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Cadmium Accumulation and its Effect on Physiological Activities and Oil Generation in Aquatic Macrophytes

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This work is dedicated to my brother

Mr. Muhammad Saeed

for their endless support and encouragement to bring the best out of me

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LIST OF ABBRIVEATIONS

NARC	National Agriculture Research Centre
BCF	Bioconcentration Factor
TF	Translocation Factor
XRF	X-ray Fluorescence
AAS	Atomic Absorption Spectrometer
EDTA	Ethylene Diamine Tetra Acetic Acid
CCI	Chlorophyll Content Index
WHO	World Health Organization
NSDWQ	National Standards for Drinking Water Quality
ATSDR	American Standards for Toxic Substances and Diseases

LIST OF TABLES

LIST OF FIGURES

Cadmium Accumulation and its Effect on Physiological Activity and Oil Production in Aquatic Macrophytes

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Table of Contents

ABSTRACT

Heavy metal pollution of fresh and marine aquatic system has increased linearly since the beginning of industrial revolution. For decontamination purpose, several aquatic macrophytes are considered as good accumulators of heavy metals and other toxic contaminants. This study investigated cadmium accumulation by water lettuce (*Pistia stratioties*), watercress (*Nastutium officinale*) and pennywort (*Cetella asiatica*). Plants were exposed to different levels of cadmium (0, 5, 10, 15 and 20 mg/L) to evaluate accumulation and its physiological effects. Plant samples were examined by atomic absorption spectroscopy for accumulated heavy metal contents. Cadmium accumulation in all macrophytes increased with the increase in metal concentrations in solution. Metal accumulation in shoots of *N. officinale and C. asiatica* was considerably higher than roots and vicversa in *P. stratioties*. Macrophytes treated with 20 mg/L died, in the mid of exposure period of 21 days, and highest accumulation was shown as 17.8. 68.7 and 67.3 mg/kg, of dry weight at 15mg/L of cadmium concertation by *P. stratiotes, N. officinale and C. asiatica,* respectively. Results also showed that all the studied macrophytes were cadmium accumulator with BCF >1. *N officinale* and *C. asiatica* are able to translocate metal into the aerial parts. This hydroponic study revealed the reduction in chlorophyll content and plant biomass upon exposure to cadmium. Plant oil generation capacity also decreased with increase in cadmium level. Furthermore, XRF results of extracted oil showed the absence of cadmium up to 10 mg/L. Keeping in view the results obtained from the study, these macrophytes have a potential to accumulate cadmium from growing medium and can be used for the treatment of contaminated water, and may also off-set the costs through bio-oil production.

Chapter 1

INTRODUCTION

1.1. Background

Water pollution issue is most concerning as the mankind and agriculture sustainability depends on it. Each creature of this planet needs water for survival, but water deterioration is increasing linearly. Almost 95% of industrial wastewater and nearly 90-95% of domestic wastewater from urban areas is discharged without any preliminary treatment (Sharma *et al.,* 2012).

Water pollution is of major social and environmental concern due to industrialization. Metals are introduced into the fresh water systems by soil//rock weathering, volcanic eruptions, pyrogenic and anthropogenic activities including metal mining, processing, direct metal usage or the use of substances containing metal pollutants. Several metals like manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) are found essential elements for plant growth. But if their concentration exceeds than threshold limit, the same chemicals become poisonous. Among the heavy metal pollutants, most concerning includes cadmium (Cd), chromium (Cr), copper (Cu), arsenic (As), nickel (Ni), mercury (Hg) and lead (Pb).

Heavy metals are naturally existing elements that are present in the earth's crust. The term "heavy metals" include the elements having specific density greater than 5 g/cm^3 and atomic number above 20, excluding alkalis, alkaline earth metals, actinides and lanthanides. Cd and Pb are among the most widespread non-nutrient heavy metals. Their contamination mainly results from four specific economic processes: burning of fuels (liquid or solid), foundry works and smelting, discharging sewage having high concentrations of Cd and Pb, and soil chemical application, including fertilizers (Yücel *et al.,* 2008).

1.2. Cadmium Sources and its Effects

Cadmium is found widely dispersed into the environment. It enters into the environment during its mining, smelting and by other anthropogenic routes i.e. by the phosphate fertilizers application to the soil, discharging of contaminated sewage sludge and during several industrial practices e.g. Ni-Cd batteries, plating, pigments and plastics (ATSDR, 1999; Sahmoun *et al.*, 2005). It may also directly enter into the air from metal production facilities (i.e. steel and iron).

Cadmium, when released by process of mining and smelting, may collide with tiny airborne particles and may carried long distances. By the process of precipitation, it again comes in contact to the soil, after that it is taken up by the plants through roots, accumulate into the plant cells and enters into the food chain. Most susceptible plants include root crops, leafy vegetables, grains and cereals. Groundwater bodies are rarely found to contain noticeable concentration of cadmium unless they are being spoiled by any hazardous site, industrial or mining wastewater discharges. According to WHO guidelines, permissible limit of cadmium in drinking water is 0.003mg/L (mille grams per liter) but for Pakistan it is 0.01 mg/L (NSDWQ, 2008). While according to ATSDR (1999), for drinking water supplies cadmium concentrations should be <1 μg/L (microgram per liter) or 1 ppb (part per billion).

Cigarettes industry is among the most prominent anthropogenic source of cadmium as tobacco plants are being sprayed by cadmium to prevent fungal growth. Cigarette papers also contain traces of cadmium, more precisely every cigarette contains about 1.4 micrograms of residual cadmium.

Cadmium may affect the higher level of food chain by the process of bio magnification. Several plants species such as tobacco, vegetables, cereals and grains are found to take up cadmium more easily than other heavy metals like mercury and lead (Satarug *et al.*, 2003). Cadmium is also detected in meat, particularly in sweetmeats i.e. meat from liver and kidney. Shellfish and mushrooms are also found to accumulate traces of cadmium. Rice crop has been found to accumulate cadmium when planted in contaminated soil (Järup, 2002). Intake of cadmium contaminated water either from rusted Zn/Cd closed water pipes or from industrial wastewater may lead to adverse health impacts. Contamination of food supplements and medicinal drugs can be a source of cadmium intake (Abernethy *et al.*, 2010).

Cadmium is a proven lethal heavy metal and do not have any recognized biological function. It inhibits the enzymatic activity and interrupts the functioning of several nutrients within the body. It disrupts the calcium metabolism in the body by replacing calcium in bone cells, and may lead to several bones related disorders including, hypercalcuria, rheumatoid arthritis, osteomalacia and osteoporosis, forming ureter and kidney stones and decreasing the active vitamin D production. On its accumulation into the joints it causes osteoarthritis. Bioaccumulation of heavy metals, like cadmium, aluminum and mercury, in the kidney may lead to several infections, formation of stones in kidney, electrolyte imbalances problem, edema and back pain. Its uptake also affects the absorption and functioning of other essential nutrient inside the human body, resulting in malnutrition of iron, manganese and copper. It is known as a possible pancreatic carcinogen in human body (Brian *et al.*, 2012). It also helps the accumulation of copper in the body tissues.

The cadmium concentration in freshwater like in Ravi River in Pakistan is about 2.46 to 8.52 mg/L which is threatening and affecting the freshwater ecosystem (Rauf *et al.*, 2009). Chemical methods for wastewater treatment are costly. So in this situation, phytoremediation appears to be a suitable and attractive choice for wastewater treatment as it has good remediation potential for polluted areas at reasonably lower costs than other available treatment methods (Eisazadeh, 2007).

1.3. Phytoremediation

Heavy metal uptake by plant was well thought as detrimental trait. Plants are basically producers, so heavy metal accumulation in plants will be blamed for nutritional uptake of heavy metals by higher trophic levels (Sary *et al.*, 2012). It is mostly known as solar driven pump because during transpiration plant release one molecule of water from aerial part and intake one molecule from root surroundings, meanwhile plant uptake the nutrients and pollutants.

Phytoremediation comprises of two words phyto (plant) and remediation (to treat), basically it is a remediation process in which several plant species are utilized to absorb, extract, translocate and stabilize the pollutant and contaminant from soil and water. It is an effective and ancient treatment strategy because there is no need of secondary energy supply. This fact makes phytoremediation a cost effective technology. There are seven different mechanisms of phytoremediation as follows;

1. Rhizosphere biodegradation
2. Phyto-stabilization
3. Phyto-accumulation
4. Rhizofiltration
5. Phyto-volatilization
6. Phyto-degradation and
7. Foliage degradation

Mostly photodegrading and phyto-stabilization process are referred for organic materials uptake, whereas phyto-volatilization and phyto-extraction are denoted for heavy metal pollution remediation (Guerinot and Salt, 2001). A brief description of different phytoremediation mechanisms is provided in the following section.

1.3.1. Rhizosphere Biodegradation

It is also known as phyto-stimulation. In this phenomenon micro-organisms such as yeast, fungi and bacteria use different hazardous organic substances as food and break them down into harmless products. Plant roots also excrete carbon containing compounds like sugar, alcohol and acids that provide food for micro-organisms.

1.3.2. Phyto-stabilization

In this mechanism, toxic pollutants are immobilized by the plants to reduce their bioavailability. For this purpose, plant should have proficient accumulation capability with extensive root system and less translocation factor.

1.3.3. Phyto-accumulation

Phyto-accumulation is also known as phyto-extraction and mostly applied to sites polluted with metals. In this strategy, plant roots uptake the pollutants from soil and water. A phyto-accumulator plant should have high bioaccumulation potential, considerably high biomass growth rate, high translocation factor and most importantly metal tolerance potential.

1.3.4. Rhizofiltration

Rhizofiltration is the adsorption, absorption, or precipitation of contaminants from nutrient solution by plant roots. This mechanism is basically a hydroponic system for water treatment. It is similar to phyto-accumulation with exception of plant growth in controlled conditions.

