APPLICATION OF INVASIVE PLANTS FOR BIOACCUMULATION OF POLLUTANTS FROM INDUSTRIAL EFFLUENT WASTE

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Invasive plants.
Phytoremediation.
Aquatic plants.

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Dedicated

To my respectable parents who made my dreams successful.

Success always solicits for two things,
"Exertion & Fortune"

If we are successful then our exertion is the effort of our beloved parents and teachers, which they made to, fulfill our wishes and our fortune is due to their prayers, hence I dedicated my success to my Beloved Parents.

DECLARATION

I hereby declare that the work present in the following report is my own effort, except where otherwise acknowledged and that the report is my own composition. No part of the project report has been previously presented for any other degree.

Date __________

Naila Yasmeen

CONTENTS

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List of Abbreviations

EPA	Environmental Protection Agency
USEPA	United states environmental protection agency
VOC	Volatile Organic Compounds
CNS	Central Nervous System
CDF	Caution Diffusion Facilitator
PEPA	Pakistan Environmental Protection Act
NEQS	National Environmental Quality Standards
BOD	Biological oxygen demand
APH	American public health
COD	Chemical oxygen demand
Cl^-	Chlorine
$CaCO_3$	Calcium Carbonate
Cd	Cadmium
Cu	Copper
Cr	Chromium
CO_3	Carbonates
DO	Dissolved Oxygen
EC	Electrical Conductivity
TDS	Total Dissolve Solids
TSS	Total Suspended Solid
mg/l	Milligram per litr
NISMP	National Invasive Species Management Plan
Co	Cobalt
HCssl	Hydrogen chloride
AAS	Atomic Absorption Spectrometer
O_2	Oxygen
Fe	Iron

H_2O_2	Hydrogen peroxide
Hg	Silver
HCO_3	Bicarbonates
NARC	National Agriculture Research Centre
$MgCO_3$	Magnesium Carbonate
NO_3 –	Nitrates
NaOH	Sodium Hydroxide
Pb	Lead
Ni	Nickle
ppm	Parts per Million
pH	Hydrogen Ion Concentration
SO_4-	Sulphates
SS	Suspended Solid
DS	Dissolved Solid
CMC	Carboxymethyl Cellulose
PVA	Polyvinyl Alcohol
NaOH	Sodium Hydrooxide
H_2O_2	Hydrogen peroxide
$NaSiO_2$	Sodium Silicon Oxide
NaH_2PO_4	Sodium Dihydrogen Phosphate
µS	Micro Simon
Sn	Tin
Zn	Zinc
FEPA	Federal Environmental Protection Agency

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

ABSTRACT

In order to estimate the pollution load of textile effluent, physico-chemical and metal analysis of textile waste water was carried out. Results indicate that the pollution load of many parameters was above the permissible limit of national environmental quality standards (NEQS). This sample was further exposed for phytoremediation to three plants named *Chenopodium ambiosoides, Mentha piperita and Xanthium stromarium.*

After treatment of textile effluent by invasive plants, results showed that pH level was slightly increased and dissolved oxygen (DO) remained constant but other parameters like electric conductivity (EC),total dissolved solids (TDS) ,and turbidity was reduced to significant amounts. Bicarbonate level in textile effluent was minimized enormously by Chenopodium as compared to the other plants and chlorides absorbed best by *Chenopodium ambiosoides* and *Xanthium stromarium* as compared to the *Mentha piperita.* Heavy metals (Cr, Cu, Cd) were present in minute quantity in textile effluent (according to the AAS results) however three plants having capacity to hyperaccumulate Cr and Cu but Cd concentration remain constant.

INTRODUCTION

1.1 INTRODUCTION

One of the most critical problems of developing countries is improper management of huge amount of wastes generated by anthropogenic activities (kenu et al, 2011). More challenging is the unsafe disposal of these wastes into the ambient environment. Water bodies especially freshwater reservoirs are the most affected. Due to these activities, natural reservoirs not suitable for both primary and secondary utilization (Fakayode, 2005).

1.2 INDUSTRIAL WASTE

Industrial effluent contamination of natural water bodies has emerged as a major challenge in developing and densely populated countries like Pakistan. Estuaries and inland water bodies, which are the major sources of drinking water in Pakistan, are often contaminated by the activities of the adjoining populations and industrial establishments (Sangodoyin, 1995). River systems are the primary means for disposal of waste, especially the effluents, from industries that are near them. These effluent from industries have a great deal of influence on the pollution of water body, these effluent can alter the physical, chemical and biological nature of the receiving water bodies (Sangodoyin, 1991). Increased industrial activities have led to pollution stress on surface water that comes from industrial, agricultural and domestic sources (Ajayi and Osibanji, 1981).

Wastes entering in water bodies are both in solid and liquid forms. These are mostly derived from industrial, agricultural and domestic activities. As a result of treated and untreated or partially treated industrial wastes converted natural water bodies into highly polluted water bodies. The resultant effects of this on public health and the environment are usually great in magnitude (Osibanjo et al., 2011).

Industries are the major sources of pollution in all environments (water, soil and air etc). Based on the type of industry, various levels of pollutants can be discharged into the environment directly or indirectly through public sewer lines. Wastewater from industries include process wastes from manufacturing, wash waters and relatively uncontaminated water from heating and cooling operations (Glyn and Gary, 1996). High levels of pollutants in natural water systems causes an increase in biological oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), toxic heavy metals such as Cd, Cr, Ni and Pb and fecal coliform (bacteria) and hence make water not suitable for drinking, irrigation and aquatic life. Industrial wastewaters range from high biochemical oxygen demand (BOD) from biodegradable wastes such as those from human sewage, pulp and paper industries, slaughter houses, tanneries and chemical industry. Others include those from plating shops and textiles, which may be toxic and require on-site physiochemical pre-treatment before discharge into municipal sewage system (Emongor et al., 2005; Otokunefor and Obiukwu, 2005).

Effluent generated by different industries as a result of their operation and processing, depending on the industry and their water use, the wastewater contain suspended solids, both degradable and non biodegradable organics; oils and greases; heavy metal ions; dissolved inorganics; acids, bases and colouring compounds as shown in (Table 1.1).

Table 1.1: Waste Effluents Generated by Selected Industries (Korsaic, 1992).

