PHYTOREMEDIATION OF HEAVY METALS FROM LEACHATE THROUGH DIFFERENT SPECIES OF GRASSES

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A thesis submitted in partial fulfilment of the requirement for the degree of

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CERTIFICATE

This dissertation submitted by **Mr. Malik Muhammad Hassan** is accepted in its present form, by the Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST), Islamabad as satisfying the partial requirement for the degree of Master of Science in Environmental Science.

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Dedicated to…….

I dedicate this thesis to my parents, family and especially my uncle Malik Rehmat Ali for their encouragement, support and fruitful advices during my study. I am also thankful to my grandmother who prayed a lot for my success.

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Malik Mohammad Hassan

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List of Abbreviations

MSW	Municipal Solid Waste
COD	Chemical Oxygen Demand
EC	Electrical Conductivity
EG	Elephant Grass
VG	Vetiver Grass
RG	Rhodes Grass
Zn	Zinc
Cu	Copper
Mn	Manganese
AAS	Atomic Absorption Spectrophotometry
WHO	World Health Organization
EPD	Environment Protection Department

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ABSTRACT

Phytoremediation is considered more cost effective than other techniques with lesser disadvantages than physical and chemical techniques. It is gaining popularity in academic as well as practical field. Grasses have been used widely to remediate contaminants from wastewater. Municipal solid waste leachate has never been treated with grasses, especially with *Rhodes grass*. A series of pot experiments was performed to investigate the contaminant uptake from municipal solid waste leachate by *Chloris gayana (Rhodes grass)* grown in combination with commonly used grass varieties i.e. *Vetiveria zizanioides (Vetiver grass)* and *Pennisetum purpureum (Elephant grass)*. Leachate used for the experiments had high values for chemical oxygen demand, pH, electrical conductivity, nitrates, and phosphates (i.e. 5,163 mg/L, 8.5, 9 mS/cm, 182.1 mg/L, and 6.4 mg/L, respectively). The results showed that all the grasses significantly reduced the values of chemical oxygen demand, electrical conductivity, nitrates, and phosphates up to 67, 94, 94, and 73% respectively. The metals showed a significant decrease too, which included Zinc (97%), Copper (89%), and Manganese (89%). Comparatively, *Rhodes grass* remained efficient for Zinc, *Elephant Grass* for Copper, and *Vetiver grass* for Manganese. Rhodes grass showed good results in diluted leachates, whereas in concentrated leachates Rhodes grass did not flower and the biomass was also decreased. In *Vetiver grass,* root and shoot lengths decreased with increasing leachate strength, but the biomass does not change. This shows that the increased uptake of contaminants enhances the mass per unit length of each plant. The study revealed that the Rhodes grass can be used for low strength leachates, especially for metals. The application of combination of these grasses can further improve contaminant removal by constructed wetlands.

Chapter 1

INTRODUCTION

Environmental pollution affects the quality of hydrosphere, lithosphere, atmosphere, biosphere and pedosphere. Great remediation efforts are being made from previous twenty years minimize the pollutants at source and remediate the contaminants from contaminated water and soil resources. Phytoremediation is considered more cost effective than other techniques with lesser disadvantages than physical and chemical techniques. It is gaining popularity in academic as well as practical field. It has been identified that more than 400 plant species have potential for remediation of pollutant from soil and water. Among them, *Brassica*, *Sedum alfredii* H., *Thlaspi*, and *Arabidopsis* species have been mostly studied of their heavy metal uptake ability (Lone *et al*., 2008).

1.1 Open Dumping of Municipal Solid Waste

Open dumping of municipal solid waste is a major problem in developing countries owing to environmental pollution (Air, land and water), health hazards, vegetation loss, and unpleasant odour. These hazards are a consequence of biodegradation of solid waste within the dumpsites, which produces leachate (Zurbrugg, 2003; Zairi *et al*., 2001, Chofqi *et al*., 2004). If the leachate goes unattended by percolation into subsoil layers, it can degrade the soil and groundwater (Oygard *et al*., 2007). Leachate is known for having high concentrations of organics, nutrients, and heavy metals (Loizidou and Kapetanios, 1993). The quantity and quality of leachate depends upon the waste type and water coming into the dumpsite (Bhalla *et al*., 2012). The contaminants get dissolved in water and leach from the bottom of dumpsite, which further percolates through soil to the ground water (Słomczyńska, and Słomczyński, 2004). The movement of contaminants through soil to ground water is affected by many factors, including properties of the contaminant itself, soil characteristics, and physic-chemical conditions (Heyer and Stegmann, 2000). Properties of contaminants that determine their fate and results to be a potential threat to water quality include water solubility, adsorption, persistency, and toxicity (Bhalla *et al*., 2013).

In Pakistan also, solid waste disposal has remained one of the most neglected areas. There is a generation of about 71,680 tonnes of solid waste per day on the basis of 0.448 kg/capita/day (Pak EPA, 2005) which amounts to 26.16 million tonnes per year. As a matter of fact, only about half of this waste is collected by the municipal authorities. For example, in city of Karachi, only 55% of the households have waste collection. These collected as well as uncollected solid wastes, due to poor management, become the solid waste dump sites, which could be observed in every city of Pakistan (NCS, 2001).

In the twin cities of Rawalpindi and Islamabad, collection and disposal of solid waste is not very much improved. The waste is being dumped in an uncontrolled manner and there are a number of solid waste dump sites at various locations in the twin cities. The major ones being H-12 dump site, H-10 dump site, dump site at Bhatta chowk on Misrial road, Losar dump site on Chakbeli road. These dump sites have been the source of unhygienic conditions and environmental degradation (NCS, 2001).

1.2 Leachate Generation

When moisture content exceeds from the waste ability to absorb, it leaches down along with all dissolved pollutants with it. Precipitation is also a major factors which increase the process of leachate production. The leachate, if not properly handled and controlled, migrates into the various phases of environment i.e. soil, water and plants that resultantly affects them badly. Just for example the number of E. coli and Streptococcus organisms, present in the leachate, is estimated to be 10^6–10^7 per 100 cm^3 (during the summer months) (Slomczynska and Slomczynska 2005).

Leachate contains organic and inorganic substances including heavy metals especially iron, mercury, lead, zinc, and other pollutants from rusting cans, discarded batteries, appliances, paints, pesticides, cleaning fluids, newspaper inks, and other chemicals (Pak EPA, State of Environment Report 2005-Draft). Their penetration into the ground poses a serious hazard to natural waters (Abu-Rukah and Al-Kofahi, 2001). For example, the groundwater found near the H-12 dump site is not fit for drinking (Ihsan, 2008). Hence, there is a dire need for the immediate treatment of leachate, in order to protect, preserve and improve our environment.

1.3 Sources of Heavy Metals in Leachate

The main sources of heavy metals in leachate are batteries, electrical equipment, electronic waste, garden pesticides, photographic chemicals, personal care products, certain detergents, fluorescent tubes, waste oil, pharmaceuticals, wood treated with dangerous substances, and paint. The organics leach from the organic material already present in waste, and the stage of decomposition. The decomposition leads to mineralization of these organic compounds and effect the solubility of these compounds. In the early stages of decomposition, the organics are usually complex (e.g. lignin and cellulose) but with decomposition these transform into humic and fulvic like substances, which are comparatively more mobile than the complex compounds (Vedillo *et al*., 1999).

Metals and nutrients adhere to the fragments of these organics and help them to remain dissolved in water (McLean and Bledsoe, 1992). The accumulation of metal in topsoil for extended time period is a major concern, as metals can be taken up by the plants and get accumulated, which further moves on to the food chain causing harmful and toxic effects to plants and humans (Raghab *et al*., 2013). The sources of different metals and their impacts on human health are listed below in Table: 1

Pollutants	Major Sources	Effect on Human Health	P. level mg/l
Arsenic	Pesticides, fungicide, metal smelter	Bronchitis, Dermatitis, poisoning	0.02
Cadmium	Welding, electroplating, pesticide fertilizers, Cd and Ni batteries, nuclear fission plant	Renal dysfunction, lung disease, lung cancer, bone defects(Osteomalacia Ostioporosis), increased blood pressure, kidney disease, bronchitis, gastrointestinal disorder, bone marrow, cancer	0.06
Lead	Paint, pesticide, smoking, automobile emission, mining, burning of coal	Mental retardation in children, developmental delay, fatal infant, encephalopathy, congenital pyrolysis, sensor neuro deafness and acute or chronic, damage to the nervous system, epilepticus, liver, kidney, gastrointestinal damage	0.1
Manganese	Welding, fuel emission, ferromanganese production,	Inhalation or contact causes damage to central nervous system	0.26
Mercury	Pesticide, batteries, paper industries	Tremor, gingivitis minor psychological changes, acrodynia characterized by pink hands and feet, spontaneous abortion, damage to nervous system, protoplasm poisoning	0.01
Zinc	Refineries, brass manufacture, metal planting, plumbing	Zinc fumes have corrosive effect on skin cause damage to nervous membrane	15
Chromium	Mines, mineral sources	Damage to nervous system, fatigue, imitability	0.005
Copper	Mining, pesticide production, chemical industries, metal piping	Anemia, liver and kidney damage, stomach and intestinal irritation	0.1

Table 1: Different kind of metals, their effects on human health and their permissible limit

1.4 Heavy Metals Effect on Environment

Heavy metals disrupt metabolic functions in two ways:

1. They gather in body and malfunction in vital organs and glands such as the brain, heart, bone, liver, kidneys etc.

2. Metals transfer the important nutritional minerals away from their original place, which causes obstructing among their biological function. It is, however, not possible to survive in an environment free of metals. These metals enter to the body through different ways such as skin exposure, consumption of food, inhaled air and beverages (Singh 2007).

Plants exposure to metals experience oxidative stress which cause cellular damage and disrupt the cellular ionic homeostasis. To reduce the harmful effects of metals exposure and their storage, plants have grew detoxification systems particularly based on chelation and subcellular compartmentalization. A principal group of metal chelator known in plants is phytochelatins (PCs), are synthesized no--translationally from reduced glutathione (GSH) in a transpeptidation reaction catalyzed by the enzyme phytochelatin synthase (PCS). Therefore, availability of glutathione is very essential for PCs synthesis in plants at least during their exposure to heavy metals (Yadav, 2010).

1.5 Leachate Treatment Options

Many treatment processes have been studied to control the pollution caused by the leachate. Biological methods including aerobic and anaerobic processes have been shown to be effective for the treatment of leachate with high BOD/COD ratio (Muller et al. 2015). However, due to the age of waste, the biodegradability of waste is different and thus is a leachate varies. Biological processes become ineffective in the treatment of old landfill leachates (Renou et al. 2008). Moreover, presence of heavy metals inhibits the biological treatment (Bashir et al. 2013). Thus the physico-chemical processes are the right option to treat an old leachate.

Different methods are currently being used for treatment of municipal solid waste leachate. These methods are adopted frequently for wastewater treatment and processing. These treatment methods include: biological treatments and physical/chemical treatments.

These methods further categorised as: (Inanç, *et al*., 2000; Raghab *et al*., 2013; Malina and Pohland, 1996).

