BIOSORPTION AND PHYTOREMEDIATION OF HEAVY METALS FROM WASTEWATER

By:

Amjad Hasnain
(62-FBAS/MSES/F09)

Supervised By:

Dr. Syed Shahid Ali
Foreign Professor (HEC)

Department of Environmental Science
International Islamic University, Islamabad
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Dr. Syed Shahid Ali
Foreign Professor (HEC)

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Department of Environmental Science
International Islamic University, Islamabad

بسم الله الرحمن الرحيم

DEDICATION

I dedicate my work

to

My beloved Parents

ABSTRACT

Fresh water resources are depleting due to anthropogenic contamination caused by ever-increasing domestic effluents, industrial discharges, and continuous use of chemicals in agriculture. Besides water conservation, wastewater treatment for its re-use is a globally adopted phenomenon. In Pakistan, Sugar manufacturing industry produces a large amount of wastewater that contains different chemicals and various heavy metals. These heavy metals are very toxic to human being and also cause water and soil pollution. The objectives of this study were: to find a cost-effective phyto-remediation and biosorption method to reduce heavy metal contamination. For this purpose, both field experiment and pot experiments were used to study heavy metals (Cd, Cr and Pb) uptake from wastewater produced from sugar-industry by using an indigenous plant species *Euphorbia prostrata*.

According to the results, Plants *Euphorbia prostrata* were grown on different concentrations (100%, 50% & 0%) of wastewater in controlled conditions of 23°C, 12hr. dark and light cycle, and same soil type. Distilled and tap water collected from the same area was used as control to grow plants in the laboratory. Experiments were set up with three treatments, and control with each treatment with three replicates. Three harvests were taken after 15, 30 and 45 days of germination of plants. Plants grown on wastewater show reduced growth on fresh weight (80%) and dry weight (50%) basis as compared to plants grown on tap and distilled water. In phyto-remediation experiment, plants germinated on wastewater in field accumulated higher amount of heavy metals from waste water (between 20-55%) over a period of 45 days. However, In Biosorption experiment, biomass collected from plants germinated in lab on tube well water adsorbed higher amount of heavy metals (>70%) as compared to heavy metal containing aqueous solution (control).

Phyto-remediation and *Biosorption* techniques are cost-effective methods for heavy metal uptake and removal from contaminated sites. This study suggests that the use of *Euphorbia prostrata* and *Biosorption* techniques by using biomass could be promising techniques to reduce amount of heavy metals from wastewater.

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"He who does not thank to people is not thank to Allah"

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Amjad Hasnain

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RESEARCH COMPLETION CERTIFICATE

It is certified that the research work entitled "**Biosorption and Phytoremediation of Heavy Metals from Wastewater**" has been completed by **Amjad Hasnain** (62-FBAS/MSES/F09) under my supervision during his **MS in Environmental Science.**

Date: January 10, 2012

Dr. Syed Shahid Ali
Foreign Professor (HEC)
Department of Environmental Science
International Islamic University, Islamabad

I. INTRODUCTION

Wastewater is a type of water that has been adversely affected in quality by anthropogenic influence and is commonly discharged by domestic residences, commercial properties, industry, and agriculture (EPA, 2000). It encompasses a wide range of potential contaminants with toxic concentrations and results in the degradation of both human health and environment. In the most common usage, it referred to as "municipal wastewater" that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources (Alves *et al.,* 1993). Wastewater may contain organic and inorganic compounds, pesticides, various salts, heavy metals and other pharmaceutical components, either individually-occurring or in a complex, depending on the source.

Industrialization and their wastewater generation have been implicated in the pollution of waterways, soil and underground water. The presence of heavy metals in wastewater poses greatest threat to both plants and animals at certain concentrations and could become part of human food chain, as well (Singh & Prasad, 2011). Sugar Industry is one of the major component of Pakistan's agricultural economy However, wastewater generated during manufacturing of sugar or other products may result in pollution due to the presence of different kinds of heavy metals (Stensel, 2003). Heavy metals as trace element are necessary for human metabolism, for example, Chromium (Cr) is important for the metabolism of fatty acids, glucose and protein (Singh *et al.*, 1998). However, their higher concentration could lead to various harmful health effects in human through food chain.

Lead (Pb), mercury (Hg), arsenic (Ar), zinc (Zn), copper (Cu), nickle (Ni) and ferrous (Fe) are the most widely reported toxic heavy metals in literature. Keskinkan (2003) suggested that heavy metals such as zinc, copper, lead and mercury are the causes of environmental problems and harmful to human health. These heavy metals are very toxic to aquatic animals and plants and are detrimental to human health, as well. Strong exposure of heavy metals causes cancer in digestive tract and lungs and may cause epigastria pain, nausea, vomiting, severe diarrhea and hemorrhage. Heavy metals such as chromium (VI) produce immediate cardiovascular shocks, and laterally, it affects kidney and blood forming organ. However, the link between cancer development and heavy metals is still under intensive investigation (Keskinkan, 2003). In another study on presence of heavy metals in wastewater, Chandra *et al.*, 1997 reported that the heavy metals presence in surface water is a problem of the entire world mostly developing countries. The persistent nature of heavy metals and their accumulation capability in any compartment of the environment makes it a major issue to all forms of flora and fauna.

Different sources of heavy metals in wastewater that include different industries, such as tanneries, textile manufacturing, paint, ink and dye industry, aluminum manufacturing industry, sugar industry, chemical industry, and urban sewage treatment plants. It is important to reduce heavy metals from water to avoid health problems. However, few studies have been conducted on the presence of heavy metals and their non-biodegradability and consequent persistence in wastewater (Soltan and Rasheed, 2003). Various techniques have been suggested for reduction of heavy metals from wastewater. It includes the use of activated carbons; chemical precipitation with lime or caustic soda; and biosorption (Wase and Forster, 1997). Ajmal *et al* (2000) conducted a study for the removal of heavy metals from wastewater by using activated carbons but method found very expensive. Other methods proposed are: ion exchange, electrolysis and reverse osmosis, although these techniques have been found effective but are suggested as costly (Ajmal *et al.*, 2000). Living organisms such as bacteria can also be used to reduce metal ions from wastewater of energy producing reactors (Friis, 1998).

Most chemical or physical engineering technologies have failed to remove heavy metals from effluents, hence; alternative techniques are being evaluated in pollution control programs all over the world. One of these techniques comprises a biological tool called as bioremediation or the use of living material such as plants and microbes or their biomass, for remediation purpose. Biologists have come up with most promising techniques, such as, biosorption and phytoremediation. These techniques are eco-friendly, cheaper and are of great importance in reduction of heavy metals from wastewater. In both techniques, natural organisms or artificially grown living organisms on inexpensive media are used (Goyal *et al.*, 2003). *Biosorption* is an emerging technique to reduce heavy metal from wastewater. The structure of outer wall of microbes, small and large plants is particularly responsible for the accumulation of heavy metals (Volesky, 1990). The capability of plant biomass to accumulate heavy metals, from water through binding from solution or irrigation water, has become an important technique to decontaminate wastewater containing heavy metals (Modak and Natarajan, 1995; Gupta *et al.,* 2001).

More recently, genetically engineered heavy metal-accumulating plants for environmental cleanup has also become an emerging technique called as *Phyto-bioremediation.* It has been proposed that Phytoremediation is an important technique to remove heavy metals especially from wastewater (Raskin and Ensley, 2000). Plants can accumulate a large amount of heavy metals from wastewater. However, most of the studies regarding to Phytoremediation of heavy metals are limited to a few species of plants, i.e., *Ceratophyllum demersum* (Foroughi *et al.,* 2011), *Durvillea potatorum* and many other species can be used in biosorption and phytoremediation (Tripathi, 2008). It is important that indigenous plants are explored for their remediation potential. Shad *et al* (2008) conducted a study and successfully used *Euphorbia helioscopia,* for phytoremediation. However, other Euphorbia species commonly found in Pakistan have not been evaluated for their phytoremediation potential.

Euphorbia prostrata (commonly known as Prostrate Sand mat) belongs to family euphorbiaceae is native to West Indies and some parts of South Africa. Now it is widely distributed all over the world and grows as a roadside weed in sub-continent and in Pakistan, as well. It is an annual herb producing slender prostrate stem up to 20 cm long. Its leaves are oval shaped 1 cm wide with finely toothed edges. Inflorescence is cyathium. Flower is with white petals. The fruit is lobed, hairy and one or 2 mm wide. It has great medicinal importance due to the presence of flavonoidal and phenolic constituents with anti-inflammatory, analgesic, haemostatic and wound healing properties. Its usefulness in the treatment of anorectal and colonic diseases, such as hemorrhoids, fissures, cracks, fistulas, abscesses and inflammatory bowel disease, has been reported as well. The novel composition of this plant has the properties to control inflammation, prevent capillary bleeding and fragility in mammals' particularly human beings (Ahmad and Abdus, 2011).

As wastewater is rich in heavy metals and due to their presence and use in irrigating sub-urban agriculture or through seepage to the ground water, hence contaminating ground water has been reported as major catastrophes. Due to industrialization and pollution of biosphere as a whole, Ar, Cd, Hg, Pb, Cr, are also present in soil. Because of increase in number chemical manufacturing industry, such heavy metals are taken up by soil from waste water released from fertilizer and pesticides factories (Hunt, 2003, water treatment plants and other human activities (Chen, 1992). Although, metals are essential micronutrients of plants, their hyper-accumulation from soil and water could be detrimental to plant health. Sugar cane crop require irrigation and need input of different fertilizers, i.e., Phosphoric fertilizers that contain significant amount of heavy metals. It has been reported that sugar-cane plants accumulate these heavy metals in their mass and during crushing these metals flow in wastewater through split wash. (McLaughlin *et al.,* 1996).

In Pakistan, no special attention has been paid to reduce heavy metals from wastewater. Heavy metals in wastewater are causing very serious health problems. Besides causing problems to human health, the presence of heavy metals in wastewater is also harmful to the ecology of aquatic life, microbes, algae and fungus. There are many techniques to reduce heavy metals from wastewater such as activated carbons, chemical precipitation with lime or caustic soda. However, all these techniques are time consuming and very costly. Biosorption is also a technique to reduce heavy metals from wastewater. This technique is simple and cheaper. In LAYYAH, the wastewater of sugar industry is contaminated with heavy metals. This wastewater is causing contamination of underground water, soil pollution and diseases to local community. The present study has been designed to investigate the potential of biosorption and phytoremediation in order to reduce the heavy metals in wastewater. For that purpose, *Euphorbia Prostrata, a*n indigenous plant species and its biomass was evaluated for its potential to remediate polluted soil and water.

Objective of Study

The objectives of the study are:

- To determine heavy metals present in wastewater released from sugar industry
- To quantify the amount of heavy metals in soil and water at specific distance from source, i.e., sugar industry
- To optimize the amount of heavy metals uptake by *Euphorbia Prostrata* from wastewater through phyto-remediation.
- To evaluate the capability of biomass from *Euphorbia Prostrata* for heavy metals uptake through biosorption technique.

Justification of Study

Heavy metals in wastewater are very harmful to human being as well as for aquatic animals and plants so their removal is must from wastewater. There are various techniques for removal but biosorption is a cheaper method for the removal of heavy metals. It is easy to treat wastewater by plants instead of other methods that are very expensive and difficult to conduct. If the study conducts at acidic pH then this process is more efficient and can remove unto 95% of heavy metals. Plants accumulate heavy metals in the cell. The chemical composition is also an important factor for absorption of heavy metals. The biosorption should be done at low pH because it is more effective in biosorption.