1.3.5. Phyto-volatilization

In this strategy, pollutants (mostly organic) are taken up by plant and liberated into the air via transpiration. According to Gosh and Singh plant can phyto-volatize the selenium, mercury and arsenic by converting them into gas (Ghosh and Singh, 2005a).

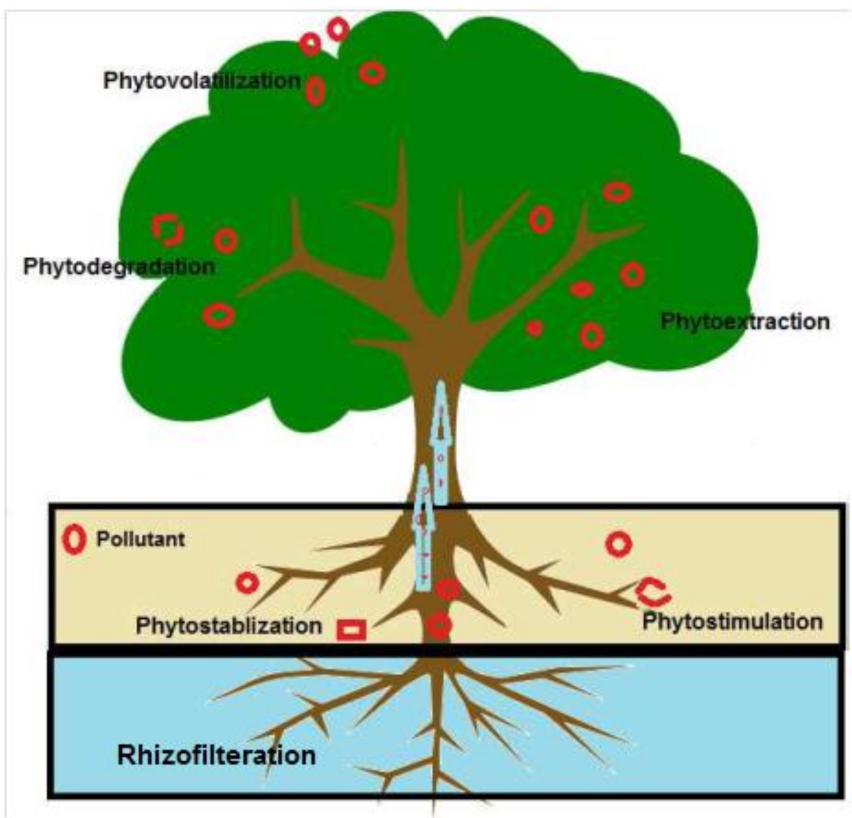


Figure 1.1: Pictorial view of Phytoremediation process (Sharma & Pandey, 2014)

1.3.6. Phyto-degradation

It is also known as phyto-transformation. In this mechanism, contaminants are first taken up by the plants and then broken down into simpler and less toxic compounds. These simpler molecules are utilized by the plants or liberated (Ghosh and Singh, 2005b).

1.3.7. Foliage Filtration

In this process, plants remove the contaminants from atmosphere through leaves stomata via transpiration.

In last few decades many studies have been carried out on aquatic macrophytes as suitable participant for toxic metal uptake and biological indicators of toxic metals in wastewater systems (Aoi & Hayashi, 1996; Bishop & Eighmy, 1989; Delgado *et al.,* 1993; Gersberg *et al.,* 1986; Jenssen *et al.,* 1993; Maine *et al.,* 1998, 1999; Martin & Coughtrey, 1982; Ozimek *et al.,* 1993; Sen & Bhattacharyya, 1994; Wolverton & McDonald, 1979). Phytoremediation has proved itself a noticeably better than other existing secondary treatment methods. Plants that are used for phytoremediation have significantly good metal uptake potential (Reddy, 1983; Gersberg *et al.,* 1986).

Most of the phytoremediation plants found to be the accumulator of heavy metals from water and are already being used in wastewater treatment systems (Abbasi *et al.,* 1999; Kadlec *et al.,* 2000).

1.4. Limitations of Phytoremediation

Every process and mechanism have some limitation and concerns. For phytoremediation limitations include;

1. The bioavailability of pollutants and contaminants is mostly unknown.
2. By-products after biodegradation may be mixed into the groundwater or bio-accumulated in animals and affect higher trophic levels.
3. Disposal of toxic accumulators is a serious concern.
4. Plant root length limits contaminant and pollutant removal. Mostly macrophytes work efficiently in shallow water systems.

5. Seasonal variation directly affects the phytoremediation potential.
6. High concentration of toxic pollutant or contaminant may become fatal for plant.
7. Phytoremediation plants after accumulation of heavy metals are considered as toxic and hazardous.
8. Most importantly, it requires large area for remediation.

1.5. Cadmium Uptake by Plant: Factors Affecting the Process

Cadmium enter into the plants from soil and water by root system (Toppi & Gibraeelli, 1999). Carboxy group of mucilage uronic acid bind the Cd^{+2} at root surface. This mucilage binding inhibits further accumulation of metal into the root and forms substantial barrier to protect the root system. Few bound metals are released due to biodegradation of mucilage (Ernst, 1998). Small proportion of cadmium is taken up by leaves as well. Most of the heavy metal are absorbed by lower leaves (Godzik, 1993). The concentration of heavy metal uptake depends upon the specific leaf morphology. Heavy metal accumulation depends on the following factors;

1. Organic matter present within soil
2. pH of soil substrate
3. Concentrations of other ions available for plant to uptake

The solubility of cadmium in the soil solution decreases by the production of less soluble molecules; which ultimately decreases the availability of soil cadmium (Salt *et al.*, 1995).

There are some studies that shows enhancement in heavy metal e.g. cadmium and lead accumulation by plant due to presence of chelating agents, i.e. EGTA and EDTA, from polluted soil. Numerous ionic species are also found to affect the heavy metal accumulation by plant. Studies have shown that the heavy metal uptake in kidney beans is increased due to presence of alkaline and alkaline earth metal as follows

Calcium (Ca^{+2}) > Magnesium (Mg^{+2}) > Potassium (K^{+}) > Sodium (Na^{+})

Similar result has been reported in case of zinc (Zn^{+2}) and copper (Cu^{+2}) cation presence (Chohdhry, 1995). However, cadmium uptake is not considerably effected by the anion species, whereas phosphorous and nitrogen fertilizers promote the uptake of cadmium in wheat plant (Zhoa et al., 2010). Another study reported the increase in cadmium uptake by birch plant due to higher concentration of Ca^{+2} in medium (Jenssen, 1995).

1.6. Fate of used Macrophytes

Phytoremediation plants after accumulation of heavy metals are considered as toxic and hazardous. But now, there is no term of waste, everything is a source. There are several methods and techniques of utilizing plant after heavy metal uptake, for example, ash of water hyacinth (*E. crassipes*), can be utilized for extraction of valuable metals from water system Mahmood *et al.* (2010). This approach opens new economic corridors instead of burden as hazardous waste. There should be prior treatment of wastewater by potential hyper accumulator at treatment pond, so that it increases the accumulation rate due to more exposure.

1.7. Objectives

Keeping in view all insights gained from the latest research, the present study was designed to assess the plant growth, bioaccumulation and translocation characteristics along with toxicity of cadmium in watercress (*Nasturtium officinale),* water lettuce *(Pistia stratiotes)* and pennywort (*Centella asiatica).*

Overall, this study was planned to achieve the following objectives;

1. Determination of the plant growth and accumulation of Cd by aquatic macrophytes
2. To determine the threshold limit of effective Cd uptake for the plant growth
3. Effects of Cd levels on oil quantity and quality of aquatic macrophytes

1.7. Significance of the Study

Phytoremediation is natural, cheaper, solar driven and environment friendly technique. Therefore, it can be applied in developing countries like Pakistan. This technique can help to harvest valuable heavy metals and metalloids from industrial wastewaters that can be reused.

Chapter 2

LITERATURE REVIEW

2.1. Water Pollution

Increasing population and its gradual adoption to luxury lifestyle ultimately resulted in an increased human impact on the environment (Asamudo *et al.,* 2005). Water pollution and industrial revolution grow side by side, water pollution by hazardous metals has increased dramatically. About 90% of the heavy metal emissions caused from anthropogenic sources occurring since 1900 AD (Nriagu, 1992). Metal ions are described as significant pollutants, due to their solubility in aquatic systems and their long term toxic effects (Demirbas, 2008)

Industries discharge their wastewater in the nearby water bodies and hazardous chemicals enter into the aquatic environment instigating toxic effects on living organisms in food chain (Dembitsky & Rezanka, 2003) by bioaccumulation and bio-magnification. Water contaminated with heavy metals from numerous industries has been a major problem for many years. Heavy metals accumulate in aquatic creatures due to its resistance to biodegradability and these metals became part of food chain due to their high water solubility, causing nausea, diarrhea, brain disorders, cancers, liver and renal dysfunctions (WHO, 2006).

Literature has reported that the use of water bodies as disposal site for toxic pollutants such as heavy metals has caused drastic effects on aquatic ecosystems (Bio-Wise, 2003; Aboulroos *et al.,* 2006). Several petrol pollutants like oil, grease, phenol, sulfate, suspended solids, dissolved solids, nitrates, were discharge directly into water bodies causing fish gills to clog and decaying of rest of aquatic flora and fauna (Asamudo *et al.,* 2005; Azeez & Sababr, 2012; Ji *et al.,* 2007; Patel & Kanungo, 2010).