- Aerated lagoons and activated sludge which is also known as Aerobic Biological Treatment
- Anaerobic lagoons or reactors such as Anaerobic Biological Treatment
- Physiochemical treatment such as pH adjustment, air stripping, oxidation, chemical precipitation and reduction
- Coagulation using lime, ferric chloride, alum, and land treatment
- Advanced techniques such as ion exchange, carbon adsorption

1.6 Phytoremediation

Phytoremediation is a green technology in which plants are used for the removal of contaminants such as, organic compounds and heavy metals which are present in waste water, sewerage, topsoil and gravels. As it is cost effective, energy efficient, environmentally sound and eco-friendly technique for remedial measures of heavy metals, therefore it is applicable on large scale.

Phytoremediation techniques are most important in which plants that bear adverse conditions of toxic pollutants are essential. One of them is Vetiver grass that has its unique ability to bear adverse climatic fluctuations, heavy metals and other topsoil conditions. The capability of Vetiver grass for degrading heavy metals and other contaminants from topsoil, wastewater, sewerage, compost leachate and mine tailing has been reported by many researchers (Shu *et al*., 2006). The efficiency of phytoremediation using vetiver grass greatly depends on the chemical and physical composition of growth media and agricultural practices. To get desired objectives proper site specific and climatic conditions must be under consideration. The plant mechanism involves following processes to uptake and remove contaminants from the polluted soil or water is showing in the following figure: 1

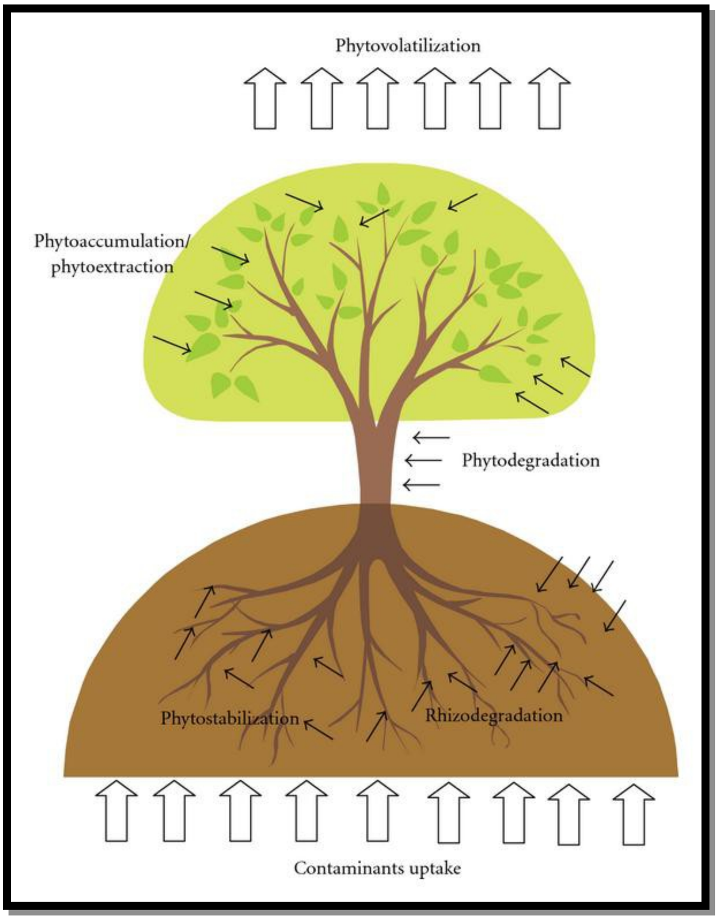

Figure 1: Processing of Phytoremediation in Plants

By introducing the phytoremediation technique using grasses it has been identified after many research scholars around the world that this technique is most useful and feasible for the removal of contaminants from polluted soil and water sources. So there is need of further investigation for grasses which uptake or accumulate the contaminants from waste water or soil efficiently. This technique is most feasible as compare to other techniques of physiochemical methods.

HYPOTHESIS

On the basis of previous statements of phytoremediation technique using plants or grasses for the removal of contaminants from waste water and contaminated soil in hydroponic environment we have developed the following statement or hypothesis to prove it.

- Vetiver grass, Elephant grass, and Rhodes grass can collectively remove significant amount of metals from municipal solid waste leachate

OBJECTIVES

The following aims were set to achieve and to approve the hypothesis of this research.

These aims/objectives are as follows:

- To evaluate concentration of contaminants in leachate and its uptake/removal particularly metals Zn, Cu, and Mn
- To compare metal uptake capacity of three grasses with their respective growth parameters

Chapter 2

LITERATURE REVIEW

Municipal sewage sludge probably does not use in agriculture due to containing high level of heavy metals and organic contaminants concentrations. The most easy and feasible way to treat municipal sewage sludge for the purpose of agriculture the phytotreatment considered suitable technique to lower the concentration of these pollutants and heavy metals from waste water. In phytoremediation technique plants species are used to uptake and reduce the contaminants and heavy metals untreated waste water. It is low cost, environmentally sound and sustainable technique that does not need high monitoring (Qiu *et al*., 2014).

2.1 Aquatic Plants in Metals Environment

In waste water or particularly leachate contaminated soil the concentration of heavy metals and other organic pollutants are very high that pose directly and indirectly effects on the human health and ultimately on the environment. Plants grown in such areas uptake these heavy metals from their roots and translocate in their shoots after harvesting of plants the heavy metals remove from them (Yang *et al*., 2009). The heavy metals present in leachate include copper, chromium, lead, iron, cadmium and mercury. The primary concern depends on the toxicity and presence of these heavy metals in particular source such as leachate (Rangsivek and Jekel, 2005).

In leachate or highly polluted water contains copper, arsenic and silicon mostly combined. Because the waste on dumpsite or landfill site is collected from various localities which comprises on different heavy metals (Nico *et al*., 2006). To remove these heavy metals such as copper, arsenic and silicon from leachate engineered wetlands and the processes which are used in these wet land for the removal of heavy metals are adsorption to sediments, chemical transformation, precipitation and the uptake of heavy metals using different species of plants. The treatment through plants is the most efficient, economical and environmental friendly technique, relatively to all those conventional remediation techniques (USEPA, 2002). That's why world is moving forward to phytoremediation and searching the plant species which can well survive in high concentrated contaminated water or leachate containing pollutant such as organic pollutants and heavy metals particularly copper, arsenic and silicon (Rofkar *et al*., 2014).

2.2 Grasses use for Phytoremediation

Phytoremediation is reduction or minimization of contaminants from contaminated soil or water by storage in plants root or translocation in shoot. Number of species have been recognized and tested for phytoremediation of metal and other contaminants from contaminated soil or water resources. In these species include duck weed (*Lemna minor* L.), water lettuce (*P. stratiotes*), water hyacinth (*Eichhornia crassipes*), calamus (*Lepironia articulate*), water dropwort [*Oenathe javanica* (BL) DC], sharp dock (*Polygonum amphibium* L.) and pennywort (*Hydrocotyle umbellate* L.) (Prasad and Freitas, 2003). It has been investigated that roots of Indian mustard are notified to be effective in the removal of Cu, Zn, Ni, Cr, Pb and Cd, and sunflower is found to reduce Cs-137, U, Pb and Sr-90 from hydroponic solutions (Zaranyika and Ndapwadza, 1995; Wang *et al*., 2002; Prasad and Freitas, 2003).

It has been found that the duck weed has potential to remove different metals such as Ni, Se, Cd, Cu and Cr from nutrient added solution and it has been in observed that it can accumulate Cu, Se and Cd more efficiently, Cr moderately and poor accumulator of Pb and Ni (Zayed *et al*., 1998). Aquatic macrophyte (*Eiochhornia crassipes*) for the removal of Pb from industrial effluents is studied in a greenhouse experiment and found efficient for Pb removal (Dos Santos and Lenzi, 2000). Water hyacinth has also fibrous root system which is well developed and has large biomass which is successfully used for the treatment of waste water to improve quality of water by reducing the limit of inorganic and organic contaminants. Water hyacinth store trace elements such as Pb, Cd, Ag, Cd, etc. and it has found efficient for phytoremediation of wastewater contaminated with Cr, Cd, Se and Cu (Zhu et al., 1999).

A research was conducted to test the efficiency of five wetland plant species, i.e., water dropwort, sharp dock, water hyacinth, duckweeds and calamus for waste water treatment in a pot experiment. Duckweeds remained efficient accumulator of Nitrgen and Phosphorus. Water hyacinth and duckweed intensely stored Cd with a concentration of 462 and 14200 mg/kg, respectively. Water dropwort accumulate the highest concentration of Hg, whereas the calamus achieved Pb (512 m/kg) and accumulate in its roots (Wang *et al*., 2002). Hydroponic research was conducted to find out the uptake of Cr, As, Hg, Pb, Zn and Ni by water hyacinth from the water solution at the concentrations which were ranging from 5 to 50 mg/L, and it has been noticed that in solutions containing 5 mg/L of As, Cr and Hg, the maximum uptake was 26, 108 and 327 mg/kg dry weight of water hyacinth, respectively (Ingole and Bhole, 2003).

In the community of ferns, *Pteris vitta* which in common language called Brake fern, it has been recognized as hyperacccumulator for toxic metal 'As' contaminated water and soil. It has ability to store up to 7500 mg As/kg from polluted site (Ma *et al*., 2001). The fern cultivar species is available for phytoremediation of

As. Fern has been effectively used for removal of As in field trials (Salido *et al*., 2003).

A small scale experiment using hydroponics conditions was conducted to identify different levels of different metals such as Cd, and effect on growth by three hydrophytes: *Echinodorus amazonicus, Gladiolous*, *Isoetes taiwaneneses*. The results of that experiment showed that the biomass of all plans which were used for experiment was reduced as the concentration of Cd increased. (Li H. *et al*., 2005). There effects were also testified for toxicity of Cd which were greater on *Echinodorus amazonicus and soetes taiwaneneses* Dwvol than to the *Gladiolous*. The storage of Cd concentration in plants was higher in *Gladiolous* than other two plants species. (Zhang *et al*., 2005) identified that the effectiveness of Cu reduction from polluted waste water by *Elsholtzi splendens* and *Elsholtzia argyi* in hydroponic environment. It has been recognized that *Elsholtzia argyi* identified good results for Cu phytofiltration than other species *Elsholtzi splendens*.

2.3 Mechanism of Metal Uptake by Grasses

Contaminant uptake by plants and its mechanisms have been being explored by several researchers. It could be used to optimize the factors to improve the performance of plant uptake. The plants act both as "accumulators" and "excluders". Accumulators survive despite concentrating contaminants in their aerial tissues. They biodegrade or biotransform the contaminants into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass (Sinah, *et al*., 2004; Tangahu *et al*., 2011).

Plants grow and nourish with specific ability to obtain nutrient efficiently and uptake essential nutrients from soil or water even present in very small amount. Plant roots uptake nutrients more efficiently when added chelating agent, pH changed and redox reactions. They also have an efficient mechanism of translocation of nutrient to shoots and also store them in roots. Another specific mechanism of plants is to translocate and store the nutrients or contaminants efficiently. Collectively all mechanisms have their specific abilities in specific plants which involves uptake, translocation and storage of nutrients and essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation (U.S. Department of Energy 1994).