Type of waste	Type of industry
Oxygen-consuming waste(organic)	Breweries, Dairies, Distillers, Packaging houses, Pulp and Paper, Tanneries, Textiles
High Suspended Solids waste (SS)	Breweries, Coal washeries, Iron and Steel Industries, Distillers, Pulp and Paper mills, Palm oil mills
High dissolved solids waste (DS)	Chemical plants, Tanneries, Water softening Oily and grease Laundries, Metal finishing, Oil fields, Petroleum refineries, Tanneries, Palm oil mills
Colored compounds	Pulp and Paper mills, Tanneries, Textile dye houses, Palm oil mills
Acidic pollutants	Chemical plants, Coal mines, Iron and Steel, Sulfite pulp
Alkaline pollutants	Chemical plants, Laundries, Tanneries, Textile finishing mills
High Temperature wastewater	Bottle washing plants, Laundries, Power plant, Textile mill effluent (Oganfowokan and Fakankun, 1998)

1.3 TEXTILE INDUSTRIAL WASTE

Textile industry is play a major part of Pakistan's economy and is one of the most important and largest industrial sectors of Pakistan with regard to production source of foreign exchange and labor employment. It alone accounts for 65% of the country's export, 46% of industrial production 38% of employed industrial work force and 9% of gross national product (Iqbal, et al., 2007).

With the increased demand for textile products, the textile industry and its effluents have been increasing proportionally, making it one of the major sources of severe pollution problems worldwide (Santos, et al., 2007).In particular, the release of dyes into waste waters by various industries is an environmental issue due to various dyes' persistent and recalcitrant nature. Textile industries are responsible for the discharge of large quantities of dyes into natural waterways due to inefficiencies in dyeing techniques. Up to 50% of dyes may be lost directly into waterways when using reactive dyes (McMullan et al., 2001).

The presence of dyes in water is easily noticeable even when released in small concentrations. This is not only unsightly, but the colour of the water by the dyes may have an inhibitory effect on photosynthesis affecting aquatic ecosystems. Dyes may also be problematic if they are broken down anaerobically in the sediment, as toxic amines are often produced due to incomplete degradation by bacteria. The breakdown products of dyes are toxic and mutagenic to life (Weisburger, 2002).

The textile industry is distinguished by raw material used and this determines the volume of water required for production as well as waste generated. Heavy metals have been associated with textile effluents (Schneider et al., 1999). The nature of the processing (sizing, dying, printing) exerts a strong influence on the potential impacts associated with textile manufacturing operations due to the different characteristics associated with these effluents shown in (Table 1.2)

Table 1.2 Effluent Characteristics from Textile Industry (Kanu et al., 2011)

Process	Effluent composition	Nature
Sizing	Starch, waxes, carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA), wetting agents	High in BOD, COD
Desizing	Starch, CMC,PVA, fats, waxes, pectins Bleaching Sodium hypochlorite, Cl2, NaOH, H_2O_2, acids, Surfactants, $NaSiO_2$ sodium phosphate (NaH_2PO_4)	High alkalinity, high SS
Mercerizing	Sodium hydroxide, cotton wax	High pH, low BOD, high DS
Dyeing	urea, reducing agents, oxidizing agents, acetic acid, detergents, wetting agents	Strongly coloured, high BOD, DS, low SS, heavy metals
Printing	Pastes, urea, starches, gums, oils, binders, Acids, Thickeners, cross-linkers, reducing agents, alkalies	Highly coloured, high BOD, .oily appearance, SS slightly .alkaline, low BOD.

1.4 TREATMENT PROCESSES

The main operational methods used for the treatment of textile industrial water involve physical and chemical processes (Shaw et al., 2002 & Liu et al., 2005). However, these techniques have many disadvantages (Mutambanengwe, 2006). These disadvantages include Sludge generation, high cost, and formation of bi – products, releasing of toxic molecules, requiring a lot of dissolved oxygen and requiring of long time.

The textile effluents could be treated biologically by using different bacteria fungi, enzymes and plants. These effluent produce different aromatic compounds and these are susceptible to biological degradation under aerobic and anaerobic conditions. Under aerobic conditions, the enzymes mono-and di-oxygenates catalyze the incorporation of oxygen from O_2 into the aromatic ring of organic compounds prior to ring fission (Madigan et al., 2003). Although azo dyes are aromatic compounds, their substituent's containing mainly nitro and sulfonic groups, are quite recalcitrant to aerobic bacterial degradation (Claus et al., 2002).The capacity of fungi to reduce azo dyes is related to the formation of exoenzymes such as peroxidases and phenoloxidases. Peroxidases are hemoproteins that catalyse reactions in the presence of hydrogen peroxide (Duran et al., 2002). Enzymes having capacity to breakdown larger compounds into smaller one e g.Laccase are copper-containing enzymes that have very broad substrate specificity with respect to electron donors, e.g. dyes (Abadulla et al., 2000). For removal of BOD and COD content from textile effluent, the sand filters was used, But sand as absorbent material not found to be efficient(Turan, 2001).The commercial lime is use as coagulant for waste water treatment, which reduce the pollution loads significantly (Helal Uddin et al., 2003)..

1.5 PHYTOREMEDIATION

Phytoremediation is cleanup technology for contaminated soils, groundwater, and wastewater, is both low-tech and low-cost. '*phytoremediation as the engineered use of green plants, including grasses,* *forbs, and woody species, to remove, contain, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water'.* Phytoremediation works on biological, chemical, and physical processes that aid in the uptake, sequestration, degradation, and metabolism of contaminants, by plants. It takes advantage of the unique and selective uptake capabilities of plant root systems, with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body. In many situations, phytoremediation can be used as an alternative to the harsher remediation technologies of incineration, thermal vaporization, solvent washing, or other washing techniques (Hinchman et al., 1998).

The generic term "Phytoremediation" consists of the Greek prefix phyto (plant), attached to the Latin word remedian (to correct or remove an evil) (Prasad, 2004). Phytoremediation is an alternative or complementary technology that can be used along with or, in some cases in place of mechanical conventional cleanup technologies that often require high capital cost, more labor and energy intensive (Cunningham et al., 1996). Phytoremediation has also been called green remediation, botano-remediation, agro remediation and vegetative remediation (Erakhrumen, 2007). It has less harmful effects to the environment, cost effective, aesthetically environmental pollutants removal approach most suitable for developing countries (Pivertz, 2001). The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time (Mudgal et al., 2010).

Plants having properties to absorb, accumulate or metabolize contaminants from the soil, water or other media in which it grows. Some metals in trace quantity are essential for plants growth, while certain hyperaccumulator plant root system absorb more metal which are apparently unnecessary for plant functions. These contaminants mechanistically are (1) stored in the roots, stem or leave, (2) changed into less harmful chemicals within the plants, and (3) changed into volatile gases through plant transpiration.

Besides this, chemicals can (1) adhere or absorb by roots and (2) can be altered into less harmful chemicals by microbial community that subsists and colonize near plant roots where enough supplies of carbon and energy requirements present for growth thereby enhance decontamination of suboptimal level of pollutants from the required media.

