, 78 % and 79%, respectively, the highest reduction being in the latter. The biomass of the freshwater hyacinth roots had the highest Pd concentration compared to the shoot. On The other hand the average Pb removal by the dry water hyacinth biomass were 78.9 %, 78.5%, 78.3 % and 78.3% for day 1, 7, 14 and 21 respectively, showing no significant difference between the different time series exposure. This suggests the instant adsorption of Pb by the dry plant material from the first day of exposure, after which the active sites for adsorption saturates to accommodate any further Pb ion uptake.

There were some plant stresses such as leaf chlorosis, and significant decrease in biomass weight and length of leaf-2 petiole in the lead-treated fresh water hyacinth plants compared to the controls (Pb-free water hyacinth plant). Thus, this study confirms that the largest removal by the dry water hyacinth biomass, occurred in the first day as opposed to the last day (day 21) by the freshwater hyacinth biomass.

**Keywords:** Biosorption Lead; Phytoremediation; Bioconcentration factor; Wastewater; Heavy metals.

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**List of abbreviations and acronyms**

| | |
|---|---|
| AMD | Acid mine drainage |
| ANOVA | Analysis of variance |
| As | Arsenic |
| BCF | Bio-concentration factor |
| $^{o}C$ | Degree Celsius |
| Co | Cobalt |
| Cd | Cadmium |
| $C_e$ | Final metal concentration |
| $C_o$ | Initial concentration |
| Cr | Chromium |
| CP | Crude protein |
| Ctrl | Control |
| Cu | Copper |
| DO | Dissolved oxygen |
| DWHB | Dry water hyacinth biomass |
| EC | Electrical conductivity |
| Fe | Iron |
| Fig. | Figure |
| FGV | Final growth value |
| FFW | Final fresh weight |
| FWHB | Fresh water hyacinth biomass |
| FTIR | Fourier transform infrared spectroscopy |
| GWHPs | Growing water hyacinth plants |
| IFW | Initial fresh weight |
| IGV | Initial growth value |
| eq | Equation |
| HSAB | Hard and soft acids and bases |
| Hg | Mercury |
| Kg | Kilogram |
| L | Litre |
| LSD | Least significance difference |
| mg | Milligram |
| Mg | Magnesium |

| | |
|---|---|
| mm | millimetre |
| NCS | Namibian Custom Smelter |
| Ni | Nickel |
| NUST | Namibia University of Science and Technology |
| Pb | Lead |
| pH | Potential of hydrogen |
| % RE | Percentage removal |
| RG | Relative growth |
| SPSS | Statistical Package for the Social Sciences |
| SQG | Sediment Quality Guidelines |
| TF | Translocation factor |
| POX | Peroxidase |
| USEPA | United States Environmental Protection Agency |
| WHO | World Health Organisation |
| XRF | X-ray Fluorescence Spectrometry |
| Zn | Zinc |

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background to the study

Water crisis is a global challenge because of periodic droughts due to climate change, rapid population growth, and pollution from large discharges of untreated or partially treated wastewaters (Newete & Byrne, 2016). The accessible fresh water for human use is less than 1% of all the water body available on earth (Dinka, 2018). The growing water stress levels globally have pushed the edge of technologies to exploit unconventional water sources like underground and sea water through desalinisation, but at a high cost (Newete & Byrne, 2016). However, this valuable resource is threatened by anthropogenic activities like urbanisation, agriculture, and mining. For instance, the global water usage by the mining industries alone was reported to be between 6 and 8 billion cubic metres per year, between 2006 and 2009 (Gunson, 2013). Globally, 80% of untreated municipal wastewater and several amounts of heavy metals, harmful sludges, and untreated effluents are disposed into the aquatic ecosystem (Mateo-Sagasta & Burke, 2010). Agriculture consumes 70% of water globally, whereas agrochemical and organic matter from fertilizers and pesticides reach the aquatic ecosystem through leaching, runoff, and spray drift (Mateo-Sagasta & Burke, 2010).

Heavy metals occur naturally and because of anthropogenic activities in the environment, and they find their way into human bodies through the water we drink and the food we consume. The presence of heavy metal contaminants in the environment, even in trace amounts, is detrimental to human health (Jaishankar et al. 2014). For instance, Mg, Hg, Pb and As can affect the central nervous system, whereas, Hg, Pb, Cd, and Cu can cause kidney or liver illnesses and Ni, Cd, Cu, and Cr affect the skin, bones, and teeth (Tchounwou et al. 2012).

Efficient water treatment is thus necessary to ensure that the ecosystem provides goods and services on which life depends (Riveeros-Perez & Riveros, 2017). Therefore, it is imperative to effectively treat effluents before they are discharged into the ecosystem to ensure a habitable environment that is void of pollution, has good water quality, and quantity to reduce health hazards and protect the ecosystem and provide a good sanitary practice that will promote sustainable development.

Namibia being the driest nation south of the Sahara has been struggling with water shortages since the 1960s and as a response to this challenge, the country has been exploiting groundwater

and employing water reclamation plant facilities (Cashman et al. 2014). The protection of this natural resource is thus of utmost importance to Namibia. The average annual rainfall in Namibia varies from less than 50 mm along the coastal part of the country to 350 mm in the central interior and 700 mm in the Caprivi, which records the highest rainfall yearly (Dirkx et al. 2008). Domestic and industrial effluents are the most common source of water pollution that may affect groundwater quality if discharged untreated. Therefore, there is a need for regular monitoring of water and wastewater before such waters are discharged into the ecosystem. In a study conducted by Vellemu and Omoregie (2014), showed that there was 47.25 mg/kg of Pb in sediment samples collected around central Namibian coast waters. Similarly, Uugwanga and Kgabi (2020), found a mean concentration range of 19-103 mg/L Pb in sediment samples of a tailing dam at Oamites mine, also in Namibia. Sediments from the Namibian Custom Smelter (NCS) at Tsumeb, Namibia's largest mining city, contained 3020 mg/kg of Pb according to the study conducted by Mapani et al. (2014), classifying the site as polluted for metals of Pb based on the USEPA and WHO (2013) Sediment Quality Guidelines (SQG). Research by Abah et al. (2016) found that due to the significant quantity of surface runoff from agricultural operations and human settlement effluents which release different levels of wastewater, the Pb level in Lisikili River in the Zambezi area of Namibia is 0.158 mg/l, which also exceeds WHO's and USEPA's maximum guideline values for water.

Therefore, it is imperative to control the level of this heavy metal in aquatic ecosystems in Namibia using economical, effective, and environmentally friendly techniques. The most common conventional methods of treatment for effluents from industries (Kanamarlapudi et al. 2018), leave environmental footprints that may be hazardous and difficult to dispose of (Newete & Byrne, 2016). Bioremediation is considered the most effective and sustainable method of abating water pollution, which includes biosorption and phytoremediation. Therefore, the passive adsorption of toxic substances into charged anions on surfaces of dead, inactive, or biologically derived materials is known as biosorption (Derco & Vrana, 2018), while the absorption and sequestration of contaminants by plant tissues rendering contaminants harmless to the environment is known as phytoremediation (Parmar & Singh, 2015).

Water hyacinth, *Eichhornia crassipes* (Mart), is one of the most infamous invasive weeds on earth (Newete et al. 2016). Its rapid growth and biomass turn over as well as its extensive root surface area, however, make it one of the most suitable aquatic plants for phytoremediation of

contaminated waters, but only if the weed is already available in the target water system (Newete & Byrne, 2016). The plant provides a high binding ability for the Pb (II) ion because it contains higher polyfunctional metal-binding sites; for both cationic and anionic metal complexes than any other aquatic weeds (Gakwavu et al. 2012).The effectiveness of water hyacinth as a biosorbent and phytoremediation will be evaluated at different time exposure in Pb treated water. This is to ensure the optimal time when the active site of the biosorbent is exhausted by Pb and when the sequestration of Pb by the active living plant is reached.

## 1.2. AIM AND OBJECTIVES

The main aim of this study was to evaluate and compare the efficiency of water hyacinth as a biosorbent using dead biomass material of water hyacinth (DWHB) and phytoremediation using actively growing water hyacinth plants (GWHPs) for Pb(II) ion removal from contaminated waters.

**The main objectives of the study are to**:

1. determine the efficiency of freshwater hyacinth biomass in phytoremediation when exposed to different time series intervals of 1, 7, 14 and 21 days of exposure to Pb-contaminated water.
2. determine the efficiency of dry water hyacinth biomass in biosorption when exposed to different time series intervals of 1, 7, 14 and 21 days to Pb contaminated water.
3. compare the efficiency of water hyacinth biosorption and phytoremediation of Pb from contaminated waters.

## 1.3 Structure of dissertation

The study is made up of five chapters. The study overview, problem statements, rationale, research questions, purpose, objectives and literature review are presented in Chapter one. The materials and procedures are presented in Chapter two; the results are presented in Chapter three and the discussion in chapter four. Chapter five is the summary of the findings and recommendations.

## 1.4 LITERATURE REVIEW

### 1.4.1 Heavy metals

Heavy metals, also known as trace elements, are elements whose specific density exceeds 5 $g/cm^3$ (Tchounwou et al. 2012a). Its physical properties such as softness, malleability, ductility,

poor conductivity, and corrosion resistance make it valuable for various industrial uses (Malar et al. 2014). Although, Pb is the most toxic heavy metal in the environment, it is an important metal with wide global distribution used in the manufacturing of lead-acid batteries, weapons, alloys, and equipment, to shield X-rays, despite its toxicity (Ali & Nas, 2018).

They are found naturally in the environment, and they enter people's bodies when drinking contaminated water and food. Heavy metals like Cu, Se, and Zn are micro-nutrients that are essential for human metabolic functions, but when these metals exceed their safe threshold in the human body, they can be harmful (Dowlatshahi et al. 2014), leading to organ impairments (Tchounwou et al. 2012).

Normally, natural Pb concentrations in surface soils are typically less than 50 mg/L and due to its non-biodegradable nature, the risk of lead poisoning through the food chain increases if soil lead levels reach above 300 mg/L (Al-Akeel, 2018, Kumar et al. 2020). A study conducted by Ali and Nas (2018) showed that there was increased Pb concentration compared to other heavy metals like Cd, Cr and Hg in 40% of the 115 participants tested for heavy metals bioaccumulation. The lead toxicity in the participants was due to ingestion of fruits and vegetables grown in Pb-contaminated soils. Ayurvedic herbs such as Ashwagandha, Boswellia, Triphala, and Brahmi Cumin, which have phytomedicinal nutrients were found to have traces of Pb concentrations with high level of risk to human health because of the Pb contaminated environment they were planted (Aggarwal et al. 2011). Pb toxicity in plants has a detrimental impact on several metabolic activities in many cellular components, which include growth reduction, dryness of roots and shoots, disruption of mineral nutrition, cell division reduction, and photosynthesis inhibition (Sharma & Dubey, 2005; Malar et al. 2014).

### 1.4.2 Heavy metals in water

Water is essential for social development and economic activities but the guarantee of this valuable natural resource for human satisfaction is of great challenge because of the unprecedented pollution level accrued daily. Thus, there is a need of sufficient information on the nature and source of the heavy metals and their composition in different lithological components of the ecosystems for an effective water management intervention (Tchounwou et al. 2012). The two sources of heavy metal pollution in the ecosystems, are natural geological withering including volcanic eruptions and anthropogenic activities (Awa & Hadibarata, 2020). The latter includes mining, agriculture, tanneries, metal plating, batteries' smelting, and

automobiles (Tchounwou et al. 2012). Most heavy metals are persistent due to their non-biodegradability in the environment (Jafari, 2010). The most common pollutants are Cr, Mn, Ni, Cu, Zn, Cd, and Pb (Ali et al. 2019; Zhou et al. 2015). They contaminate water sources by leaching or percolating into groundwater, or by runoff into rivers and lakes. They are ingested by living organisms and bio-accumulate in living tissues and cause carcinogenic effects in humans. Amongst such metal pollutant of great concern includes Cd, Cu, Cr, Ni, Co, Zn, Hg, and Pb (Fu & Wang, 2011).