The ion uptake and translocation mechanisms plants are known as the transport mechanisms or the plants cell plasma has specialized proteins embedded in it which is important in translocation of nutrients these mechanisms includes (1) proton pumps (″-ATPases that consume energy and generate electrochemical gradients), (2) co- and antitransporters (proteins that use the electrochemical gradients generated by ″-ATPases to drive the active uptake of ions), and (3) channels (proteins that facilitate the transport of ions into the cell). Every transport mechanism has capability to take specific amount of ions. But there is a problem in interaction of ionic species while high concentration of different metals uptake. The uptake by roots is further desired to translocate to the shoots. Because the biomass of shoots can be harvested whereas roots are not feasible to harvest. Little is known regarding the forms in which metal ions are transported from the roots to the shoots (U.S. Department of Energy 1994).

Plant uptake-translocation mechanisms are likely to be closely regulated. Plants generally do not accumulate trace elements beyond near-term metabolic needs. And these requirements are small ranging from 10 to 15 ppm of most trace elements suffice for most needs (U.S. Department of Energy 1994). The exceptions are “hyperaccumulator” plants, which can take up toxic metal ions at levels in the thousands of ppm. Another issue is the form in which toxic metal ions are stored in plants, particularly in hyperaccumulating plants, and how these plants avoid metal toxicity. Multiple mechanisms are involved. Storage in the vacuole appears to be a major one (U.S. Department of Energy 1994).

The evaporation of water from plant leaves into the air work as pump which make room and respiration in which plant root uptake more nutrient from the rhizosphere which consist of water and soil. In general sense this process of release and uptake nutrient is called evapotranspiration. Under this process the contaminants present in soil or water translocate into the shoots of plant. When contamination moved to the shoots from root then the shoots harvested and contamination removed from plant. In this process the soil remained unspoiled. These plants also go through the process of phytoextraction mechanism that is named as “hyperaccumulators.” In this term the plant shoot or root get the maximum concentration stored or accumulate into one of them. In the mechanism of Nonaccumulating, plant species naturally accumulate contaminant more or less in shoots or roots. Preferably, hyperaccumulators plants should increase their plantation in hazardous environments, because they required little check and balance and also generate high biomass, though some plants species completely fulfil these requirements (Salido, *et al*., 2003).

Metal accumulating plants species can accumulate metals like Cd, Zn, Co, Mn, Ni, and Pb more than 1000 times from those accumulate by the mechanism of nonaccumulator (excluder) plants species. In numerous cases, bacteria present in the rhizosphere environment also contribute their role to move the nutrients and metals to transfer in plants which also translocate to the shoots from roots. They remove organic contaminant more efficiently than inorganic contaminants (Erdei, *et al*., 2005).

2.4 Vetiver Grass Considered Best Accumulator of Metals

Vetivrer grass (Vetiveria Zizanioidous) is considered as versatile grass. It is used for various purposes on the unique capabilities it possessed by nature. By the research studies in china the grasses have particularly abilities to grow rapidly, deep root for resilient to the harsh environment, larger biomass and stabilization of soil erosion (Gilbert, 2000; Loch, 2000; Ye, *et al*., 2000). Therefore vetiver grass used to control the land degradation and soil erosion in most effected parts of the china. It has also been studied well that the Vetiver grass (Vetiveria zizanioides) can grow and tolerate adverse environment and can well survive in abundance of heavy metal (Xia and Shu, 2001). Vetiver grass has successfully survived in very alkaline (pH 9.8), saline coal and very acidic (pH 2.5) in gold mine area. Same research results are extracted from China with similar application (Xia, 2001; Xia and Shu, 2001). For the purpose of strong resistance against harsh climate and to identify the ability of Bahia grass (Paspalum notatum Flugge) Vetiver grass (Vetiveria Zizanioides), St.augustinc Grass (Stanotaphrum Secundatum) and Bana grass (Pennisetum Glaucum) to uptake and accumulate heavy metal from oil shale dump. In comparison study the vetiver grass show best results to survive in adverse environment and accumulate heavy metals in shoots and roots whereas Bahia grass shows best in control of land degradation and strong resistant against harsh environment (Xia and Shu, 2001, Xia. H. P. 2004). The effectiveness of vetiver grass also confirmed by the research conducted in Nigeria where soil erosion and land degradation is wide spread that vetiver grass is a strong resistant against adverse environment high biomass and survive in highly concentrated polluted land (Babalola, *et al*. 2007).

In Burkina-Faso a research was conducted to check the capability of decontamination of polluted soil and waste water from pesticides by growing vetiver grass around the cotton growing fields (Abaga *et al*. 2014). It has been researched by many scholars that the Vetiver grass (Vetiveria zizanoides) has capability to fulfil all the criteria and requirements which are essentials for phytoremediation technology. Vetiver grass (Vetiveria zizanoides) is native from the Indian subcontinent (Danh *et al*. 2009). Undeniably vetiver grass is a miracle grass having unique qualities such as fast growing, non-invasive, need low maintenance after plantation and perennial grass (Srivastava *et al*. 2008). Vetiver grass bears extremely dangerous climatic circumstances and also raises in tropical areas like Burkina Faso. The vetiver grass accumulates heavy metals especially zinc and lead (Truong 1999; Antiocha *et al*., 2007).

The number of plant species that are reported to have hyperaccumulation traits (metal concentration >1000 mg/kg dry weight). There are different species used for uptake of metals such as 4 species has been identified for the removal of As, 1 specie for Cd, Co 34, Cu 34, Pb 14, Ni >320 and Se 20 species (Reeves, 2003).

Vetiver grass (Chrysopogon zizanioides) has extraordinary qualities among all plant species used for phytoremediation (Lomonte *et al*., 2011). Due to unique composition, morphology and symbiotic combination vetiver grass has ability to bears environmental pressures (Srivastava *et al*., 2008). Vetiver grass is a tall (3-4 m), having high biomass, aerenchymatous and complex root structure. The complex root system of vetiver grass percolate deep under the soil and attach itself with the soil strongly. Vetiver grass has very strong physiological and morphological advantages to survive under very harsh and adverse climatic conditions and environmental fluctuations, like sever temperature (−25 to 65◦C), fire, storms, submergence, frosts and droughts (Truong, 2000; Lomonte *et al*., 2014).

The positive and outstanding outcomes of vetiver grass has confirmed by many scholars due to its different applications in different parts of the world. The application of vetiver grass for phytoremediation on heavy metal polluted soil can be promoted by initializing the economic incentives to them (Danh *et al*., 2011). The oil extract from vetiver grass grown on contaminated soil with heavy metals it is possible to return the cash and cost to the producer. But high concentration of Pb present in soil cause adverse effect on both the chemical composition of oil extracted from the roots of vetiver grass, its yield amount and particularly the existence of vetiver grass (Danh *et al*., 2010).

2.5 Sources and Impact of Heavy Metals

Anthropogenic activities becoming worsen to the environment as a whole with the passage of time and damaging it adversely. The pollutants which contaminate the environment extremely the heavy metals play an important role (Evangelou *et al*., 2007). The continuous industrial development, rapid increase in global economy and increase in world's population cause major anthropogenic activities in all sectors of life for better living style of the human being. These man made activities bring a dramatic change into the environment and make it worse (Gerhardt et al. 2009). Toxic chemicals exposure and heavy metals release in to the environment are of major concerns. The major sources of heavy metal exposure into the environment are gas exhausting, burning of fossil fuels, fuel generation and energy production, manure slush treatment, warfare, military training, mining, use of fertilizers, pesticides application and electroplating. The largest heavy metals leftover are produced by the process of hard rock mining. The hard rock mining industry is now working in all regions of the world except Antarctica (Mendez and Maier 2008).

The heavy metals occurring in the environment can cause a major threat to the human and environment by entering in to the food chain. The important heavy metals particularly mercury (Hg), cadmium (Cd), lead (Pb), and arcenic (As) these are identified as Potentially Toxic Elements (PTEs) which can probably cause chronoic poisoning and hazardous diseases (Ang *et al*., 2010).

Heavy metals are the most dangerous element in the environment which directly and indirectly harm the environment by different ways. There is no other option to remove the heavy metals except phytoremediation through those plants which are harvestable. Consequently, phytoremediation is basically to extract, uptake and decontaminate the polluted sources by using plants. It has been intensively confirmed because of their influences, non-intrusive, cost effective, an environmentally sound and socially acknowledged source to remediate pollutant from contaminated soils and waste water (Alkorta and Garbisu, 2001; Garbisu *et al*., 2002; Alkorta *et al*., 2004; Nunez-Lopez *et al*., 2008).

The heavy metal accumulation in plants is categorised and elaborated in two types: Translocation Factor (TF) it is described as the particular amount of heavy metals present in shoots to roots. The translocation ability of plants to relocate the heavy metals from roots to shoots (Roongtanakiat, 2009). The second type is the Bioconcentration Factor (BF) it can elaborate as the amount of heavy metals concentration into the shoots of plants at the time of harvesting and the amount of heavy metals concentration present in the external environment (Zayed *et al*., 1998). The values of these two factors (BF) and (TF) are very important for the estimation of plants potential using the phytoremediation technique (Selamat *et al*., 2014).

As heavy metal is polluting the topsoil and ground water it is severely damaging the aquatic environment as well. In heavy metal pollution includes copper, cadmium, nickel, arsenic, lead, zinc, mercury etc. The heavy metals are non biodegradable persistent in environment and having ability to accumulate in different environmental constituents particularly in living things unlike the organic pollutants. That is the biggest issue related with the heavy metals. The main sources of heavy metals exposure into the environment is through various man made sources such as fertilizers, discharge of industrial effluent, small level industries, fuel generation sources, electronic waste dumping, mining, manufacturing industries etc (Chaudhuri *et al*., 2014).

Heavy metals are major threat to human health, animals and environment. It enter to the food chain and directly and indirectly disturb all segments of environment. Most of the heavy metals and other organic pollutants are mainly caused by anthropogenic activities, the arsenic is one of the toxic heavy metal produced by mining, using pesticides in fields, industrial effluent and burning of fossil fuels and become a part of the environment (Muntean *et al*., 2009). Therefore, its individual sources are rocks, minerals, topsoil, living organisms and water (Alvarado *et al*., 2008).

Reasonably, the use of plants and trees with repetition cycles, the accumulated heavy metals will reduce to an acceptable level from contaminated soil and waste water (Ang *et al*., 2010). The Plant species with the efficiency of having large biomass, fast growth and efficient heavy metals accumulation are essential for major-scale phytoremediation of heavy metal from polluted soils (Visoottiviseth, 2002).

Furthermore, the heavy metals present in the environment do not reduce like the organic pollutants do. These heavy metals translocate from one place to another. It is transferred from contaminated soil and water to plants and then become the part food chain (Lomonte *et al*., 2010). Heavy metals which are commonly exist in polluted zone, mercury (Hg) is the biggest environmental threat to the public health and environment. Because it has ability to accumulate on the surface. It is very toxic in both inorganic and organic forms even very small quantity (Oliver *et al*., 2005). For the removal of Hg it has been confirmed that the phytoremediation is the most efficient and environmental friendly technique for degradation of mercury Hg (Kelly *et al*., 2006).