II. REVIEW OF LITERATURE

In order to discover new methods and techniques for the reduction of toxic metals from wastewaters, attention has been focused to alternate methods other than chemical or physical techniques. Phytoremediation through bioaccumulation and biosorption by using biomass are natural processes that are fast becoming the method of choice in remediation science. Both of these techniques have shown promising results with fewer falls out when compared with other techniques, i.e., chemical precipitation, ion exchange or other conventional physical methods.

A. Biosorption Technique

Biosorption is the capability of plant biomass to accumulate heavy metals from source i.e., wastewater, through accumulation or sorption process, (Roux and Fourest, 1992). In biosrption technique, two phases are involved: one is solid phase and the other is liquid phase. Sorbent accumulate heavy metals from liquid phase until equilibrium is reached. Due to the chemical make-up of the microbial cells-walls, some microorganisms have also shown strong biosorbent behavior towards metallic ions. Plant scientists have been using easily available biomass as a first choice for biosorption and have been developing genetically engineered plants with extra capabilities for biosorption, as well. Such biosorption techniques are being used for remediating contaminated soils, waste materials and waste in general. It has been reported extensively that biosorption of heavy metals is an important technique for the treatment of wastewater. Various organisms involved in biosorption are discussed here:

a. Biosorption by microorganisms

Microorganism can be used for biosorption because living or dead microbial cell or its products are good accumulators for both soluble and particulate heavy metals. Surface of microbial cell is negatively charged therefore, microorganisms have the capability to bind heavy metals.

Investigation on recombinant bacteria for removal of heavy metals is also carried out. For example a genetically changed *E.coli,* which have mercury transport system and metallo-thionin (specific protein which bind metal ions), and can reduce mercury by its cell wall. The presence of chelating agents, i.e., Na^{+}, Mg^{2+} and Ca^{2+}, did not affect bioaccumulation (Ahalya, *et al.*, 2003). Biomass of micro organisms have very fine size and possess a charge, which increase their ability to adsorb metals ions from wastewater. A study was conducted by Veglio *et al.*, 1996 on sorption of different toxic heavy metals such as Cu, Mn, Ni and Pb by using *Arthrobacter* sp. It was reported that this organism can take up to Mn (406mg/g), Cu (148mg/g), Ni (13 mg/g) and Pb (130mg/g).

b. Biosorption by using algae

Algae are found in abundance in fresh & surface waters and is considered a cheaper raw material for Biosorption, i.e., seaweeds of ocean. Gupta *et al.,* 2001 conducted a study on Filamentous algae *Spirogyra* as a biosorption source and concluded that it is very useful for biosorption. It was found that sorption of chromium was rapid on initial time and became constant after 120 minutes. The adsorption of chromium by 5g algal dose was 12 to 30 % and an increase up to 98 % was achieved with further addition of biomass. Biosorption is a very low cost method for removal of metallic ions. This method can be used for municipal wastewater and for industrial wastewater. Various studies have concluded that in biosorption the metabolically mediated or pure physiochemical pathway techniques of plants can also be utilized.

c. Biosorption by using fungi

Fungus has also been selected by different researchers to investigate its potential for biosorption from wastewater. Higher fungi, yeast, bacteria, seaweeds and plant bark are present in nature abundantly and can be used for Biosorption as an efficient alternative to conventional methods (Srivastava and Thakur, 2007). Del'Mundo & Babel (2008) used *Asperigllus niger* for biosorption of heavy metals from sludge. It was reported that factors that affected the Biosorption were pH, contact time with biomass and form of metal. Furthermore, Cr & Zn were removed to 100% wheareas 94% Ni was biosorbed from industrial sewage. Among *Aspergillus niger*, *Aspergillus versicolous* and Hirsitulla species the ability of *A. niger* to remove chromium from waste water was found better. Hirsitulla remove chromium up to 60%. *A. niger* uptake chromium in mycelium 11.6 mg/g dry weight (Srivastava and Thakur, 2005).

d. Biosorption by using macrophytes

The use of Aquatic macrophytes and their biomass have been in use for biosorption purpose since time immemorial. It has been reported, extensively that aquatic plants can accumulate thousands times more heavy metals than the surrounding aquatic environment (Schneider *et al.,* 1999). Similarly, Plants have also been reported as an effective accumulator of heavy metals from wastewater. Veglio and Beolchini (1997) conducted a study and concluded that removal of metals by biosorption follow the pathway of extracellular accumulation, cell surface sorption and intracellular accumulation. Schneider (1999) conducted a study on biosorption in order to determine whether it was exchanged, adsorbed or precipitated. It was reported that plants accumulate metal ions on their surface and metals could attach with cell wall, in roots of plants or could be hyperactively accumulated. In a study on *Ceratophyllum demersum* plants for their biosorption capability of heavy metals, it was found that accumulation largely found in the aerial parts, i.e., leaves. (Faroughi *et al.*, 2011). Mishra and Tripathi (2008) reported the use of aquatic macrophytes for heavy metals removal and assesed their removal capacity. They reported that heavy metal can be removed up to 77 to 95 % by using *Euphorbia crassipes*. Keskinkan (2003) conducted a study on the biosorbtion capacity of Cu, Zn and Pb on submerged aquatic plant *Myriophyllum spicatum*. The adsorption capabilities recorded were 46.69 ppm for Pb, 15.59 ppm for Zn and 10.37 ppm for Cu.

In another study, agricultural biomass residues such as rice straw, wheat straw and aquatic plant, *Salvinia,*t biomass has been proposed for the removal of heavy metals such as Cr, Ni, and Cd (Dhir and Kumar, 2010). According to the study, different combinations, i.e., *silvinia* alone, *silvinia* with rice straw or wheat straw showed variable capability to reduce heavy metals. It was reported that removal efficiency was more at low metal concentration (35 mg/L). *Salvini*a biomass possessed the highest capacity for the reduction of these metals followed by the combination of all three materials (rice straw, wheat straw, *Salvinia* biomass) as compared to other combinations (Dhir and Kumar, 2010)

e. Biosorption by using biomass

Biosorption of chemicals by using biomass is another technique that has been used for decades. Pagnanelli*et al.,* 2002 conducted a study on the 'residues of olive mill for biosorption and reported that Cu could be biosorbed in 5.0 to 13.5 ppm range under different experimental conditions. In another study, Hammaini *et al.* (2003) compared the simultaneous biosorption capability of activated sewage sludge for copper, cadmium and zinc. The results showed that the biomass had significant copper uptake followed by cadmium and zinc. It was also suggested that pH had played an important role in biosorption and was found most favorable at acidic pH (Hammaini *et al.,* 2003).

Bygi *et al* (2004) used carrot residues in his study to remove heavy metals from waste water and concluded that different functional groups such as carboxylic, phenolic and other functional groups of cellulose, hemicelluloses and other constituents of cell wall absorb the heavy metals. Audu and Lawal (2005) conducted a study and reported that all the vegetables and herbs accumulate heavy metals in roots, leaves and stem in various concentrations from wastewater. We can use microorganisms or microbial biomass to remove heavy metals. It is very difficult to search for microbial biomass, and it is very easy and useful to look plant biomass that is present in nature abundantly.

Different kinds of non-living biomass can also be used for the reduction of heavy metals from wastewater (De Rome and Gadd, 1991). Removal of pollutants by using different types of plants is a good technique to remove heavy metals from aqueous solution (Miretzky *et al.*, 2004). These macrophytes can be use to remove heavy metals from effluents of any source. Biosorption is a rapid phenomenon of uptake of heavy metals by biomass (Beverdige and Doyle, 1989). Wase and Foster (1997) reported that biosorption ability of certain biomass for reduction of heavy metals is equal to commercial synthetic cation exchange technique. Furthermore, Volesky *et al* (1995) reported the loss of microbes and their metal binding capacities and reported that biosorption of multi ions is a complex process. Sorption of one metal should be compare with other because most of effluents contain more than one metals, however, most of the studies conducted on microbes are about sorption of one metal at one time. He also reported that many factors such as physical and chemical conditions also affect biosorption capacity. Chang *et al* (1997) reported that microbes have the ability to reduce heavy metals from wastewater; however, their capability depended on the type, of microbe, biomass produced and their carrying capacity.

f. Mechanism of biosorption

The mechanism of biosorption is slightly complex. It includes: ion exchange capacity of biosorbent, chelation property, physical adsorption, and entrapment in the spaces of structural polysaccharide network. It works on the phenomenon of difference in concentration and diffusion of metals through cell membrane. By these mechanisms, different chemical groups in biomass can absorb and accumulate the metals (Ercole *et al.*, 1994). Few examples include: *Acetamido* groups of chitin, polysaccharide structures of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphhydryl groups of amino acid in proteins. Different groups of polysaccharides, i.e., carboxyl, hydroxyls in polysaccharide and mainly carboxyl and sulphates in structure of biomass are important for biosorption mechanism (Erocle *et al.*, 1994).

The structure of cell wall provides sites for various mechanisms for the accumulation of heavy metals from wastewater. However, no specific mechanism of biosorption has been fully understood so for. According to cell metabolism criteria, such mechanisms have been classified into two types: metabolism-dependent and non-metabolism-dependent mechanisms. Based on the location of removed material, this uptake or accumulation mechanism can be classified into three types: accumulation on extra cellular surface, accumulation on cell surface and intracellular accumulation. (Hall, 2002)

In some cases, heavy metals can transport through cell membrane and accumulate inside the cell, a well. It is connected with a defense system of the microorganism, the active defense system react in presence of these heavy metals. In non-metabolism dependent mechanism of biosorption, the heavy metals are accumulated on physiochemical bases on the functional group of cell wall. However, cell metabolism is not involved in this accumulation process. Similarly polysaccharides, proteins and lipids are present in cell walls of microbial biomass, which have metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of sorption is independent of cell metabolism and is a rapid reversible process (Volesky, 1990).

g. Factors affecting biosorption

Biosorption process is facilitated by various chemical and physical factors, i.e., temperature, pH, concentration of sorbent and sorbate, etc. These factors affect both the chemistry of solution and the ability of functional group to accumulate the heavy metals from wastewater. On the other hand, concentration of biomass also affects the process as with more biomass more heavy metals will be reduced (Roux and Fourest, 1992). Gadd (1988) concluded that due to an increased biomass, the active binding site for biosorption increaseed as well, hence the process becomes faster for heavy metal uptake.

It has been reported that biosorption process show better performance in the temperature range of 25-30 °C (Aksu *et al.*, 1992). In a study conducted by Mohan *et al* (2005) to evaluate different temperatures for Biosorption capability, It was observed that the capacity of kraft lignin to adsorb Cu^{2+} and Cd at 25 ◦C was 87.05 and 137.14 mg/g, respectively, whereas, adsorption of Cu^{2+} was 68.63 mg/g at 10 ◦C and 94.68 mg/g at 40◦C. Adsorption of Cd^{2+} was 59.58 mg/g at 10◦C and 175.36 mg/g at 40◦C. This showed that adsorption increases with increase in temperature (Mohan *et al.*, 2005).

Few studies (Gupta *et al.*, 2000; Sag and Kutsal, 1996) were conducted for the understanding of the mechanism by which micro organisms accumulate heavy metals from aqueous solution. It was concluded that: in case of non-living biomass, metabolism independent mechanism is important for reduction of heavy metals from wastewater. Metabolism-independent sorption involves adsorption process such as chemical and physical accumulation and also ion adsorption. In case of fungi different chemicals such as ligands are present on cell wall, involved in adsorption process. Carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups are present in ligands. Metals ions adsorbed by binding with negatively charged reaction site of cell wall. It was further reported that cell walls of microbes contain more polysaccharide and glycoprotein such as glucans, chitin, mannans and phosphor-mannans and these polymers were reported as a source of metal binding ligands (Erocle *et al.*, 1994).