2.2. Phytoremediation

The main indication that the plants can be utilized for removal of soil and water pollutants is primitive; however, a chain of several scientific researches with an interdisciplinary approach helped the expansion of phytoremediation. Phytoremediation is considered as solar driven remediation of pollutants from soil and water. Phytoremediation of toxic metals is an economical 'green' approach that depends upon the plants to accumulate or render toxic metals from adjacent environment. In recent times phytoremediation has gained strong public and scientific interest (Salt *et al.*, 1995 and Cunningham 1996).

Environment contaminated with heavy metals has a critical threat to environmental and human health. Most important environmental metallic pollutants are divided into two categories i.e. nonradioactive metal like cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), lead (Pb) and mercury (Hg) and radioactive metals such as cezelium (Cs), uranium (U), and strontium (Sr) (Raskin *et al.,* 1997).

Phytoremediation has many advantages over other remediation techniques as it is economic, less toxic to environment, has sufficient ability to remove and render pollutant and most importantly it is a solar driven technique (Maine *et al.*, 2001; Xue *et al.*, 2005; Agunbiade *et al.*, 2009).

Several studies have shown that few plants have capability to accumulate heavy metals via different methodologies (Maine *et al.,* 2001; Soltan & Rashed 2003; Yaowakhan *et al.*, 2005; Hasan *et al.*, 2007). Though, the selection of desire plant species for phytoremediation critically influence the phytoremediation efficiency. Numerous researches have shown that the type of metal accumulation plants would considerably vary in pollutants uptake (Gersberg *et al.,* 1986; Maine *et al.,* 2001; Alvarado *et al.,* 2008).

The plants sensitivity to heavy metals uptake depends on numerous physiological activities and molecular processes like metal accumulation by binding to root exudates or cell wall, modification in internal cell chemistry, activation or modification of antioxidant enzyme and quick restoration of injured cell structures. (Hall, 2002; Cho *et al.*, 2003). Numerous studies have reported effect of cadmium on cellular activities of plants including DNA alteration, disruption of transportation of electron, membrane damage and activation/inhibition of enzymes (Smeets *et al.,* 2005, Benavides *et al.,* 2005, Semane *et al.,* 2007).

2.3. Phytoextraction

Inspiration for phytoextraction ideology expansion came from the discovery of wild plants that are naturally present on polluted areas and accumulate the excess amounts of essential and nonessential, but toxic, heavy metals in their aerial parts. (Bakers & Brooks 1989). Cunningham 1995 reported 50mg/g (mille grams per gram) of lead accumulation from highly contaminated site due to extremely insoluble characteristics of lead. Even *B. juncea* which has genetic capability to absorb lead not able to accumulate this much of lead from lead contaminated soil.

Several studies have reported an increase in metal accumulation from soil on addition of chelating agent. Most important chelating agent is EDTA (ethylenediaminetetraacetic acid) that assist the phytoextraction of heavy metals like Cd, Zn, Cu, Pb and Ni. Study shows 1.6% accumulation of lead from soil contaminated with 1200 mg/kg (mille grams per kilo grams) of lead by addition of 10 mM/kg (mille mole per kilo grams) of lead by *B. juncea* (Blaylock, 1997).

2.3.1. Biological Mechanisms of Phytoextraction

For long term and effective utilization of phytoextraction strategy, better understanding of biological mechanisms concerning metal uptake and transportation to foliage is very important. A study conducted by Marschner in 1995 reported a lot of work regarding uptake of metals by plant cells through roots. Several other studies have also been conducted in this concern, that assist the understanding of essential nutrients accumulation methodology like that of nitrogen (N), sulfur (S), phosphorous (P), iron (Fe), potassium (K), calcium (Ca) and possibly chlorine (Cl) by plants/ However, some studies also reported the uptake and transportation phenomenon of toxic heavy metals like Cd, Pb, Cu, Zn, Sr, Cs and U.

For accumulation of soil bound toxic metals, phytoextracting plants have to liberate them from soil by secreting metal chelating molecules. These molecules are released into the rhizosphere by plant roots to solubilize the soil bound metals. Major success of phytoextraction was achieved by addition of synthetic chelating agents. It is believed that iron-chelating compounds, termed as phytosiderophores, are released due to deficiency of iron and helps mobilizing Cu, Zn and Mn bound to the soil (Raskin *et al.,* 1997 and references there in).

Microbial activity is reported as an enhancer in metal accumulation process (Kramer *et al.,* 1998). Past studies had revealed the importance of assistance of organic acid such as citrate for metal accumulation and transportation in phytoextractors (Sende *et al.,* 1992). Latest study regarding cadmium accumulation and translocation, has shown the presence of CdSJ complex in in *B. juncea* roots. Cadmium mostly accumulates in trichrome in leaves and bind with oxygen or nitrogen ligands in xylem (Salt, 1995).

2.3.2. Cadmium and Phytoextraction

Several studies have reported the use of aquatic macrophytes for heavy metal uptake. These macrophytes include floating plants, such as water hyacinth (*Eichhornia crassipes*) (Mishra *et al.,* 2008) duckweed (*Lemna minor*) (Mishra & Tripathi, 2008), and water lettuce (*Pistia stratiotes*) (Mishra *et al.,* 2008). These macrophytes have shown the ability to accumulate cadmium (Badr & Fawzy 2008; Bunluesin *et al.,* 2004; Mishra *et al.,* 2008). Most of the studies were carried out in greenhouse or controlled condition (Bunluesin *et al.,* 2004; John *et al.,* 2008; Maine *et al.,* 2001; Mishra and Tripathi, 2008), showing metal uptake of up to 90% (Mishra and Tripathi, 2008).

2.4. Bioavailability of Cadmium

A critical factor affecting the toxicity of a metal is its bioavailability. The term "bioavailability" is the amount of total available pollutant in the adjacent environment of plant. According to Fischerová *et al.* (2006) heavy metal uptake is directly depend upon the initial concentration of metal available.

2.4.1. Cadmium Tolerance in Plants

Plants with bio-concentration value >1 are known as hyper accumulator. These plants accumulate excess concentration of metals from environment, and transfer it to their aerial parts, particularly leaves. Rascio and Navari-Izzo (2011), observed 450 species of plants as hyper accumulators for Cd, Cu, Co, As, Mn, Ni, Zn, Sb, Se, Pb, and Ti. Hyper accumulating plants have built in resistance to toxicity as their detoxification mechanism comprises on chelation or exudation that helps the plant to grow in stress conditions

(Yadav, 2010). Translocation of heavy metals like Cd, in hyper accumulating plants is different. This mechanism first restricts the mobilization of heavy metal ions from roots, followed by its detoxification in cytoplasm by excretion of chelates and transferring it to the aerial parts. (Rascio and NavariIzzo, 2011).

2.4.2. Effects of Cadmium on Macronutrient and Micronutrient

Numerous studies have shown that cadmium affects the plants by reducing the calcium concentration in leaves (Sandalio *et al.*, 2001). Cadmium accumulation in plant reduces the uptake of nutrient as follows; Mn (47%), Zn (41%), Cu (30%), Ca (27%), Mg (20%), Fe (19%) and it also increases the sulfur uptake up to 3 folds, while the Na was found to have no effect. In second phase of experiment $Ca(NO_3)_2$ was added, that causes 30% decline in cadmium accumulation in plant leaves (Rodríguez-Serrano *et al.*, 2009).

2.4.3. Effects of Cadmium on Plant Growth

Cadmium restrained lateral root growth while the top roots become mucilaginous, and causes the plant length to decrease (root + shoot) and hinders the formation of new leaves (Rascio *et al.*, 2008). It also results in abnormal growth of epidermal and cortical cell layers, meanwhile causing damage to the leaf structure due to low contents of chlorophylls, chlorosis and ending up on inhibition of photosynthesis (Miyadate *et al.*, 2011). Tran & Popova *et al.*, (2013) observed the disorders in root growth and the mitotic process due to cadmium toxicity. The effects of heavy metals on plants are different in different growth stages of plants. The reduction of root elongation was mainly due to cadmium induced de-polymerization of microtubules of cell's cytoskeleton, causing chromosomal aberrations and lowering the mitotic activity of meristematic cells (Fusconi *et al.*, 2006; Seth *et al.*, 2008). Cadmium effect on root elongation affect nutrient uptake (Chen *et al.*, 2003).

2.5. Effects of Heavy Metals

2.5.1. Effects on Cell Division

Cadmium directly affects the leaf cell division process (Tran & Popova *et al.*, 2013). It also results in abnormal mitosis (Aery *et al.*, 2012). Mo *et al.* (1992) showed the abnormal cell division under low concentration of 0.01, 1.0 and 10 ppm of Cd, Pb and Zn, respectively. Similar results have been reported by Zhang (1997) while investigating the effects of Cd, Hg and Pb on barley (*Hordeum vulgate*).

2.5.2. Effects on the Form of Chromosome

Heavy metal accumulation in plant causes genotoxicity during synthesis of DNA and chromosomes. Zhang (1997) described that cadmium combines with nucleic acid and damages the nucleolus's structure, causing chromosomal fragmentation, followed by aberration, conglutination and ending on its liquefaction. When beans, onion and garlic are treated with cadmium their chromosomal bridges break, chromosomal rings split, different chromosome fusion occurs followed by micro-nuclei and nuclear decomposition (Duan & Wang 1995).

2.5.3. Effects on Cell Membrane

Cadmium affects the enzymatic system of plant and increases the invasion of cell membrane (Li *et al.*, 1992). It triggers the uptake of O_2, H_2O_2 and malondialdehyde (MDA) in wheat (*Triticumaestivum L.*) leaves that result in liberation of electrolyte of the leaf cells. This indicate that the lipid peroxidation of cellular membrane was stimulated by active oxygen radicals (Luo, 1998).

2.5.4. Effects on Photosynthetic System

Photosynthesis is an essential mechanism for plant survival. Cadmium accumulation decreases the chlorophyll pigment at the leaf surface and directly alters the chloroplast functioning (Tran & Popova *et al.,* 2013). Chlorophyll pigment decreases significantly due to enzymes malfunction caused by cadmium contamination (Vassilev *et al.*, 1998).