Different traditional techniques are being used for the removal of this toxic heavy metal from water. In phytoremediation technique the plants are used to clear the pollutants and heavy metals from polluted sites such as contaminated soil, water and sediments (Khataee, 2012). Phytoremediation is used for the extraction of pollutants from the contaminated sites to clean up the environment. It can give extraordinary results when it applies on large area to clean up the environment. It is publically very accepted due to eco-friendly, sustainable and very cost effective technique for the treatment of contaminants (Bennicelli *et al*., 2004). It has ability to clean up extensive range of contaminants such as, organic compounds, heavy metals etc (Alvarado et al. 2008). The variety of plants are used to test the efficiency of arsenic removal and uptake from the waste water by authors. The accumulation of arsenic in plants shoots and roots depends mainly on the morphological and physiological structure of plants. Vetiver grass have these characteristics even grow well in hydroponic conditions (Anderson and Walsh 2007, Favas et al. 2012, Goswami et al. 2014).

2.6 Phytoremediation

Phytoremediation involves the processes of extraction, stabilization, and volatilization. Phytoextraction is a sub process of phytoremediation in which plants remove dangerous elements or compounds from soil or water, most usually heavy metals, metals that have a high density and may be toxic to organisms even at relatively low concentrations (Roongtanakiat, 2009). Phytostabilization is the use of metals- tolerant plants to inhibit the mobility of metals, thus reducing the risk of further environmental degradation by leaching into ground water or by airborne spread (Salt *et al*. 1995; Neuman and Ford 2006). A form of phytoremediation from the soil, are released into the air, sometimes after being broken down into volatile components. These processes of phytoremediation are mentioned in figure 2 (Chen *et al*., 2004; Khan *et al*., 2004).

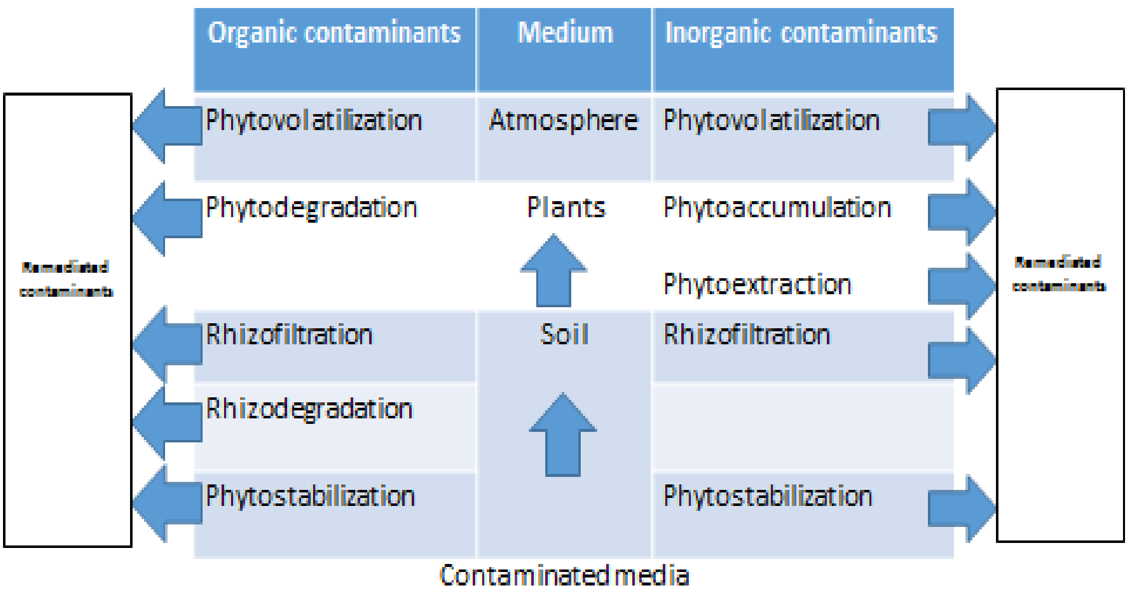

Figure 2: Process of Contaminants Removal through Different Techniques of Phytoremediation

Phytoremediation is a green, cost effective and sustainable technology to decontaminate the polluted soil and uptake and accumulate the heavy metals from waste water and contaminated soil. This technology protect the environment where all other conventional technologies such as ion exchange columns, alkaline precipitation, and electrochemical removal, filtration, and membrane technologies. Though, these traditional technologies may cause sever effects on the environment particularly aquatic ecosystems (Ahmad et al. 2014). For the objective of phytoremediation of heavy metals from waste water and contaminated soil many grass species have been used. In this regards the plants of wetland have also been used but their capacity to uptake and accumulate heavy metals fluctuate greatly (Deng et al. 2004; Mishra et al. 2007; Chandra and Yadav 2011). So, the heavy metal uptake ability of a particular plant species must be investigate for appropriate and certain heavy metal uptake and decontamination of soil.

For phytoremediation of heavy metals from soil of mined lands different plants species are used. Vetiver grass (Chrysopogon zizanioides and Vetiveria zizanioides) is an outstanding species that grows in the excessive presence of heavy metals in harsh environment. It can grow and tolerate worse climatic condition. It accumulates high concentrations of heavy metals into its roots and shoots particularly includes copper, zinc, lead and chromium (Rotkittikhun et al. 2007; Antiochia et al. 2007; Castillo et al. 2007). Besides the purposes of environmental protection (Truong and Baker, 1998) there are other uses of vetiver grass such as control soil erosion with its massive and long deep roots system, flood control and droughts (Truong and Baker, 1998; Yang *et al*., 2003; Chiu *et al*., 2006).

Furthermore, the oil is extracted from roots of vetiver grass. This oil has organic and perfumed characteristics and also have conventional application in aromatic and medication field (Zhu *et al*., 2001). Vetiver grass fractions are extensively used for unification in perfumes and cosmetics, whereas, Vetiver Grass root extracts occupying various and unique biological characteristics such as, antioxidant, antifungal, anti-inflammatory, antibacterial and anticancer (Sridhar *et al*., 2003; Hammer *et al*.,1999; Chen *et al*., 2003; Jagtap *et al*., 2004; Kim *et al*., 2005).

2.7 Efficiency of Grasses

Vetiver grass *(Vetiveria zizanioides*) lately named as (*Chrysopogon zizanioides*) is widely employed in heavy metals removal especially from MSW leachate (Troung 2000). It is tall (1 – 2.5m), fast in growth, tolerant to heavy metals, and with the help of lengthy roots, it creates massive root system beneath soil. (Truong and Scattini, 1990; Troung 2001; Ibezute et al., 2014). It is also used to produce pesticide oil repellents (Truong et al., 1995).

Elephant grass (*Penisetum purpurium Schumach*) is also used for Phytoremediation purposes, owing to its excessive production of cellulose biomass, tolerance to heavy metals (Copper) and high growth rates (Ito and Oi 1990; Woodward *et al*., 1991; Ekpenyong *et al*., 1995; Mesa-Perez *et al*., 2005; Prine *et al*., 2007; Wang *et al*., 2002; Liu *et al*., 2009). It is also being used as growth medium for edible fungus and feedstock for papermaking along with as conservation media to lessen down soil erosion. (Zhou and Li, 1999; Kabi et al., 2005; Adekalu *et al*., 2007). EG, which originates from Africa, has been grown successfully in China for nearly 80 years. It has a potential biomass yield of 5×104– 6×104 kg/ha (about 20–25 tons/acre) over a long period (about 180 days) with a linear growth rate (Xie, 1995).

However phytoremediation capacity of comparatively a less known grass was also investigated under the same conditions i.e. Rhodes grass (*Chloris guyana*), which is a crop tolerant to adverse climates, developed in Australia, like barley (*Hordeum vulgare*) (Greenfield, 2002; Danh *et al*., 2009). In fact it has not been used before the date for phytoremediation purpose as per literature reviewed. However, in fact the Rhodes and Vetiver grass belong to the same group of highly salt tolerant species, encouraged the author to believe that this grass could be of real use as well. As discussed earlier it is adaptable to various ranges of soil and climatic conditions along with being moderately tolerant to alkaline and saline soil (Suttie, 2000).

Furthermore, the experiments were carried out under hydroponic environment, which has been widely used to evaluate the accumulation ability w.r.t. heavy metals of grasses (Khabaz-Saberi *et al*., 2010; Stoyanova *et al*., 2009). Also it has been confirmed through research comparisons that there occurs a significant correlation of results under hydroponic conditions to that of real time conditions (Zhivotovsky *et al*., 2010).

2.8 Hydroponic Culture

Hydroponics may simply be described as growing plants with nutrients and water, and without soil. The water must be delivered to the plant root system. The root system may hang directly in the nutrient solution, be misted by it, or can be enclosed within a container or a trough which is filled with a substrate [a replacement for soil]. The substrate may consist of many different types of materials, such as perlite, sand, sawdust, wood chips, pebbles, or rockwool.

The application of hydroponic experiment has been widely used to evaluate the accumulation ability and tolerance against heavy metals (Khabaz-Saberi et al. 2010; Stoyanova et al. 2009). The hydroponic condition is also considered the best method to speed up the experiment in particular environment. It has also been reported by Zhivotovsky et al. 2010 that results are correlated well which obtained from field experiment and in hydroponic condition.

2.9 Benefits of Hydroponics

Hydroponic system has some advantages over the soil environment. The plant grown in hydroponic environment grow 35-55 percent faster than plant grown in soil, under the same environment and conditions. The yield and biomass of the plant is also greater than the later one. Scientists observe that there are many causes for differences between hydroponic conditions and soil plants. And one of the major difference is the plenty of oxygen present in rhizosphere. This extra oxygen in hydroponic system help the root growth efficiently. Plants with ample oxygen in the root system also absorb nutrients faster. In hydroponic system nutrients precipitate and mixed with water and become available for roots uptake. In this mechanism the nutrients remains readily available for plants root and roots do not search for nutrients to uptake. The frequency of nutrients to deliver the plant per day increases several times more than in soil. The plants grown in hydroponic system do not need high energy to find out food or energy. Extra energy saved by plants help to grow the plant faster and produce extra fruits. Hydroponic plants also have some disadvantaged but such as bug infestations, funguses and disease. In general, plants grown hydroponically are healthier and happier plants than grown in soil.

Hydroponic gardening provides many advantages to environment. In hydroponic gardening less water is used significantly than soil gardening, the reason behind id that the nutrient solution is constantly reused. Small amount of pesticides are used in hydroponic fields and they do not considered necessary to use more pesticide in crops. The issue of topsoil eliminated because the soil is not used in hydroponic system. Ultimately, it will not wrong to elaborate that it agricultural practices continued to cause soil erosion and generate waste water, hydroponic system may take over and will be our only sustainable solution.

2.10 Growing Mediums

The main aim of growing medium is aeration for the support of plants growth and particularly root system. Scientists believe that plants grow better in hydroponic environment because presence of ample oxygen. They uptake nutrient efficiently in such conditions. Different type of hydroponic systems work very well in presence of different growing mediums. These growing mediums includes fast draining medium, like Hydrocorn or expanded shale it works efficiently in an ebb and flow kind of system. It consist of light and aerated type of growing medium that permits plenty of oxygen to penetrate the plant's root system. These growing condition is very stable and rarely effect the pH of the nutrient solution.

Rockwool is another growing medium which has become very famous growing medium. Rockwool growing medium contains 10-15 times more water than soil contains and has 20 percent of holding capacity of air in it which can be used for hydroponic system for better performance. Though the gardeners should be extra careful about the pH, Rockwool has a pH of 7.8 it can raise the pH of the nutrient solution. Rockwool cannot be used indefinitely and most gardeners only get one use per cube. It is also commonly used for propagation.