Gupta *et al* (2000) conducted a study and reported that in different types of fungi cell wall is different in its chemical composition so its absorbing capacity of metals is also different. Previously, it was reported that cell wall of fungi contains 90 % of their dry biomass amino or non-amino polysaccharides which are source of metal binding in fungi (Gupta *et al.*, 2000). Similarly, another study on cellular pathway that helps to detoxify heavy metal has reported that capacity of cell to hold heavy metals varies among plant species could dependent on site on uptake capability (Erocle *et al.*, 1994).

Mullen *et al* (1989) reported that Ag^{2+} is present on cell wall of bacteria, which is responsible for biosorption. Muraleedhran & Venkobachar (1990) conducted a study on cooper sorption by wood rotting fungus *(Ganoderma lucidum)* and reported that interaction between protein and metals do not play significant role in binding of metal but there is some other mechanism. Luef *et al* (1991) conducted a study for removal of Zn from aqueous solution by using different type of waste material of *Asperigllus niger, Phanerochaeta chrysogenum* and *Caviceps paspali* from industrial plants were used and reported that under normal conditions *A. niger* and *P. chrysogenum* acccumulate more metals than *C. paspali.* Volesky *et al* (1995) conducted a study and reported that dead cells of *Saccharomyces cerevisiae* remove 40% more uranium or zinc than living culture and biosorption of zinc and uranium reach up to 60% with contact time of 15 min.

Modak *et al.*, 1996 use attached to wheat bran non-living waste biomass of *Aspergillus niger* and concluded that these are good material for reduction of zinc and copper from aqueous solution. In the presence of co-ions metal uptake decreased, which was dependent on the aqueous solution. In another study, Euef *et al* (1991) reported the use of fungal mycelium to reduce heavy metal from wastewater and similar to other microbes, the capacity of heavy metal uptake depended on the biomass produced by the mycelia.

B. Phytoremediation Technique

The release of heavy metals in biologically available forms by human activity could destroy or change both natural and man-made ecosystems. Phytoremediation, the use of living plants for removal of pollutants from environment, is an important technique of the present day. In this technique, various plants can be used for removal of heavy metals from wastewater and soil. In phytoextraction higher plants can be used for removal of pollutants. These plants can also accumulate various types of inorganic and organic pollutants. Plants have the ability to absorb these pollutants because these are the important components of their growth.

Phytoremediation is becoming as one of the most effective and cheaper technological solutions for removal of heavy metals from wastewater and from polluted soil. This technique is a topic of global interest due high efficiency and cost effective. In the last two decades, several plants have been identified as being most effective in absorbing and accumulating various toxic heavy metals. Other plants are being evaluated for their role in the phytoremediation of heavy metals from polluted soil and water.

For wetland remediation, the value of metal-adsorbing plants has recently been realized. It has been reported that this capacity is useful in reducing toxic heavy metals from contaminated water and soil (Tang *et al.*, 2001). Industrial discharge, agriculture runoff, or acid mine drainage can also be treated by this process. Pb, Cd, Cu, Ni, Zn and Cr that are primarily retained in roots are most adsorbed by the plants (Chuadhry *et al.*, 1998). Pinto *et al.*, 1987 demonstrated that silver could easily removed by using water *hyacinth*, an aquatic plant.

The bio-accumulation of some other heavy metals and trace elements in many species of wetland plants has also been demonstrated (Zhou *et al.* 1991) Water hyacinth a plant has been successfully used in reducing level of heavy metals, organic and inorganic pollutants from water to improve its quality (Delgado *et al.*, 1995). Concentration of metals in water is also responsible amount of metals accumulated in plants (Ismail *et* *al.*, 1996). Phyto-extraction a type of phytoremediation, also called as phytoaccumulation, is a best method to remove contaminations primarily from soil and water. By this technique, isolation of heavy metals without destroying the structure and fertility of soil has been reported (EPA, 2000).

Alkorta and Garbisu (2001) reported that phytoremediation is the application of plants for in-situ or ex-situ treatment for removal of contaminants from soils, sediments and water. The green plants degrade, assimilate, metabolize, or detoxify various inorganic and organic pollutants from the environment or make them harmless. It is a cost effective 'green' technology based on the use of specially selected metal-accumulating plants to reduce toxic metals from soils and water.

Plant based technologies for metal decontamination includes extraction, volatilization, stabilization and rhizofiltration. Various factors such as soil's physical and chemical properties, metal bioavailability, plant's ability to uptake, accumulate, translocate, sequester and detoxify metal amounts for phytoremediation efficiency. Use of transgenics to enhance phytoremediation potential is also a good technology. Sadowsky, 1999 reported that water contaminated with heavy metals has been treated in the past by the use of ion exchange and activated charcoal filter methods. The disadvantages of these process are costly and not ecofriendly for heavy metal removal. As a result, phytoremediation, a low cost, effective, and efficient method is now being used. Terrestrial plants have being used now a day.

Raskin *et al* (1997) reported that metal ions could move freely in vascular system of plant by symplast or apoplast pathway. Metals ions also transported as a non-cationic metal chelate. The plant accumulates toxic metals such as Cd, Pb and Zn, directly from water, which serves as sources of nutrients. Verma and Shukla (2000) reported that biomass of stem of wheat and bark of babul can be used for reduction of nickel from industrial wastewater. The removal of nickel was reported 2-10% less than to synthetic solution under similar conditions. Prasad and Freitas (2000) conducted a study on *Quercus ilex* phytomass to remove Ni, Cd, Pb, Cu, and Cr. He reported that order of root for removal is Ni > Cd > Pb > Cu > Cr, for stem Ni > Pb > Cu > Cr and for leaf Ni > Cd > Cu > Pb > Cr.

Chandrasekhar *et al* (2003) reported that plant biomass of Indian saraprilla can remove toxic heavy metals like As, Se, Zn, Ni, Co, Pb, Mn, Hg, Cr and Cu. Pb was preferentially reduced followed by Cr and Zn at concentration less than 250 mgL^{-1} and with biomass quantity above 2g. Presence of co-ions affects the uptake of copper and zinc but not affect the other metals.

Ajmal *et al* (2003) reported that when phosphate treated rice husk was used adsorption of Cd and Ni was greater than Cr and Zn. Bishnoi *et al* (2004) reported that temperature, contact time and pH of the solution affect the process of biosorption. Park *et al.*, 2004 reported that Cr (VI) to Cr (III) reduction by Ecklonia species biomass increased with decreasing pH, which appeared in the solution or was partly bound to biomass.

III. RESEARCH METHODOLOGY

A. Study Area:-

To investigate "Biosorption of Cadmium (Cd), chromium (Cr) and Lead (Pb) by *Euphorbia Prostrata* and their effect on some vegetative and biochemical attributes" two areas of District Layyah was select for sampling (Figure 3.1). First Area was irrigated with tube well water near sugar mill; second area was irrigated with wastewater. The samples were collected from both the areas. Layyah is a district of southern Punjab. Climate of the area is hot in summer and very cool in winter season. Soil of the district is mostly sandy but near the river is mostly clay soil. Layyah is situated 472 feet above the sea level.

B. Materials

The material used in the study is given under the following section.

a. Water samples

The tube well water was collected from tube well away from sugar industry and wastewater collected from a pond next to sugar industry.

b. Plant samples

Plants were collected from tube well water irrigated area and from wastewater irrigated area. Plants grown in the lab were also used as sample.

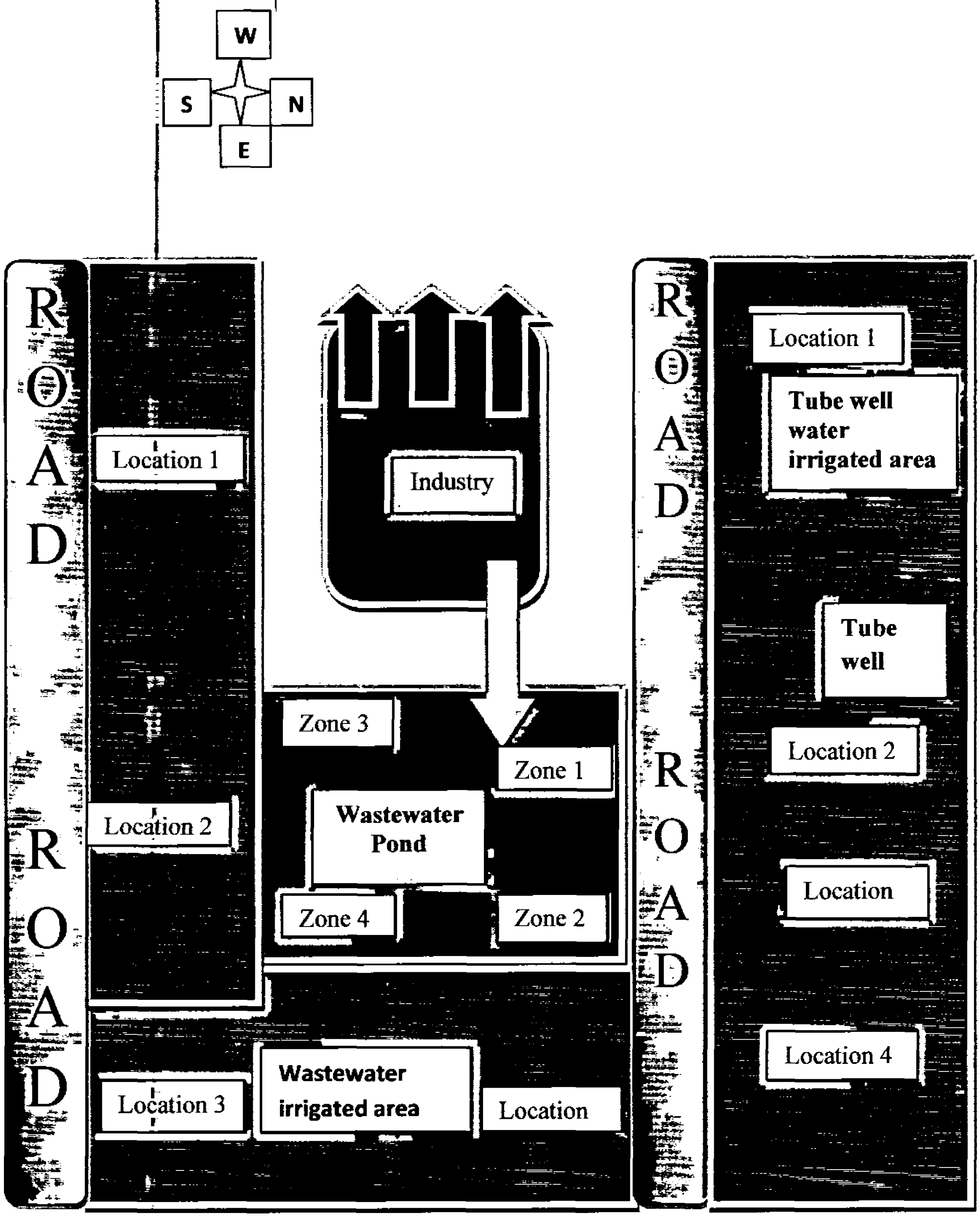

Figure 3.1 Map of sampling location

c. Chemicals

The following chemicals were used in the study. HNO_3, $HClO_4$, $K_2Cr_2O_7$, $CdSO_4$, and $Pb(CH_3OO)_2$. All chemicals used, were procured from Merck chemicals, Germany.

d. Equipments:-

Equipments used in the study were, atomic absorption spectrophotometer (Hitachi A-1800 Japan), pH meter (CD 640 digital, USA), analytical electrical balance, oven (Gallenhamp Hotbox oven size 1), hot plate (ARIKO Co. Ltd Japan) etc.