2.5.5. Effect on Enzymes

Cadmium accumulation in leaves affects the enzymatic activity due to reduction in nitrate reductase in plant leaves (Sharma *et al.,* 2012). Plants have a protective enzymatic system that includes vital enzymes; peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) that helps plants to adapt the environmental stresses. Tran & Popova *et* *al.* (2013) observed variation in SOD, POD and CAT activities with increase in cadmium concentration.

2.5.6. Effect on Proteins and Amino Acids

Protein synthesis is reduced by cadmium accumulation in plants. There are two possibilities of less protein production i.e., either cell becomes unable to synthesize new protein or protein degrading rate becomes high. Balestrasse (2003) reported the decrease in protease activity at 200uM by cadmium accumulation. Cadmium stimulate the release of reactive oxygen species (ROS) in plant (Sobkowiak *et al.,* 2004). Shanthala *et al.* (2006) investigated that the total protein and glutathione decreases with an increase in heavy metal concentration.

2.5.7. Effect on Water and Sugar Contents Uptake

Farouk *et al.* (2011) observed that due to increase in cadmium concentration up to 150 mg/kg in soil, significantly affects water and sugar content of radish plants.

2.5.8. Effect on plant-water status

Absorption of cadmium inhibit root hair growth that ultimately reduces the water uptake by plant. Gouia *et al.* (2000) reported different plant species that shows the similar results. According to Barceló & Poschenrieder (1990), water uptake reduction occurs due to following reasons;

1. Reduced root growth
2. Reduction in permeability and conductivity
3. Inhibition of water movement and
4. Loss of cell structure

2.6. Aquatic plants for phytoremediation

Fatih *et al.* (2009) studied the watercress (*Nasturtium officinale*), for bioaccumulation characteristics and growth, exposed to cadmium, chromium and cobalt, and found that the most efficient uptake of Cd, Cr and Co occurred at external solution concentrations of 0.5, 5 and 10 mM, respectively. Narain *et al.* (2011) concluded that variety of toxic metals likes Cd, Cr, Pb and Fe mostly exceeds the threshold limits of

(WHO, 2006). Results shows the 80.26% and 71.28% removal efficiency for chromium and cadmium respectively.

Mokhtar *et al.* (2009) investigated the Phytoremediation of copper by pennywort (*Centella asiatica*) and water hyacinth (*Eichhornia crassipies*) and concluded that he pennywort plants remains healthy and survived well in wastewater at copper concentration of 2 mg/L which indicated the suitability of the plant as an accumulator in phytoremediation.

Maine *et al.* (2001) observed the linear correlation between initial concentration of cadmium and its bioaccumulation rates while studying the cadmium uptake by floating macrophytes. Cadmium was mostly accumulated in plant roots and it was linearly correlated to the cadmium concentration added.

Qin *et al*. (2011) investigated water lettuce (*Pistia stratiotes*) plant for uptake and distribution of metals by and suggested that its growth reduces Mn, Fe, and Al concentrations in water by > 20%, Cu and K by > 10% and Zn, Ca, Na, Zn, and Mg to a small extent.

Suchismita *et al.* (2014) conducted a study on water lettuce (*Pistia stratiotes*) for phytoremediation potential of cadmium, and concluded that water lettuce is a good hyper-accumulator of cadmium.

Bhat (2016) studied phytoremediation of iron contaminated soil by pennywort (*Centella asiatica*) and reported that it is hyper-accumulator plant species which efficiently accumulate iron metal in higher concentration at harvestable parts of the plant.

Chapter 3

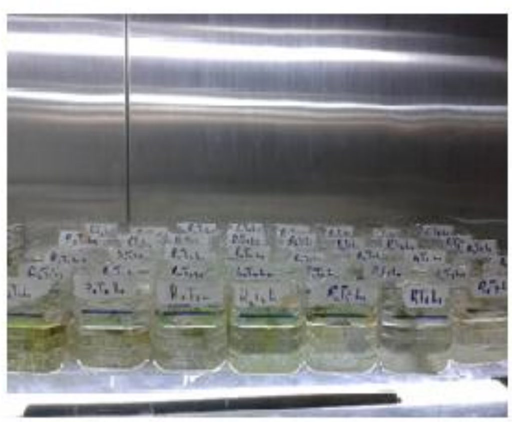
EXPERIMENTAL SET UP AND METHODOLOGY

3.1. Experimental Setup and its Preparation

Water lettuce (*Pistia stratiotes*) and pennywort (*Centella asiatica*) with about the same weight, length and size were collected from National Agriculture Research Centre (NARC) Islamabad, Pakistan. Similarly, Watercress (*Nasturtium officinale*) was collected from Rawal lake stream, Islamabad. The plants were thoroughly washed with tap water former to the experimentation. Watercress and pennywort were grown in 0.5 L experimental bottle while water lettuce were grown in jars. All of these bottles and jars were filled with 0.5 L of distilled water along nutrients solution and specific quantity of Hoagland solution was added in each bottle.

Pennywort (*Centella asiatica*)

Watercress (*Nasturtium officinale*)



Water lettuce (*Pistia stratiotes*)

Figure 3.1: Pictorial view of experimental setup inside the plant growth chamber

It was basically lab scale experimental study that was conducted in plant growth chamber. The given conditions were 25 - 30^{0}C temperature and 14 h light period with 30 - 40% humidity.

Figure 3.2: Pictorial view of control box of plant growth chamber

3.2. Composition of Hoagland solution

Reformed Hoagland solution for experimental setup was prepared as described by Taiz & Zeiger (2002). For each reagent stock solution was separately prepared by adding appropriate quantities of desired salts and mixed together to form the final nutrient solution. Plant needs two type of nutrients for survival i.e. macro (nutrients required in excess quantities) and oligo nutrients (required in lesser quantities). Composition of Hoagland's solution is given in table 3.1 and detailed preparation is described in later section.

Table 3.1: Proportions of composites of Hoagland's solution

Solution	Nutrient Salt	Amount of salt	Amount added in Experimental setup
Macro Nutrients			
Reagent A	Potassium nitrate (KNO_3)	101.1	5ml/L
	Calcium nitrate tetra hydrate ($Ca(NO_3)_2.4H_2O$)	236.15	
Reagent B	Potassium dihydrogen phosphate (KH_2PO_4)	13.61	5ml/L
	Magnesium phosphate hepta hydrate ($MgSO_4.7H_2O$)	49.29	
Oligo Nutrients			
Reagent C	Manganese sulfate hydrated ($MnSO_4.H_2O$),	1.69	1ml/L
	Zinc sulfate hepta hydrate ($ZnSO_4.7H_2O$)	0.57	
	Copper sulfate penta hydrate $CuSO_4.5H_2O$	0.25	
	Boric acid H_3BO_3	2.47	
	Sodium molybdenum oxide dehydrated $Na_2MoO_4.2H_2O$	0.024	
Reagent D	Disodium EDTA (Na_2EDTA)	33.3 (in 300mL)	1ml/L
	Iron sulfate hepta hydrate $FeSO_4.7H_2O$ + H_2SO_4	4.9 + 4mL (in 300mL)	

3.2.1. Macro nutrients

Macro nutrient solution comprise of two reagents namely, reagent A and reagent B.

3.2.1.1. Reagent A

For preparation of reagent A approximately 101.10 grams of potassium nitrate (KNO_3) and 236.15 grams of calcium nitrate tetra hydrate ($Ca(NO_3)_2.4H_2O$) were added in 1L volumetric flask and thoroughly dissolved in distilled water and the flask volume was filled with distilled water up to the mark.

3.2.1.2. Reagent B

Precisely 13.61 grams of potassium dihydrogen phosphate (KH_2PO_4) and 49.29 grams of magnesium phosphate hepta hydrate ($MgSO_4.7H_2O$) were measured and dissolve in 1L volumetric flask in distilled water and the flask volume was made up to the mark.

3.2.2. Oligo Nutrients

Oligo nutrient solution also comprises of two reagents namely, reagent C and reagent D.

3.2.2.1. Reagent C

For reagent C, 1.69 grams of hydrated manganese sulfate ($MnSO_4.H_2O$), 0.57 grams of zinc sulfate hepta hydrate ($ZnSO_4.7H_2O$), 0.25 grams of copper sulfate penta hydrate $CuSO_4.5H_2O$, 2.47 grams of boric acid H_3BO_3 and 0.024 grams of dehydrated sodium molybdenum oxide $Na_2MoO_4.2H_2O$ were dissolved in 1L volumetric flask containing distilled water and the volume was made up to the mark.

3.2.3. FeNaEDTA

3.2.3.1. Reagent A

For preparation of reagent A for FeNaEDTA, 33.3 grams of disodium EDTA (Na_2EDTA) were dissolved in 500ml of distilled water in 500 mL flask.

3.2.3.2. Reagent B

For preparation of reagent B for FeNaEDTA, 24.9 grams of iron sulfate hepta hydrate $FeSO_4.7H_2O$ were dissolved in 300ml of hot distilled water, then 4ml of 1N sulfuric acid H_2SO_4 was added.

After preparation of reagent A and B, reagent B was thoroughly added into reagent A and resulting solution was vigorously aerated for 12 h and final volume of the solution was made up to 1L by adding distilled water.

3.3. Preparation of Final Sample Bottles

Cadmium sulfate was added in distilled water along with Hoagland solution to get the required concentration level of 1, 2, 3, 4, 5, 10, 15 and 20 mg/L. Control level was also setup, by growing plant without adding cadmium. All the experimental sets had ten replicates. Samples were harvested after 21 days of exposure.