Phytoremediation process comprises of four functions

1. Absorbing and accumulating hazardous substances.
2. Degrading and detoxifying it.
3. Stabilizing it around the roots.
4. Activating microbes around the roots to degrade and detoxify it (Fulekar M.H., 2008)

There are several methods of phytoremediation summaries into two major types organic and metal contaminants are stated in table 1.3.

Table 1.3 Mechanism of phytoremediation (Chandra, 2010)

Types of pollutants	Phytoremediation techniques	Mechanism	Media
Metals	Phytoextraction/ Phytoaccumulation	Uptake of contaminants by plants roots and its accumulation in plant parts	Surface water and water driven through
	Phytostabalization	Precipitation of metals by roots and its immobilization	Ground water,soil and mine tailings
	Rizofiltration	Removal of toxic compounds in water through filtration by root mass. The pollutants remain absorbed to the roots	Roots
Organic	Phytodegradation/ Phytotransformation	Uptake and subsequent degradation of organic contaminants by plants	Surface water and ground water
	Phytovolitalazation	Uptake of certain metal ions and and subsequent modification to volatile organics that are evaporated through plants leaves	Soil and ground water

	Rhizodegradation	Uptake and degradation of contaminants in plant roots	Surface water and ground water

1.5.1 PHYTOREMEDIATION OF METAL CONTAMINATION

Metal contaminations may be removed either physically from system and transform into a biologically inert form (Cunningham and Ow 1996). In biological means plants having different phenomenon for metal uptake and removal.

Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants. It is the best approach to remove contaminants from soil, sediment and sludge (Raskin and Ensley, 2000).Rhizofiltration is a mathod use by plants, both terrestrial and aquatic, to absorb, concentrate and contaminants from polluted aqueous sources in their roots (Jadia and Fulekar, 2009). Phytostabilization another phenomenon use of plants to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into food chain (Cunningham et al., 1996). Uptake contaminants from soil and waste water by plants, transforming them into volatilized compound and then transpiring into the atmosphere is known as Phytovolatilization (Pivertz, 2001).

1.5.2 REMEDIATION OF ORGANIC CONTAMINANTS

Removal of organic pollution with the help of plants utilizes three different methods. One of these is phytodegradation /phytotransformation process in which contaminants are taken up from soil / water, metabolize in plant tissues and subsequently broken down to less toxic or non toxic compounds within the plants by several metabolic processes through the action of compounds produced by the plant (Macek *et al.*, 2000; Meaghar, 2000).In phytovolatilization plants uptake water soluble contaminants and release them into the atmosphere through transpiration (Singh and Jain, 2003). Rhizodegradation mainly refers to the biodegradation of contaminants in the soil by interaction between edaphic microbes and the inherent character of the rhizosphere itself (Susarla et al., 2002).

Using different forms of phytoremediation there are many positive and negative aspects to consider (table 1.4).

Table 1.4 The advantages and disadvantages

Methods	Advantages	Disadvantages
Phytoextraction	1. Cost of phytoextraction is fairly inexpensive. 2. The contaminant is permanently removed from the soil (Henry, 2000).	1. Metal hyperaccumulators are generally slow- growing with a small biomass and shallow root systems. 2. Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass (Prasad, 2004).
Rhizofiltration	1. The ability to use both terrestrial and aquatic plants for either in situ or ex situ applications. 2. The contaminants do not have to be translocated to the shoots (Henry, 2000).	1. The constant need to adjust pH. 2. Plants may first need to be grown in a greenhouse or nursery (Henry, 2000).
Phytostabilization	1. The disposal of hazardous biomass is not required. 2. The presence of plants also reduces soil erosion and decreases the amount of water available in the system (Henry,2000).	1. Contaminant remaining in soil. 2. Application of extensive fertilization or soil amendments, mandatory monitoring is required (Henry, 2000).
Phytovolatilization	1. Contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas. 2. Contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation (Prasad, 2004).	1. The contaminants or a hazardous metabolite might accumulate in vegetation such as fruit or lumber. 2. Low levels of metabolites have been found in plant tissue (Prasad, 2004)

1.5.3 AQUATIC PLANTS: AS PHYTOREMEDIATOR

Aquatic plants are very effective in removing heavy metals from polluted water. Plant assimilation of nutrients and its subsequent harvesting are another mechanism for pollutant removal. Low cost and easy maintenance makes the aquatic plant system attractive to use (Kanabkaew and Puetpaiboon, 2004).

Thus, aquatic plants are increasingly applied as a viable treatment for industrial wastewater. The accumulation of metals in various parts of aquatic plants is often accompanied by an induction of a variety of changes in cellular level, some of which directly contribute to metal tolerance capacity of the plants (Prasad et al., 2001). However, there are some difficulties with using aquatic plants such as the requirement for large area of land, the reliability on pathogen destruction, and the types and end-uses of aquatic plants (Kanabkaew and Puetpaiboon, 2004). One reason of using aquatic plants for remediation, that the aquatic plants are able to easily remove the heavy metals from the water than terrestrial plants uprooted in soil where metals are not present in soluble form before plants can absorb them. In an aqueous solution, metals are already present in soluble form so, accumulation by the plants can be achieved much easier. Recently, there has been growing interest in the use of metal-accumulating roots and rhizomes of aquatic and semi-aquatic vascular plants for the removal of heavy metal from contaminated stream (Gallardo et al., 1999).

1.5.4 INVASIVE AQUATIC PLANTS

In this research different type of invasive aquatic plants have been used for the remediation of pollutants from industrial waste. Invasive species as "*an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.*" In the Executive Summary of the National Invasive Species Management Plan (NISMP) the term invasive species is further clarified and defined as "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health."

Invasive species affects the biological diversity second only to direct habitat loss and disintegration (Pimm and Gilpin, 1989). Introduction of these plants cause variety of effect on aquatic habitat that alter ecosystem processes such as nutrient cycling, hydrological cycles and sediments deposition (Antonio and vitousck, 1992).These invaders change the game of survival and growth, placing many native species at a severe disadvantage (Vitousek et al.1992), these plants may reduce or eliminate the water habitat (Brothwerson and Field,1987). Some invasive plants completely alter the structure of the vegetation in which they invade (LaRoche1994), such changes in the community structure may be expected to be followed by changes in ecosystem function. Some non-native plants hybridize natives and effectively eliminate native genotype (Ayes et al., in press).

The invasive plant are biotic resistance (Maron and Vilá, 2001), superior competitor (Bakker and Wilson, 2001), and have allelopathic advantage over indegenous species (Callaway and Aschehoug, 2000; Bais et al., 2003; Callaway and Ridenour, 2004). However, many invasive plants have shown greater abilities to use water and nutrients under sewere environmental stress (Blicker et al., 2002, 2003). It means that the adaptation or tolerance to stressful environments (water and nutrient limitation,soil pollution) may be a significant trait of invasive plant species.