C. Methods

Following methods were followed in the study.

a. Water sampling methods

Samples of water were collected in sterilized plastic bottles. For sterilization, the plastic bottles were cleaned by washing with detergent then rinsed with tap water and again soaked in 10% HNO_3 for 24 hours. Finally, these bottles were rinsed with de-ionized water just before use. During sampling, bottles were rinsed with sampling water followed by filling it with water sample. The bottles were sealed and stored at 4°C until water analysis and further use. Tube well water was collected from tube-well irrigated region (divided in 4 zones) three times during 45-day study after regular intervals of fifteen days. The samples collected comprised of four zones and collected in replicates during the whole period of research study. Similarly, wastewater was collected from all four zones of wastewater disposal pond of sugar industry after regular intervals in replicates, each time during the whole research period (Sharma and Prasad, 2010).

b. Plants sampling methods

A site irrigated with tube well water was selected and all old plants were removed while new germinating plants were allowed to grow. Four samples of plants were collected randomly from tube well/tap-water irrigated area three times after regular intervals of fifteen days during the research period. Similarly, a site irrigated with wastewater of sugar industry was also selected and all old plants were removed while new germinating plants were allowed to grow. Four samples of plants were collected randomly from wastewater irrigated area three times after regular intervals of fifteen days during the whole period of research. Plant samples collected and wrapped in black calico bags were properly labeled and taken to the laboratory for further analysis. Before drying process, each sample was washed with distilled or deionized water to remove dirt and packed in labeled envelops followed by drying at 105 C^0 in the oven. The dried samples were removed from the oven and milled into powder using Author Thomas milling machine (maker unknown) and stored in an airtight container for future analysis of heavy metals (Sharma and Prasad, 2010).

c. Germination of plants

Seeds of plant (*Euphorbia prostrata*) were collected from mature plants in field irrigated with tube well water and were grown in pots having filled with soil. In order to drain or remove extra water, a hole was carved at the bottom of each pot. The planting pot arrangement comprised of two treatments in a randomized complete block design (RCBD). Plants in first treatment (T1) were irrigated with tap water whereas in the second treatment (T2), plants were irrigated with waste water of sugar industry, when required. There were 12 pots in each treatment, with three (3) harvests were collected from each treatment during 45-day study and in each harvest four plants were taken for digestion and further analysis. After each harvesting, samples of plants were collected, rinsed with de-ionized water and wrapped in properly labeled black calico bags until further analysis. Before analysis, plant samples were packed in labeled drying envelops and dried at 105 °C in a drying oven. The samples were milled into powder using milling machine as described earlier and stored in airtight containers.

d. Digestion of samples

In order to prepare samples for atomic absorption spectroscopy (AAS), digestion of wastewater and plant samples was carried out by the following procedures.

i. Digestion of wastewater

Wastewater (50 ml) was taken in beakers and 2ml HNO_3 (1M) was added and allowed to evaporate on a hot plate. After evaporation beakers were removed and brought to room temperature. Another 2ml HNO_3 (1M) was added to the beaker, and were covered with watch glass and transferred to hot plate until solution started to appear colorless. The solution was filtered by Whatman porcelain filter paper no 41 (pore size 20-25 um) in a conical flask. The volume was adjusted to 50ml of by adding de-ionized water (Sharma and Prasad, 2010)

ii. Digestion of Plants:-

Dried and crushed samples were taken in beakers and 5ml mixture of concentrated HNO_3 (1M) and $HClO_4$ (70 %) with a ratio of 2:1 was added. The mixture was heated on hot plate until the production of fumes, followed by cooling. 5 ml distilled water was added and heated until clear solution was obtained. The solution was filtered by whatman porcelain filter paper no 41 (pore size 20-25 um) in a conical flask and the volume was adjusted to 50ml by adding deionzed water (Sharma and Prasad, 2010).

d. Biosorption

Stock solutions of each $K_2Cr_2O_7$ (6M), $CdSO_4$ (3M), and $PbNO_3$ (5M) were prepared. 50 ml of each solution was taken in 300 ml conical flask separately. An amount of 0.2 gram dry biomass collected from field irrigated with tube well water was added to each flask with control pH 7.0. Then dry biomass was mixed thoroughly in solution for specific time of one hour. The solution was filtered and concentration of Cd and Pb were again measured to determine absorbed amount. Then again 50 ml of solution from each of the three solutions were taken in separate 300 ml flasks and 0.2 gram of biomass from plants grown up in lab on tap water was added to each flask on control pH 7.0. Then dry biomass was mixed thoroughly in solution for one hour. The solution was filtered, and concentration of Cd and Pb were measured to determine absorbed amount (Gupta *et al*, 2001). The following chemical and physical parameters were evaluated (Table 3.1 & 3.2).

Table 3.1 Parameters for water analysis

Tubewell water	Wastewater
pH	pH
Cd concentration	Cd concentration
Pb concentration	Pb concentration
Cr concentration	Cr concentration

Table 3.2 Parameters for plant analysis

Tubewell irrigated plants	Wastewater irrigated plants
Growth of plants	Growth of plants
Fresh biomass	Fresh biomass
Dry biomass	Dry biomass
Cd accumulation	Cd accumulation
Cr accumulation	Cr accumulation
Pb accumulation	Pb accumulation

D. Statistical Analysis

Mean and standard deviation were calculated by using M.S excel (2007) and subject to manual ANOVA (Co-stat Package version 6.3) for finding significance of difference among different treatments.

IV. Results & Discussion

The wastewater of sugar industry contains heavy metals, which are toxic to plants, animals and human being. Although different chemical techniques to reduce heavy metals from wastewater have been reported, however, such techniques are either costly or time-consuming or could result in the production of more persistent secondary pollutants (Ajmal *et al.,* 2000). Phytoremediation and biosorption are two techniques without any negative effects; however, their application has to be investigated (Sag *et al.,* 1998). The current study was designed to investigate the phytoremediation and biosorption capacity of plant *Euphorbia Prostrata* on wastewater of sugar industry. Furthermore, the effects of wastewater on some physical attributes of the same plant such as growth of plant, fresh biomass and dry biomass.

A. Determination of Heavy Metals in Wastewater

For initial part of this study, heavy metals were determined in the wastewater and tube well water samples and were compared with National Environmental Quality Standards (NEQs) (Pakistan EPA, 1999). According to results, the pH remained in the range of 6.70 to 7.20 irrespective of water sample (Table 4.1). The heavy metals were determined in wastewater samples for 15, 30, and 45 days. Concentration of Cd determined was in the range of 2.03 to 2.58 ppm, which was not significantly different for all samples collected at 15, 30 and 45 days. The presence of Cr was in the range of 2.39 to 4.50 ppm, but not in a significantly different range except for two samples. On the other hand, the concentrations of Pb were found in the range of 1.4 to 4 ppm (Figure 4.1). It was expected that sampling of wastewater would show similar results since wastewater is produced and released throughout the manufacturing season and would release similar wastewater. The results were compared with NEQs for Cd (0.1ppm); Cr (1.0ppm) and Pb (0.5 ppm), and all samples were found above NEQs Standards (Pakistan EPA, 1999).

Table 4.1 Amount of heavy metals in wastewater

		pH mean	Atomic absorption spectrophotometer results		
			Cadmium Concentration (ppm)	Chromium Concentration (ppm)	Lead concentration (ppm)
Control		6.5	0	0	0
15 Days	S1	7.03±2.1	2.29±0.12	2.49±1.29	2.26±0.1
	S2	7.23±1.92	2.32±0.24	2.39±1.35	2.30±0.2
	S3	6.70±2.48	2.03±0.63	3.98±1.2	1.40±1.3
	S4	6.90±.58	2.46±0.53	4.55±1.13	2.25±0.1
	Mean	6.97	2.28	3.35	2.05
30 Days	S1	6.97±0.5	2.26±0.5	2.53±1.1	1.73±1.2
	S2	6.75±0.9	2.58±0.41	3.53±1.3	4.51±2
	S3	7.20±.84	2.39±0.32	2.97±1.2	2.27±0.8
	S4	6.90±1	2.46±0.21	3.83±1.5	2.54±0.5
	Mean	6.96	2.42	3.21	2.76
45 Days	S1	7.15±0.9	2.17±0.5	2.32±1.2	3.85±1.61
	S2	7.00±0.3	2.46±0.2	2.83±0.9	3.14±1.5
	S3	6.80±0.9	2.32±0.1	4.26±1.61	1.95±1.03
	S4	6.95±0.37	2.49±0.3	3.04±0.2	1.65±1.05
	Mean	6.98	2.36	3.11	2.64

S1=wastewater collected from location-1: S2= wastewater collected from location 2
S3=wastewater collected from location 3: S4=wastewater collected from location 4

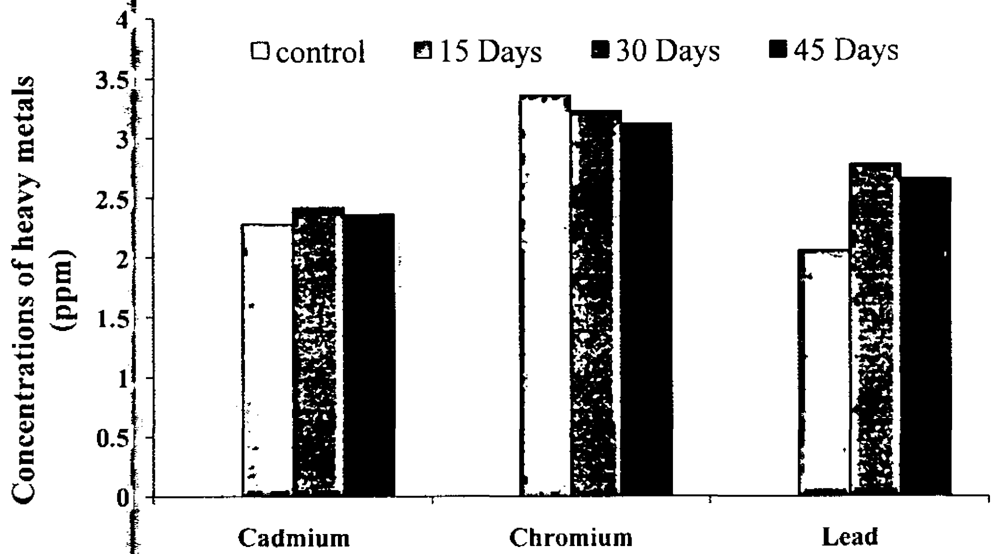

Figure.4.1 Amount of heavy metals in wastewater

Previously, Rath *et al* (2010) conducted a study on wastewater from sugar industry and concluded that wastewater of sugar industry contain concentrations of Cd (0.036 ppm), Cr (0.067 ppm), Pb (0.19 ppm) and other heavy metals more than permissible level based on International standards. Similarly, it has been reported previously that wastewater of sugar industry contain heavy metals more than permissible level in India, as well (Bhardwaj and Singh, 2009). The research findings of current study confirm the same since sugar industry waste water was found to contain heavy metals above International standards, as well.