3.4. Cadmium Analysis of Shoots and Roots

For removal of moisture content first plant samples were separated into root and shoot (Figure 3.2) and oven dried at 60°C. Oven dried samples were then milled to powder with the help of pestle and mortar and passed through 2 mm nylon sieve.

Figure 3.3: Sampled plants separated into root and shoot

One gram of each sample was digested with Nitric acid (HNO_3) – Hypocaloric acid ($HClO_4$) in 2:1 ratio (v/v) using hotplate, and heated until the digester became clear. This digested material was then allowed to cool and filtered using Whattman filter paper 41. This filtrate was collected in 50 ml volumetric flask and diluted up to the mark with distilled water for preparation of 1, 2, 4, 6 and 8 mg/L solutions. These digested plant samples were analyzed using Atomic Absorption Spectrophotometer to detect concentrations of cadmium. The lamp wavelength used for Cadmium was 228.8 nm. Each sample was run thrice. Results are shown as mean ± Standard Error in results section.

Figure 3.4: Atomic absorption spectroscopy

3.5. Calculation of Bio-concentration Factor

Bio-concentration Factor (BCF) of metal acts as a tool to find out the content of heavy metals absorbed by the plant from the water/soil. This is an index of ability of plant to accumulate a specific metal with respect to its previous concentration in the water/soil (Ghosh and Singh, 2005a). It is determined using the following formula,

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue (whole plant)}}{\text{Initial concentration of metal in substrate (water)}}$$

If BCF value is greater than 1, it is considered that the plant is suitable for phytoextraction with higher BCF values.

3.6. Calculation of Translocation Factor

Translocation factor (TF) is a critical parameter for the assessment of the plant's potential for phytoextraction. It is a ratio that signifies the plant's potential to translocate metals in the plant, from the roots to the shoots (Marchiol *et al.*, 2004).

It is determined using the following formula,

$$TF= \frac{\text{Metal concentration in aerial part (shoot + leaves)}}{\text{Initial concentration of metal in substrate (root)}}$$

Metals after being accumulated by the plants, translocation factor values < 1 indicates the largely stored metals in plant roots, while TF values > 1 indicates the metal storage in plant shoots.

3.7. Chlorophyll Estimation

Chlorophyll content of fresh leaves was measured using the CCM-200 plus Chlorophyll meter in CCI units.

Figure 3.5: Chlorophyll estimation using CCM-200 plus Chlorophyll meter

3.8. Biomass Determination

Sampled plants were thoroughly washed with distilled water then oven dried at 80°C for 24 h to determine the biomass of each sample (in g $plant^{-1}$).

Figure 3.6: Pictorial view showing samples being dried in oven

3.9. Oil Extraction

For oil extraction samples were taken at intervals of 0, 6 and 12 days. These samples were oven dried at 60°C and were ground to fine powder. These processed samples were then added in Soxhlet apparatus for 5 hrs. at the boiling points of the solvent (n-hexane 69°C).

Figure 3.7: Soxhlet apparatus for oil extraction (Gerhardt - 1712-08-0069) at IESE-NUST

The ratio of solvent to solid is 10:1 (Flora *et al.,* 2011). In order to separate oil from n-hexane samples were run into rotary evaporator at 70°C and 30 rpm (rotations per minute). Then in order to separate pigments from oil 0.2 g of bleaching activated earth powder was added to bind pigments and centrifuged at 6000 rpm for 10 min.

The qualitative analysis of oil samples was carried out using energy dispersive X-ray Fluorescence Spectrometer (JEOL JSX-3202-M, Japan). Oil samples were directly analyzed by XRF without any sample preparation. The instrument software allowed simultaneous multi-element spectral measurement and qualitative elemental analysis.

Figure 3.8: X-ray Fluorescence Spectrometer (JEOL JSX-3202-M, Japan)

Chapter 4

RESULTS AND DISCUSSIONS

4.1. Comparison of Cadmium Effect on Plant Length

In comparison with control, slight increase in plant growth at 1mg/L and 2mg/L cadmium concentration has been shown in figure 4.1. After 2mg/L growth of all studied macrophytes start to decline. Increase in chlorophyll content at low metal concentration of cadmium on *Linum usitatissimum* has been reported in literature (Kavulicova *et al.*, 2012), that ultimately result in retarded plant growth.

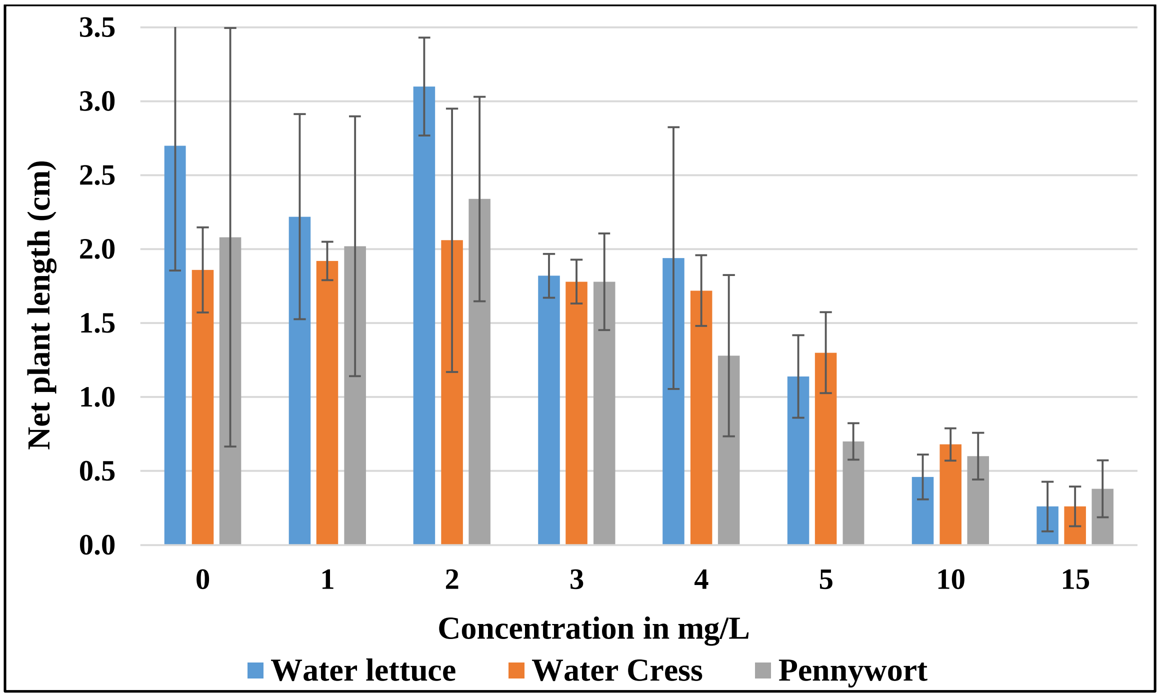


Figure. 4.1: Effect of cadmium over plant length of Water lettuce (blue), Watercress (orange) and Pennywort (grey)

Present study showed contradiction with literature as an increase in plant growth and decease in plant biomass has been observed. This may be because of following reason;

1. Present study was conducted in plant growth chamber with less light intensity, which lead to plant stability and growth in early days.

2. According to Arshad *et al.* (2015) photosynthetic pigment tend to increase at cadmium concentration of 1mg/L in first three days and after that it starts declining. This result in increase of plant growth in first few days.

Above mentioned mechanisms caused reduction in plant biomass due to wilting and necrosis of leaves but its length remained the same. Macrophytes used in this research, responded well to higher cadmium concentrations, this behavior authenticates the available previous literature. Mishra & Tripathi (2008) observed stunned plant growth due to cadmium effect on nutrient uptake and chlorophyll synthesis. Aslan *et al.* (2003) reported inhibition of plant growth at higher concentration of cadmium while treating *N. officinale* with 0.5mg/L and 5mg/L cadmium concentration in Hoagland solution for 2 weeks. Aydin & Coskun (2013) also observed 21% increase in chlorophyll at 1mg/L chromium level that resulted in retarded plants growth. Faith *et al.* (2009) reported increase in *N. officinale* growth at 1M arsenic concentration and effects like chlorosis at high concentrations. The results of the present study revealed that an initial exposure of cadmium up till 2mg/L helps increasing the plant growth but on increasing concentrations plant growth stats decreasing gradually.

4.2. Comparison of Cadmium Effect on Plant Biomass

The biomass production of macrophytes observed to decrease significantly with increasing cadmium concentration. Figure 4.2 shows typical response of biomass reduction by cadmium exposure. Watercress (*N. officinale*), water lettuce (*P. stratiotes*) and pennywort (*C. asiatica*) showed 28, 37 and 14 minimum and 70, 78 and 46% maximum biomass reduction respectively at 1 mg/L and 15 mg/L of cadmium concentration. The acquired results are well supported by existing literature.