After consulting numerous articles of this particular field it has confirmed that for purpose of phytoremediation of heavy metals from contaminated soil or water grasses play a vital role. Hydroponic culture experiment also studied and scientists believe that hydroponic system using water as growth medium has extra oxygen and in the presence of ample of oxygen plant uptake nutrients faster. So it is quite evident that phytoremediation of contaminants particularly metals can be removed efficiently by plants or grasses in hydroponic environment.

Chapter 3

MATERIALS AND METHODS

3.1 Plants and Leachate Collection

Three types of grasses Vetiver Grass, Elephant Grass and Rhodes Grass were taken from the nursery of National Agriculture Research Council (NARC) Islamabad, Paki... of Rawalpindi city) Rawalpindi, Pakistan. ...al landfill site, extends over 75 acres. At prese... s is dum... was colle...

Figure 3: A glimpse of Dumpsite ...m where leachate was collected

Plants and Leachate were collected from NARC and Losar

Pots were prepared with different leachate concentrations

Analysis of leachate were examined before Plantation

Pots trial was installed for four months (Sep.-Jan).

Samples of leachate and plants were analysed

Plants Oven Dried and crushed in mortar and pestle

Leachate and plant prepared for heavy metal analysis after digestion process at Atomic Absorption Spectroscopy (AAS)

...rom material collection to samples analysis. ... steps which were taken to the end of resea...

Figure 4: Flow Chart Diagram of Experimental Work to Analysis

3.2 Leachate (growth media) Preparation for Plantation of Grasses

The pattern for leachate dilutions was followed according to the Figure 5 below:

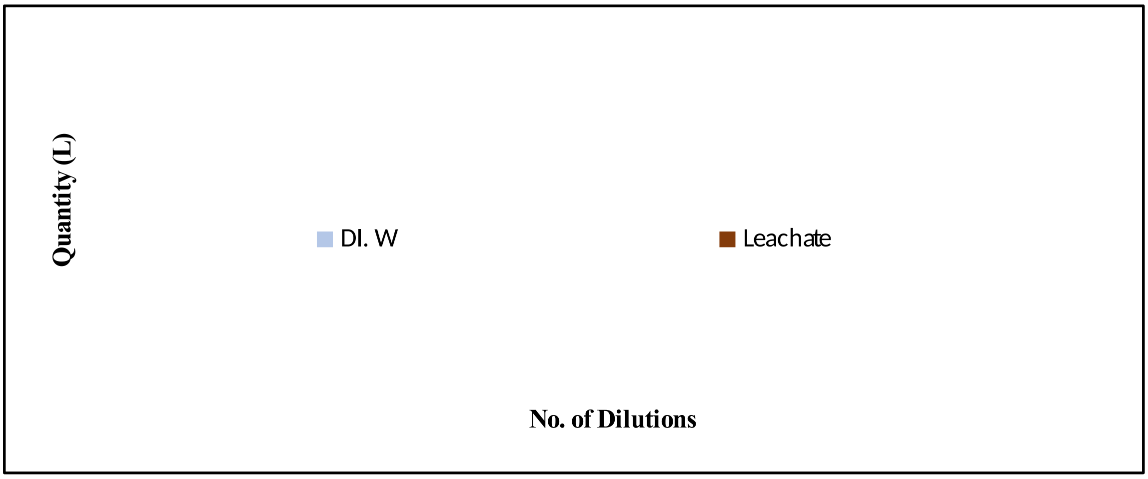

Figure 5: Pattern of Leachate Dilutions used for treatment

Experiment Design

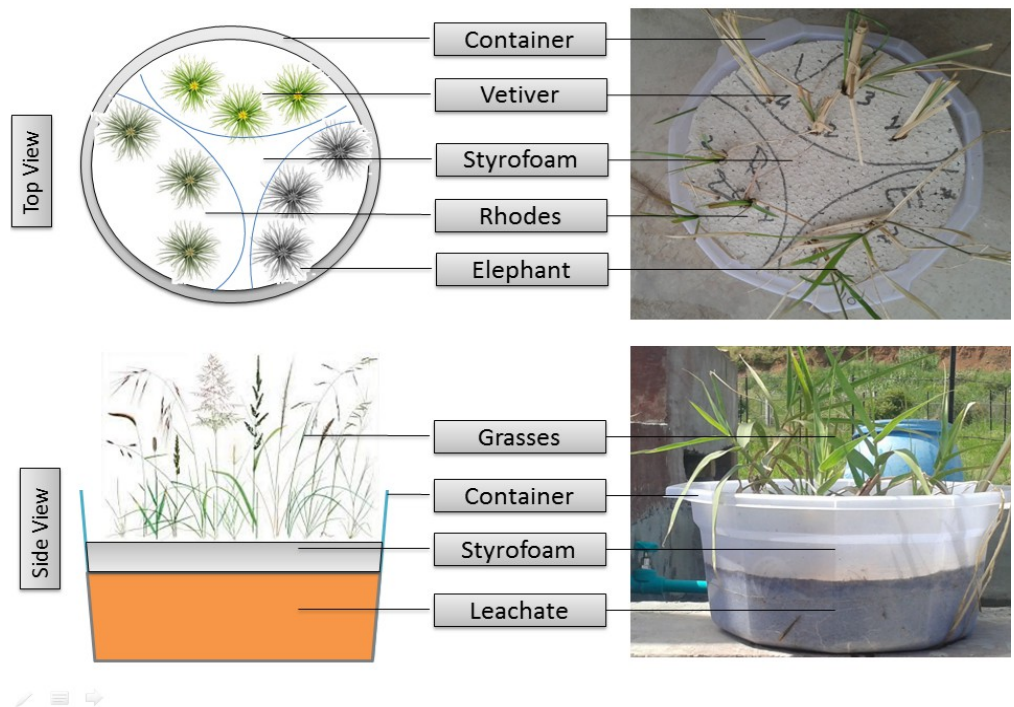

Figure 6: Experimental setup by Top and Side View

3.3 Design of Pot Experiment

Eleven plastic pots (12 cm diameter and 15 cm height) were filled with six litre leachate samples each. Different grasses were grown in separate pots in hydroponic environment in tap water as control experiment showing in above Figure 6. Each pot received two-month old grasses (each containing three grasses) with shoots and roots of 15 and 9 cm length respectively for Vetivre grass, 12 and 5 cm for *Pennisetum purpureum* (Elephant grass) respectively and in case of *Chloris gayana* (Rhodes grass) the shoot and root were 10 and 6 cm long respectively. Also the plants in pots were grown in different leachate strengths (10, 20, 30,40,50,60,70,80,90 and 100%) to compare the effect of each leachate strength on plants growth. This preparation of leachate is explained through a graph which is shown in above Figure 5.

3.4 Analysis of Metals in Leachate

All chemical used in this research was of analytical grade. Sulphuric acid (H_2SO_4) 98%, Potassium dichromate ($K_2Cr_2O_7$) 99.5%, Hydrochloric acid (HCl) 37% of Scharlau Spain. Mercuric Sulfate ($HgSO_4$) 98%, Silver Sulfate ($AgSO_4$) 99.5%, Hydrogen per Oxide (H_2O_2) and Farious Ammonium Sulfate (FAS), Ferroin Indicator, Vanadate-molybdate.

Leachate sample (20g) was digested by acidifying the sample with 10ml HCl (37%), 2ml H_2O_2 and 10ml of 2% HNO_3 in a microwave digestion system (MV 700) at 120^oC for 2 hrs. The digested samples were analyzed for Cr, Pb, Cu, and Cd by Flame Atomic Absorption Spectrometer (AAnalyst 800).The metal concentration in leachate samples was expressed in mg/l.

3.5 Leachate Analysis for other Contaminants

Experiments were conducted to spotlight the basic parameters of leachate samples. The electrical conductivity (EC) and pH of the leachate was measured using conductivity and pH meters (inoLab pH/Cond 720). Nitrates and phosphates were also calculated before and after the treatment of leachate using UV-Visible Spectrophotometer (T-60 PG Instruments: Wavelength Range: 190-1100 nm) (Clescerl et al. 1999). Atomic Absorption Spectroscopy (AAnalyst 800, Perkin Elmer Precisely) was used to measure heavy metals concentration present in leachate. Chemical Oxygen Demand (COD) was calculated using standard Open Flux method which involved followed steps. 1.5 ml of $K_2Cr_2O_7$ (99.5%) & 3.5 ml of H_2SO_4 reagents were added to 2.5 ml of leachate sample. The samples in the PTFE vial were heated in COD reactor (HACH) at 120ºC for 2 hrs. Ferrous Ammonium Sulfate (FAS) was used as titrant in the presence of fermion indicator (5220 B. Open Reflux Method). COD was calculated using the following formula:

$$COD = \frac{(A - B) \times M \times 8000}{Volume\ of\ sample\ (mL)}$$

Where,

A= Volume of FAS used to titrate the blank in mL

B= Volume of FAS used to titrate the sample in mL

M= Molarity of FAS solution which is 0.1

For nitrates and phosphates analysis digestion of leachate samples were made. For nitrates analysis of leachate samples UV-Spectrophotometer (T-60 PG Instruments: Wavelength Range: 190-1100 nm) was used to find out absorbance of sample at the wavelength of 220 and 275 nm (4500 NO_3^- B. Ultraviolet Spectrophotometric Screening Method ASTM). The absorbance was used to put in standard curve readings to get final concentration of nitrates. To find out the concentration of phosphates Microwave Digester (AURORA Instruments) used for digestion of sample. The digestion of sample converts the organic contents in to the orthophosphate which is detectable by UV Spectrophotometer. Vanadomolybdo phosphoric acid colorimetric method (4500-P C ASTM) was used to measure the absorbance by UV- Visible Spectrophotometer. For phosphate determination 470 nm wavelength was used. The absorbance at this wavelength used to put into the standard curve reading to get the concentration of phosphate in mg/L. in Table 2 the methods and instruments used for analysis are listed in Table 2 below:

Table 2: Tests of Leachate Analytical Analysis Using Different Methods and Instruments

Parameters	**Symbol**	**Units**	**Method/Type**	**References**
pH	pH	--	pH/Cond 720 inoLab	4500 H+ B
Conductivity	EC	µS/cm	pH/Cond 720 inoLab	2510 B
Chemical Oxygen Demand	COD	g/L	Open Flux Method	5220-B.
Phosphates	PO_4	mg/L	Vandomolybdo Phosphoric acid colorimetric method	4500-P C.
Nitrates	NO_3	mg/L	Ultraviolet Spectrophotometric Screening Method	4500 B. NO_3^-
Copper	Cu	mg/L	Direct Air-Acetylene Flame Method	3111 B.
Zinc	Zn	mg/L	Direct Air-Acetylene Flame Method	3111 B.
Manganese	Mn	mg/L	Direct Air-Acetylene Flame Method	3111 B.

3.6 Plant sampling and analysis

The plants were given growth time of four months (Sep. to Dec.). The plant samples were washed with tap water followed by deionized water and their shoots and roots were cut and placed separately. Plants height was measured at the time of plantation and harvesting whereas length of roots and shoots were measured separately. Moreover weight of shoots and roots was measured before and after drying grasses in oven at 60ºC for 72 hrs. Then the dried biomass was weighed and fine ground in pestle and mortar (Ash, and Troung, 2014).