The wide use of (Pb) pipes for wine distribution in ceramics that has been laced with Pb had in the past caused a decrease in fertility and an increase in psychosis among the Roman aristocracy (Delile et al. 2017).

### 1.4.3 Methods of wastewater treatment

Most wastewater treatment methods are costly and labour-intensive (Aniyikaiye et al. 2019). Chemical precipitation and ion exchange are among the most common conventional methods used for removal of heavy metals from contaminated waters (Barakat, 2011). The limitation of these processes are, however, their exorbitant cost, and the residual traces of contaminants they leave behind limit their use and efficiency (Igiri et al. 2018) as shown in Table 1.1. Thus, bioremediation is considered as more feasible, efficient, cost-effective, and safer option for removal of heavy metals even at trace concentrations (Dhankharand and Hooda, 2011; Demirbas, 2008). The techniques involve the use of fresh or dead plant biomass depending on the nature of the environmental (soil, water and air) contamination. By carefully selecting the appropriate plant for the type and concentration of contaminants as well as the characteristics of the polluted site, it is possible to achieve an acceptable abatement of the contaminants with relatively lower cost. Efficiency of plethora of aquatic micro and macrophytes in phytoremediation of heavy metal-contaminated waters and other organic and inorganic pollutants have been proved by many studies. Additionally, a high biomass output, rapid growth and harvestable plant shoots, as well as a strong ability to absorb and accumulate contaminants are pre-requirement for effective phytoremediation (Newete & Byrne, 2016; Basile et al. 2012; Chamier et al. 2012). Similarly, biosorption involves the rapid and reversible binding of ions from aqueous solutions onto functional groups that are present on the surface of biomass (Michalak et al. 2013). Biosorption is different from bioaccumulation because the latter relies on active metabolic transport as opposed to biosorption which is dependent on the affinity between the biosorbent and the sorbate (Volesky, 2007).

**Table 1.1:** Benefits and drawbacks of the conventional techniques used for remediation effluents.

| Procedure | Benefits | Drawbacks | Reference |
|---|---|---|---|
| Rainfall and purification | Inexpensive | High concentration becomes strenuous for separation, thereby producing sludge | (Englande et al. 2015) |
| Chemical oxidation and reduction | Enhances metal precipitation and retrieval of metals | Chemicals are needed and used in high concentrations of pollutants. It is very costly | (Tünay, 2010) |
| Biological oxidation and reduction | Inexpensive | The biological process is very poor, and it is affected by weather conditions | (Lynd et al. 2022) |
| Reverse osmosis | Produces pure effluent which can be recycled | Pressurised system and very costly | (Scholes et al. 2021) |
| Ion exchange | Effective and enhances metal recovery | Reacts to the presence of particles and requires expensive resins | (Czupryński et al. 2022) |
| Evaporation | Produces clean effluent. | Requires a source of energy, is quite expensive, and generates sludge | (Snyder & Wyant, 2002) |

### 1.5 Mechanisms of heavy metal biosorption

Among aquatic species, water hyacinth (*Eichhornia crassipes*) has shown a great capacity for removal of organic and inorganic contaminants from water, including its dry material though absorption. It is, therefore, important to understand the functional groups responsible for the sorption process. The metal removal occurs in the active groups of the biosorbent by the affinity between the biosorbent and biosorbate (Crini et al. 2018). Optimum biosorption occurs in temperatures between 20 to 35 ℃, but above 45 ℃ the active groups in the proteins of the biosorbent gets compromised and reduces the adsorption of the heavy metals (Shamim, 2018). The mechanism of metal biosorption involves phase separation of two distinct phases between the charged biomass with metal ions and water free of contaminants. The reuse of biosorbents and metal recovery are two important processes that could be employed to manage the

"contaminated" biomass (Kanamarlapudi et al. 2018). This is a useful strategy since the biomass can be used again to remove metal ions from other polluted effluents (Barakat, 2011).

Complex processes are involved in the sequestration of metals, and some of these mechanisms operate concurrently to various degrees depending on the biomass, the metal ions, and the solution environment (Razzak et al. 2022). Ion exchange is an important process in biosorption (Davis et al. 2003). In the initial stage of biosorption, the biosorbent is kept in an aqueous suspension that contains the biosorbate (metal ions) (Demirbas, 2008). Equilibrium is attained following the exposure time it will take the adsorbent to adhere to the adsorbate. At this point, the biosorbate that the biosorbent has captured is separated (Chojnacka, 2010). The biosorption mechanisms may be classified by the different criteria discussed below.

### 1.6 Types of adsorption mechanism

#### 1.6.1 Physical adsorption

In physical adsorption the intermolecular interactions between the molecules of the biosorbent and the biosorbate cause the adsorbent process to trigger. Physical adsorption is reversible in nature because of the weak binding force between adsorbate and adsorbent and the low heat involved (Chojnacka, 2009). Reversibility of this phenomenon enables the recovery of the adsorbate and biosorbent for reuse. A study conducted by Volesky (2007), indicates that in the chitin structure of the cell wall, the physical adsorption technique provided the basis for thorium and uranium adsorption using the fungal biomass of Rhizzopus arrhizzus. Through electrostatic interaction between ions in water and the cell walls of the biosorbent, uranium and thorium were absorbed by the dead biomass of yeast, fungus, and algae.

#### 1.6.2 Chemisorption

Chemisorption is the residual effect of a chemical reaction between the sorbent and the sorbate which is very relevant in catalysis (Webb, 2003). Compared to physical adsorption, the adhesive force and heat released are significantly higher. Often, the process is irreversible. While certain chemical interactions only exhibit physical adsorption at low temperatures, others exhibit chemisorption at high temperatures. Sometimes, both types of adsorptions may take place at the same time (Demirbas, 2008).

### 1.6.3 Metal transportation across the cell membrane

The process of metal transportation across the cell membrane of microorganism such as bacteria, fungi and algae biosorbent involves cell metabolism, and it takes place in the cell walls of the biosorbent. The process may be mediated by the transport of heavy metals via the microbial cell membrane that conveys essential ions such as K, Mg, and Na (Igiri et al. 2018). This mechanism often takes place on the cell surface of the biosorbent that is linked to the metabolic activity because of distinguishing features like chitin, lipids, polyphosphate (Violante et al. 2010). The metabolic activity follows two steps: firstly the metabolism process that independently binds to the cell walls of the fungal spores on root surfaces and the metabolism process that depends on intracellular uptake where the metal ions are conveyed through the cell membrane into the cell (Alberts et al. 2002), Additional metabolism may occur in which some of the metal ions are entrapped in the form of an insoluble micro-deposit that enhances metal ions biosorption by the biomass cell wall (Volesky, 2007).

### 1.6.4 Ion exchange

The heavy metal biosorption process essentially involves ion exchange between the adsorbate and adsorbent. Ion exchange takes place either by anionic or cationic mechanism (Kuyucak & Volesky, 1989). An adsorbent is capable of exchanging both cations and anions from an adsorbate and releases counter-ions of similar charge in a chemically equivalent amount (Davis et al. 2003). Electrostatic or London-van der Waals forces are the ion exchange techniques of chemical binding used in adhering Pb on the active sites of the dry water hyacinth biomass (Tran et al. 2019; Wang et al. 2022) (Fig. 1.1).

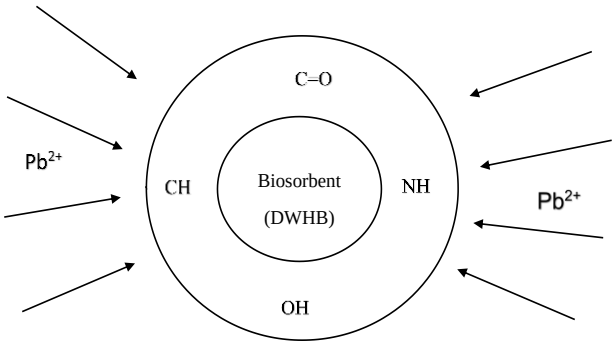



**Figure 1.1:** Illustrative representation of an ion-exchange mechanism for dry water hyacinth biomass and Lead ($Pb^{2+}$).

### 1.6.5 Complexation

It is described as the association of two or more species that results in the formation of a complex (Kanamarlapudi et al. 2018). The process of complexation involves the removal of biosorbate from water through the formation of complexes on the biosorbent's cell surface due to the interactions between the cell surface and biosorbate (Varma et al. 2004). Based on the hard and soft acids and bases (HSAB) theory, the affinities of interactions between metal ions and organic molecules (sulfur, nitrogen) on the surface of the biosorbent produce a complex of higher stability than the biosorbate (Ali Redha, 2020). This implies that metals binding on the surface of the active site of the biosorbent are regarded as the hard or soft acids while the polyfunctional groups of the active site of the biosorbent are regarded as the hard or soft bases. In this study, Pb which is the adsorbate is the soft acid that binds covalently to organic ligands (nitrogen or sulphur) of the biosorbent which is the soft base.

### 1.6.6 Precipitation

The process of precipitation occurs simultaneously on the cell surface of the biosorbent and water (Ali Redha, 2020). It is also dependent on the cellular metabolism in the presence of the biosorbate and the absorbent. However, when cellular metabolism is not a factor, precipitation may occur from the chemical reaction between the biosorbate and the biosorbent's cell surface (Kuyucak & Volesky, 1989). This reaction is also referred to as the redox reaction. For instance, using the husk from a green tomatoes, Fe (III) ions can be removed from an aqueous solution using only precipitation and ion exchange (Ali Redha, 2020).

Both physical and biological mechanisms are involved in the ion uptake by aquatic plants (Win, 2003). Some of the physical processes include ion exchange, complex formation, osmosis, diffusion, and others. Cations enter plant cells through diffusion. According to Win (2003), cation uptake is hindered by osmosis, which invariably aids the movement of water from the plant or vice-versa. Since osmosis and diffusion occur through the cell wall, the permeability of the cell membrane had a significant impact on the Pb absorption by the freshwater hyacinth biomass. Ion exchange modifies the rates of absorption by aquatic plants and the formation of large complex ions may impact the assimilation of $Pb^{2+}$ ions from getting into the plant cell. However, biological processes may promote or impede the movement of $Pb^{2+}$, depending on the degree of assimilation of $Pb^{2+}$ ions that the plant requires. Because of these two kinds of opposing processes, there was a quick rising of initial traces of $Pb^{2+}$ in the plant indicating the dominance of uptake mechanisms. However, towards the latter day of the trial experiment (day

21), the plant cells became saturated, thereby impeding the continuous absorption of $Pb^{2+}$ by the plant cells. This process is similar to a study conducted by Win (2003) on the absorption of Pb by fresh water hyacinth. Therefore, the number of exposure days affects how much Pb accumulates in the water hyacinth plants.

### 1.7 Morphological, biological and physiological properties of water hyacinth

Water hyacinth is originally from the Amazon Basin in South America, specifically Brazil, and was introduced to many parts of the world as an ornamental plant (Dagno et al. 2012). It is a perennial, aquatic plant from the family Pontederiaceae. The average height of water hyacainth is about 40 cm, but may grow as tall as 1 m under exceptional conditions. They have attractive lavender flowers, round, glossy, dark green leaves, and occasionally inflated, spongy stalks (Van Driesche et al. 2010). It grows well at optimal pH values from 4 to 10, optimal temperatures between 21-30 ℃ and low air humidity from 15 - 40 % (Van Driesche et al. 2010). The plant grows favourably in nutrient-enriched waters, and can also adapt to a low-nutrient aquatic system. Water hyacinth is sensitive to salinity, thus it does not grow in coastal areas (Jafari, 2010).

Water hyacinth is a noxious weed because of its rapid multiplication and invasion of water systems (Tegene & Ayele, 2014). It is found in lakes, rivers, dams, reservoirs, irrigation and drainage structures in temperate, tropical and sub-tropical climates (Mujere, 2015). Water hyacinth infestation in Namibia was first recorded in the 1960s in the Kafue River of the lower Zambezi Basin (Kurugundla et al. 2016). Its invasion compromises of a healthy aquatic ecosystem by depleting oxygen, forming dense mats of biomass that reduces light to submerged vegetation and endangers the survival of living organisms in the system (Chamier et al. 2012).