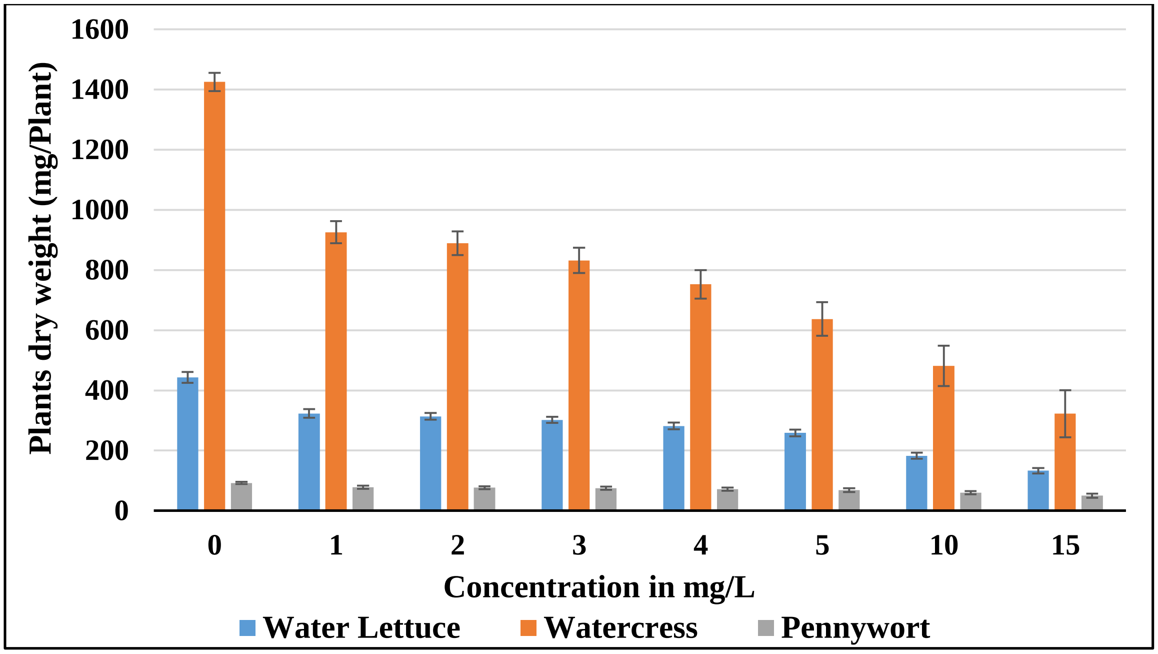


Figure. 4.2: Effect of cadmium over plant biomass of Water lettuce (blue), Watercress (orange) and Pennywort (grey)

Paivoke & Simola (2001) and Kara (2005) reported the remarkable biomass reduction during long term cadmium exposure. Aydin & Coskun (2013) also observed biomass reduction in plant biomass with increase in chromium concentration from 1 to 10 mg/L.

According Barceló *et al.* (1993) and John *et al.* (2012) bioaccumulation of pollutants cause reduction in plant biomass by

1. Lipid peroxidation and protein fragmentation due to ROS liberation and
2. Disturbance in nitrogen and carbohydrate metabolisms

4.3. Comparison of Cadmium Uptake by Macrophytes

Plants mechanism of metal uptake includes absorption of heavy metal in roots, transport to xylem tissue, translocation to aerial parts or sequestering in root after detoxification (Lombi *et al.*, 2002). In present study, highest cadmium accumulation was observed at 10 mg/L for water lettuce and 15 mg/L for watercress and pennywort.

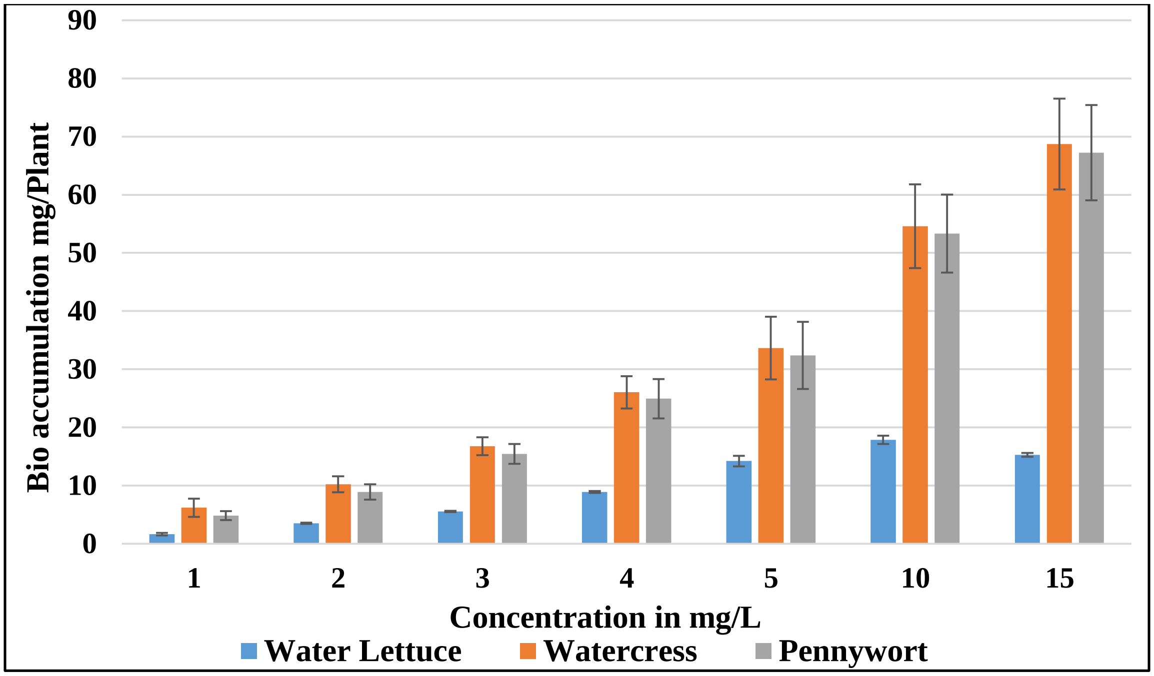


Figure. 4.3.1: Cadmium uptake by macrophytes Water lettuce (blue), Watercress (orange) and Pennywort (grey)

According to Muramoto & Oki (1983), maximum uptake of 36 mg/kg (dry weight) cadmium uptake was observed in *Eichonnia crasipies* while Liu *et al.* (2007) showed minimum cadmium accumulation ranging from 4.98 (*P. cumminis*) to 36.3 mg/kg of dry weight (*M. vaginalis*) after 2 months' treatment. Zayed *et al.* (1998) found 13g/kg metal uptake at 10mg/L cadmium level. Similarly, 0.39, 0.31 and 0.25 mg/g cadmium accumulation was observed in *P. stratiotes*, *E. crassipies* and *S. polyrhiza* (Mishra & Tripati, 2008). Peng *et al.* (2008) showed the cadmium uptake of 202 and 178 mg/kg of dry weight by *P. pectinatus* and *P. malainus* respectively.

The difference between accumulation by root and shoot shows the important inhibition of sequestration or mobility of pollutant or contaminant from root to shoot. Maximum cadmium uptake has been showed at 10mg/L for *P. stratiotes* and 15mg/L for *N. officinale* and *C. asiatica* of 13, 24.2 and 13 mg/kg of dry weight respectively. According to Das & Goswami (2014), *P. stratiotes* inhibit the cadmium uptake at higher concentration due to saturation. Turgut *et al.* (2004) reported that metal uptake depends upon type of species, type of chelator and its initial concentration.

Maximum bioaccumulation in shoot was observed at 15mg/L treatment. The macrophytes plants would directly affect the available biomass for storage and translocation as well as susceptibility to toxicity and resistance. Bioavailability was influenced by the chelator type and source as well. Sivaci (2004) reported the maximum bioaccumulation of 80 (*M. spicutum*) and 150 mg/kg of dry weight (*M. triphyllum*) in shoot.

4.4. Bio-concentration Factor of Metal in Macrophytes

As described in chapter 3, bio-concentration factor value is the ratio of metal accumulated by plant to initial concentration of metal in solution. It is a basic parameter that shows the ability of plant to remove pollutant. BCF values of water lettuce (*P. stratiotes*), watercress (*N. officinale*) and pennywort (*C. asiatica*) are mentioned in figure 4.4.

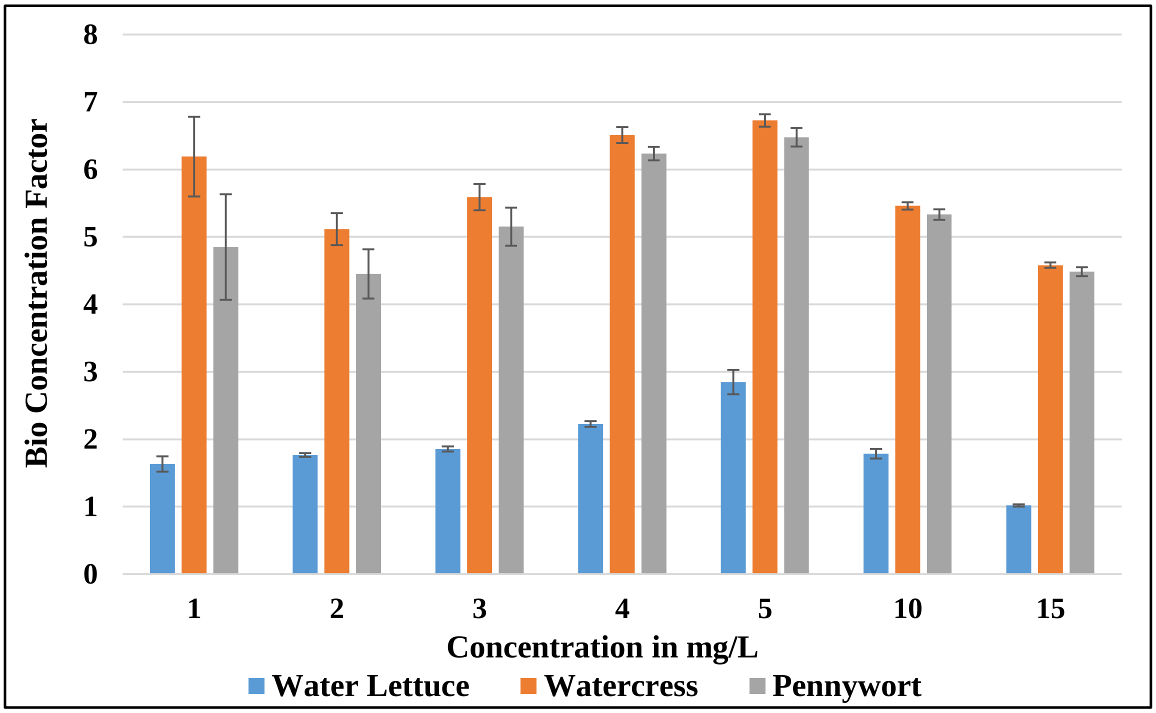


Figure. 4.4: Bio-concentration factor of macrophytes Water lettuce (blue), Watercress (orange) and Pennywort (grey)