For Cu, Zn and Mn extraction, 100 mg of plant material was digested with a mixture of 97.9 % $HClO_4$ & 69% HNO_3 (2:1 v/v) in a microwave digester then solution heated until the brown fumes disappear. It was then cooled 5mL of diluted (1:1) HCL (density 1.18 g/mL) added, and finally diluted with H_2O up to 25 mL solution. Then the sample was filtered using the filter paper of 1µm and the filtrate was diluted for Atomic Absorption Spectroscopy (Danh *et al.,* 2009). Standards of different metals were analysed to make the standard line straight for all metals using Flame Atomic Absorption Spectroscopy. Then samples of plants and leachate were run to find out the concentration of different heavy metals. Concentration of Cu, Zn and Mn were found through Flame Atomic Absorption Spectrometer. Moreover, the shoot/root metal concentration ratio, was also calculated.

Chapter 4

RESULTS AND DISCUSSION

4.1 Leachate strength (%) and its Characteristics

Leachate strength was started from 10% to 100%. Total volume of solution was 6 L (6000mL). Tap water was used to make the %age dilutions (Strength) of leachate. The parameters of tap water was analysed before making solution with the leachate for leachate dilutions which is explained in Table 3.

Table 3: Analysis of Different Parameters for Leachate Dilutions

Parameters	Units	Treatments (%)		
		Control	10	100
COD	g/L	0.00	3.5	6.9
Ph	-	7.2	8.0	8.9
EC	mS/cm	0.855	3.1	14.3
NO_3^-	mg/L	0.00	92.9	295.0
PO_4^{3-}	mg/L	0.00	2.4	11.3
Mn	mg/L	0.001	45.0	190.0
Cu	mg/L	0.01	51.9	156.0
Zn	mg/L	0.03	73.9	378.0

4.2 Municipal Solid Waste Leachate Characteristics

The analysis for both treated and untreated leachate of each MSW treatment were performed. An average pH values of all leachate strength of untreated leachate remained alkaline (pH 8.5) and slightly reduced to 3% after treatment at (pH 8.25). Electrical conductivity was also reduced by almost 73 % from its initial concentration. The average concentration of COD before treatment was calculated 5163.6 and reduced to 1715.2 mg/L after treatment which reduced 66.8 % of COD. Similar outcome was reported by (Khai and Trang 2012; Ibezute *et al.,* 2014) which were 61 and 63.11%. In a hydroponic experiment, (Xia *et al*. 2000) investigated that vetiver had ability to survive in leachate with COD of 1120.1 mg/L and we have found that vetiver could still survive in the 100% leachate strength in which the COD value was 6,930, which was extremely high. It has also been proven here that different grasses in comparative hydroponic environment can survive even in high concentrated leachate such as this study has proven by using these three type of grasses vetiver, Elephant and Rhodes grass. These are an excellent pollution resistant plant and could be used to alleviate the problem of contaminated soil.

Nitrate and phosphate were reduced by 93.7 % and 29 %, respectively after the treatment of leachate. The average removal of heavy metals such as zinc, copper and manganese were from 184.952 to 5.833 mg/L, 98.098 to 0.103 mg/L, and 115.209 to 1.709 mg/L respectively. The average removal of Zn, Cu and Mn from MSW leachate was 96.85%, 99.9 % and 98.52 % respectively are shown in Figures 7 and 8:

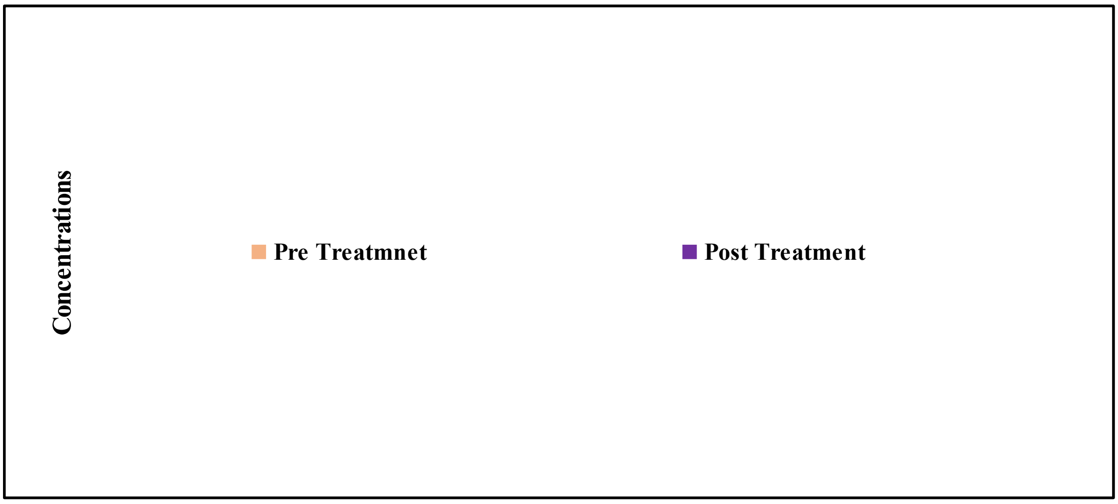

Figure 7: Characterization of Different Parameters of Leachate before and after the Treatment

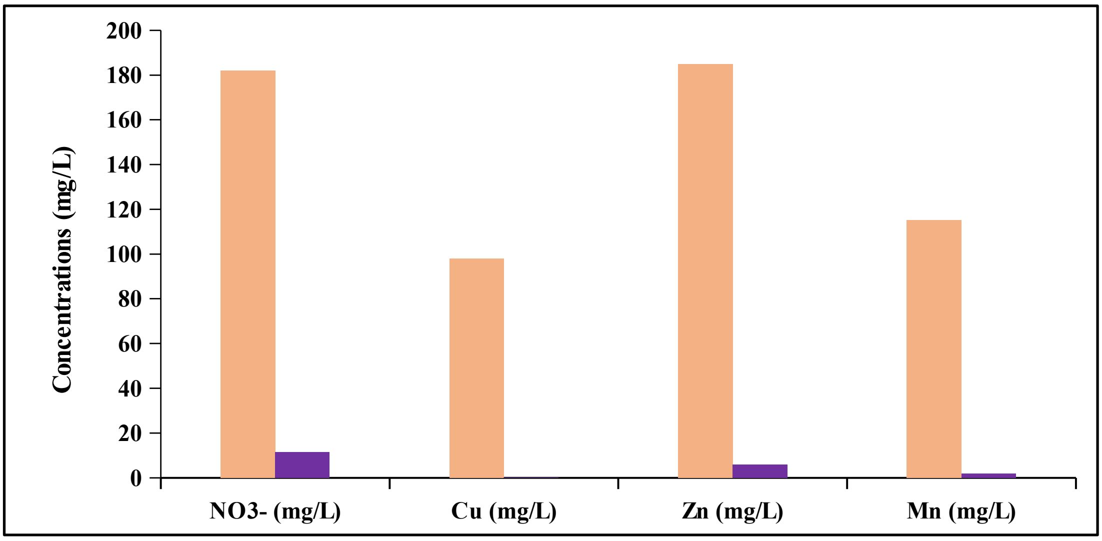

Figure 8: Characterization of Different Parameters of Leachate before and after the Treatment

The control was filled with water only. For the other dilutions such as 10%, 5400mL of water and 600mL of leachate was used. Same behaviour was kept with other 20, 30, 40… 100% dilutions by increasing and decreasing the amount of leachate and water respectively. The 6 L solution was selected due to the capacity of pots which were used for experiment. The size of pot was 11 inches diameter and 6 inches height.

4.3 Growth Measurement of Plants

The height of plants shoots and roots were kept same with their species type. Such as height of Vetiver grass roots and shoot in all leachate strength or treatments was same. There initial heights of shoots and roots were 15, 10, 12 (cm) and roots 9, 6 and 5(cm) Vetiver, Rhodes and Elephant grass respectively. After treatment the height was increased three folds as shown in Figure 9;

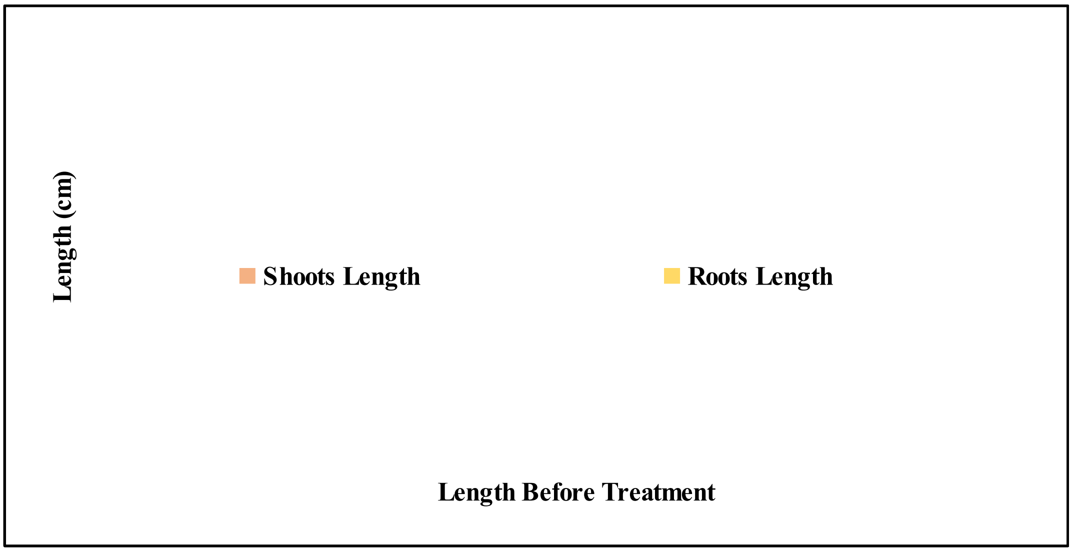

Figure 9: The initial length of Shoots and Roots

4.4 Length of grasses Shoots and Roots

Vetiver is characterized as fast growing, high (1-2.5 m) and perennial grass and its roots are also extraordinary long and create a complex web and massive root system under the ground (Truong and Scattini, 1990; Danh *et al.,* 2009). The maximum shoot height was measured 30 inches. In other leachate strength except 100% Vetiver shoots showed increasing height and a similar growth pattern while 100 % treatment plants had the lowest plant height. The average weight of dry shoot and root was not different among plants treated with other than 100% leachate strength. Growth performance of roots of vetiver, elephant and Rhodes grass are shown in these graphs. The initial length of grasses roots are also along with final length as shown in Figure 10.