Despite its invasive characteristics, water hyacinth is considered a potential plant for phytoremediation because of its dense mats and rapid biomass production and sorption ability for heavy metals (Okunowo & Ogunkanmi, 2010). Studies have shown that water hyacinth roots have a strong affinity for aqueous heavy metals in solution with sorption performances that depend on the metal species, concentration and aqueous chemistry (Gupta & Balomajumder, 2015). For example, a study by Newete et al. (2016), showed that the roots of water hyacinths accumulated over 80% of eight different heavy metals from polluted waters. The water hyacinth adopts a tolerance strategy of coping with the toxicity of the accumulated

metal ions by storing them at the root cell walls and intracellular spaces as a result of the absorption of heavy metals into its cytosol (Yan et al. 2020).

### 1.8 Chemical components of fresh and dry water hyacainth

The freshwater hyacinth has 95.5% water content, 3.5% of organic compound, while nitrogen, ash, phosphorus pentoxide and other substances represent 0.04, 1.0, 0.06 and 0.20%, respectively (Jafari, 2010a). Nitrogen, organic matter, ash, potassium oxide, sodium oxide, calcium oxide, chlorine and phosphorous pentoxide in dry water hyacinth are 1.5, 75.8, 24.2, 28.7, 1.8, 12.8, 21, and 7%, respectively (Jafari, 2010). However, methionine, phenylalanine, threonine, lysine, isoleucine, valine, and leucine in crude protein (CP) of dried water hyacinths are 0.72, 4.72, 4.32, 5.34, 4.32, 0.27 and 7.2 g respectively (Muhammed et al. 2021). The special chemical components of fresh and dry water hyacinth enable them to adsorb metals like Pb, emerging pollutants, organic and inorganic molecules that are carcinogenic from the aqueous meedium (Jafari, 2010).

### 1.9 Pathways of heavy metals uptake

Heavy metal mobilisation, root uptake, xylem loading, root-to-shoot transport, cellular compartmentation, and sequestration are few of the techniques used by plants to absorb heavy metals (Pasricha et al. 2021). However, heavy metals mainly exist in an insoluble form in the water which is not bioavailable to plants. Therefore, plants can increase their bioavailability by releasing a range of root exudates which can alter the pH of the rhizosphere and increase heavy metal solubility (Yan et al. 2020).

The two channels water and solutes can reach all the parts of the plants through the root system are apoplast and symplast. Cell walls, intercellular space, and xylem make up the apoplast, while the fluid component that facilitates the osmotic passage of solutes such as heavy metals (Pb) into and out of the cell wall is called cytosol, and it is found in the symplast, also known as the cell cytoplasm, which is made up of a chain of cytoplasts. The apoplastic and Symplast channels' main function is to deliver water and other solutes such as Pb to the xylem for later delivery to the plant's shoot system. Therefore, these are the channels by which plant roots absorb heavy metals (Siciliano and Germida, 1998).

Water contaminants are absorbed by the roots, through the carboxyl groups present in the cell membrane of the root systems (Gakwavu et al. 2012). The absorbed contaminants, water and minerals are transported into the root hair by osmosis, then pass through the root hair cortical cells, either along cell walls or into the cytoplasm of each cell, until they reach the central stele which contains the xylem and phloem. The suspended solids and bacteria are not only trapped by the fibrous and feathery roots but also offer anchor sites for microbial and fungi growth. The pollutants get biosorbed to the root surface via the bacteria such as *herbaspirillum* and *enterobacter* present at the roots as a result of an ionic imbalance in the cell membrane (Legorreta-Castañeda et al. 2020).

Plants also absorb small amounts of pollutants through foliar absorption in addition to root absorption. The actual conditions that influence foliar absorption of heavy metals by water hyacinth are the physical structure, polarity, and hydrophobicity (Fernandez & Brown, 2013). Most aerial plants have an extracellular layer called a cuticle that protects the epidermal cells and serves as a barrier between the environment and plant organs. The cuticle has specialised stomata cells which facilitate foliar nutrient (heavy metals) uptake in water hyacint (Fernandez & Brown, 2013). Heavy metals infiltrate through the stomata and go through the diffusion track of the pore walls since the stomata cells are less selective than the rest of the cuticle (Shahid et al. 2017).

# CHAPTER TWO

# METHODS AND MATERIALS

The potted water hyacinth trials were conducted at the Water Research Laboratory of the Namibia University of Science and Technology (NUST) in Windhoek, Namibia. All the chemicals and reagents used were of analytical grade purchased from Medlab (Windhoek, Namibia). All glassware used were thoroughly washed with a detergent, rinsed with water and soaked overnight with 1 % $HNO_3$, then rinsed with distilled water and autoclaved for 20 minutes before use.

## 2. Experimental design

### 2.1. Phytoremediation trial using freshwater hyacinth plants (FWHPs)

The experiment was conducted over a three-week period during summer in January 2022, when the water hyacinth was in the midst of its active growing phase. Only short, green, and healthy water hyacinth plants were collected from Liambezi Lake in the Zambezi region of Namibia and transferred to the Water Research Lab at NUST, Windhoek for the laboratory trial. Six young water hyacinth plants of same size with an average weight of 1.2 kg per tub were washed thoroughly using tap water to clean all associated debris. The plants were then allowed to grow and acclimatize for two weeks in water modified with half strength (500 mL) of 1 L of Hoagland's solution, before the actual exposure of the plants to the Pb treatments in tubs of 10 L in volume. Hoagland solution provides all essential nutrients to support normal growth of a plant (Li & Cheng, 2014).

### 2. 2 Preparation of Hoagland's solution

The Hoagland solution composition was prepared based on Kaur et al. (2016). The following standard prepared solutions were mixed with distilled water to make 1L of Hoagland's solution as shown in table 2.1.

**Table 2.1:** Hoagland solution composition

| Vol. (ml) | Compound's name | Conc. of the stock solution |
|---|---|---|
| 1 | Magnesium sulphate hepta hydrate ($MgSO_4.7H_2O$) | 2446.5 g/L |
| 1 | Mono potassium phosphate ($KH_2PO_4$) | 136.1 g/L |
| 1 | Potassium nitrate ($KNO_3$) | 101.1 g/L |
| 1 | Calcium nitrate tetra hydrate ($Ca(NO_3)_4. H_2O$ | 236.1 g/L |
| 1 | Zinc sulphate hepta hydrate ($ZnSO_4.7H_2O$) | 0.2 g/L |
| 1 | Copper sulphate penta hydrate ($CuSO_4.5H_2O$) | 0.1 g/L |
| 1 | Sodium molibdate ($NaMoO_4$) | 0.025 g/L |
| 1 | Boric acid ($H_3BO_3$) | 2.8 g/L |
| 1 | Manganese chloride tetra hydrate ($MnCl_2.4H_2O$) | 2.8 g/L |
| 1 | Ethylene dia amine tetra acetic acid $[CH_2N(CH_2CO_2H)_2]_2$ | 10.4 g/L |
| 1 | Potassium hydroxide (KOH) | 7.8 g/L |
| 1 | Iron sulphate hepta hydrate ($FeSO_4.7H_2O$) | 56.1 g/L |

**2. 3 Experimental Design**

A total of 1.615 g of 99% $Pb(NO_3)_2$ were dissolved in deionized water to prepare a stock solution of 1000 mg/L $Pb(NO_3)_2$. Half strength (500 mL) from 1 L of Hoagland's solution, which provides nutrients to FWHB was added to each tub using a plastic syringe by carefully lifting the plants out of the water to limit immediate absorption by some roots of the fresh plants before the mixture was homogenised using a glass rod.

The equation 2.2, was used to prepare the solution that was used for this trial as described below:

$C_1V_1 = C_2V_2$.

Therefore,

$1000 \times V_1 = 4 \times 1000$

$V_1 = 4$ mL

Therefore, 40 ml of $Pb(NO_3)_2$ solution was added to to the 10 L tubs to get a Pb concentration of 4mg/L(Fig.2.1).

The Pb-treated plants were positioned in completely random quartet rows of sextuplicate of a total of 24 tubs for each of the treatments and sextuplicate of a total of 24 tubs for each of the control experiments, making a total of 48 tubs for the FWHB trial.

The tubs were positioned at random in order to take into account, the differences in light exposure direction and other environmental factors (humidity, temperature, sunlight, air movement, pressure and precipitation). Hoagland's solution was the only item added to the control; no Pb treatment was added. Prior to starting the experiment with the addition of Pb treatment, an X-ray Fluorescence (XRF) Spectrometer (Rigaku NEX CG EDXRF) instrument was used to evaluate the concentration of Pb. The outcomes demonstrated that the plants contained no detectable Pb.

The water hyacinth plants were exposed to the Pb solutions for 1 day, 7 days, 14 days, and 21 days in order to evaluate the optimal exposure (equilibrium time) prior to taking any samples to ascertain the amount of Pb removed by plants. Prior studies showed that water hyacinth could withstand concentrations of heavy metals like Pb, Hg, Cr, As, Zn, and Ni up to 5 mg/L before experiencing plant stress and symptomatic interveinal chlorosis (IVC), which denotes a decrease in chlorophyll biosynthesis and causes the plant to wilt. Therefore, the Pb concentration of 4 mg/L was chosen for this study (Fahruddin, 2020; Pereira et al. 2014).

The plants were watered at an interval of 3 – 4 days with tap water to make up for the loss of water due to evapotranspiration. The phytoremediation and biosorption experiment were conducted for a maximum of 21 days, beginning from day 1 when the 4 mg/L of Pb concentration was first administered into the tubs.



**Figure 2.1**: Pb treatment on freshwater hyacinth biomass.

## 2. 4 Preparation of water hyacinth samples for analysis

The freshwater hyacinths plants were taken out of each tub after being exposed to the treatment at various time-series intervals, this is to evaluate the equilibrium time of biosorption and phytoremediation of the biosobent and active living plant respectively. After which they were rinsed with running water to get rid of any undesired dust and debris. The exterior leaves (the petiole and lamina) for each plant sample were removed, keeping only the youngest three leaves of the water hyacinth rosette. This is due to the concentration of nutrients and Pb in the middle of the fresh burgeoning leaves of the water hyacinth plant (Malar et al. 2014). The water hyacinths shed their older leaves and develop new ones as they continue to grow because of Pb stress that causes leaf chlorosis and necrosis (Newete et al. 2016). One of the tolerance mechanisms to reduce metal phytotoxicity is the ability of the water hyacinth to absorb heavy metal better when its leaves are young and fresh than when they are older (Sharma et al. 2012, Tangahu et al. 2011). To avoid external contamination, plants were cut into root and shoot sections using a disposable knife. Plants were split into root and shoot samples with a plastic knife to avoid any external contamination and then washed thoroughly three times in deionised water and sun-dried for five days in an inert environment to remove plant water content and later oven-dried at a temperature of 40 ºC for 5 hrs. This process is to ensure that any residual moisture left is removed before pulverising with Essa LM2 Pulverising Mill into powder as shown in figure 2.2. The powder was later sent to the Water Research Lab at NUST for analysis.



**Figure 2.2**: Pulverising dry water hyacinth biomass with Essa LM2 Pulverising Mill.

### 2. 5 Characterisation of dry water hyacinth biomass (DWHB)

The DWHB was characterised by Fourier transform infrared (FTIR) spectroscopy for possible functional groups responsible for Pb uptake (Fig. 2.3). The strong and broad peaks of the dry water hyacinth biomass's FTIR spectra between 3000 $cm^{-1}$ and 3700 $cm^{-1}$ indicate the overlapping stretching vibrations of hydroxyl, phenols, amines, carboxylic acids, and aromatics. The N-H stretch of amines is 2959.52 $cm^{-1}$, while a strong and broad peak between 2690 and 2840 $cm^{-1}$ is attributed to aldehydes and ketones. The strong peaks at 1605.85 $cm^{-1}$ and 1385.20 $cm^{-1}$ were attributed to the N-H bend of primary amines and the S=O stretching of sulphate, respectively. The O-H band of carboxylic acids and C-Cl stretch of alkyl halides were attributed to the strong peak between 927.27 $cm^{-1}$ and 749.04 $cm^{-1}$ respectively (Dixit, 2015).