1.5.5 PHYTOREMEDIATION AND GENETIC ENGINEERING

Genetic engineering approach has successfully facilitated to alter the biological functions of plants through modification of primary and secondary metabolism and by adding new phenotypic and genotypic characters to plants with the aim of understanding and improving their phytoremediation properties (Davison, 2005). Many reports have supported the increase of valuable natural products through the over expression of biosynthetic genes with a strong promoter and a suitable signal sequence to control the preferred subcellular localization (Ohara et al., 2004).

Metal-hyperaccumulating plants and microbes with unique abilities to tolerate, accumulate and detoxify metals and metalloids, represent an important reservoir of unique genes (Danika and Norman, 2005). These genes could be transferred to fast-growing plant species for enhanced phytoremediation (De Souza et al., 1998). It has been established after a number of thorough genetic studies, that the adaptive metal tolerance has been shown to be governed by a small number of major genes and perhaps contribution of some minor modifier genes (Schat et al., 2002). Probably it is this adaptive metal tolerance that gears a plant species for hyperaccumulation.

For example a genetic analysis of copper tolerance with Cu-tolerant and susceptible lines of *Mimulus guttatus* showed that a modifier gene that is active only in presence of the tolerance gene is responsible for the difference in Cu-tolerance in this species (Smith and McNair, 1998). Similar studies with Zn-hyperaccumulator *Arabidopsis halleri* and the nonaccumulator *Arabidopsis petrea* suggested that Zn-tolerance is also controlled by a single major gene (McNair et al., 2000). Therefore the desired characters for phytoremediation can be improved by identifying applicant protein, metal chelators, and transporter genes for transport and/or over expression of particular gene. Through genetic engineering modification of physiological and molecular mechanisms of plants heavy metal uptake and resistance is successfully achieved by implanting bacterial gene or mutant cells on the basis of desired phenotype in plant genome which enhances the very process of uptake of metals.

One promising approach for manipulation of plants character is through recombinant DNA technology. It has vastly proven its potential in phytoremediation process and many modifications are already made to change the property of plants. Recombinant DNA technologies combine the potentially more powerful ability to more selectively and proactively choose the traits to be introduced into the plant cell, via the introduction of DNA encoding enzymes or other proteins from other living organisms, or even completely synthetic genes designed to encode enhanced enzymes. DNA or gene of interest is spliced into a small, circular carrier DNA molecule known as a vector. The vector is introduced into plant cells either by physical means (electroporation or via high-velocity microprojectiles shot inside the cell), or biological means (utilizing natural biological systems where bacteria such as *Agrobacterium* can insert DNA into plant cells, and cause the DNA to be incorporated into plant chromosomes). Upon entry into the cell and mixing into the plant chromosome, the desired gene is "expressed" in a subset of the cells (that is, its genetic code is read by the plant cell to cause the synthesis of a protein encoded by the gene); these cells are selected in tissue culture and used to redevelop whole plants for subsequent breeding. Enzymes have a vital role in development of plants for interaction with environment. Introduction of genes encoding enzymes to alter the oxidation state of heavy metals, like the bacterial merA gene encoding mercuric oxide reductase (Rugh et al., 1996), or that converts metals into less toxic forms, such as enzymes that can methylate Se into dimethylselenate (Hansen et al., 1998). In both of these cases, the resulting form of the metal is volatile, so that one can produce plant capable of metal remediation by phytovolatilization.

1.6 PROBLEM STATEMENT

It has been reported that about 9000 million gallons of waste water is being discharged daily in water bodies from the industrial sector of Pakistan (saleemi,1993),polluted water is responsible for cost of Rs.112 billion /year on the consequences of health (national drinking water policy,2009).Hence, there is need to develop a technique to remove contaminants from water at low cost .For this purpose, use of invasive plant species as pollutant and metal hyperaccumulator/ phytoremidiator which benefit the environment by two ways. One is removal of pollutants from water using cheaper and efficient technique and the other is to use invasive species those having dangerous effects to native species.

1.7 OBJECTIVES OF STUDY

- Screening of invasive plants for phytoremediation
- Generation of stock of best 2-4 plants, their propagation, cultivation, multiplication and preservation of phytoremediator species
- Estimation of contamination load in the industrial effluent
- Reduction in the contamination load of effluent through phytoremediator plant species

LITERATURE REVIEW

Developing countries like Pakistan are dumping huge amount of domestic and industrial wastewater into streams, lakes and rivers without any treatment. Poor management and without implementation of environmental legislation is making the condition worse day by day (ziai, 1987). Water quality of rivers and streams are becoming poor due to mixing of untreated wastewater. It is necessary to examine and formulate different strategies for water quality management which would sustain acceptable water quality standards for irrigation and public use (Balfour report, 1987; cheema, 1991). Considering these issues, it is required to deal with all natural rivers and streams properly. In Pakistan, most of effluent receiving rivers like Ravi are being used for irrigation and livestock purposes (WWF, 2001; Qazi, 1991). Due to lack of proper monitoring plan most of the streams in Pakistan are losing their environmental values so quickly. Unfortunately at present there is no monitoring for assessment of river water quality in Pakistan. According to the environmental standards, steams in the world are classified on behalf of DO concentration. The standard DO level must not be less than 5 mg/L for a good water quality, ecology and marine life. Most of the marine species cannot survive when DO concentration decreases less than 5 mg/L in a natural stream (Ziai, 1987; Qazi,'1991). The limitations set by Pak-EPA (Pakistan Environmental Protection Agency); the NEQS does not permit the effluent discharge of any domestic and industrial effluent into any receiving bodies which has (BOD, COD) more than 80 and 150 mg/L, respectively. Similarly the NEQS does not permit the wastewater having TSS concentration more than 150 mg/ L to be discharged in any water receiving body without proper treatment (Naeem, 2010).

Discharge of toxic effluents in water and that water used by human can cause cancer, delayed nervous damage, malformation in children, mutagenic changes, neurological disorders etc. Various acid manufacturing industries discharge acidic effluent, which not only make the land infertile. But make the water of the river acidic also. The high acidity causes stomach diseases and skin ailments in human beings (Kumar, 2009).

Industrial waste like heavy metals and other pollutants causes serious risk to nature as they are non- biodegradable and accumulate at high levels. Heavy metal pollution is a global problem, although severity and levels of pollution differ from region to region. At least 20 metals are classified as toxic with half of them releases into the environment that poses great threat to human health (Akpor and Muchie, 2010). The common heavy metals like Cd, Pb, Co, Zn and Cr etc. are toxic at both low concentration as well as very high concentration.If these metals are presented in sediments that they reach in food chain through plants and aquatic animals. In small quantities, certain heavy metals are nutritionally essential for health but large amounts of any of them may cause acute or chronic toxicity to human and environment (Dviya et al;2012).