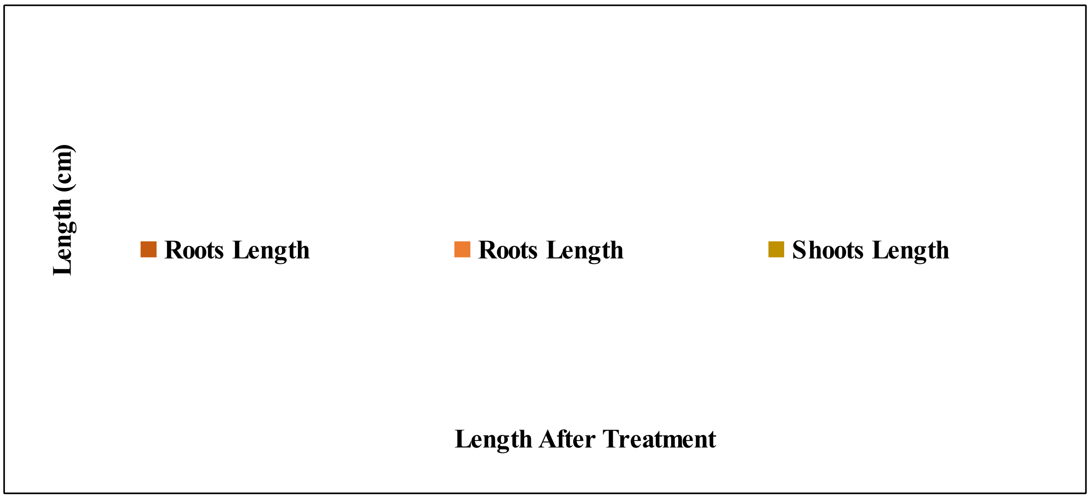

Figure 10: The length of Vetievr, Rhodes and Elephant grass shoots and roots for the period of four month in hydroponic environment

The roots of Vetiver grass spread very rapidly in hydroponic environment. It is tolerant to heavy metals, and with the help of lengthy roots, it creates massive root system beneath soil (Ibezute *et al.,* 2014). Due to its unique characteristic the roots of Vetiver is used for different purposes such as for medicinal use (Balasankar *et al*., 2013), in aromatic and cosmetic industries and particularly the vetiver is used to prevent soil erosion in different region of the world (Troung, and Loch, 2004). In hydroponic environment of pot experiment

Roots were circulating in the bottom of pot. The maximum length of Vetiver roots was observed in 40% leachate strength which was 56 inches (5ft) as resulted by (Troung, 1999). After 40% leachate strength the roots length was decreased with increasing leachate strength whereas, in 100% leachate strength, it remains 38 inches as shown in Figure 11 below.

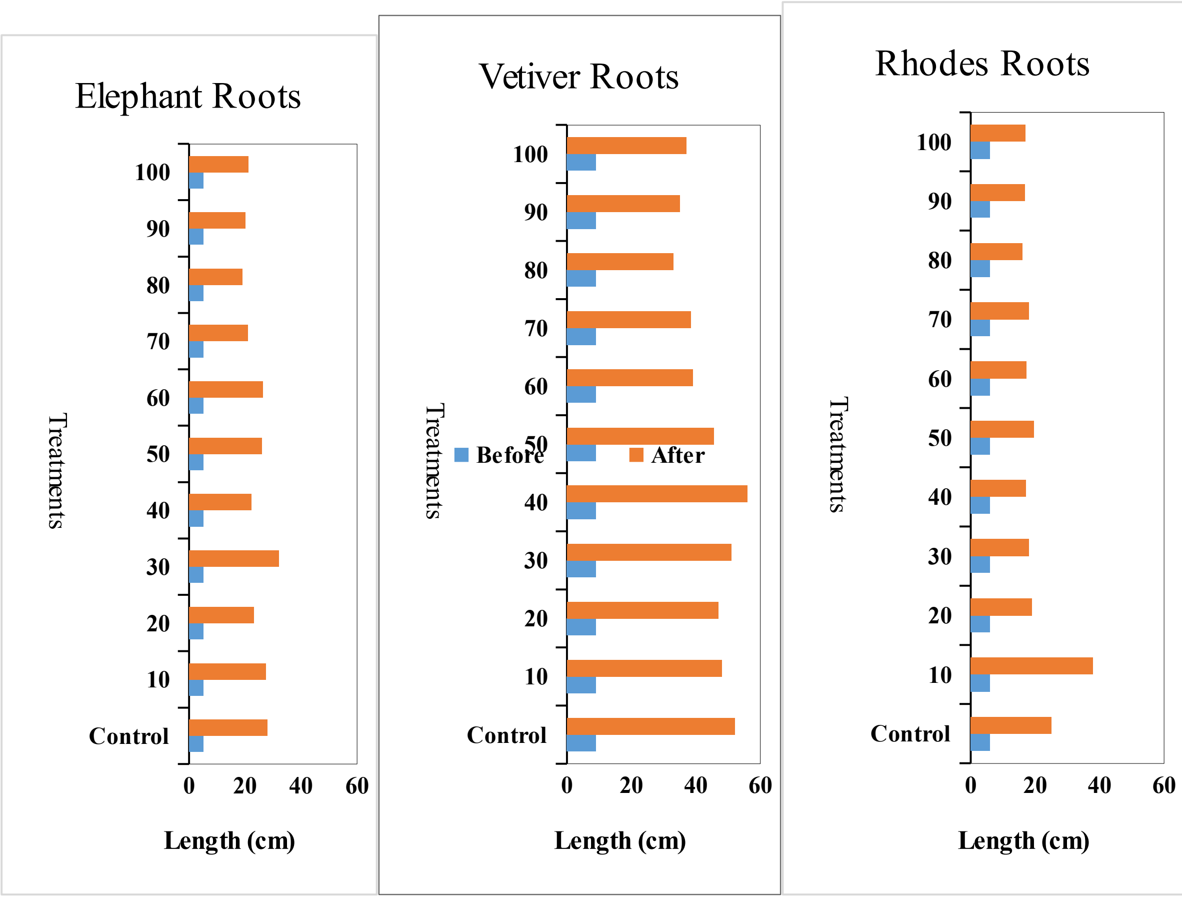
Elephant Roots
Vetiver Roots
Rhodes Roots
Treatments
100
90
80
70
60
50
40
30
20
10
Control
0
20
40
60
Length (cm)
Before
After

Figure 11: Initial and Final Root Length of Vetiver, Elephant and Rhodes grass

The shoots height of Vetiver was maximum in 50% leachate strength due to less metals stress on roots and its accumulation and translocation system. In case of roots the most feasible stage was 40 % leachate strength where roots of Vetiver grass showed maximum growth and length.

Roots of three grasses were also spread in length and these roots were in spiral shape due to bottom of the pot shown in Figure 13. The roots spread more rapidly than shoots and the length of roots is three to four time greater than height of shoots in same period.

In these graphs vetiver shoots are showing the highest growth. In literature we have already go through growth performance of vetive which were 3.6 meter in 12 months. The maximum growth of roots was measured in the leachate strength of 40% which had contained 3600mL of water and 2400mL of leachate. The main reason for maximum root length in 40% leachate strength was moderate level of contaminants and concentration of metals.

Shoots of Rhodes grass cut in same height before plantation. The maximum height of Rhodes grass was observed in 10% and 20% leachate strength as shown in figure 2. Where it was flowering and blooming quit charmingly. Its height at 10% was 36 inches (3ft). In 100% leachate strength it survived quite well but low in height of 10.5 inches. In case of Rhodes grass the maximum length of roots was measured in 10% of leachate strength which was 35 inches (2.9ft). 100% leachate strength pot was lowest root length as 18 inches (1.5ft) which stand on average root. These comparison of shoots of all grasses are explained in Figure 12.

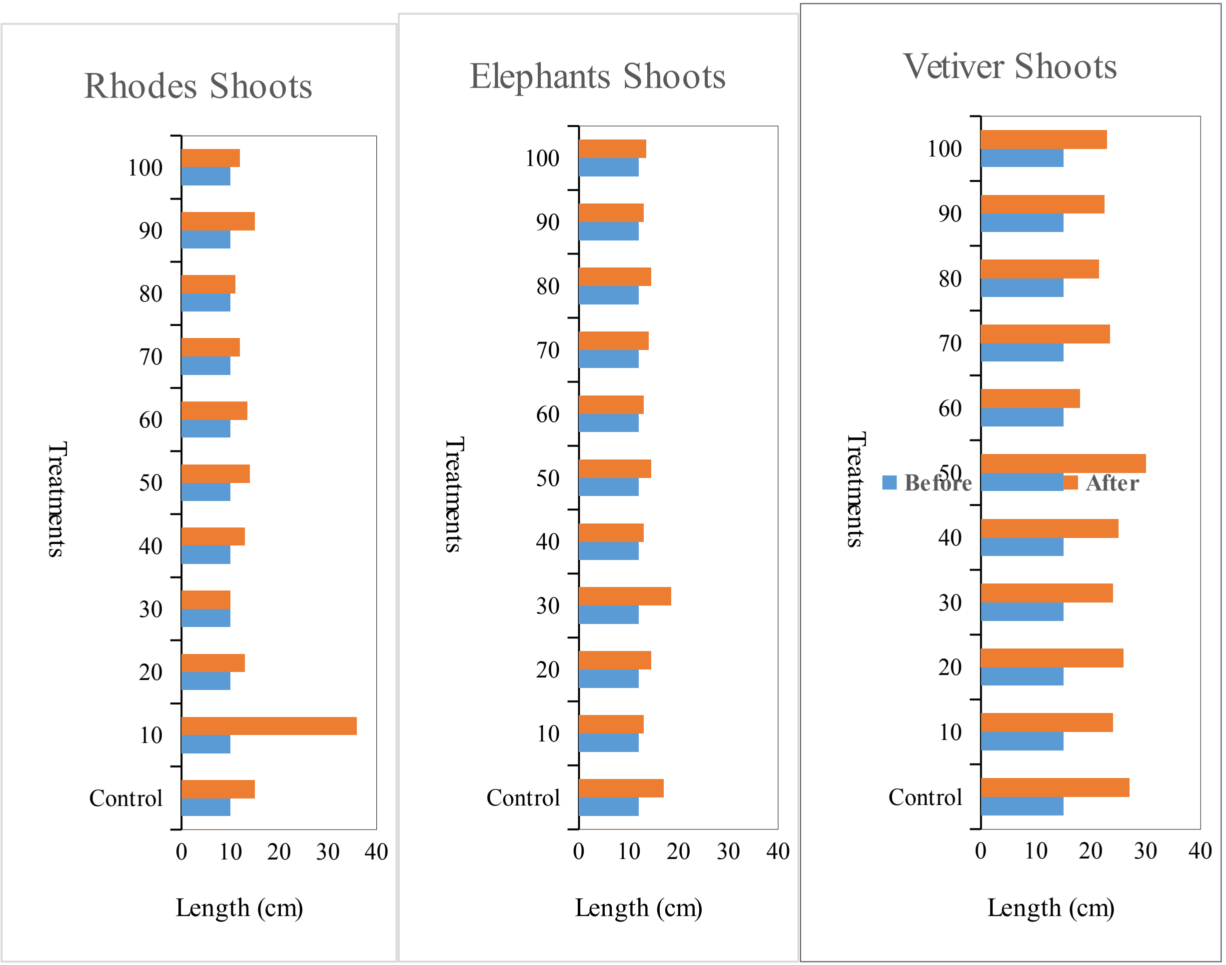
Rhodes Shoots
Elephants Shoots
Vetiver Shoots
Treatments
100
90
80
70
60
50
40
30
20
10
Control
0
10
20
30
40
Length (cm)
Before
After

Figure 12: Shoots Length of All Three Grasses Before and After the Treatment

Rhodes grass: maximum length of shoots and roots was 36 and 32cm and also flowering leachate strength 10 %

Elephant grass: maximum length was 19 and 36 cm leachate strength of 30 %.