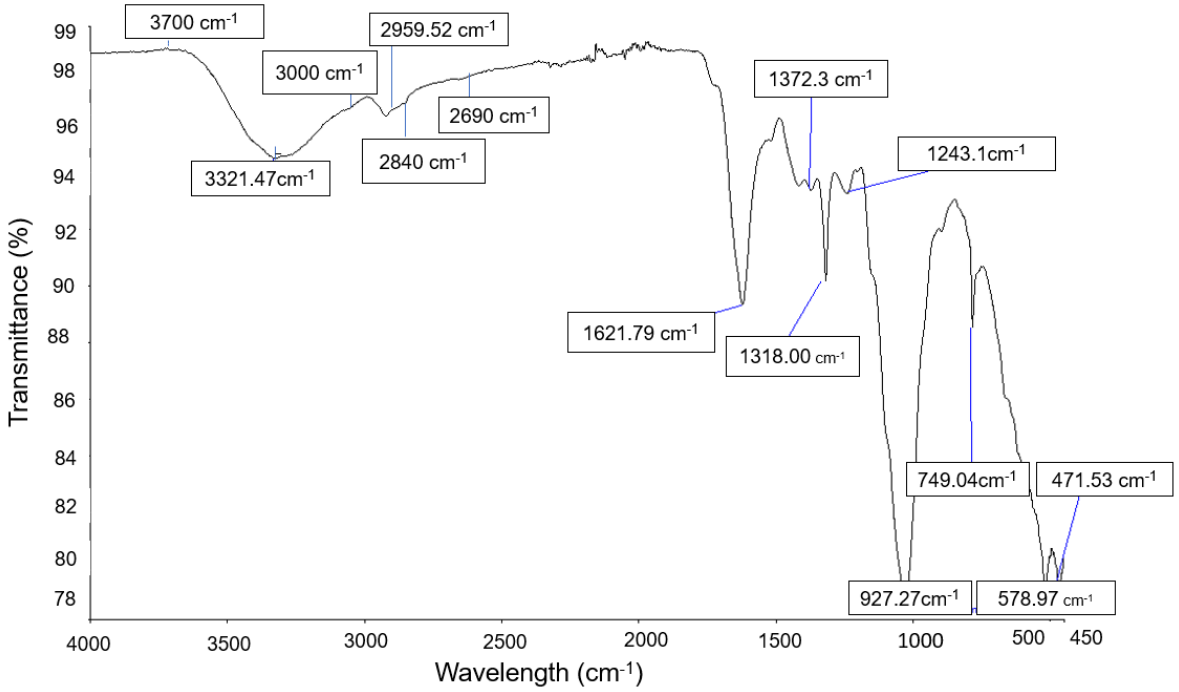



**Figure 2.3**: FTIR Spectra of DWHB showing the possible functional groups responsible for Pb uptake.

### 2. 6 Biosorption of Pb experiment with Dry Water Hyacinth Biomass (DWHB)

All the freshwater hyacinth plants were properly washed with tap and deionised water and dried in the sun for seven days for the biosorption of the Pb experiment. The dried and ground plant was sieved through 76-millimetre laboratory test sieves to standardize the plant material size and remove unwanted materials (Fig. 2.4a). The DWHB was stored in an airtight plastic at room temperature to reduce or avoid moisture gain before the biosorption experiment. Figure 2.4 below provides a schematic representation of the preparation of dead water hyacinths biomass"

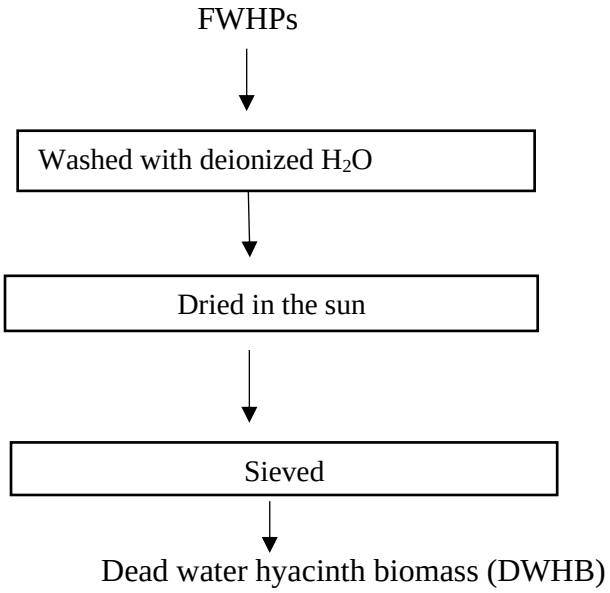



**Figure 2.4:** Schematic representation of the preparation of dead water hyacinths biomass.

The experiment was conducted in 30 tubs of 10 L volume each for four different time-series Pb exposure treatments and a control experiment with six replicates each. To take into account the surrounding environment (humidity, temperature, sunlight, air movement, pressure and precipitation), the tubs were arranged randomly in four different rows. The equation 2.2, was used to prepare the solution that was used for this trial as described below:

$$C_1V_1 = C_2V_2.$$

Where:

$C_1$ = Initial concentration of Pb

$V_1$ = Initial volume

$C_2$ = Final concentration of Pb

$V_2$ = Final volume

Therefore,

$$1000 \text{ x } V_1 = 4 \text{ X } 1000$$

$$V_1 = 4 \text{ mL}$$

Therefore, 4 ml of Pb solution was used in every 1L. However, 10 L tub was used for this experiment. Hence, for 10 L, 40 mL volume of Pb solution was added.

The Pb concentration of 4 mL was pipetted to get 40 mL volume of Pb solution, which was added to each tub containing 55 g of DWHB in 10 L water tub. The biosorbent material of 55 g of DWHB was used based on similar study conducted by Wolverton and McKow (1976).

Since dry water hyacinth biomass floats on water, it may not entirely be immersed for a complete Pb exposure of the surface for effective biosorption, the chopped dry water hyacinth materials were well mixed for 30 mins by using a glass rod to increase exposure to the Pb in the water. The biosorbent material was sampled for analysis at four different time series intervals of 1 day, 7 days, 14 days and 21 days. The sample was then taken to the lab for Pb analysis after filtration using Whatman filter paper of a 0.45 m pore size.




**Figure 2.5:** *(*a) Clean dry water hyacinth biomass, (b) Mixing of the Pb treated and control dry water hyacinth biomass.

**2. 7. Data collection from the FWHB (phytoremediation) and DWHB (biosorption) trials**

The height, weight, number of petioles, root length, and length of the petiole number two (Petiole-2) of all the water hyacinth plants were measured before and after the time series Pb exposure trials to avoid destruction or disturbance of the trail during any other time. To measure the rate of leaf production per plant (leaf turnover) over the course of the trial, the youngest petioles in the middle of each plant in each tub were marked at the start of the experiment, and the position on the plant was recorded every week as per the duration of each time series treatments (Newete et al. 2016). The number of ramets was counted from all the plants in each tub at the end of the experiment. The fresh weight of the growing young water hyacinth plants was evaluated at the beginning and last day of the trial.

Other parameters such as the electrical conductivity and dissolved oxygen and the initial pH of the treatment water were recorded immediately at the beginning of the trial, middle and at the end of the trial using HQ4100 (HACH) Portable Multi-Meter which was standardised with a buffer solution. Days of exposure were used as the determinant factor for the removal efficiency of Pb by water hyacinth plant as biosorbent and phytoremediating plant in Pb-contaminated waters. The Pb analysis from the aliquot of the phytoremediation and biosorption trial was done with an Optima 8000 inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer) while analysis of Pb in water hyacinth tissue was done with X-ray Fluorescence (XRF) Spectrometer (Rigaku NEX CG EDXRF).

The experiment was based on comparison of DWHB (biosorption) and FWHB (phytoremediation) in Pb removal from contaminated water at the same experimental conditions. Therefore, it would be difficult to vary temperature, pH, and contact time. Therefore, the experimental conditions for the two techniques were kept the same.

Percentage of Pb that was removed by the DWHB and FWHB from the Pb water contaminant was calculated using eq. 2.1

$$\textbf{\% Removal (\% R)} = \frac{C_o - C_e}{C_o} \times 100 \qquad (2.1)$$

Where:

Co indicates the initial metal concentration (Co) in the experimental trial solution.

Ce indicates the final metal concentration at the end of the metal in the trial solution and

The quantity of Pb sorbed by DWHB was reported as a percentage removal (% RE).

The volume of the Pb concentration that was used for the trial was measured using equation 2.2

$$C_1V_1 = C_2V_2.$$

Where:

$C_1$ = Initial concentration of Pb

$V_1$ = Initial volume

$C_2$ = Final concentration of Pb

$V_2$ = Final volume

## 2. 8 Bio-concentration factor

A measure of plant metal accumulation in relation to the grwoth substrate metal concentration is called the bio-concentration factor (BCF). According to Basile et al. (2012), macrophytes are considered to be good hyperaccumulators of heavy metals if they can accumulate 5000 mg /kg of dry plant biomass or if their BCF value is greater than 1000 mg/kg. The BCF is an important indicator in determining how efficient a plant can accumulate metals and metalloids (Zayed et al. 1998). The formula for the BCF is:

$$\mathbf{BCF = (P/E)_i} \qquad (2.3)$$

Where:

i indicates the relevant heavy metal, which is Pb.

The dimensionless bioconcentration factor is known as BCF.

P=concentration of trace elements in plant tissues (mg/L),

E= initial metal concentration in water (mg/L).

## 2. 9 Translocation factor (TF)

The translocation factor (TF) is the ability of the plant to transfer metal contaminants from the roots into the shoots. It is calculated as:

$$TF = \text{Pb concentration in shoot/Pb concentration in root} \qquad (2.4)$$

TF values < 1 indicate that the species has the potential for phytostabilization of water or soil, which is one of the characteristics of a Phytoremediating plant (Saraswat & Rai, 2009).

## 2. 10 Plant growth measurement

At the start of the experiment with the addition of the Pb treatment in each tub and at the end of the experiment (day 21), a number of plant growth parameters were measured, including the weight of all six plants in each tub, the lengths of the roots and Petiole-2 for three of the six plants in each tub. Six plants in each tub were marked with a celotape on the youngest petioles at the cneter of the rosette (petiole number 1), and the position of that leaf was recorded at the end of the experiment to determine the rate of leaf production per plant per week, and the number of ramets of each plant in a tub was counted. A ruler (with a precision of 0.1 cm) was used to measure the length of two leaves (reference leaves) from each treatment tub (total of 12 leaves) from the apex of the blade to the base of the petiole of the leaf, and the areea was measured at the widest point perpendicular to the longitudinal axis of the leaf at the beginning

and the end of the experiment. To assess the impacts of Pb concentrations on the growth of GWHPs, the relative plant growth was computed as:

$$\mathbf{RG = FFW/IFW} \quad (2.5)$$

Where:

RG connotes the relative growth (dimensionless) of water hyacinth plants over the course of the experiment. FFW connotes the water hyacinth plants' final fresh weight, which was measured in grams at the conclusion of each study period, and IFW connotes the water hyacinth plants' initial fresh weight, which was measured in grams before the experiment began.

Also, percentage growth of the plant was calculated using the formula below:

$$\mathbf{PG = [(FGV - IGV)/IGV] \times 100} \quad (2.6)$$

Where:

PG connotes percentage growth of the water hyacinth plant during the experiment. FGV connotes the water hyacinth plants' final growth value and IGV connotes the water hyacinth plants' initial growth value before the experiment.

### 2. 11 Data analysis

One-way analysis of variance (ANOVA) followed by the least significant difference (Fisher's LSD) test were used to determine the water hyacinth efficiency as a biosorbent and phytoremediating plant for the removal of Pb from water. A p-value of $< 0.05$, then the difference between the different treatments or parameters were considered significant. The statistical features of SPSS were used for the statistical analysis.