B. Effects of Wastewater on Plant Growth

Plant growth of *Euphorbia prostrata* was monitored throughout its germination. The size, growth pattern and its fresh and dry biomass has been recorded and is presented under the following sections.

a. Effect of wastewater on growth area of plants

According to the initial results, plant growth on tap water (control) in the laboratory show similar growth when compared with field irrigated plants on tube well water (T1) throughout study period. However, when compared after 15 days of germination, plants irrigated with tube well water in field (T1) and plants irrigated on wastewater in field (T3) showed greater growth (3.5 - 3.8 cm^2), whereas, the plants germinated on wastewater in the laboratory (T2) showed a reduced growth (2.68 cm^2) but similar to control in the laboratory (C) (Figure 4.2). Similar results were recorded after 30 and 45 days of germination for control and T1. Plants irrigated with tube well water in field showed greater growth, which was 5.98 cm^2, whereas, plants irrigated with wastewater germinated in lab or in the field continued to show reduced growth, in the range of 4.40 - 4.45 cm^2 Whereas, after 45 days of germination, plants irrigated with tube well water in field (T1) showed greater growth, which was 9.0 cm^2. Plants irrigated with wastewater germinated in lab (T2) showed reduced growth, which was 6.52 cm^2 and was even below 7.60 cm^2 as in case of plants irrigated on wastewater in field (T3) (Figure 4.2).

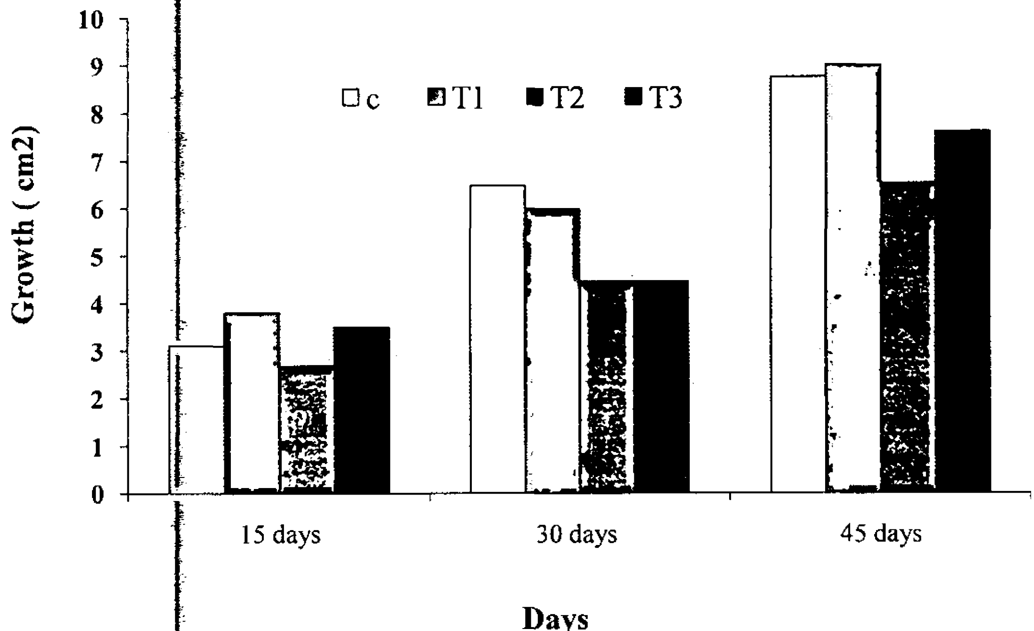

Figure 4.2 Effect of wastewater on growth area of plants (cm^2)

C=Plants germinated in lab on tap water: T1=plants collected from field (tube well water)

T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

As a whole, tube well water showed significant plant growth when compared with wastewater-irrigated plants, irrespective of their location after 30 and 45 days. However, there was no significant difference among plants after 15-days of growth.

b. Effect of wastewater on fresh biomass of plants

According to the results recorded, after 15 days of germination plants irrigated with tap water in lab (C) and in the field (T1) showed slightly higher fresh weight because of more branching area, which was 0.3g when compared with T2 or T3 (0.19 – 0.22 g), however, it was not significantly different (Figure 4.3).

After 30 days of germination plants irrigated with tube well water in field (T1) and control (C) showed greater fresh weight because of greater branching area (0.61 - 0.66

g) respectively, when compared with T2 & T3. Plants irrigated with wastewater germinated in lab (T2) or field (T3) showed lower fresh weight, which was (0.35 -0.38 g). Similar results were observed for control and T1 after 45 days and after germination plants irrigated with tube well water in field (T3) showed higher fresh weight (1.03 g) than all other samples However, Plants irrigated with wastewater in the lab (T2) showed lesser fresh weight, which was 0.50 g and was found even lower than field irrigated plants (T3) with 0.86 g of fresh biomass (Figure 4.3).

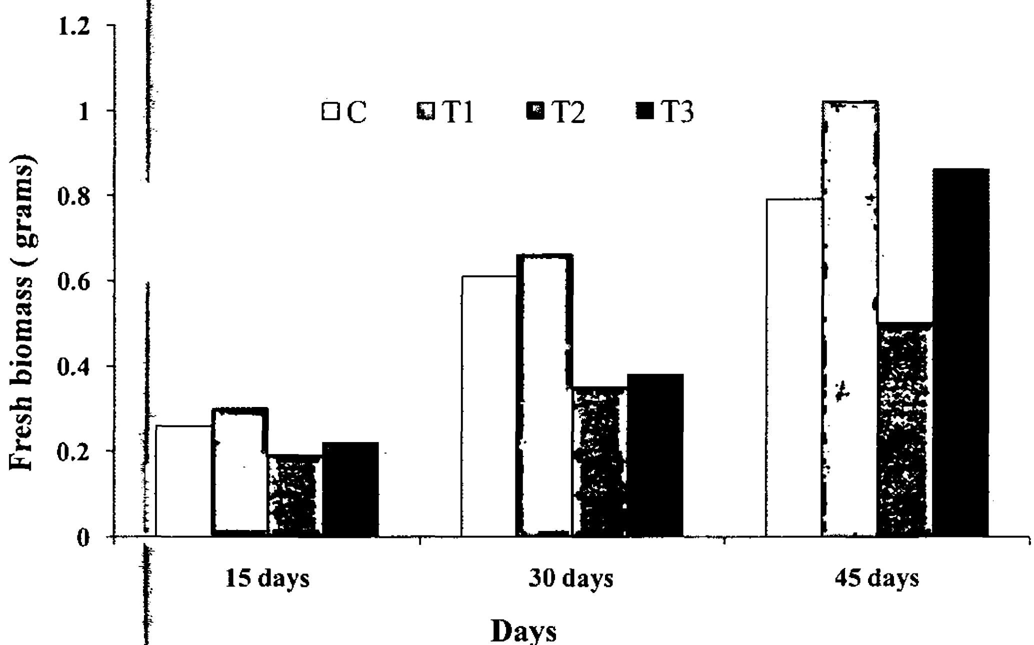

Figure 4.3 Effect of wastewater on fresh biomass of plants (grams)

C= (control) Plants germinated in lab on tap water: T1=plants collected from field (tube well water)
T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

As a whole, tube well water showed significant fresh plant weight when compared with wastewater-irrigated plants irrespective of their location after 30 and 45 days period. Whereas, plant collected after 45 days of germination showed higher growth comparable with tube well water. Previously, Rath *et al.*, (2010) documented that plants grown on 100 % pure spilt distillery wash (wastewater of sugar industry) showed 60 % less growth and decrease in physical growth was recorded due to accumulation of heavy metals. Similar results were confirmed by the findings of current study.

c. Effect of wastewater on dry biomass of plants

According to the results, plants collected after 15 days of germination on tube well water in field (T1) showed greater dry weight because of greater branching area, which was 0.18 g when compared with control and T2 and T3. Plants irrigated with wastewater germinated in lab (T2) showed lowest fresh weight (0.13 g) (Figure 4.4). However, the difference was not significant among all treatments.

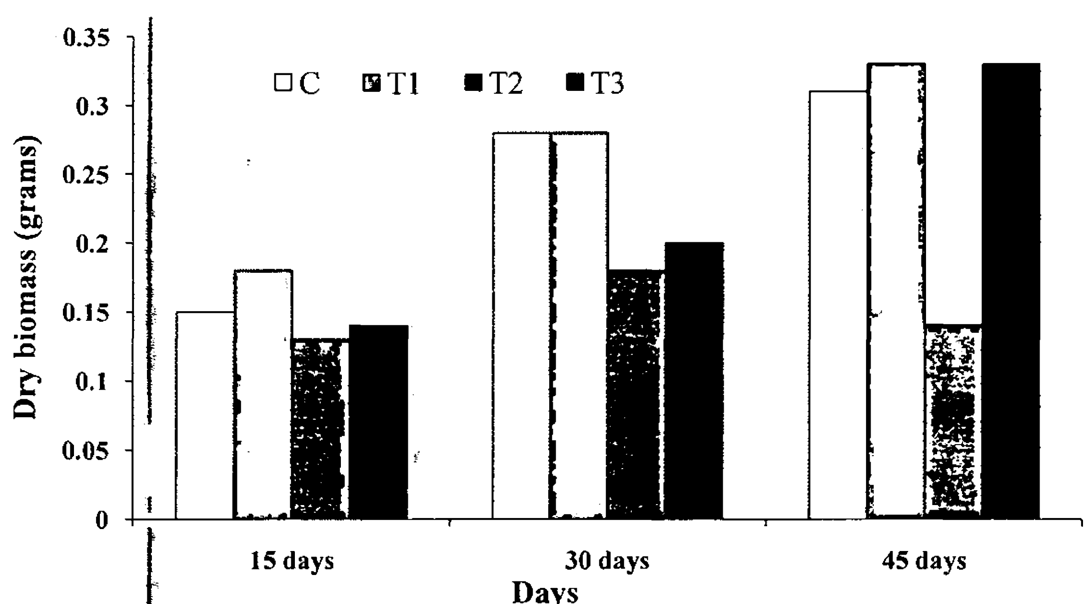

Figure 4.4 Effect of wastewater on dry biomass of plants (grams)

C= (control) Plants germinated in lab on tap water: T1=plants collected from field (tube well water)
T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

On the contrary, after 30 days of germination plants irrigated with tube well water in the field (T1) and control showed significantly higher dry weight (~ 0.28 g) as compared to wastewater-germinated plants T2 (0.18g) and T3 (0.20 g) irrespective of germination in laboratory or field (Figure 4.4). Whereas, after 45 days of germination plants irrigated with tube well water in field (T3) showed higher dry weight (0.33g) comparable with control (0.30g) and T1 (0.32g) with greater branching area. However, the plants irrigated with wastewater germinated in lab (T2) showed significantly lower fresh weight, which was 0.14 g (Figure 4.4).

As a whole, results indicate that dry weight of plants germinated on waste water remains less than that of tube well water-germinated plants during 45 days period. Plants collected from wastewater irrigated area showed dry weight almost equal to tube well water irrigated plants that may be due to experimental error. It has been reported previously (Rajendran, 1990; Ramana *et al.,* 2001) on studies conducted on sunflower (*Helianthus annus*) plants that an increased concentration of wastewater (spent wash) of sugar industry caused reduction in seed germination, seedling growth and chlorophyll contents of plants. Similarly, bacterial biomass has also been reported to adsorb heavy metal (Chang *et al.*, 1997). However the uptake of heavy metal was found below plant biomass uptake (Rajendran, 1990; Ramana *et al.*, 2001). It could be, therefore, concluded that if wastewater of sugar industry is applied to plants, their height, leaf length, leaf breath, stem girth, number of leaves and leaf area index will decrease.

C. Uptake of Heavy Metals by Plants in Phytoremediation.

The most important aspect of this study was to determine uptake of heavy metals by *Euphorbia prostrata* both in the field and laboratory conditions. The results of this trial are presented and discussed in the following sub-sections:

a. Uptake of cadmium in phytoremediation by plants

According to the results of study after 15 days of germination, plants irrigated with wastewater in field (T3) showed accumulation of cadmium (0.50g), whereas, all other treatments did not show any accumulation (Figure 4.5). However, after 30 days of germination, plants irrigated with wastewater in field (T3) and in lab (T2) showed the higher accumulation of cadmium, which was between 0.77- 0.88 ppm. Whereas, plants irrigated with tube well water in field (T1) accumulate 0.18 ppm Cd. Plants irrigated with tap water germinated in lab (C) showed no accumulation of cadmium at all or was below detection limit of AAS.