Key environmental issues associated with textile industry are water use, treatment and disposal of liquid effluent. The risk factors are associated with the wet processes desizing, scouring, bleaching, mercerizing, dyeing and finishing. Desizing and bleaching processes produce large quantities of wastewater. Treatment for color removal can increase the risk of pollution. For instance, treating azo-dyes results in production of amines which could be a greater environmental risk than the dye itself (Navarro et al, 2001). Dyes contributed to overall toxicity at all process stages. Also, dye baths could have high level of BOD, COD, color, toxicity, surfactants, fibers and turbidity, and contain heavy metals (EPA, 1998). They generally constitute a less amount of total liquid effluent, but may contribute a large proportion of total contaminants. Textile effluents are highly colored and saline, contain non-biodegradable compounds, and are high in Biochemical and Chemical Oxygen Demand (BOD, COD) (Wynne et al, 2001).

USEPA(United states environmental protection agency) reported that the pollution parameters in textile effluents are suspended solids, BOD, COD, nitrogen, phosphate, temperature, toxic chemicals (phenol), chromium and heavy metals, pH, alkalinity-acidity, oils and grease, Sulphide, and Coliform bacteria (EPA, 1974).Textile effluents are high in BOD due to fiber residues and suspended solids (EPA, 1998). These effluents contaminate water with Oils, Grease and waxes contain heavy metals such as chromium, copper, zinc and mercury (PEPA, 2000) Dyeing process releases chromium, lead, zinc and copper to wastewater (Benavides, 1992) Copper poses threat to aquatic plants at concentrations below 1.0 mg/l while concentrations near this level can be toxic to fishes (Sawyer and McCarty, 1978 and Nergis et al, 2005).

Aquatic plants are heavy metal accumulators and the use of aquatic plants for the removal of heavy metals from wastewater gained high interest (Kuyucak and Volesky, 1989: Lacher and Smith, 2002). Some freshwater macrophytes including *Potamogeton lucens, Salvinia herzogoi, Eichhornia crassipes, Myriophyllum brasillensis, Myriophyllum spicatum, Cabomba sp.and Ceratophyllum demersum* have been investigated for the removal of heavy metals (Wang et al; 1996: Schneider et al; 1999).

Heavy metals cause a serious threat to aquatic environments, as they do not degrade but accumulate in aquatic plants, and then enter into the food chain. Aquatic plants are useful in decreasing heavy metal concentrations in water (Dhote and Dixit, 2009). They having the ability to accumulate metals in to their tissues (Vardanyan and Ingole, 2006), with the greatest accumulation of most of the heavy metals occurring in the root system. Two plant species *Elodea canadensis* and *Elodea nuttallii* accumulate pollutants (Kähkönen et al., 1997).

Textile industrial production involves a number of wet processes. Each process generates waste water containing different types of pollutants. Finishing and drying processes generate volatile organic compound (VOC). Dyeing and printing processes produce waste water-containing toxic organic compound such as phenols and also having highly concentrated color and copper, chromium metals. Bleaching of fibers adds halogen, makes the waste water alkaline. Desizing step in textile process contributes 50 % increase of BOD load. Wool processing may release pathogenic germs. Sometimes pesticides are used for the maintenance of natural fibers. These pesticides are discharged into waste water during washing and scouring process. Chemicals present in finishing waste water are highly variable due to the broad range of finishers available, which generate pollutants of natural and synthetic polymers. The biodegradable organic compound can cause shortage of dissolved oxygen in receiving water bodies and have a direct effect on aquatic life (Dos Santos et al., 2006).

Phytoremediation is the use of living green plants to fix or adsorb contam inants, and cleaning the contaminants. The phytostabilization, phytovolatilization and phytoextraction are the main types of phytoremediation (Shen and Chen,2000).Phytostabilization is fixing heavy metals by plants through the adsorption, precipitation and reduction of root, and thus reducing their migration and bioavailability and preventing them migrating into the groundwater and food chain (Wang et al;2009). Phytovolatilization is transferring the heavy metals into volatile state or adsorbing the metals and transferring into gaseous matter (Watanabe, 1997).

Phytoextraction is adsorbing the heavy metals by plants. Studying adsorption characterization of plants and screening high uptake plants is the key of this technology (Phytoextraction). According to the rules of U.S. department of energy, the high uptake plants screened should have the following characterizations: 1) Have high accumulating efficiency under the low contaminants concentration; 2) Accumulate high concentrations of the contaminants; 3) Accumulate many different kinds of heavy metals; 4) Grow fast and with large biomass; 5) Have pest and disease resistance ability (Wang,2001).

Chromium metal is toxic to mammals, plants, aquatic lives and microorganisms. It causes diarrhea, nausea, low blood pressure, lung irritation, CNS (central nervous system), cancer, dermatitis etc. Some submerged aquatic plants *Hydrilla sp.* and *Chara sp.* Having the ability to uptake and remove chromium from waste upto 99.70% of chromium and *Chara sp.* remove

91.70% at a concentration of 2mg/L. These two species use as bioabsorbant material for removal of chromium (Arjun and Perveen, 2011).

Lead hyperaccumulator plants in the world are14 taxas and 7 families (Ensley, 2000), while *Chenopodium ambrosioides, Pennisetumclandestinum* and *Bidens humilis* are the only metallophytes(plant that can tolerate high level of heavy metals like lead), they are reported as lead metal tolerant (Bech et al., 2001, 2002).The lead is a toxic heavy metal and *Chenopodium ambrosioides* is one of few metal-tolerant and hyperaccumulator species that have been reported(Huang and Cunningham,1996).

Some weed species have hyperaccumulator properties, and they can survive in highly polluted soils and marshy areas and exclude metals from the soil. Compared with crops, weeds often possess stress resistant properties and can maintain their growth under poor water and fertilizer conditions as well as heavy metal polluted soils (Wei et al., 2005).

Plants can be classified into three categories in relation to their ability to absorb, accumulate and tolerate heavy metals within their tissues as hyperaccumulator, indicators and excluders (Wagner & Yeargen, 1986; Ghosh & Singh, 2005). Hyperaccumulator plants more often produce low biomass. Indicator plants have lesser metal bioaccumulation in comparison with the hyperaccumulators but have at least 10 times more biomass production, so that the actual amount of extraction is relatively higher (Ghosh & Singh, 2005). Excluders produce rather high biomass but accumulate lower amount of metals. They produce biomass up to 30 tons per hectare (Robinson et al., 2000). *Xanthium strumarium* commonly grows in summer, reported that *X. strumarium* is more tolerant and produces huge biomass per unit area and tolerant to Cd (Abe et al., 2006).