Vetiver grass: maximum length was 30 and 56 cm observed in 50 and 40 % leachate strength respectively.

Figure 13: A pictorial view of roots of different grass after treatment of four months. Roots were round and round into the bottom of the pots

The Elephant grass shoots height was maximum in leachate strength of 20%. It was about 30 inches (1.8ft) as shown in Figure 2. Similar results were measured by (Liu *et al.,* 2009). In other leachate strength, it was fluctuating from 15 to 17 cm. In 100% leachate strength the height was 15.5 inches. In different leachate strength the Elephant grass survived and also enhanced its biomass in all leachate strength including 100%. Elephant grass roots showed noticeable growth. The maximum root length was observed in 30% leachate strength which was 33 inches (2.75ft). The 100% leachate strength was containing roots length of 22 inches which was slightly below the average length of roots.

4.5 Heavy Metal uptake by Grasses

The removal of heavy metals concentration from MSW through different grasses is eco-friendly and sustainable application. Plants which can accumulate heavy metals have ability to convert the toxic heavy metals into less toxic forms (Ensley, 2000).

In case of copper, less proportion was absorbed and translocated in roots and shoots of vetiver where Elephant grass uptake copper more efficiently than Rhodes and Vetiver grass. Elephant grass is more tolerant against copper than Vetiver and Rhodes (Liu *et al*., 2009) and large portion accumulates in root system. The average uptake of Elephant grass for copper was 28.495 mg/kg in roots whereas Vetiver and Rhodes grass uptake 25.23 mg/kg and 22.44 mg/kg respectively. In Shoots of Elephant grass the trend of average uptake was the same as 10.12 mg/kg maximum then shoots of Vetiver and Rhodes grass as 7.9 mg/kg and 4.855 mg/kg respectively.

The highest concentrations of Zn was found in Rhodes grass both in shoots and roots as mentioned in figure The mean concentration of Zn in roots of Rhodes grass was 36.39 mg/kg whereas in Vetiver and Elephant grass the average concentration was 25.37% mg/kg and 16.85% mg/kg in roots respectively. The Rhodes grass is not used for heavy metal removal before so the roots of Rhodes grass uptake high concentration of Zn than the shoots of Vetiver and Elephant grass. In case of Rhodes grass shoots the uptake was four times less than shoot of Vetiver and Elephant grass. The proportion of Zn in shoots and roots of Elephant grass was almost same and maximum translocation happen in shoots than Vetiver and Rhodes grass but lesser accumulation in roots than others these metals accumulation by different grasses are also elaborated graphically in Figure 14 below.

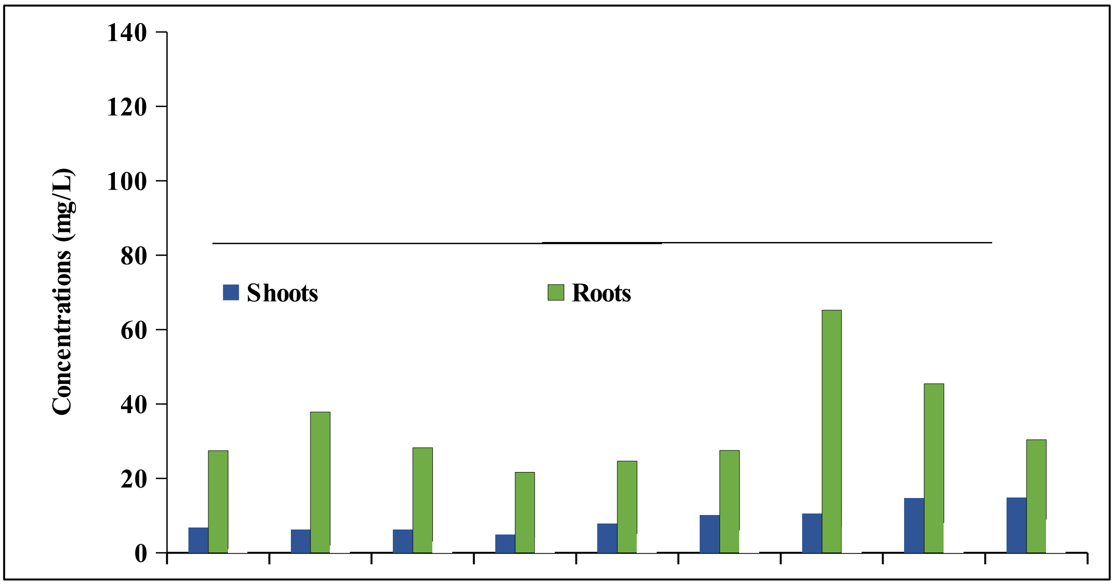

Figure 14. Graphical representation of different Metals uptake by Shoots and Roots of different grasses

The accumulation of many heavy metals in Vetiver roots is much higher than in shoots (Zhuang *et al*., 2005; Troung, 1999; Yang *et al*., 2003; Xia *et al*., 2004; Lai and Chen 2004; Danh *et al*., 2009; Roongtanakiat, *et al*., 2007). Vetiver is highly tolerant to many heavy metals (Troung, 2001). Mn was found maximum in roots of Vetiver grass than that of shoots. The concentration of Mn in roots of Vetiver, Elephant and Rhodes grass was 33 mg/kg, 25.205 mg/kg and 24.45 mg/kg respectively.

4.6 Dry weight of shoots and roots after treatments

Weight of dry plants was also measured. Roots and shoots were measured separately. All plants were alive and full of chlorophyll content. Which was the actual reason for their higher weight and it is explained in Figure 15.

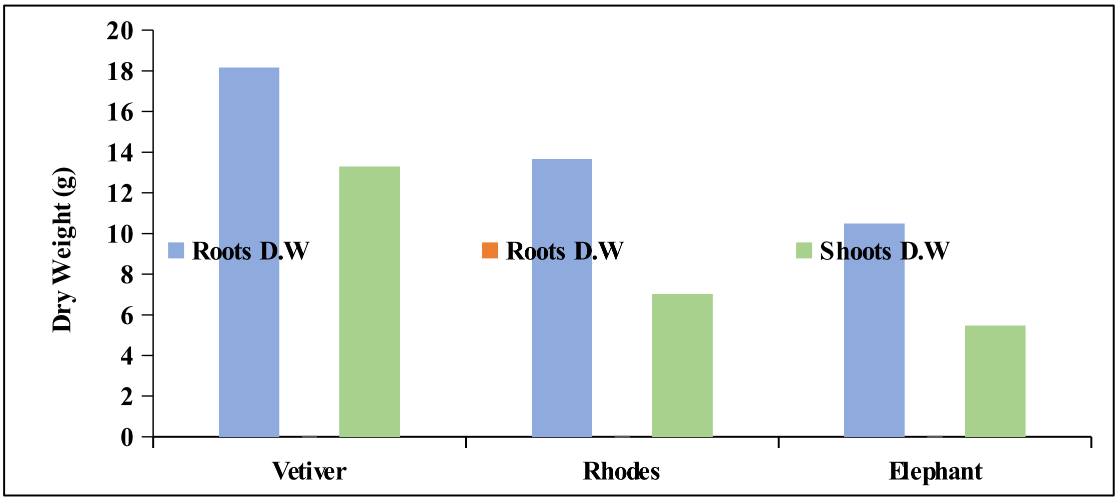

Figure 15: Dry weight of Plants Shoots and Roots after the treatment

The maximum biomass of Vetiver grass shoots and roots were at 50% leachate strength which is 37.44 grams. The dry weight of root and shoot part is 21.56 and 15.65 respectively. In Elephant grass the maximum biomass produced in 30% leachate strength and bloom very well. The dry weight of biomass is 22.76 grams whereas the mass of shoot and root is 8.35 and 14.41 grams respectively. Rhodes grass was grown and flowering at 10% leachate strength with dry weight of 30.63 grams where roots and shoots contain 11.87 and 18.86 grams respectively. The shoots remains low in length but high in biomass due to load of nutrient and metals and they increase its weight of biomass. Their length of shoots in 80, 90, and 100% leachate strength was low as compare to the other 8 dilutions which but their biomass at that stage was greater than other dilutions.

When these plants were installed they were of equal weight but with slight variation of weight but after the treatment in polluted water full of contaminants and micronutrients there was great variation of dry weight among each other. The dry weight was measured before and after the oven dry. The sample were oven dry at 105 ºC for 72 hours. The dry mass subtracted from the previous mass when washed with distilled water and net weight was calculated. The above grass shows the variation among the net weight of each grass. Standard deviation explains the variation in individual plant of each species to their average value.

Maximum dry weight with respect to biomass was weighed of Vetiver grass whereas, Rhodes grass remained after Vetiver grass with both shoots and roots. Elephant grass dry mass was lowest of all other Vetiver and Rhodes grass with shoots and roots.

4.7 Mass Balance of Metals

The inflow and out flow were analysed before and after the treatment. The inflow of Mn, Cu, and Zn was measured as 115.21, 98.10, and 184.95 mg and outflow remained 3, 1 and 4 mg respectively. The maximum percentage uptake of Mn, Cu and Zn done by Rhodes, Vetiver and Elephant grass which were 39%, 39%, and 41% respectively. It is also explained in Table 4 below:

Table 4: Mass Balance of Metals and their Uptake efficiency in mg and Percentage

Metals		**Mn**	**Cu**	**Zn**
Inflow (mg)		115	98	185
Uptake (mg)	Rhodes	34	27	76
	Vetiver	44	33	60
	Elephant	34	38	45
Outflow (mg)		3	1	4
Percentage uptake	Rhodes	30	27	41
	Vetiver	39	34	33
	Elephant	30	39	25

CONCLUSIONS

At the end the outcome of this research is concluded by the following statements:

- In this study all three grasses Rhodes, Elephant and Vetiver grass remained effective for different metals like Zn uptake by Rhodes grass (33%), Cu efficiently uptake by Elephant grass which was (31%), and Mn efficiently uptake by Vetiver grass (35%).
- Uptake of metals at high concentrations significantly
- Rhodes grass was used very first time for phytoremediation from municipal solid waste leachate and it "Uptake Zn greater than other two grasses. And also grew very well particularly at the leachate strength of 10%.
- This grass has been used for the first time for metal uptake/phytoremediation from Municipal Solid Waste Leachate"
- Vetiver grass, Elephant grass, and Rhodes grass can collectively remove significant amount of metals from municipal solid waste leachate under hydroponic conditions. They compete with each other and such competition lead them to display great efficiency of their particular ability.

RECOMMENDATIONS

- Plant species are more feasible for the removal of contaminants which is environmentally friendly and cost effective. Phytoremediation reduce the land degradation and land remain reversible.
- It has been discovered through this research that Rhodes grass uptake metals efficiently from municipal solid waste leachate particularly Zinc. So, Rhodes grass should be used for phytoremediation of different metals
- This experimental process is very helpful for exploration of new species that can remove metals efficiently. Such exploration leads us to the phytoremediation of contaminants efficiently in that case the exploration of new grasses must be encouraged.
- The phytoremediation technique must be applied in areas highly contaminated with metals and other contaminants to reclaim the degraded land area.

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