On the other hand, plants with 45 days of germination on wastewater in the field (T3) and in lab (T2) showed significant accumulation of cadmium in the range of 1.12 - 1.97 ppm. However, the plants germinated in lab with tap water (C) and plants collected from tube well irrigated area (T1) did not show any accumulation of Cd (Figure 4.5). It could be concluded that wastewater significantly affects the accumulation of cadmium in plants. These results also prove that plants could accumulate cadmium from wastewater immediately (Figure 4.5).

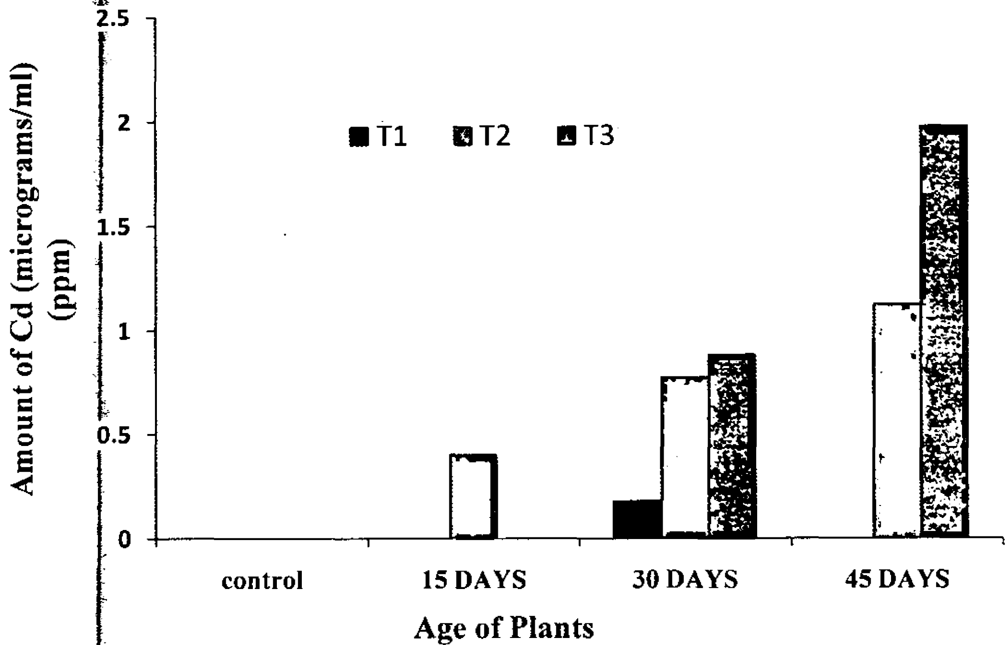

Figure 4.5 Uptake of cadmium in phytoremediation by plants (ppm)

C=Plants germinated in lab on tap water: T1=plants collected from field (tube well water)
T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

Previously, Akan *et al* (2008) reported that spinach accumulates 0.03ppm Cd, onion accumulates 0.56ppm Cd and carrot accumulates 2.01ppm Cd from polluted wastewater. Similarly, Sharma *et al.,* (2010) conducted a study on accumulation of heavy metals from wastewater and reported similar results that these vegetables collected from polluted areas accumulate heavy metals in their body. Rath *et al* (2010) conducted a study and concluded that sugar cane grown in polluted area accumulate heavy metals in their various parts of body. Same results are also confirmed by the findings of current study.

b. Uptake of chromium in phytoremediation by plants

After 15 days of germination plants irrigated with wastewater in field (T3) and laboratory trial (T2) showed higher accumulation of chromium, which was determined as 0.12 and 0.25ppm, respectively (Figure 4.6). Whereas, plants irrigated with tube well water irrespective of their germination in the lab (Control) or in the field (T1) showed no accumulation of chromium. Similar results were observed after 30 days of germination as plants irrigated with wastewater in field (T3) and lab (T2) showed more accumulation of chromium, which was 0.15 and 0.41ppm, respectively. Plants irrigated with tap water germinated in lab (C) as well as in field (T1) showed no accumulation of chromium.

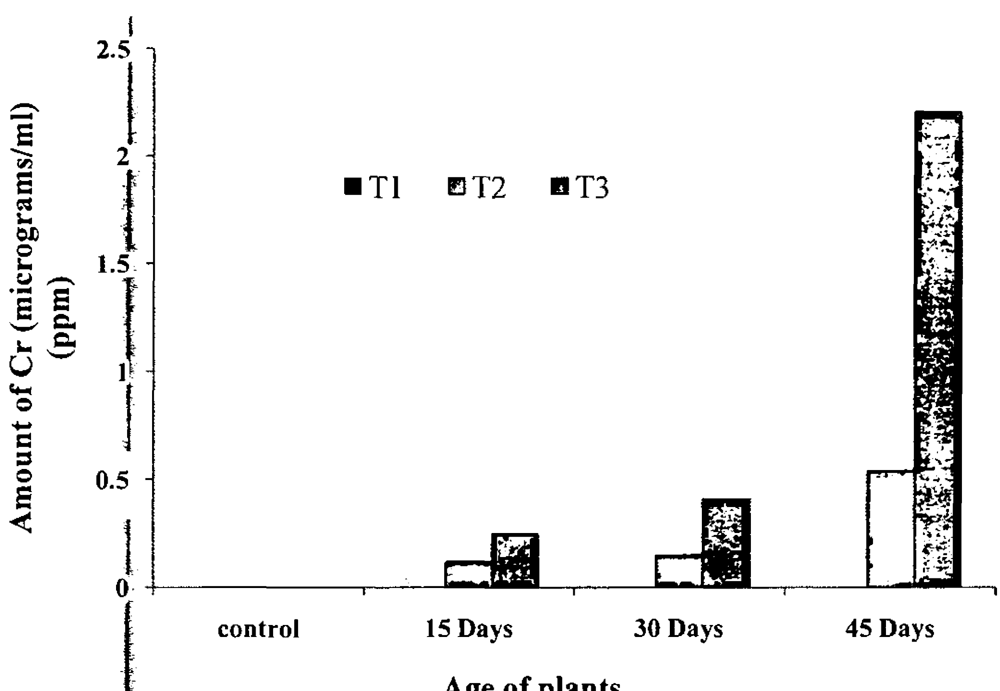

Figure 4.6 Uptake of chromium in phytoremediation by plants

C=Plants germinated in lab on tap water: T1=plants collected from field (tube well water)

T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

However, after 45 days of germination plants irrigated with wastewater in field (T3) showed significant accumulation of chromium, which was determined at 2.20ppm, followed by plants germinated in lab on wastewater (T2) (0.54ppm). This significant uptake of Cr is inexplicable, as time period increased and biomass increased the uptake exponentially increased. However, the plants irrigated with tap water germinated in lab (C) as well as in field (T1) showed no accumulation of chromium (Figure 4.6). These results showed that plants germinated on wastewater were significantly higher in chromium uptake (2.2 microgram) whereas, plants germinated on tube well water showed no accumulation of chromium.

Previously it was reported that watermelon accumulated 0.019ppm Cr, egg-plant accumulated 0.18ppm Cr, and carrot accumulated 0.54ppm Cr when irrigated with polluted wastewater after the plant has settled their roots (Akan *et al.*, 2008). Shad *et al* (2008) reported that 0.18ppm chromium was accumulated by *Euphorbia helioscopia* when grown in polluted area near road side and showed increased uptake a earlt stage of germination. Lujan *et al* (1994) conducted a study and showed similar results that carrot residues could accumulate 10.3ppm Cr from waste water and from sludge, as well. The results of current study confirmed the findings of previous studies.

b. Uptake of lead in phytoremediation by plants

According to results of the study after 15 days of germination plants irrigated with wastewater in field (T3) showed accumulation of lead (0.21ppm), whereas, plants germinated in lab on wastewater (T2), as well as in field (T1) or control samples showed no accumulation of lead (Figure 4.7). However, after 30 days of germination plants irrigated with wastewater in field (T3) were the only group that showed accumulation of lead (0.50ppm) (Figure 4.7) whereas plants in all other trials did not show any accumulation of lead. On the contrary, after 45 days of germination plants irrigated with wastewater in field (T3) and plants germinated in lab on wastewater (T2) showed significant accumulation of lead, which was determined as 0.36 and 0.90 ppm, respectively. No accumulation of lead was observed in tube well irrigated plants (Figure 4.7).

These results showed that wastewater significantly affect the accumulation of lead in plants but after the age of plants reach a certain stage. Akan *et al.*, (2008) reported that carrot accumulates 0.032 ppm Pb, water-melon accumulate 0.25 ppm Pb, egg-plant accumulates 0.26 ppm Pb spinach accumulates 0.67 ppm Pb, and lettuce accumulates 1.34 ppm Pb from polluted wastewater after they had matured its fruit. Similarly, Shad *et al* (2008) reported that more lead was accumulated by *Euphorbia helioscopia* after 30 day trial, which was recorded as 2.10mg/kg that is much greater than unpolluted area which was 0.20mg/kg. Sharma (2010) conducted a study on accumulation of heavy metals from waste water and concluded similar results that these vegetables collected from polluted areas accumulate heavy metals in their fruit. Mishra and tripathi (2008) conducted a styudy on *Euphorbia crassipes* and reported it can remove upto 77 % metals from wastewater. Same results are also confirmed by the findings of current study.

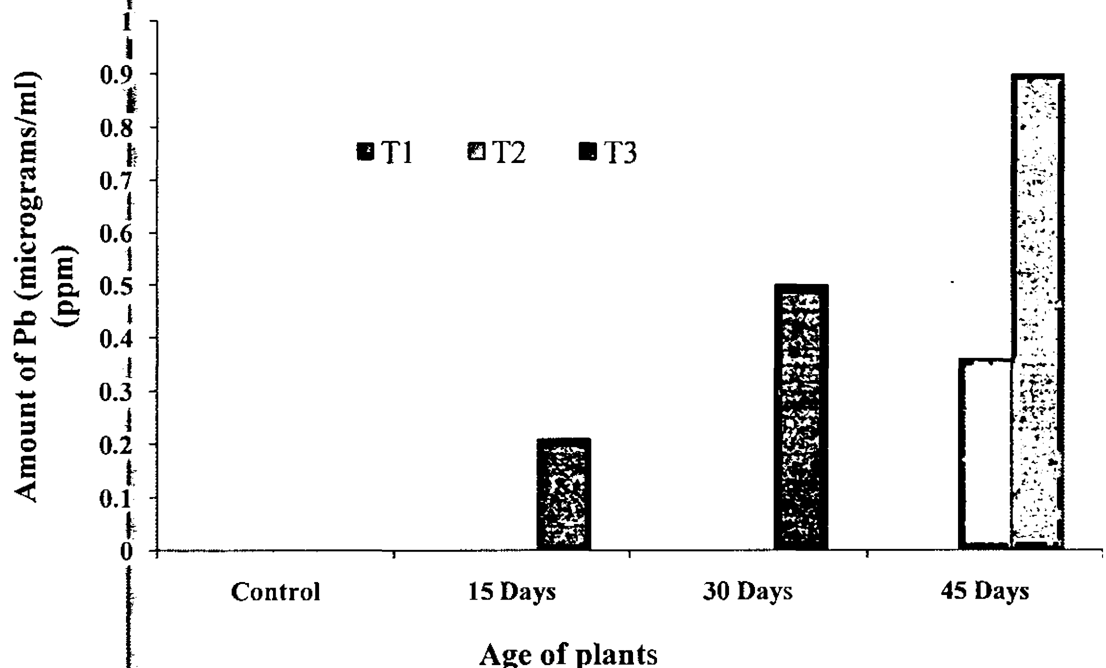

Figure 4.7 Uptake amount of lead in phytoremediation by plants (ppm)

C=Plants germinated in lab on tube well water: T1=plants collected from field (tube well water)
T2=plants germinated in lab on waste water: T3=plants collected from field irrigated (waste water)

These results showed that all the plants collected from wastewater-irrigated area accumulated Pb. However, no Pb was detected in plants-germinated in lab after 15 and 30 days. Whereas, plants germinated in lab on wastewater started to show accumulation after 30 days only. This accumulation of Pb was attributed to the aging of plants or increase in the biomass. However, this phenomenon needs to be researched.