Aquatic plant like *Mentha piperita* is known to be accumulate excessive amount of arsenic (As), up to 88 mg/kg on a fresh weight basis (Robinson et al., 2005).

MATERIAL & METHODS

MATERIALS AND METHODS

Invasive plant species for the purpose of phytoremediation were isolated, screened and utilized for the treatment of textile waste water. Details are given below.

3.1 COLLECTION OF EFFLUENT

The sample of textile effluent was collected from washing and drying unit of Munir and Sons private limited, Maqbool Road Faislabad in April 2012; through Grab Method using Polyethylene containers. The containers were properly cleaned forehand with non-ionic detergents and thorough washing with tap water followed by rinsing with de-ionized water. Later the sample was stored at 4°C in the Bioremediation Department at NARC. Synthetic complex chemicals and dyes are used in this industry.

3.2 PHYSICO-CHEMICAL CHARACTERIZATION OF WASTE WATER

In-situ measurement of the textile effluent was done for Dissolved oxygen. The effluent was then stored in pre-washed reagent bottles and was stored at low temperatures. Rest of the parameters including hydrogen ion concentration, electrical conductivity, total dissolved solids, turbidity, chlorides, carbonates, bicarbonates and heavy metals were assessed later in the laboratories.

Analytical work was carried out at the National Agriculture Research Center (NARC) Islamabad and International Islamic University Islamabad (IIUI). The physico-chemical tests were carried out according to the standard procedure (APHA, 1998).

3.2.1 HYDROGEN ION CONCENTRATION (pH)

The pH of sample was measured with the help of multimeter (MM 40+). Calibration of pH meter was performed with two standard buffer solutions; 6.86 (25°C) and 7 (25°C).

For performing calibration of electrodes, pH meter was dipped in buffer with pH 6.86 and then in buffer of pH 7 after washing with de ionized water between the intervals, hereafter, the pH meter was calibrated and ready for use. Then the electrode was dipped in the respective samples and readings of samples were taken (Manjare, 2010).

3.2.2 ANALYSIS OF ELECTRICAL CONDUCTIVITY (EC) AND TOTAL DISSOLVED SOLIDS (TDS)

Electrical conductivity and total dissolved solids were measured by multimeter (MM 40+) by simply dipping the electrode in a 100 ml beaker filled with the effluent.

3.2.3 ANALYSIS OF DISSOLVED OXYGEN (DO)

Prior to the analysis, DO meter was calibrated as per standard method (Kodarkar, 1992). The DO was measured by using DO meter (OXI 45+) at the spot when the effluent was collected from the textile industry.

3.2.4 TURBIDITY MEASUREMENT

Turbidity is a good parameter to measure the degree of pollution of water (Ali et al., .2006). The turbidity meter (TN-100/T-100) is a portable instrument that allowed measurement of an aqueous sample of water in the field and operated on the Nephelometric principle; hence turbidity is reported in Nephelometric turbidity units (NTU).

3.2.5 ESTIMATION OF CARBONATES AND BICARBONATES

The concentration of carbonates was determined by titrometric method (Manjare, 2010). 5ml sample was taken and 2-3 drops phenolphtheline were added as an indicator. The solution was titrated against standard 0.02 N HCl till the achievement of end point (i.e. change of color from purple to colorless). The formula used for calculating carbonates is as below:

CO3 (mg/L as CO3 ion) = 60009×A×CF/V Eq. 3.1

The concentration of bicarbonates was determined by the same titrometric method as for the carbonates. 5ml of the sample was taken and methyl orange was used as indicator. The sample was titrated against standard 0.02 N HC till the achievement of end point (i.e. change of color from orange to pink). The formula used for calculating bicarbonates in mg/l is as:

Bicarbonates (mg/l) = 61017(B – 2A) Ca. CF/Vs Eq. 3.2

A= volume of acid titrant added from initial pH to the carbonate equivalent point (near pH -8.3) in ml.

B= volume of acid titrant added from initial pH to the bicarbonate equivalent point (near pH -4.5) in ml.

CF= correction factor

Vs= volume of sample in milliliter

Ca= concentration of acid (HCl) = N

3.2.6 CHLORIDES

The chemicals used for the measurement of chlorides by titrometric method (Trivedy and Goel, 1986) were potassium chromate (indicator) and silver nitrate. 5ml samples were taken in a wide mouthed flask. Silver nitrate solution was poured into the burette as titrant. 3-5 drops of potassium chromate were added in the sample. Samples were titrated with silver nitrate solution until color changes from yellow to reddish pink. This was the end point and the reading was noted down from the burette.

The procedure was repeated three times to take the average of the silver nitrate used which showed the results.

Equation to calculate the chloride ion is as:

Cl^- (g/l) = volume of titrant used×normality×35.45 (atomic mass of Cl^-) ×1000/volume of sample Eq.3.3

3.2.7 ESTIMATION OF HEAVY METALS

Industrial effluent was analyzed through atomic absorption spectrophotometer for the determination of lead, chromium, copper and cadmium (Kodarkar, 1992)

A series of standard metal solution were prepared in the optimum concentration range by appropriate dilution of the following stock metal solutions. In general, commercial stock solutions of the highest purity were used.

Lead: Taken 0.5, 1.0, 1.5 and 2.0 ml of 1000 ml lead of commercial stock solution were diluted in 100 ml of demonized water and get 5, 10, 15, and 20 ml of lead.

Copper: Taken 0.1, 0.2, 0.4 and 0.5 ml of 1000 ml copper of commercial stock solution were diluted in 100 ml of deionized water and get 1, 2, 4, and 5 ml of copper.

Cadmium: Taken 0.5, 1.0, 1.5 and 2.0 ml of 1000 ml cadmium of commercial stock solution were diluted in 100 ml of deionized water and get 0.5, 1.0, 1.5, and 2.0 ml of cadmium.

Chromium: Taken 0.5, 1.0, 1.5 and 2.0 ml of 1000 ml chromium of commercial stock solution were diluted in 100 ml of deionized water and get 0.5, 1.0, 1.5, and 2.0 ml of chromium.