### 2. 12 Disposal of phyto-biomass and biosorbent

The removal of heavy metals from phyto-biomass can be done safely using several methods that have been reported in the literature. Composting, compaction, and pyrolysis are among the pre-treatment procedures used to reduce the biomass before incineration; ashing, liquid extraction, solarisation, gasification, and direct disposal of phytobiomass before final disposal. However, in the current study, all plant materials and water used were safely disposed by a registered waste management company in Namibia called “RENT A DRUM”.

# CHAPTER THREE

# RESULTS

The results of the analysis of water hyacinth's efficiency as a biosorbent and phytoremediating plant in the removal of lead from contaminated waters are explained in this section.

## 3.1 Pb Removal from Water by Freshwater Hyacinth Biomass (FWHB) vs. Dry Water Hyacinth Biomass (DWHB)

The lead concentration in water only started to significantly decline from day 14after the water exposure to FWHB ($F_{(3.20)}$ = 12,3293. p = 0.00009) (Table 3.1) ("F" is simply the test statistics and statistical results of test that is done in order to compare the two variables) . The Pb water concentration in day 1 and day 7 were significantly different from those in day 14 and day 21 which were not significantly different from each other. Nor, were the first two. The initial Pb water concentration of4 mg/l dropped by 40% in day 1, 56% in day 7, 78% and 79% in days 14 and 21, respectively in the water with FWHB treatment, the highest reduction being in the latter. The highest reduction of Pb concertation. This indicates. The Pb concentration in the water exposed to DWHB, however, showed no any significant difference between the different time of exposure ($F_{(3.20)}$ = 0,2011. p = 0.8944). (Table 3.1). Although, the control waters were not treated with Pb, there was a trace of Pb concentration of about 0.8 mg/kg.

**Table 3.1:** Effect of exposure time on the absorption by FWHB and adsorption by DWHB of Pb from standard solution. Similar letters in the same columns are statistically non-significant (p > 0.05; LSD test). Data are means (n = 6, ±SE).

| Time | FWHB (mg/l) | FWHB (% removal) | DWHB (mg/l) | DWHB (% removal) |
|---|---|---|---|---|
| 1 Day | 2.37 ±0.10 b | 40 | 0.83 ±0.18 | 78.9 |
| 7 Days | 1.77 ±0.23 b | 56 | 0.84 ± 0.16 | 78.5 |
| 14 Days | 0.87 ± 0.33 a | 78 | 0.87 ±0.13 | 78.3 |
| 21 Days | 0.84 ±0.84 a | 79 | 0.88±0.11 | 78.3 |

## 3.2 Pb accumulation in water hyacinth tissues and the BCF and TF of FWHB vs DWHB

The Pb concentration in the roots was significantly greater ($F_{(3.20)}$ = 46,965. p = 0.00000) than those in the shoots and increased significantly with the increase of exposure time to the

treatments (Table 3.2). The Pb sequestration in the roots of FWHB over the exposure period of day 1, day 7, day 14 and day 21 were 39.7 mg/kg, 2007 mg/kg, 2512 mg/kg and 2913 mg/kg, respectively. This accounts for 77%, 92%, 91%, and 90% or the total Pb concentration of the whole plant (roots and shoots), respectively. (Fig. 3.1). However, there was no Pb detected in the roots or shoots of the freshwater hyacinth in the control plants.

The Pb uptake by FWHB increased significantly ($p < 0.01$) with the increase of exposure time to the treatments (Fig. 3.1). The BCF was the lowest (12.9 mg/kg ±1.06) on day 1 and the highest on day 21 (807.3 mg/kg ± 170.50) followed by day 14 (690.2 mg/kg ±103.70) with increasing trend of Pb bioconcentration along the time series exposure gradient (Table 3.2). On the contrary, the highest TF of 30 % was recorded on day 1, and thereafter it dropped to 9 %, 10 % and 11% on day 7, 14 and 21, respectively (Table 3.2). The translocation factor from the roots to the shoots on days 1, 7, 14 and 21 were 0.31, 0.088, 0.10 and 0.11, respectively, indicating that the largest amount of the Pb removed by water hyacinth was retained in the roots.

Unlike in the FWHB, the Pb bioconcentration in DWHB showed no significant difference between the time series exposure treatments from day 1 to day 21, although the highest was observed on day 1 (846 ±84.14) followed by day 7 (845.42 ±125.28) (Table 3.2).

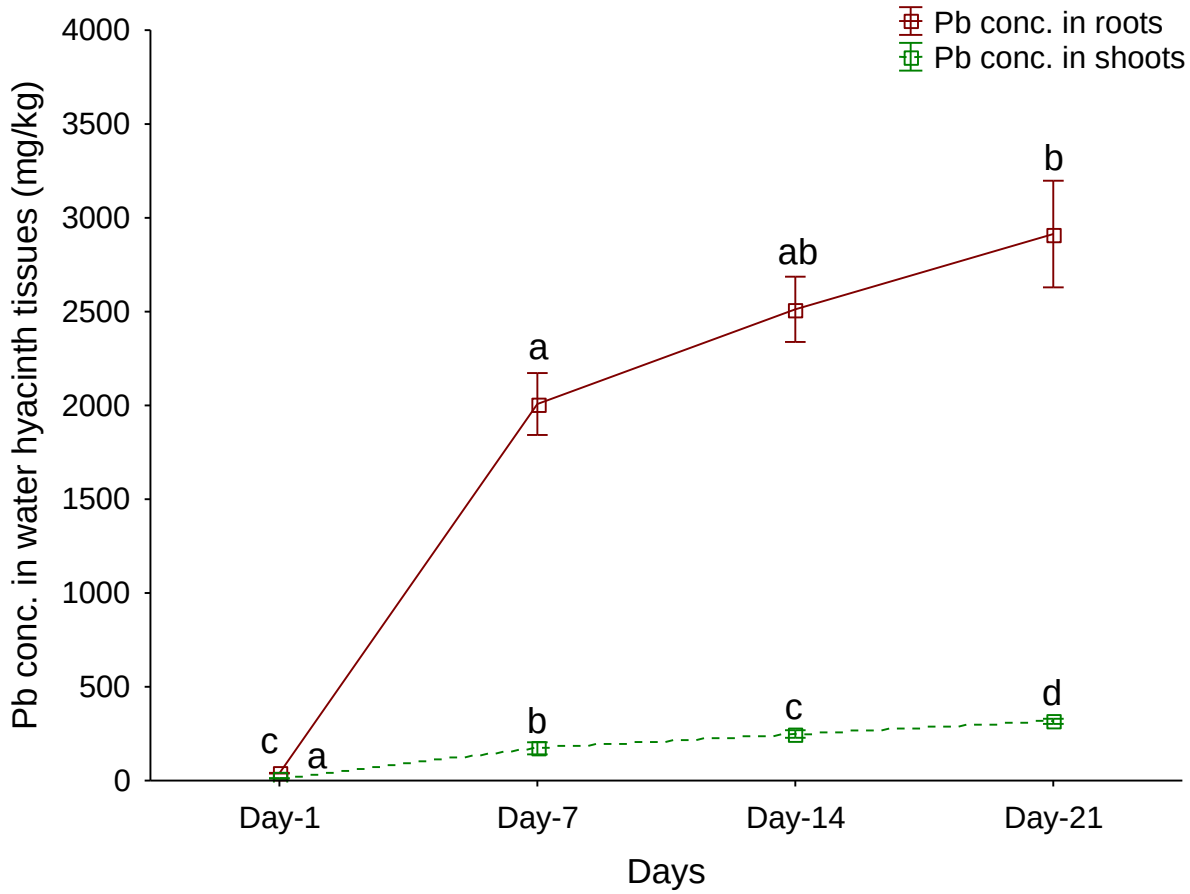



**Figure 3.1:** Pb sequestration in water hyacinth root and shoots. Means compared by one-way ANOVA and those sharing the same letter(s) are not significantly different (P > 0.05; Fisher's LSD test.

**Table 3.2:** Pb bio-concentration (BCF) and translocation (TF) factors of Fresh water hyacinth biomass (FWHB) and dry water hyacinth biomass (DWHB) at different time series exposures. Data represents mean ± SE of n = 24. Different letter(s) shows that they are significantly different ($P < 0.05$; Fisher's LSD test).

| | FWHB | | | | | DWHB | |
|---|---|---|---|---|---|---|---|
| **Exposure time** | Pb conc. in roots (mg/kg) | Pb conc. in shoots (mg/kg) | Total Pb conc. in whole plant (mg/kg) | BCF of Pb in FWHB | Translocation factor (TF) | Pb conc. in DWHB (mg/kg) | BCF of Pb in dry water hyacinth |
| 1 Day | 39.69± 4.53c | 11.88 ±3.19a | 51.57 ±4.25a | 12.89 ±1.06a | 0.31 ±0.09a | 3384.32 ±336.57 | 846 ±84.14 |
| 7 Days | 2007.34 ±404.22a | 172.37 ±78.56b | 2179.71 ±422.70b | 544.93 ±105.69b | 0.088 ±0.04b | 3381.69 ±501.11 | 845.42 ±125.28 |
| 14 Days | 2512.09±425.99ab | 248.67 ±49.99c | 2760.76 ±414.78c | 690.19 ±103.70b | 0.10 ±0.02b | 2818.71 ±196.70 | 704.68 ±49.17 |
| 21 Days | 2913.40±696.16b | 315.90 ±33.18bd | 3229.30 ±681.88d | 807.33 ±170.47bc | 0.11 ±0.04b | 2846.78 ±112.93 | 711 ±28.23 |

### 3.3 Impact of Pb on plant growth parameters

#### 3.3.1 Weight of freshwater hyacinth biomass (FWHB)

The percentage in fresh weight of the Pb-treated FWHB increased significantly ($F_{(3.20)}$ = 39.6141. p = 0.00000) with the increase of exposure days from day 1 to day 21 (Fig. 3.2). The average weight of the freshwater hyacinth recorded at the start of the experiment was noted. However, this increased significantly with the increase of the plants' time of exposure to the treatments by 4 %, 15 %, 34 % and 48 % on days 1, 7, 14 and 21, respectively (Fig. 3.3). Similarly, the increase in percentage weight of the FWHB in the control trial showed a significant difference from day 1 to day 14 which was not significantly different from that of day 21($F_{(3.20)}$ = 68.7802. p = 0.0000) of 7 %, 32 %, 64 % and 70 % in days 1, 7, 14 and 21, respectively (Fig. 3.3). There was a percentage increase of between 31 – 53% in the controls than in the Pb treated FWHB.