All studied macrophytes have BCF > 1, so they may be utilized for phytoremediation in future. According to Blaylock *et al.* (1997), plant is considered suitable for phytoextraction if its BCF vale is > 1. Heavy metal accumulation may vary with in plant species of same genus (Singh *et al.*, 2003). BCF values for *E. crasipies* was reported as 0.62 (Lu *et al.*, 2004) and 0.65 (Zhu *et al.*, 1999) which shows variation in BCF value by experimental conditions even in same plant. Zayed *et al.* (1998) concluded that BCF greater than 1 is a sign of phytoremediation potential. BCF value of cadmium is 0.65 in *L. polyrrhiza* (Jain *et al.*, 1990), 1.7 in *E. nuttalli* (Nakada *et al.*, 1979), 2.4 in *A. pinnata* (Sela *et al.*, 1989), 2.7 in *E. aquaticum* (Miller *et al.*, 1983), 6 in *M. exalbescens* (Franzin & McFarlane 1980), 4 in *B. monneri* (Sinha & Chadra 1990) and 1.5 in *E. aciculari* (Ha *et al.*, 2011).

4.5. Translocation Factor of Metal in Macrophytes

Furthermore, the plant's potential to translocate metal from root to aerial parts is measured by translocator Factor, which is metal ratio in root to shoot. According to Luo *et al.* (2005) TCF >1, then plant is able to translocate metal from root to shoot and leaves.

Figure 4.5 illustrates that *P. stratiotes* had TCF < 1, while other two macrophytes were able to translocate metal from root to aerial parts. Jayaweera (2008) reported that some physiological activities inhibit the metal translocation.

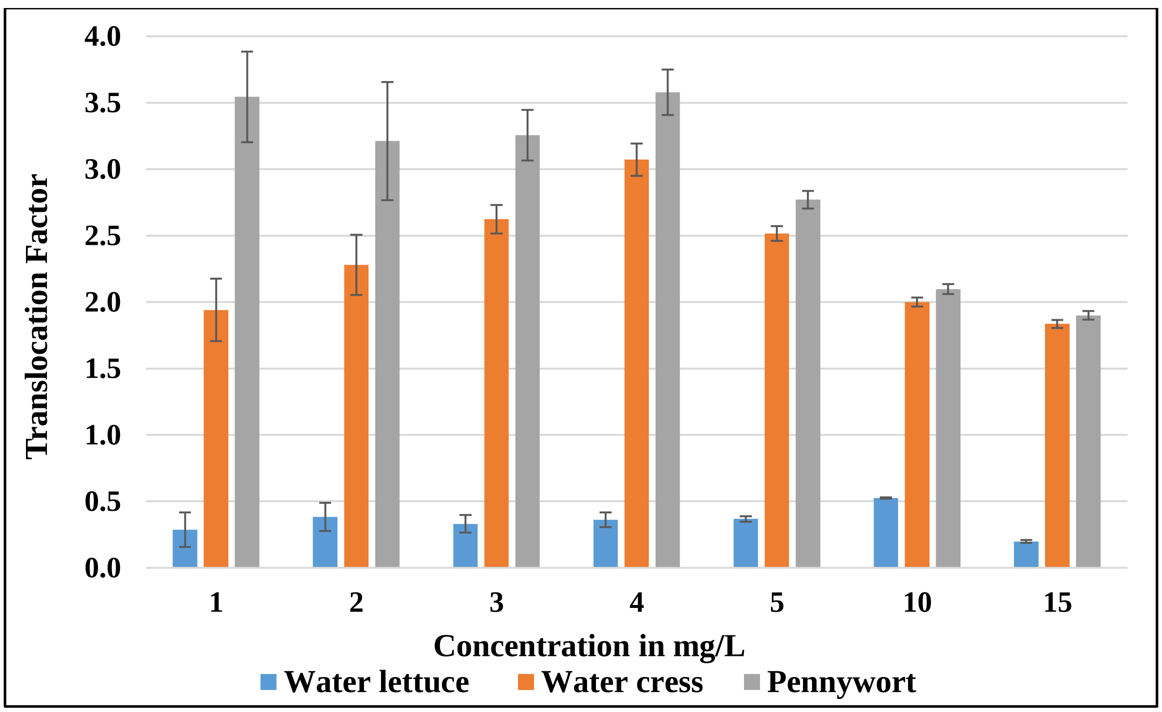


Figure. 4.5: Translocation factor of macrophytes Water lettuce (blue), Watercress (orange) and Pennywort (grey)

4.6. Comparison of Cadmium Effect on Photosynthetic Pigment: Chlorophyll

All of three macrophytes expressed considerable sign of heavy metal toxicity on chlorophyll content. Chlorophyll was measured using CCM-200 plus Chlorophyll meter on 21st day, as described in section 3.6. Cadmium concentration of 15 mg/L showed maximum and 1 mg/L showed minimum effect on photosynthetic content of the plants.

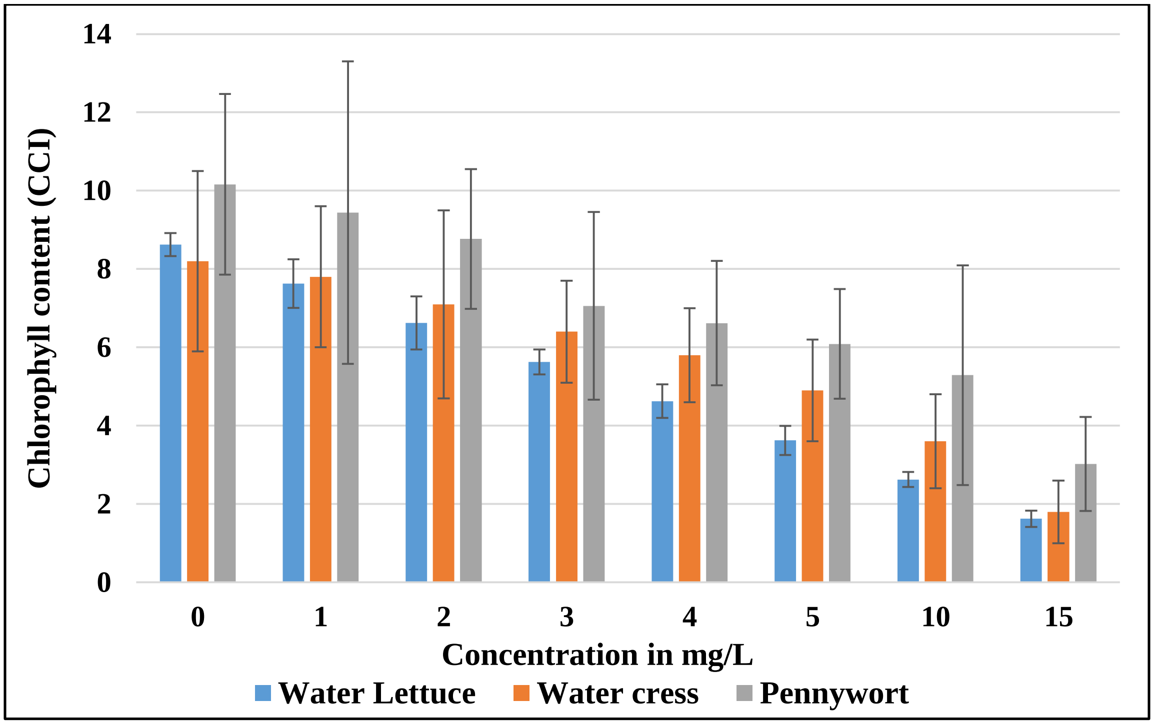


Figure. 4.6: Effect of cadmium over chlorophyll content of Water lettuce (blue), Watercress (orange) and Pennywort (grey)

Physiological parameter of all studied macrophytes were affected by the cadmium even at low concentrations. *Nasturtium officinale*, *Pistia stratiotes* and *Centella asiatica* showed negligible effects at 1mg/L, that is slightly different from previous research works because of low light intensity in plant growth chamber. Guimarães *et al.* (2011) reported prominent decrease in total carotenoid and chlorophyll content as one of the primary symptoms of toxicity of plant being exposed to various stress agents, including toxic metals and metalloids. Dhir & Srivastava (2013) showed similar results while studying *Salvinia* *natanus* exposed to Cd, Cu, Fe, Zn, Co and Cr at 10mg/L but the decrease in chlorophyll synthesis was reported only due to cadmium accumulation.

Rolli *et al.* (2014) suggested that the introduction of phyto-chelatin helps increasing chlorophyll synthesis. Moreover, at 25 mg/L concentration of cadmium, chlorophyll inhibition and yellowing of leaves occurred (Sanitta & Gibberelli, 1999) due to inhibition of stomatal closure and conductance (Marchiol, 1996). Plant leaves treated with higher concentrations of cadmium were observed to become smaller, curled and dis-colored. Whereas a slight stimulation in other plant organs have also been recorded at the lower cadmium concentrations. This result is consistent with previous studies as in research reported by Aslan *et al.* (2003) reported a significant reduction in chlorophyll concentrations in *N. officinale* leaves after 14 days of exposure to 1.0 and 5.0 ppm cadmium.