3.3 COLLECTION AND SCREENING OF PLANTS

About 9 different species of invasive plants (Figure 3.1) were collected from around a stream at I-8/4. The plants were then identified according to the taxonomical characteristics e.g. shape of the leaf, stem, type of leaf veins etc (Flora of Pakistan, 2002: Flowers of Himalaya, 1998)

Table 3.1 Indigenous Plants Collected and Screened for Phytoremediation

Sr. No.	Common Name	*Botanical Name*	Characteristics	Growth and Survival	Known Phytoremediation potential
1.	Giant salvinia	*Salvinia molesta*	Buoyant,broad Fronds with bristly surface to form egg beater shape	-	Nickel, Heavy metal accumulatorn (Gosh & Singh. 2005)
2.	Narrow leaf cattail	*Typha angustofolia*	Distinctive stalks topped with sausage shaped head	-	Metal accumulator (Ram & Sangeeta, 2010)
3.	Moss verbena	*Verbena tennuisecta*	Ferns like leaves, low ground hugging herbaceous cover	-	Arsenic treatment (US EPA Project, 2002)
4.	White top weed	*Parthenium hysterophorus*	Alternate leaves profoundly cut in narrow segments	-	Heavy metals (Hadi et al., 2014)
5.		*Verbena offinalis*	Lobbed leaves, delicate spines holds mauve flower	-	Nickel (Cullaj et al., 2004)
6.	Mexican tea	*Chenopodium ambiosoides*	Alternate dissected leaves	+	Heavy metals (Salas-Luévano et al., 2009)
7.	Prickly lettuce	*Lactuca serriola*	Leaves grow along spiny stem	-	Heavy metals (Porebska &

			that progressively smaller at the top		Osterowska, 1999)
8.	Pepper mint	*Mentha piperita*	Fleshy leaves having fibrous roots	+	Tolerate and accumulate heavy Metals (Khoramivafal et al 2012)
9.	cocklebur	*Xanthium stromarium*	Alternate, opposite hairy, irregularly lobbed leaves	+	Phytoaccumulation of organochlorine metabolites and reduce pesticide concentrations (Nurzhanova et al., 2011)

Collected plant samples were exposed to fresh water for acclimatization during months of March and April 2013. They were then submerged in the collected effluent. Most of the plants were dying and were under stress so the survived three plants i.e. *Chenopodium ambiosoides, Mentha piperita* and *Xanthium stromarium* were transferred to 10% diluted sample.

Aquatic plants were selected on the basis of their phytoremediation potential. Plants of uniform size were taken for experimentation in duplicate.

Figure 3.1. Nine different species of invasive plants for screening

3.4 TREATMENT OF TEXTILE WASTE WATER BY INDIGENOUS PHYTOREMEDIATORS

The textile effluent sample was highly polluted (NEQS, 2000) as it was discharged without treatment. Presently this textile waste water was treated by environment friendly means using *Chenopodium ambiosoides*, *Mentha piperita* and *Xanthium stromarium*.

The pots (30 cm) were partially filled with waste water (8000 ml), labeled (plant number, date and replication i.e. R1 and R2 and exposed for three weeks (May 23^{rd} to June 13^{th}) in the field. Samples were collected (500 ml) at 0 day, 7^{th} day, 14^{th} and 21^{st} day, respectively in tagged bottles and were stored at room temperatures.

Figure 3.2. Survived Plants; *Chenopodium ambiosoides, Mentha piperita and Xanthium stromarium*

Figure 3.3. May 30th First Sampling Date; *Xanthium stromarium* and *Mentha piperita survived, Chenopodium ambiosoides*

Figure 3.4. June 6th (Second Sampling Date); ***Xanthium stromarium*** **and** ***Mentha piperita*** **survived,** ***Chenopodium ambiosoides*** **has wilted**

Figure 3.5. June 13th (last sampling date); *Chenopodium ambiosoides* and *Mentha piperita* survived, *Xanthium stromarium* died

3.5. DETERMINING CONTAMINATION LOAD AFTER THE TREATMENT

All the samples were analyzed for the contamination load by standard procedure using respective instruments (Table 3.3). Physiochemical analysis was done again in order to determine the contamination load expected to be reduced by the hyper accumulator activity of the plants.

Table 3.2 Analytical Equipments used in the Study

S/ N	Equipment	Manufacturer	Model
1	pH meter	CRISON Barcelona	MM 40+ 5059P
2	TDS meter	CRISON Barcelona	MM 40+ 5059P
3	EC meter	CRISON Barcelona	MM 40+ 5059P
4	Turbidity meter	EUTECH Singapore	OXI 45+
5	DO meter	CRISON Spain	TN-100/T-100
6	Atomic Absorption	AAnalyst 700	PerKinElmer

3.6. STATISTICAL ANALYSIS

The data was added in Microsoft Excel to calculate descriptive statistics and their graph were drawn.

RESULTS &DISCUSSION

RESULTS AND DISCUSSION

4.1 AIM OF THE STUDY

The study was conducted for the estimation of contamination load in textile waste water by analyzing selective water quality parameters after that reduction in pollution load of effluent with the help of three selected plant species (*Chenopodium ambrosoides, Mentha piperita and Xanthium stromarium*).

4.2 PHYSICO-CHEMICAL CHARACTERIZATION OF TEXTILE WASTE WATER

In order to estimate the pollution load of a textile industry, samples of textile waste water were taken from Munir and son's private limited, Faisalabad. As it is the important industrial sector contributing in water pollution. The physico-chemical analysis of the selected parameters (pH, EC, TDS, DO, Cl^-, turbidity, CO_3^-, HCO_3^- and metal contents) were measured according to the standard procedure (Manjare et al., 2010). Research study was carried out (in the months March April, May, and June of 2013) at NARC and in the wet lab of DES, IIU, Islamabad.

Results of contamination load (Table 4.1) in the textile waste water showed that some of physico chemical parameters (Textile industry of Munir & son's private limited, Faisalabad) were violating the PEPA, 1997 which stated that "Subject to the provisions of this Act and the rules and regulations made there under no person shall discharge or emit or allow the discharge or emission of any effluent or waste or air pollutant or noise in an amount, concentration or level which is in excess of the National Environmental Quality Standards".