D. Uptake of Heavy Metals by Plants in Biosorption

For this part of study, dried biomass of *Euphorbia prostrata*, as produced previously, was used to biosorb heavy metals. This section discusses the finding of two trials conducted with a mixture of all three metals on tap-water irrigated and tube well-water irrigated plants.

a. Biosorption of heavy metals by tap-water irrigated plants in lab

Plants germinated in lab on tap water for 15 days, adsorbed cadmium up to 27.4 % and whereas, plants of 30 days age adsorbed 53.2% cadmium and plants for 45 days, adsorbed 59.1% cadmium (Figure 4.8).

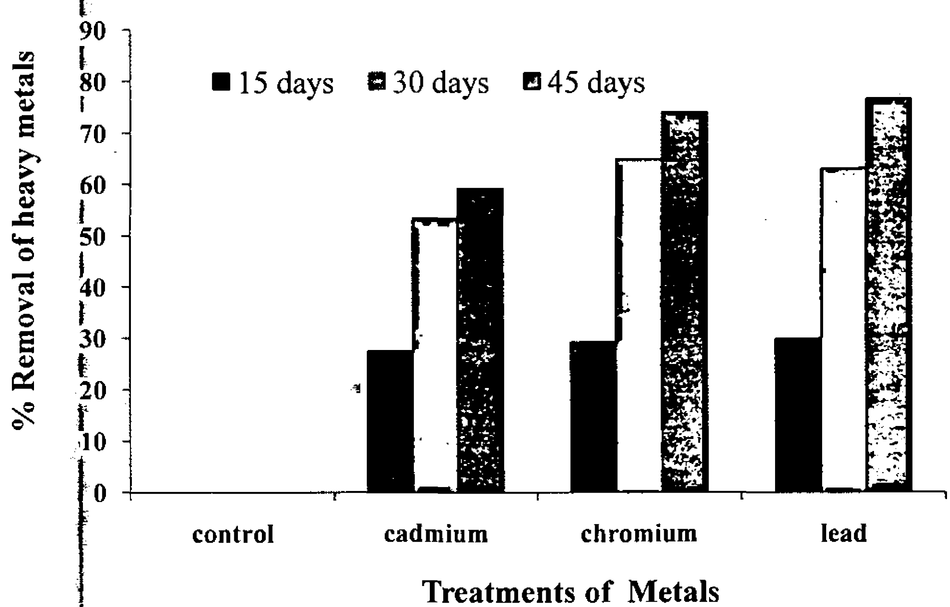

Figure 4.8 Biosorption of heavy metals by plants germinated in lab by tap-water

Plants germinated in lab on tap water for15 days, adsorbed chromium up to 29.1 %, plants for 30 days, adsorbed 64.7% chromium and plants for 45 days, adsorbed 73.9% chromium. Plants germinated in lab on tap water for 15 days, adsorbed lead up to 29.9 %, plants for 30 days, adsorbed 62.9% lead and plants for 45 days, adsorbed 76.4% lead

(Figure 4.8). These results represent that sorption capacity of plants increases significantly with their age and could be due to increase in their biomass. When the data was compared with phytoremediation experiment, a significantly higher amount of heavy metals were absorbed by the dried biomass. Dried biomass of plants, dead plant material, microbial biomass and synthetically produced fiber have been investigated for its biosorption properties. Mishra *et al.,* (2008) has reported previously that plants biomass could adsorb significantly higher amount of cadmium and other heavy metals from wastewater up to 70-80% and adsorption increased with increase in biomass. Furthermore, the use of aquatic macrophytes *i.e., E. crassipes,* was also reported to remove up to 77% to 95 % heavy metals. Similarly, The capacity of kraft lignin, a synthetic cellulose, to adsorb Cu^{2+} was 87.05 mg/g and for Cd^{2+} was137.14 mg/g. (Mohan *et al*., 2005). The results of current study reports the similar range of accumulation in biosorption experiment. However, this phenomenon needs to be further investigated.

b. Biosorption of heavy metals by tube-well irrigated plants in field

Results in Table 4.9 and Figure 4.9 show that plants collected from tube well irrigated area adsorbed more cadmium than that of lab. Plants after 15 days of growth adsorbed 35.9%, for 30 days, adsorbed 59.6% and for 45 days, adsorbed 69.0% cadmium. Plants collected from tube well irrigated area adsorbed more chromium than that of lab. Plants after 15 days of growth adsorbed 25.1%, for 30 days, adsorbed 70.5% and for 45 days, adsorbed 77.7% chromium.

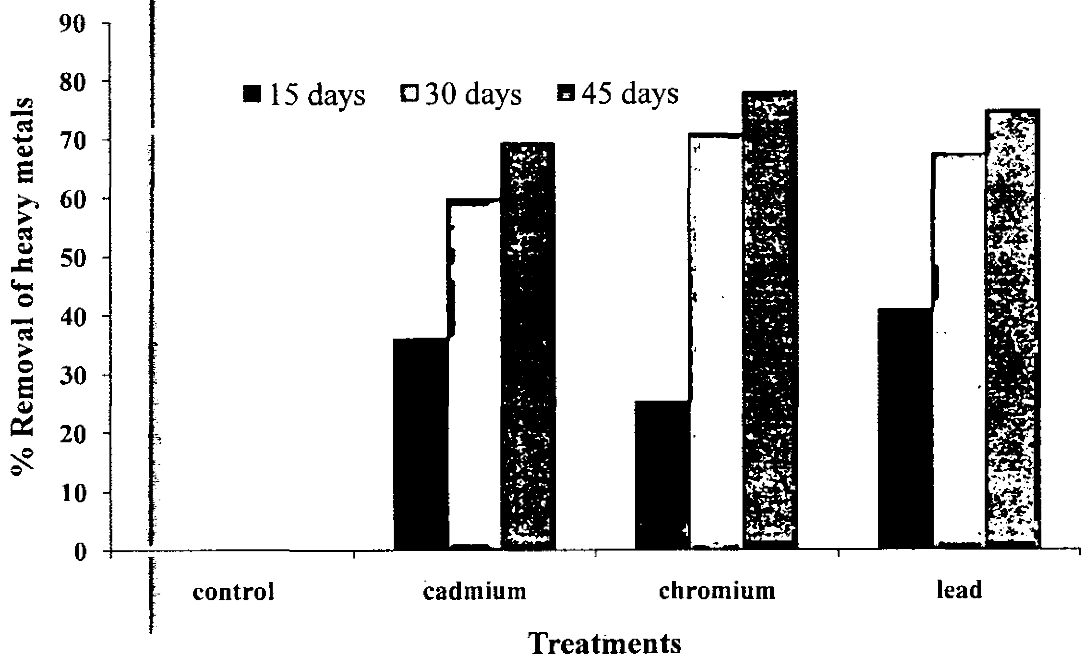

Figure 4.9 Biosorption of heavy metals by plants collected from field irrigated by tube well water

Plant biomass could absorb chromium and other heavy metals from wastewater and adsorption increased with increases in biomass. Gupta *et al.*, (2001) reported that biosorption of chromium could be increased up to 98 % by increasing available biomass. According to the results, plants after 15 days of growth adsorbed 40.7%, for 30 days, adsorbed 67.0% and for 45 days, adsorbed 74.3% lead (Figure 4.9). Same results are also confirmed by the findings of the current study. It is represented in the results that sorption capacity of plants increased significantly with their age due to increase in their biomass.

Based on the results of current study and similar techniques used previously by other researchers, it is concluded that biosorption and phytoremadiation have the potential to remediate soil and water. However, the use of *Euphorbia prostrata* and *Biosorption* techniques by using biomass could be promising techniques to reduce amount of heavy metals from wastewater.

V. Conclusion and Recommendations

From the current study, it is concluded that wastewater of sugar industry contain different types of heavy metals above permissible level. These heavy metals are toxic and can be transported to human and other animals through food chains. These heavy metals cause different types of acute health disorders in human, i.e., epigastric pain, nausea, vomiting, severe diarrhea and hemorrhage, and more complex diseases after chronic exposure i.e., cancer in digestive tract and lungs. Heavy metals such as chromium (VI) could produce immediate cardiovascular shocks and laterally it affects kidney and blood forming organ and has been suspected as carcinogenic, as well (Singh *et al.*, 1998).

These metals also effect the growth of plants by reducing growth of plant up to 60% as compared to plants grown on tube well water and ultimately affect the yield of plants. However, these heavy metals can be reduced by using different techniques such as ion exchange, activated carbons and many other chemical techniques but all these techniques have harmful effects on environment by producing secondary pollutants and are expensive and time consuming. Based on the findings of current study, indigenous plants and their biomass could be a solution to overcome wastewater containing heavy metals through natural remediation. Wastewater should be treated before its disposal as this pollute soil, underground water and various types of plants, animals and ultimately human being. Phytoremediation and biosorption are two promising techniques based on the ability of living organisms or their biomass to adsorb heavy metals from water and soil. Both of these techniques are cheaper and time efficient when compared with conventional techniques, i.e., chemical precipitation and ion-exchange. However when compared, It could be concluded from the current study that: Biosorption (70-80% uptake) results in a significant reduction in heavy metals in wastewater as compared to phyto-remediation (40-50%). By using these techniques, we can reduce heavy metals from wastewater up to 80% as plants have greater ability to absorb heavy metals by the functional groups in their cell wall. Biosorption and bioremediation techniques are affordable for any economy or industry to be adopted as it is cheap and has less fallout. Use of unwanted indigenous plants and weeds are more suitable for this purpose because these have no direct role in human food supply, as well. *Euphorbia Prostrata* has shown the capability to reduce the heavy metals through both biosorption and phytoremediation techniques in this study. This weed is present abundantly in the nature, so it can be used for wastewater treatment instead of other conventional methods based on chemical treatment, that translate into more harmful effect on the environment, as a whole.

However, further investigation for finding unwanted indigenous species of different geographies in Pakistan and their use and potential to remediate has require more insight. In this connection, it is recommended that local flora and fauna could be further investigated for their potentials in bioremediation. Furthermore, other industrial effluents should be treated with *E. prostrata* in order to compare more complex feedstock (wastewater). Although, this study suggests that the use of *Euphorbia prostrata* and *Biosorption* techniques by using biomass could reduce the amount of heavy metals from wastewater. However, other more complex industrial sewage or effluents should also be treated with *E. prostrata* to find out the comparative reduction in heavy metal amount.

More importantly, It is also recommended for the future study that the biomass waste produced in the form of slurry or else, from both Biosorption and phytoremediation experiments, should be investigated for metal ion recovery; or for its re-use or for proper disposal, as well.