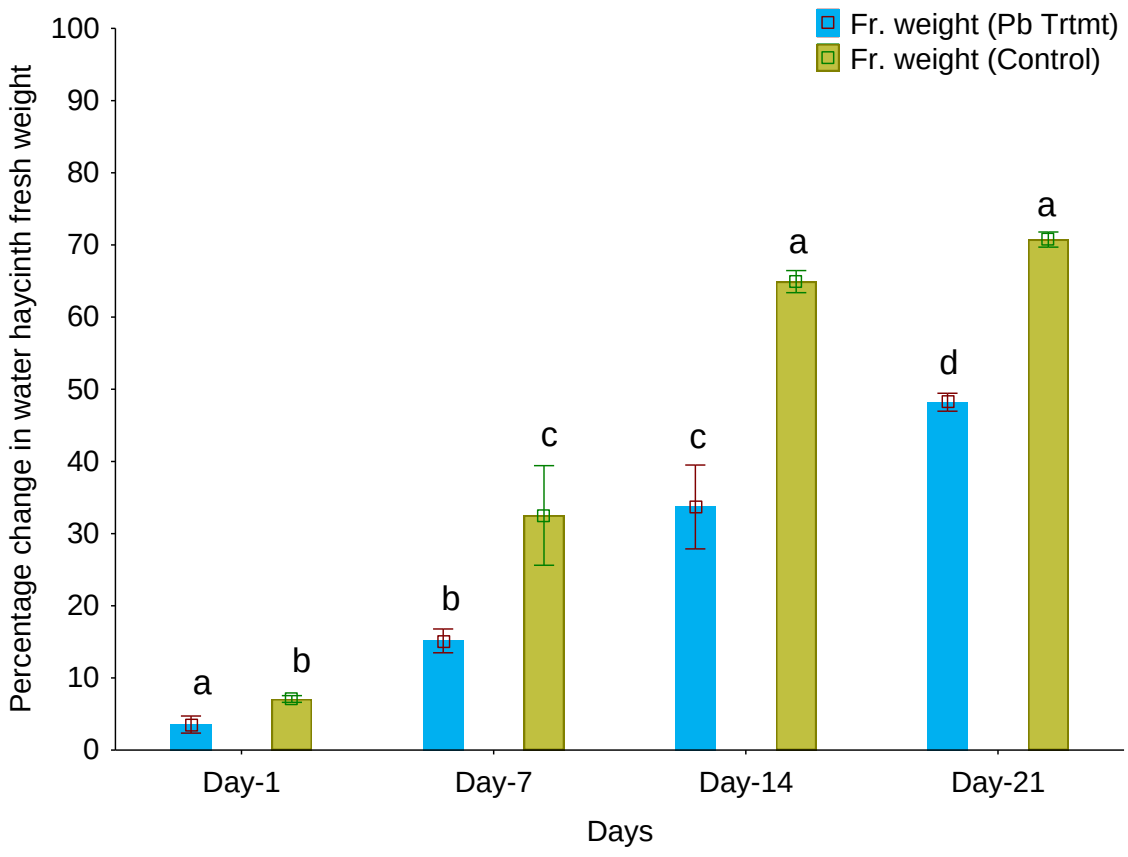



**Figure 3.2:** Fresh weight of water hyacinth plants with and without Pb treatment. Means compared by one-way ANOVA and those sharing the same letter(s) are not significantly different (P > 0.05; Fisher's LSD test).

### 3.3.2 Area of leaf-2 in freshwater hyacinth biomass (FWHB)

The percentage change of the leaf-2 area in the Pb-treated plants showed a no significant difference between the different times of exposure from day 1 to day 21 in the Pb treated plants ($F_{(3, 20)} = 3.0525$, $p < 0.0522$) (Fig. 3.4). However, the average area of leaf-2 in the control plants, increased significantly with the increase of the exposure time from day 1 to day 21, ($F_{(3, 20)} = 80.9333$, $p < 0.001$) showing increases of 3 %, 17 %, 41 % and 55 % on days 1, 7, 14 and 21, respectively (Fig. 3.4).

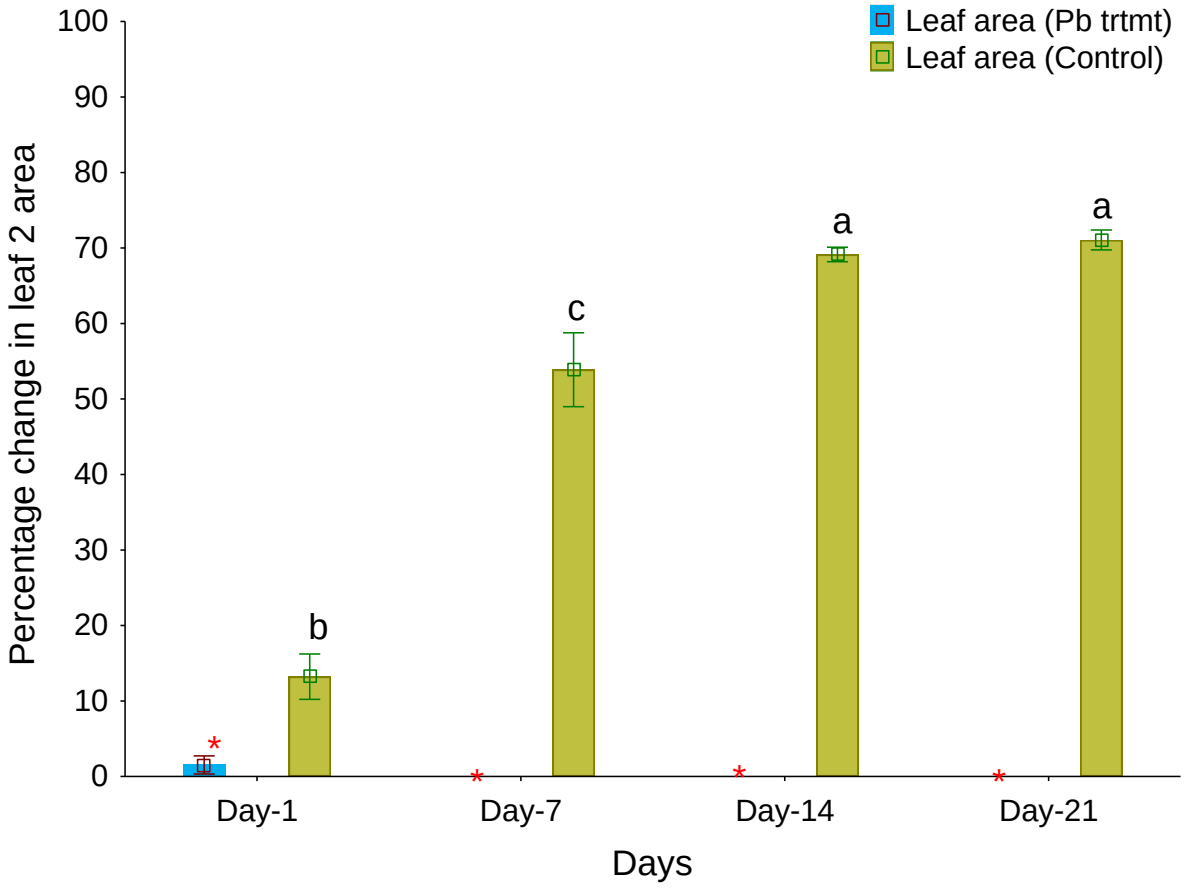



**Figure 3.3:** Area of Leaf-2 of water hyacinth plants with Pb treatment and control in tubs. Means compared by one-way ANOVA and those sharing the same letter(s) are not significantly different (P > 0.05; Fisher's LSD test).

### 3.3.3 Root length of the freshwater hyacinth biomass (FWHB)

The percentage changes in the root length showed significant differences between the different time-response exposure in both the Pb-treated and control water hyacinth plants (Fig. 3.5a). The only significant change observed in the roots of the Pb-treated plants was on the day 7

which was greater than in day 1 $F_{(3,20)} = 3.3$, $p = 0.0414$). In the control plants, however, the root length increased significantly with the increase of the exposure time to the Pb treatment from day 1 to day $F_{(3,20)} = 87.9399$, $p = 0.0000$). a progressive increase of 12%, 31% and 35% were reported from day 7 to day 21, respectively compared to their corresponding exposure times in the Pb treatments.

### 3.3.4 Length of leaf- 2 petiole of the freshwater hyacinth biomass (FWHB)

The length of the leaf -2 Petiole in the Pb-treated plants increased significantly ($F_{(3,20)} = 36.218$, $p = 0.00000$) after day 7 (Fig. 3.5b). There were not any significant changes observed between day 1 and day 7, which were significantly different from day 14 and day 21 (Fig. 3.5b). A progressive increase of 3%, 12%, 29% and 31% were found in the control plants with the increase of the exposure days from day 1 to 21, respectively the length of petiole -2 increased by 0.1 %, 4 %, 12 % and 24 %, respectively compared to the corresponding exposure times in the Pb-treated plants (Fig. 3.5b).

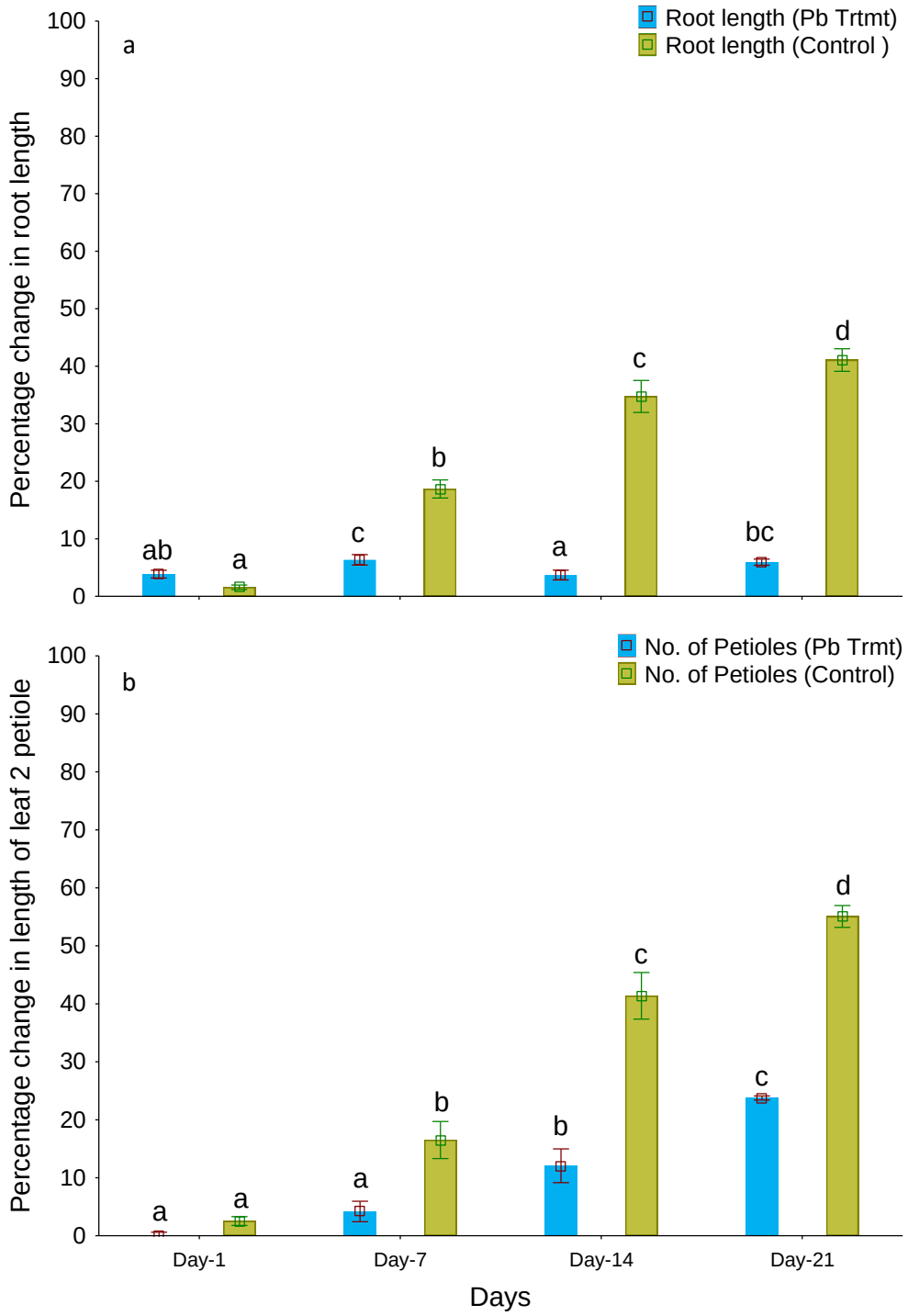



**Figure 3.4:** The percentage changes of the initial and final (a) area of Leaf 2 (b) length of Leaf-2 Petiole in Pb-treated and control water hyacinth plants. Means compared by one-way ANOVA and those sharing the same letter(s) are not significantly different (P > 0.05; Fisher's LSD test).

### 3.3.5 Number of ramets produced in the freshwater hyacinth biomass (FWHB)

The number of plants ramets increased significantly in both Pb- treated ($F_{(3, 20)} = 9.47$, $P < 0.001$) and control ($F_{(3, 20)} = 27.0741$, $p < 0.001$) plants between the different time exposure stretching from day 1 to day 21 (Fig. 3.6). The average number of ramets produced in Pb treated plants were 0.35 1.17 and 2.17 ramets in day 7, day 14 day 21, respectively. but no ramets were produced in day 1. Similarly, ramet production in control plants only started from day 7 with an average number of 1.33, 1.83, and 3 ramets produced in day 7, 14 and 21, respectively (Fig. 3.6).