According to Pätsikkä *et al.* (2002) the decline in chlorophyll pigment in plants is due to the following three factors;

1. Inhibition of chlorophyll synthesis enzymes
2. Peroxidation of chloroplasts due to heavy metal stress
3. Generation of metal substituted chlorophyll

4.7. Comparison of Oil Production

4.7.1. Oil Quantity

Macrophytes samples were treated with similar cadmium concentrations and oil extracted in a Soxhlet apparatus using n- hexane. By acquiring data, significant reduction in oil amount was observed.

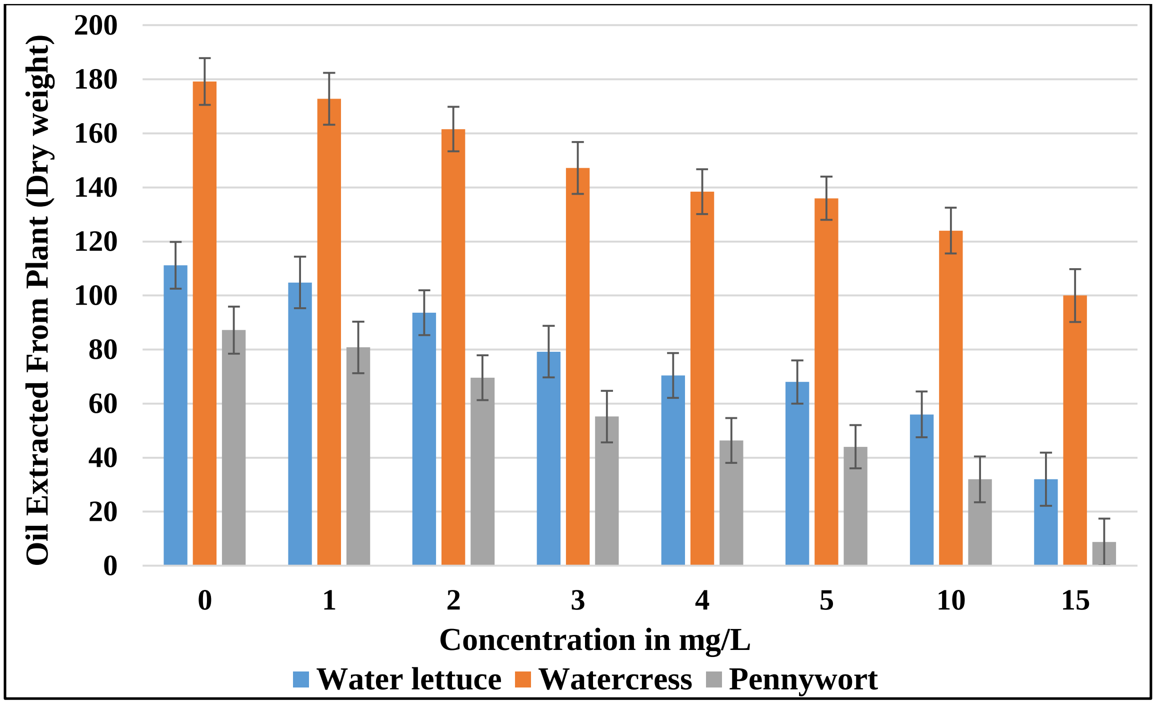
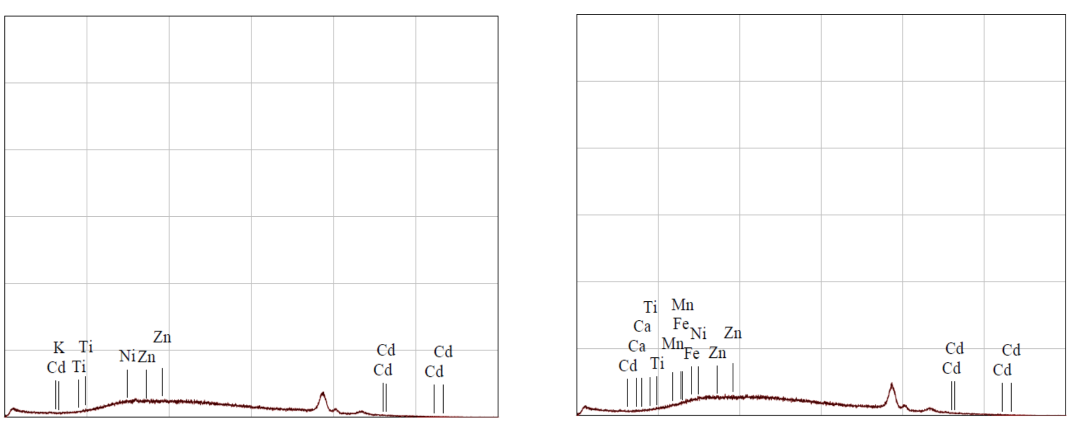


Figure. 4.7: Quantity of oil produced from macrophytes Water lettuce (blue), Watercress (orange) and Pennywort (grey)

4.7.2. Oil Quality

Cadmium was not detected in oil extracted from all studied macrophytes up to 10 mg/L of cadmium concentration. But at 15 mg/L, 42, 27 and 22% cadmium was detected in water lettuce (*P. stratiotes*), watercress (*N. officinale*) and pennywort (*C. asiatica*) respectively. XRF out puts of all three macrophytes at 15 mg/L are shown in figure 4.8.

(a) Water lettuce (*P. stratiotes*) **(b) Watercress (*N. officinale*)**



(c) Pennywort (*C. asiatica*)

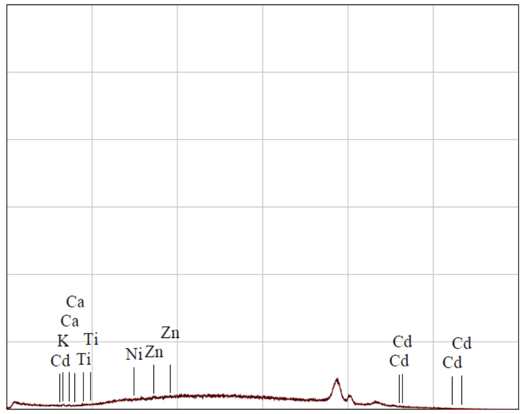


Figure 4.8: XRF outputs of oil samples of macrophytes showing nutrient composition (a) Water lettuce, (b) Watercress and (c) Pennywort

XRF analysis showed the presence of cadmium with other elements including potassium, titanium, nickel and zinc in water lettuce (*P. stratiotes*), calcium, nickel, manganese, iron, titanium and zinc watercress (*N. officinale*) and potassium, calcium, titanium, nickel, and zinc in pennywort (*C. asiatica*) at 15mg/L cadmium concentration oil samples. Since the oil extracted from all macrophytes contains cadmium at 15mg/L thus it cannot be utilized for oil production while phytoremediation at high concentration.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Industrial wastewater containing heavy metals like mercury, lead, zinc, chromium, cadmium, silver, arsenic, copper and iron are discharged into fresh water without any primary treatment. In present study *P. stratiotes, N. officinale and C. asiatica* has been tested for their ability to uptake cadmium. Results showed that the phytoremediation potential of used macrophytes increased with increasing Cd treatment level up to 17.8, 68.7 and 67.3 mg/kg of dry weight at 15mg/L for *P. stratiotes, N. officinale and C. asiatica* respectively. Remarkable inhibition in length growth was also observed. Moreover, by the end of three weeks tested macrophtes showed signs of remarkable stress by reduction in biomass from 29, 37 and 15% to 70, 78 and 47% at 1mg/L and 15mg/L Cd treatment for *P. stratiotes, N. officinale and C. asiatica* respectively. Similarly, chlorophyll content was observed to decreas from 2.4, 2.6 and 7% to 49, 60 and 43% in *P. stratiotes, N. officinale and C. asiatica* at I mg/L and 15 mg/L. *P. stratiotes, N. officinale* and *C. asiatica* showed minimum BCF value 1.02, 4.58 and 4.48 at 15mg/L which are greater than 1, so these plant can be used for toxic heavy metal accumulation. Meanwhile *P. stratiotes* showed maximum TF value 0.5 at 10 mg/L that is less than 1, while *N. officinale* and *C. asiatica* has minimum TF value of 1.8 and 1.9 at 15mg/L, which indicate less harvesting of metal from aerial parts in *P. stratiotes* as compared to other tested species. Oil extracted from all examined macrophytes showed cadmium contamination of 42, 27 and 22% at 15mg/L Cd concertation. Hence, it is apparent from this work that phytoremediation of Cd is promising by all studied macrophytes. Numerous studies highlighted the importance of use of aquatic plants as phytoremediation for polluted water and soil sites. Even though several aquatic plant has shown high bioaccumulation of Cd but final disposal of those plants after phytoextraction is a critical concern. There should be proper solution of this problem for the successful implementation of solar driven technique like valuable metal extraction by ash method.

5.2. Recommendations

Phytoremediation is effective and ancient treatment strategy because there is no need of secondary energy supply. This fact makes phytoremediation a cost effective technology. It is mostly known as solar driven pump because during transpiration plant release one molecule from aerial part and intake one molecule from root surroundings, meanwhile plant uptake the nutrients and pollutants. Phytoremediation plants after accumulation of heavy metals are considered as toxic and hazardous. Now a day, there is no term of waste, everything is a source.

There are several methods and techniques of utilizing plant after heavy metal uptake, for example, ash of water hyacinth (*E. crassipes*), can be utilized for extraction of valuable metals from water system Mahmood *et al.* (2010). This approach opens new economic corridors instead of burden as hazardous waste. There should be prior treatment of wastewater by potential hyper accumulator at treatment pond, so that it increases the accumulation rate due to more exposure.

Observing the findings of this study, few recommendations can be given as;

1. There should be further investigation of cadmium effect on plant growth and stress.
2. Oil quality should be examined thoroughly for cadmium contamination and threshold limit identification.
3. Phytoremediation trend should be encouraged for heavy metal contamination removal.
4. Further research should be carried out for the proper disposal of used macrophytes.

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