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Appendix

Appendix - A

A-1 Effect of wastewater on growth in of plant at age of 15 days (cm^2)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	3.10±0.00	3.40±0.80	2.30±0.80	3.20±0.80
S2	4.60±1.96	4.00±0.70	2.50±0.70	3.80±0.90
S3	2.20±1.25	3.80±0.00	3.00±0.80	4.00±0.90
S4	2.50±1.26	4.00±0.70	2.90±0.80	3.00±1.00
Mean	3.10	3.80	2.70	3.50

A-2 Effect of wastewater on growth in of plant at age of 30 days (cm^2)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	5.50±2.00	5.50±1.40	5.00±1.30	5.00±1.30
S2	5.50±2.00	6.00±0.30	4.00±1.10	4.80±1.20
S3	7.00±1.50	6.50±1.50	5.00±1.30	4.00±1.00
S4	5.90±1.50	5.90±1.10	3.80±1.30	4.00±1.00
Mean	6.50	6.00	4.50	4.50

A-3 Effect of wastewater on growth in of plant at age of 45 days (cm^2)

Sample name	45 days old plants			
	C	T1	T2	T3
S1	7.00±3.00	9.00±0.00	5.90±1.50	7.00±1.70
S2	8.00±2.00	8.20±2.00	7.00±1.60	7.20±1.40
S3	8.00±2.00	8.80±1.00	7.00±1.60	8.00±1.50
S4	10.0±0.80	10.0±2.50	6.20±1.00	8.20±1.50
Mean	8.80	9.00	6.50	7.60

C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)
T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)
S1= plants collected from location-1: S2= plants collected from location 2
S3= plants collected from location 3: S4= plants collected from location 4

Appendix - B

B-1 Effect of wastewater on fresh biomass of plants at the age of 15 days (grams)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	0.29±0.07	0.25±0.09	0.17±0.01	0.19±0.05
S2	0.30±0.08	0.35±0.20	0.18±0.01	0.22±0.00
S3	0.21±0.20	0.28±0.05	0.20±0.01	0.28±0.10
S4	0.26±0.00	0.32±0.01	0.20±0.01	0.20±0.03
Mean	0.26	0.30	0.19	0.22

B-2 Effect of wastewater on fresh biomass of plants at the age of 30 days (grams)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	0.70±0.20	0.60±0.16	0.40±0.11	0.42±0.11
S2	0.55±0.15	0.68±0.09	0.35±0.00	0.38±0.00
S3	0.65±0.18	0.72±0.01	0.35±0.00	0.34±0.01
S4	0.55±0.20	0.65±0.11	0.30±0.11	0.36±0.01
Mean	0.61	0.66	0.35	0.38

B-3 Effect of wastewater on fresh biomass of plants at the age of 45 days (grams)

Sample name	45 days old plants			
	C	T1	T2	T3
S1	0.80±0.08	1.08±0.20	0.45±0.13	0.80±0.18
S2	0.72±0.10	0.83±0.36	0.53±0.10	0.81±0.17
S3	0.77±0.10	1.02±0.00	0.54±0.11	1.00±0.21
S4	0.90±0.25	1.18±0.34	0.48±0.10	0.85±0.10
Mean	0.79	1.02	0.5	0.86

C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)
T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)
S1= plants collected from location-1: S2= plants collected from location 2
S3= plants collected from location 3: S4= plants collected from location 4

Appendix - C

C-1 Effect of wastewater on dry biomass of plants at the age of 15 days (grams)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	0.15±0.00	0.15±0.05	0.10±0.05	0.14±0.00
S2	0.19±0.07	0.21±0.01	0.11±0.05	0.15±0.01
S3	0.12±0.05	0.15±0.05	0.15±0.02	0.17±0.05
S4	0.14±0.01	0.19±0.01	0.14±0.01	0.13±0.01
Mean	0.15	0.18	0.13	0.14

C-2 Effect of wastewater on dry biomass of plants at the age of 30 days (grams)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	0.30±0.05	0.24±0.03	0.21±0.06	0.23±0.03
S2	0.26±0.02	0.31±0.05	0.16±0.01	0.20±0.00
S3	0.29±0.01	0.32±0.06	0.17±0.01	0.17±0.02
S4	0.25±0.02	0.25±0.02	0.16±0.01	0.19±0.01
Mean	0.28	0.28	0.18	0.20

C-3 Effect of wastewater on dry biomass of plants at the age of 45 days (grams)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	0.32±0.01	0.35±0.01	0.11±0.02	0.28±0.03
S2	0.24±0.01	0.28±0.02	0.16±0.02	0.29±0.01
S3	0.28±0.01	0.32±0.01	0.16±0.02	0.40±0.06
S4	0.38±0.01	0.35±0.01	0.12±0.01	0.35±0.05
Mean	0.31	0.33	0.14	0.33

C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)
T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)
S1= plants collected from location-1: S2= plants collected from location 2
S3= plants collected from location 3: S4= plants collected from location 4

Appendix - D

D-1 Uptake amount of cadmium in phytoremediation by plants at the age of 15 days (ppm)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	N.P*	0.92±0.43	0.36±0.11	0.50±0.00
S2	N.P*	0.09±0.30	0.36±0.11	0.39±0.18
S3	N.P*	0.57±0.01	0.37±0.12	0.67±0.25
S4	N.P*	0.59±0.02	0.47±0.12	0.41±0.15
Mean	-	0.53	0.40	0.50

D-2 Uptake amount of cadmium in phytoremediation by plants at the age of 30 days (ppm)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	N.P*	0.14±0.20	0.79±0.08	0.80±0.21
S2	N.P*	0.21±0.20	0.83±0.17	0.98±0.25
S3	N.P*	0.23±0.10	0.80±0.08	0.77±0.20
S4	N.P*	0.16±0.01	0.65±0.18	0.97±0.25
Mean	-	0.18	0.77	0.88

D-3 Uptake amount of cadmium in phytoremediation by plants at the age of 45 days (ppm)

Sample name	45 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	1.1±0.01	1.68±0.50
S2	N.P*	N.P*	1.10±0.01	1.97±0.00
S3	N.P*	N.P*	1.10±0.01	2.2±0.56
S4	N.P*	N.P*	1.17±0.19	2.0±0.10
Mean	-	-	1.12	1.97

N.P= Not Present
C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)
T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)
S1= plants collected from location-1: S2= plants collected from location 2
S3= plants collected from location 3: S4= plants collected from location 4

Appendix - E

E-1 Uptake amount of chromium in phytoremediation by plants at the age of 15 days (ppm)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	0.08±0.15	0.02±0.13
S2	N.P*	N.P*	0.16±0.14	0.56±0.28
S3	N.P*	N.P*	0.12±0.00	0.22±0.07
S4	N.P*	N.P*	0.10±0.10	0.23±0.08
Mean	-	-	0.12	0.25

E-2 Uptake amount of chromium in phytoremediation by plants at the age of 30 days (ppm)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	0.34±0.17	0.63±0.01
S2	N.P*	N.P*	0.09±0.13	0.60±0.25
S3	N.P*	N.P*	0.13±0.01	0.41±0.01
S4	N.P*	N.P*	0.04±0.14	0.60±0.21
Mean	-	-	0.15	0.41

E-3 Uptake amount of chromium in phytoremediation by plants at the age of 45 days (ppm)

Sample name	45 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	0.50±0.11	2.0±0.50
S2	N.P*	N.P*	0.55±0.02	1.94±0.60
S3	N.P*	N.P*	0.53±0.01	3.03±1.20
S4	N.P*	N.P*	056±0.03	1.84±0.70
Mean	-	-	0.54	2.20

N.P= Not Present
C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)
T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)
S1= plants collected from location-1: S2= plants collected from location 2
S3= plants collected from location 3: S4= plants collected from location 4

Appendix - F

F-1 Uptake amount of lead in phytoremediation by plants at the age of 15 days (ppm)

Sample name	15 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	N.P*	0.20±.03
S2	N.P*	N.P*	N.P*	0.38±.18
S3	N.P*	N.P*	N.P*	0.25±.50
S4	N.P*	N.P*	N.P*	0.03±.10
Mean	-	-	-	0.21

F-2 Uptake amount of lead in phytoremediation by plants at the age of 30 days (ppm)

Sample name	30 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	N.P*	0.66±0.25
S2	N.P*	N.P*	N.P*	0.86±0.40
S3	N.P*	N.P*	N.P*	0.04±0.28
S4	N.P*	N.P*	N.P*	0.46±0.11
Mean	-	-	-	0.50

F-3 Uptake amount of lead in phytoremediation by plants at the age of 45 days (ppm)

Sample name	45 days old plants			
	C	T1	T2	T3
S1	N.P*	N.P*	0.31±0.05	0.56±0.40
S2	N.P*	N.P*	0.45±0.15	1.03±0.30
S3	N.P*	N.P*	0.51±0.36	1.6±0.70
S4	N.P*	N.P*	0.19±0.20	0.41±0.46
Mean	-	-	0.36	0.90

N.P= Not Present

C= (control) Plants germinated in lab (tap water) T1= plants collected from field (tube well water)

T2=plants germinated in lab (waste water) T3=plants collected from field irrigated (waste water)

S1= plants collected from location-1: S2= plants collected from location 2

S3= plants collected from location 3: S4= plants collected from location 4

Appendix - G

G- Biosorption of heavy metals by plants germinated in lab on tube well water

Name of sample	Control at 15, 30 and 45 days	Amount of Cd biosorbed (%)			Amount of Cr biosorbed (%)			Amount of Pb biosorbed (%)		
		15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R1	0	29.3 ±6.6	46.5 ±15	64.5 ±15	12.6 ±15	86.8 ±33	98.1 ±37	10.7 ±16	98.0 ±43	36.8 ±22
R2	0	32.0 ±9.5	61.0 ±17	40.8 ±24	32.0 ±7.8	53.5 ±21	63.4 ±22	27.6 ±6.6	12.8 ±55	44.6 ±35
R3	0	20.6 ±10	69.1 ±25	49.9 ±18	11.0 ±15	73.2 ±19	40.6 ±35	42.2 ±17	79.1 ±27	100 ±37
R4	0	27.3 ±1.2	35.4 ±23	81.0 ±32	10.3 ±15	45.2 ±8.4	93.7 ±11	35.5 ±11	61.5 ±7.0	100 ±37
Mean	0	27.4	53.2	59.1	29.1	64.7	73.9	29.9	62.9	76.4

R1= Replicate 1, R2= Replicate 2, R3=Replicate 3, R4=Replicate 4

Appendix - H

H- Biosorption of heavy metals by plants collected from field irrigated with tube well water

Name of sample	Control at 15, 30 and 45 days	Amount of Cd biosorbed (%)			Amount of Cr biosorbed (%)			Amount of Pb biosorbed (%)		
		15 days	30 days	45 days	15 days	30 days	45 days	15 days	30 days	45 days
R1	0	31.7 ±9.7	41.6 ±54	61.3 ±18	13.8 ±12	71.4 ±6.5	100 ±36	13.7 ±2	83.3 ±28	47.3 ±33
R2	0	42.7 ±13	66.1 ±16	69.5 ±14	38.9 ±17	61.2 ±20	78.6 ±7	37.2 ±9	24.5 ±35	54.9 ±28
R3	0	36.3 ±3.0	81.6 ±32	76.8 ±19	24.3 ±3.6	100 ±40	51.7 ±3	55.8 ±22	93.8 ±37	100 ±38
R4	0	32.9 ±8.0	49.2 ±19	68.3 ±10	23.4 ±5.1	49.4 ±30	80.5 ±12	56.0 ±22	66.5 ±4.6	100 ±38
Mean	0	35.9	59.6	69.0	25.1	70.5	77.7	40.7	67.0	74.3

R1= Replicate 1, R2= Replicate 2, R3=Replicate 3, R4=Replicate 4