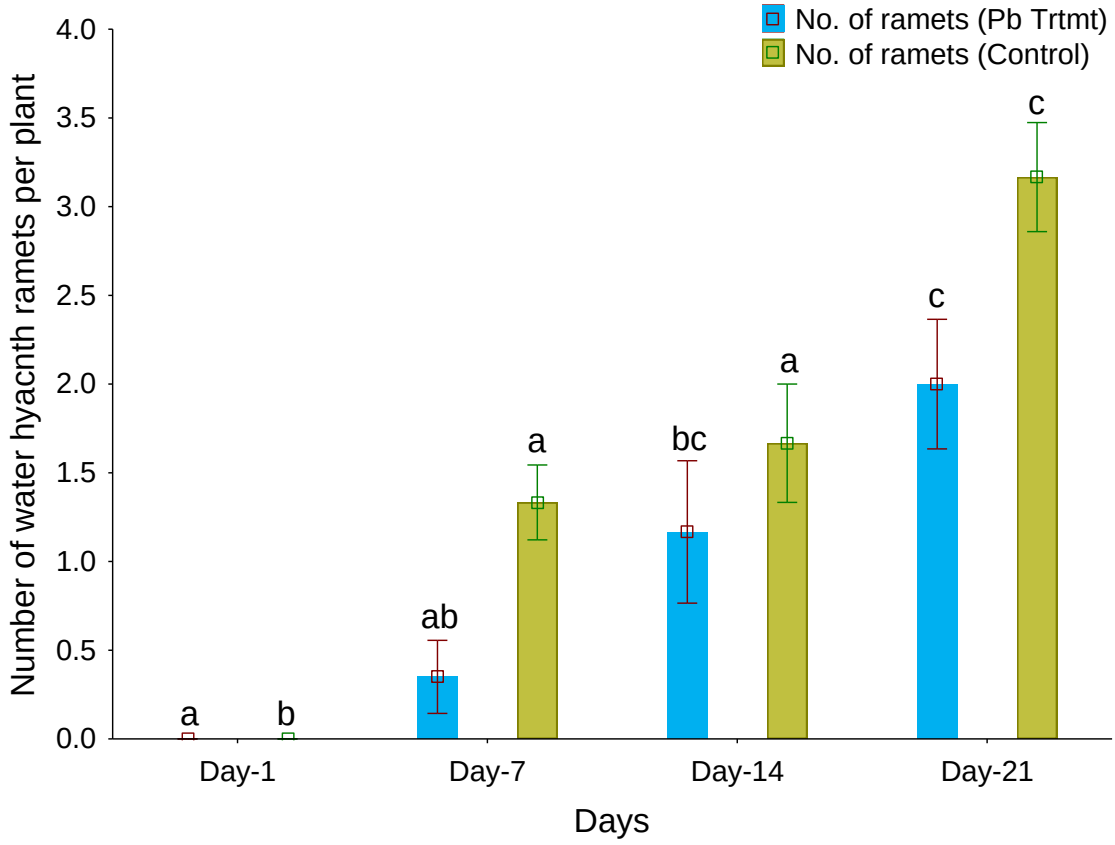



**Figure 3.5:** The number of ramets produced in the Pb-treated and control plants of water hyacinth grown in tubs. Means compared by one-way ANOVA and those sharing the same letter(s) are not significantly different (P > 0.05; Fisher's LSD test).

### 3.4 Water quality measurement before and after the addition of Pb treatment

The average initial pH of water recorded at the beginning of the experiment was 7.4. However, the pH of the Pb-treated water with FWHB in all the tubs increased significantly ($F_{(3,18)} = 45.4299$, $p = 0.00000$) as the days of exposure increased from day 1 to day 21 (Table 3.3). The

average pH over the time series exposure 7over the period of day 1, day 7, day 14 and day 21 were 7.6 ± 0.1, 7.7 ± 0.15, 8.1 ± 0.19 and 8.2 ± 0.12 respectively. In the Pb-treated waters with DWHB, however, the pH was significantly greater that in day 21 in the other days, but there was no significant difference between day 7 and day 14. from day 1 to day 21 ($F_{(3,18)}$ = 21.9702, p = 0.00000) with average pH recorded were 7.6 ± 0.08, 1, 7.9 ± 0.18, 8.1 ± 0.14 and 8.4 ± 0.16 mg/L, respectively.

The average initial electrical conductivity (EC) of water recorded at the beginning of the experiment was 345 µs/cm. The EC of the Pb treated water in tubs with FWHB showed significant increase ($F_{(3,18)}$ = 16.4816, p = 0.00002) on day 7 and day 21, but there was not any significant difference between day 7 and day14. The highest EC recorded in day 21 (531.5 ±19.89 µS/cm) and the lowest (351.3 ±9.99) in day 1(Table 3.3). On the contrary, the EC of the Pb-treated waters with DWHB increased significantly from day 1 to day 21 ($F_{(3,18)}$ = 37.9536, p = 0.00000). with average EC results of 353.7 ±11.67 in day 1, 439 ± 19.09b in day 7, 493 ±12.98 in day 14 and 577.8 ± 9.74 µS/cm in day 21.

The average initial dissolved oxygen (DO) of water recorded at the beginning of the experiment was 7.98 mg/L. The DO of the Pb-treated waters with FWHB showed significant decrease ($F_{(3,18)}$ = 52.8331, p = 0.00000) with the increase of the exposure days of the plants to the Pb treatment from day 1 to days 7, 14 and 21, the DO results were 8.41 ±0.17, 6.23 ±0.57, 5.96 ±0.27 and 5.02 ±0.52, respectively (Table 3.3). a similar trend of DO was also reported in Pb-treated waters with average EC results of 7.45 ±0.39 in day 1, 4.77 ±0.49 in day 7, 4.53 ±0.57 in day 14 and 4.03 ±0.51 in day 21.

**Table 3.3:** Parameters of water quality before and after the addition of Pb treatment. Data are means (n = 6, ±SE).

| **Exposure time** | **FWHB** | | | **DWHB** | | |
|---|---|---|---|---|---|---|
| | **pH** | **EC (µS/cm)** | **DO (mg/L)** | **pH** | **EC (µS/cm)** | **DO (mg/L)** |
| 1 Day | 7.58 ±0.1 a | 351.33 ±9.99 b | 8.41 ±0.17 a | 7.55 ±0.08 b | 353.67 ±11.67 a | 7.45 ±0.39 a |
| 7 Days | 7.71 ±0.15 b | 420.83 ±15.41 a | 6.23 ±0.57 b | 7.89 ±0.18 a | 439 ±19.09 b | 4.77 ±0.49 b |
| 14 Days | 8.10 ±0.19 c | 457.17 ±16.24va | 5.96 ±0.27 c | 8.07 ±0.14 a | 493 ±12.98 c | 4.53 ±0.57 c |
| 21 Days | 8.20 ±0.12 d | 531.5 ±19.89 d | 5.02 ±0.52 d | 8.42 ±0.16 d | 577.83 ±9.74 d | 4.03 ±0.51 d |

# CHAPTER FOUR
# DISCUSSION

## 4.1 Lead accumulation in water hyacinth roots and shoots

The largest portion of heavy metal removed by plants is sequestrated in the roots because of the precise position, morphology, and extent of roots in the contaminant's uptake by the process of diffusion (Remans et al. 2012). The possible functional groups mainly the carboxylic and phenolic functional groups components that are associated with cellulose such as lignin and hemicellulose as described in fig. 2.3 that are negatively charged identified by FTIR spectroscopy in the DWHB and the FWHB biosorb the $Pb^{2+}$ contaminant that was present in the contaminated water.

Thus, there is an exchangeable cation fraction electrostatically bonded to negatively charged sites in the free space of roots, which are most likely the free carboxyl groups of the pectic cell wall matrix components (Haynes, 1980); Usman et al. 2020; Sharma & Dubey, 2005). In the current study, as the exposure days of the freshwater hyacinth plants to the lead treatment increased, the accumulation of Pb in the plant tissues increased (Fig. 3.1). The highest Pb accumulation in the roots (92 %) was reported on day 7 followed by day 14 (91%), day 21 (90%) and day 1 (77%). The response of the water hyacinth plants to the Pb treatment and its toxicity was evaluated using several plant growth parameters including, *inter alia*, root length, number of ramets and fresh biomass weight and these parameters were not grossly affected. Previous studies on the Pb toxicity mechanism suggest that the aggregation and segregation of chromatin in plants aid in Pb binding to nucleic acids and this mechanism facilitate the transcription and replication process that allows cell division and plant growth (Malar et al. 2014). The current study showed that the average number of ramets produced in Pb treated plants were 0.35 in day 7, 1.17 in day 14 2.17 in day 21 but no ramet was produced in day 1. Therefore, water hyacinth plants exposed to high concentrations of Pb may suffer significant disorders such as changes in the permeability of the cell membrane. Moreover, the growth, number of ramets, and flower production of chromatin in water hyacinth plants may be affected by the reaction of sulphydryl (-SH) groups that contribute to the structures of protein with cations such as Pb (Ali et al. 2013).

Sharma and Dubey (2005), explained that roots have developed different mechanisms to minimize Pb uptake and transfer to the aboveground parts of the plant and limit its harmful effects. Lead is toxic to the leaves, where the processes of photosynthesis, chlorophyll production, and antioxidant enzymes occur. The translocation factors for heavy metals in plants should not be > 1 for non-essential elements like Pb (Tangahu et al. 2011). The highest translocation factor in the current study was 0.31 which suggests that most of the Pb were sequestered in the roots out of reach from the sensitive photosynthetic apparatus of the plant as a means of tolerance to the Pb-induced metallotoxicity (Takarina & Tjiong, 2017). Thus, the containment of the Pb in the cell walls and cell vacuoles in the roots than in the shoots is evidence of the strategic mechanism adopted to shield the photosynthesis mechanism from toxic levels of Pb contamination. Considering that the water hyacinth plant possesses the important characteristics required for an effective heavy metal up take from water, it is considered as one of the best candidates for phytoremediation of contaminated water bodies.

### 4.2 Plant relative growth rate

One of the main responses of water hyacinth plants to Pb toxicity was plant growth inhibition. There was significant disorder in plant growth in the lead-treated plants compared to the control plants. The number of ramets produced and the length of Leaf -2 petiole were unaffected under the Pb treatment with an average of one ramet per week which was the same as those in the control plants. However, other symptoms such as drying of the leaf edge and chlorosis were noticed during the trial experiment, which was similar to a study indicated by Hadi and Aziz (2015) on *Sesbania drummondii* (poison bean). In the current study, the symptoms of the drying of the leaf edge and chlorosis were due to the decrease in the absorption of nutrients and essential minerals (Mg, K and Ca) because Pb was competing for the absorption channels replacing the ions of the nutrient elements (Piotrowska et al. 2009, Collin et al. 2022). Presence of Pb in water poses antagonistic and competitive nature in nutrient and essential mineral absorption in aquatic plants (Ali et al. 2019, Drzewiecka & Mleczek, 2017). However, the weight of the plants increased with the increase of the plants' exposure time to the treatments by 4%, 15%, 34% and 48% on days 1, 7, 14 and 21 respectively. The control plants had similar weight increases of 7%, 32%, 64% and 70% on days 1, 7, 14 and 21 respectively over the time series exposure period, although relatively greater (Fig. 3.3). Furthermore, the weight of the plant biomass can be used to characterise how effectively a plant will develop in the presence of heavy metals. Malar et al. (2014) and Piechalak et al. (2003) indicated in their studies that

various plants responded similarly to lead treatment. Thus, this study confirms that the water hyacinth plants showed significant disorders in their growth such as the withering and shrinking of the leaf-2 area with 3% reduction on day 1 and 55% on day 21. The petiole length increased by 24% on day 21, indicating that plant continued to thrive well even under the Pb treatment suggesting their level of tolerance to the contaminant.

The excessive production of reactive oxygen species (ROS) which severely stresses plants and disrupts their metabolic processes may be another factor related to a decline in plant development because of the adverse effect of presence of Pb that might have led to the drying of the leaf edge and chlorosis of the studied plant, which is related to a similar study conducted by Newete et al. (2016). Previous studies conducted by Usman et al. (2020), support the hypothesis that Pb impairs cellular homeostasis by substituting vital cations and modifying the function of metal-containing enzymes. Cellular homeostasis impairment by Pb might have impeded the development of ramets as shown in the present study. Therefore, electron transport which is the main sources of ROS production in Chloroplasts, mitochondria, and peroxisomes in plants may be disrupted because of Pb toxicity. This phenomenon has a detrimental effect on plant health and may even cause it to die because of the drying of the leaf as shown in the present study. Although plants have developed a complex regulatory system that boosts the production of inhibitory enzymes like peroxidase (POX) and peroxidase activity to reduce ROS creation, most of them are unable to cope with the stress of excessive ROS produced as a result of very high Pb or other toxic organic absorptions (Sharma et al. 2012). The stimulation of POX activity is thought to be responsible for ROS content detoxification in water hyacinth plants, which makes it to flourish in the presence of Pb. Malar et al. (2014) indicated that increased POX activity levels have been associated with water hyacinth plant adaptation to severe Pb heavy metal stress as shown by the adaptation features of the plant in the present study.

### 4.3 Biosorption of Pb using Dry Water Hyacinth Biomass (DWHB)

The dried water hyacinth biomass (DWHB) was used as a biosorbent for adsorption of Pb from water. The percentage removal of Pb by DWHB on days 1, 7, 14 and 21 were 78.9 %, 78.5 %, 78.3 % and 78.3 %, respectively showing no significant difference between the different time series exposure to the Pb treatment. This shows that the equilibrium of Pb removal was attained on day 1 showing no further changes afterwards, which could be due to the large surface area

of the water hyacinth material that comes directly in contact with the Pb ions instantly with the start of the trials in the first day. Thus, due to the competition for the availability of active sites in the dry water hyacinth biomass, the adsorption rate declined right after the first day as the binding sites became exhausted and saturated in the first day leaving no further action with the increase of exposure days in (Dixit et al. 2015). Li et al. (2018) also found similar trend in their study for removal of heavy metals by dry water hyacinth and dead plant biomass. This biosorption procedure uses two techniques: biosorbent's surface biosorption and absorption of molecular accumulation within the pre-existing biosorbent (Thompson & Goyne, 2012). The vast sorptive surface that contains different organic functional groups (carboxyl, amino, imino, hydroxyl, sulfhydryl, and keto groups) makes these mechanisms efficient (Dirkx et al. 2008). In the current study, this shows that the surface of the DWHB is made of small heterogeneous pores which favours the adsorption of Pb by physisorption process. According to Davis et al. (2003), the macromolecules that make up these functional groups have the capacity to biosorb metal ions through ion exchange or complexation phenomena that take place on the surface of the material as a result of the interaction between the metal and the functional groups present. Mostly, the biosorption process is quick and occurs at room temperature (Araujo et al. 2013). After the phase separation procedure, a biomass that biosorbed a larger amount of Pb was obtained from the Pb water contaminant.

But it's important to understand that biosorption process doesn't depend on just one mechanism. Metals can be dissolved as free hydrated ions, complex ions chelated with inorganic ligands like hydroxide, chloride, or carbonate, or they can be complexed with organic ligands like amines, humic or fulvic acids and proteins and can be present in the aqueous environment in dissolved or particulate forms (Namieśnik & Rabajczyk, 2010). Therefore, ion exchange and complexation are the complex mechanisms that are possibly involved in the Pb adsorption from an aqueous system.

### 4.4 Water Hyacinth Efficiency as Biosorbent

Different exposure days to Pb treatment were used to examine the Pb removal efficiency of freshwater hyacinth biomass and dried water hyacinth material. It was evident that the fresh and dried water hyacinth biomass responded to the Pb exposure differently in terms of its removal (Table 4.3). On day 1, the removal of Pb by the dried water hyacinth biomass was significantly greater than the freshwater hyacinth plants. Among other factors such as pH,

exposure time, initial metal concentration, biosorbent dosage, the high biosorption of Pb on the first day might be because of the modification of the plant cell membrane by sun drying, which exposes the active binding sites in the cell wall for adsorption process (Nedukha, 2022). The dry water hyacinth biomass can be categorised as lignocellulosic adsorbents because they mostly contain cellulose, hemicellulose, and lignin (Tanpichai et al. 2019). The functional groups of the macromolecules that make up cellulose have the capacity to biosorb Pb ions through ion exchange or complexation processes. This often occurs in the first 24 hours, after which no any significant adsorption occurs with the continued exposure days to the Pb treatment and this could be due to the particle interactions, such as aggregation, resulting from high sorbent concentration (Rao et al. 2009). This aggregation effect led to the saturation of the possible active surface area of dry water hyacinth biomass. The same mechanism in the adsorption of heavy metals from contaminated water was revealed in similar studies (Li et al. 2018; Kuyucak & Volesky, 1989; Dirkx et al. 2008; Jayabalan et al. 2010). According to Li et al. (2010), more functional groups were linked to the biosorption technique of dead biomass than fresh plant biomass, which invariably increased the efficiency of heavy metal removal in their studies.

The overall biosorption process is significantly influenced by the pH and electrical conductivity of the aqueous system, particularly the solution chemistry of the metal and the activity of the functional groups in the biomass (Jones et al. 2018). Jayaweera et al. (2008), suggested that the general range within which the plant can grow and thrive is between 3.5 and 9, which will allow metal ions to adhere to the roots of water hyacinth. In both the freshwater and dry water hyacinth experimental tests, the average pH was well within the range of between 7.58 and 8.42.

Therefore, the dry water hyacinth (biosorption) and freshwater hyacinth (phytoremediation) processes were generally effective in the removal of Pb from the contaminated water.

One of the most important indicators of water quality is dissolved oxygen (DO). High levels of DO improve the taste of drinking water (Zhong et al. 2021). The quality of contaminated water that is treated by freshwater hyacinth (phytoremediation) has enough dissolve oxygen (DO) (Table 3.3) which is essential for aquatic life to survive. When it falls too low to support aquatic life, it seriously impairs the quality of the water.

### 4.5 Bioconcentration factor

Plants with a BCF >1 are considered to possess metal-tolerance capacity which could potentially be useful for phytoremediation purposes and water hyacinth is one of such plant that meet these criteria. The results of the study showed that water hyacinth possesses tolerance to metal toxicity as the BCF on day 21, which was the highest was 807.33. The bioconcentration factor of dry water hyacinth biomass reported on day 1 was 846 which was greater than that of the freshwater hyacinth reported (12.89) for the same day, though gradually decreases from day 7 to day 21 probably because of desorption. From the data, dry water hyacinth is more efficient than freshwater hyacinth.

# CHAPTER FIVE
# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusion

In this study, water hyacinth was used as a biosorbent and phytoremediating plant for removal of Pb from contaminated water. The highest accumulation absorbed in the roots of the phytoremediating plant was 92 % that was reported on day 7, followed by day 14 with 91 %, day 21 with 90% and 77 % on day 1, the highest reduction being in the latter.

This high accumulation of Pb in the roots suggests that freshwater hyacinth has physiological characteristics and some defence mechanisms against heavy metal toxicity. Like many aquatic macrophytes, the water hyacinth's tolerance mechanism mostly depends on locating heavy metals that have been taken from water in their roots and preventing their movement to the aerial system, especially to the most susceptible part of the plant.

The percentage removal of Pb by dried water hyacinth biomass (DWHB) on days 1, 7, 14 and 21 were 78.9, 78.5, 78.3 and 78.3 % respectively. The largest adsorption was observed on the first day (day 1) and showed no significant difference thereafter with the increase of exposure days to the Pb treatment until day 21. This shows that the equilibrium of Pb adsorption was attained on day 1 suggesting that all adsorption sites were occupied reaching saturation level in the first day of the experiment. on the contrary, the removal of Pb by the fresh water hyacinth biomass (FWHB) increased with the increase of the exposure days to the Pb treatment. The translocation factor from the roots to the shoots was < 1 for all the exposure days to the treatments, suggesting why the plant survives the highly toxic heavy metals by retaining it in the roots far away from the sensitive photosynthetic apparatus of the plant.

Therefore, it can be concluded that the studied plant can be considered ‘effective’ for phytoremediation and biosorption process because it has most of the important characteristics and features that may be used to uptake and adsorb the heavy metals from the environment.

The main conclusion as per the objectives set for the study were as follows:

1. determine and compare the efficiency of water hyacinth biosorption and phytoremediation of Pb from contaminated water:

   The removal of Pb from the water was effective in both the fresh and dry water hyacinth. Nevertheless, the largest removal in the dry water hyacinth biomass occurred in the first day as opposed to the last day (day 21) in the freshwater hyacinth biomass.
2. determine the efficiency of 1 day, 7 days, 14 days, and 21 days of exposure of water hyacinth weeds in phytoremediation of Pb in contaminated water:

   Freshwater hyacinth efficiency was a gradual process as it involves gradual absorption of Pb from day 1 to day 21. Generally, the efficiency with which freshwater hyacinth biomass (FWHB) removes Pb from water increased with the increase of the plant's days of exposure to the Pb treatment.

3. determine the efficiency of 1 day, 7 days, 14 days, and 21 days of exposure to water hyacinth as a biosorbent for Pb removal from contaminated water:

The largest adsorption was observed on the first day (day 1) which remained almost constant with no significant increase with the increase of exposure days to the Pb treatment. This shows that the equilibrium of Pb removal was attained on day 1 showing no further change afterwards.

Therefore, the cost, accessibility and environmental impact of the clean-up solutions provided by phytoremediating plants can be contrasted to those of employing chemicals and other techniques, which may not be as efficient as the novel technology. Hence, the water hyacinth physical characteristics are suitable for the application of phytoremediation due to its unusually long, fine, and hairy root structure, the enormous associated surface area, and its prolific growth rate, which can double its biomass in just two weeks under optimal growth conditions.
However, the limitation of Water hyacinth is that, the plant may also be a water pollutant but this disadvantage can be turned to benefit by using it for bioremediation. However, such suggestion should be cautiously implemented to avoid unnecessary spreading of this water pollutant.

## 5.2 Recommendations

Biosorption and phytoremediation techniques are effective tools for removal of heavy metals from water. Depending on the level of contaminants in the contamination site, the target contaminant, and the efficacy of the target plant, special consideration such as the rapid growth and biomass turn over as well as its extensive root surface area be taken while employing this technique.

The following areas are recommended for further studies:

- In order to have a balanced mixing ratio between the biosorbent, phytoremediating plant, and the adsorbate, it is important to study and understand the mechanism of action of the DWHB and FWHB.
- An investigation of the effects of temperature, pH, varying concentration of adsorbate, contact time, sorbent mass to water volume ratio, Pb concentration, presenc e of competitive ions in water, surface area and the sorption capacity of DWHB in brackish water could be investigated in future. This is because in water treatment, varying temperatures may have an impact on autoionization and functional groups on DWHB, and this may destabilise the sorption capacity of the dry plant on metal ions. Therefore, it is recommended to investigate the effects of temperature.
- An investigation on the Water Hyacinth efficiency as biosorbent and phytoremediating plant in management and remediation of acid mine drainage (AMD) is recommended for future research

## 5.3 Limitations to the study

- Non-availability of chemicals for preparation of standards and characterisation instrument for analysis was a challenge.
- Non availability of some functional instruments for analysing of the aliquot (Pb remaining in water) posed a serious limitation to this study.
- Accessibility to Water hyacinth plant posed a serious challenge in the study because the researcher had to go to the village that is at the outskirt of the place of the study to source for the plants.
- The challenge in development of the standard process for Pb removal from contaminated water due to the requirement of large amount of biosorbents, extra chemicals, and equipment posed serious challenges in the study.

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