Table 4.1 Contamination Load of the Textile Effluent

Parameters Studied	Estimated pollution load	NEQS
pH	6.66	6-9
TDS	469.6 mg/l	2000 mg/l
DO	6.66	NA
Turbidity	24 NTU	75 NTU
EC *	665mS/cm	200mS/cm
Carbonates	NP	100mg/l
Bicarbonates*	1134.8 mg/l	20mg/l
Chlorides*	4040592 mg/l	1000 mg/l
Pb	NP	0.5 mg/l
Co	0.009ppm	1.0 mg/l
Cd	0.048 ppm	0.1 mg/l
Cr	0.081 ppm	1.0 mg/l

According to Arun (1999) textile wastewater contain pollutants which are often non-biodegradable. It includes oils, dyes, lubricants, hydrogen peroxide, chlorine compounds, acids, bases, nitrites, water softeners and low molecular polymers. Wastewater contains acids used in desizing, dyeing bases like caustic soda used in scouring and mercerization. It also contains inorganic chloride compounds and other oxidants, e.g. hypochlorite of sodium, hydrogen peroxide and finishing chemicals for bleaching and other oxidative applications. Organic compounds are also present, e.g. dyestuff, optical bleachers, starch and related synthetic polymers for desizing and thickening. Surface active chemicals are used as wetting and dispersing agents and enzymes for desizing and degumming. Salts of heavy metals are also present, e.g. of copper and zinc, and iron chloride used as printing ingredients (Ohioma *et al.*, 2009). Reactive dyes have become a default choice for colouration of cotton textiles, because they provide a vast range of inexpensive bright colours with brilliant washing fastness (King, 2007). The inorganic salt, such as sodium chloride or sodium sulphate, for dye transfer to and penetration into the fiber, and inorganic alkali, such as sodium bicarbonate, sodium carbonate or sodium hydroxide, for dye-fiber reaction, are required in substantial quantities to accomplish the dyeing process. Irrespective of the dyeing method using reactive dyes, just about all of the salt and alkali is worn out to effluent. Such effluents are characterised by high levels of dissolved solids which is environmentally undesirable (Bide, 2007; Dolby P. J., 1977).

4.3 TREATMENT OF WASTE WATER BY ENVIRONMENT FRIENDLY APPROACH (PHYTOREMEDIATION)

Lots of treatment technologies are available (Wang et al., 2011 ; Sengupta, 2007) but during the present study phytoremediation strategy was selected because it is environmental friendly green technology, esthaticalicaly pleasing, easily implemented and cost effective as compare to the conventional treatment technologies (Henry,2000). Test plants (*Chenopodium ambrosoides, Mentha piperita and Xanthium stromarium*) were acquired from polluted site of I-8/4 for experimentation. Plants were identified by taxonomical characters (Flora of Pakistan, 2002: Flowers of Himalaya, 1998). *Chenopodium ambrosoides, Mentha piperita and Xanthium stromarium* (test plants) was introduced in the collected effluent (ml volume) for 21 days of retention period in order to reduce the pollution load. Details of results are given below.

4.3.1 HYDROGEN ION CONCENTRATION

Our results regarding pH of the industrial effluent was found slightly acidic. Slightly acidic nature of effluent might be due to the utilization of acetic acid, This compound is present very often because of its general use in cotton fiber dyeing (Kos et al., 2012). Different acids, alkalis and buffer solutions (Hussain et al., 2004) used in textile water to maintain a pH 6.5 to 8.0 at processing; this pH of the effluent affects the physico-chemical properties of receiving water which in turn adversely affects the aquatic life and human beings (Ramamurthy et al., 2011; Henry, 1971; Mount, 1973). Pollutants in textile wastewater are weak organics acid or base that can influence the property of pollutants available in wastewater (Nordin et al; 2013). At low pH, most of the metals remain persistent in the environment while at high pH, and most of the metals get insoluble and accumulate in the sludge or settle down as sediments (WHO, 1993).

After treatment it reaches to the level of neutrality by *Xanthium stromarium* (pH 6.82) and *chenopodium ambiosoides* (pH 6.76) within 7 days. Whereas *Mentha piperita* gives its best after 14 day (pH 6.715) a shown in Fig.4.1.It showed that all test plants have the capacity to reach the level near to neutrality at different time interval.

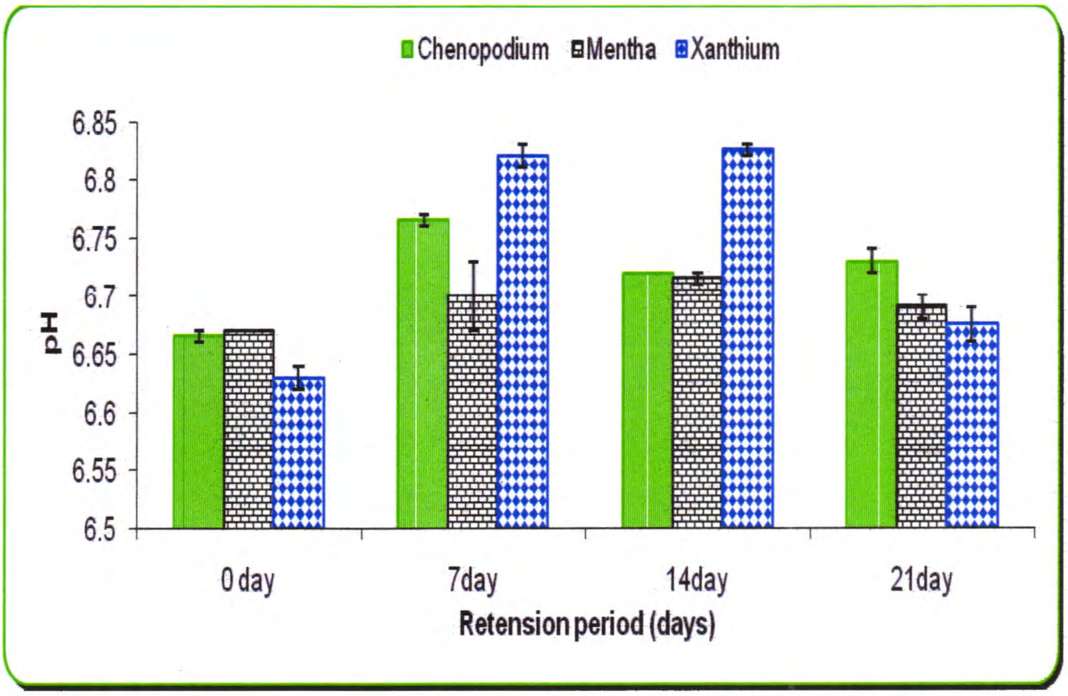

Figure 4.1. Effect of invasive plants on pH of the effluent

4.3.2 DISSOLVED OXYGEN (DO)

In the present study dissolved oxygen (DO) of textile waste water was recorded with the DO meter. Initially the concentration of dissolved oxygen was 6.66 mg/l. The amount of oxygen dissolved in water is affected by salinity and temperature, higher salinity reduces the amount of oxygen in water (Campbell and Wildberger 1992). Water temperature also affects lakes indirectly by influencing DO concentrations. Warm water holds less oxygen in solution than cold water (Kelly and Linda, 1997).

DO values reduced from 6.6 mg/l by *chenopodium ambiosoides* and 3 i.e., 6.15 mg/l and 6.19 mg/l respectively, it might be due to the oxygen gets into water by diffusion from the surrounding air, by aeration, photosynthesis (Walker et al 1980). The low value of dissolved oxygen leads to a serious threat to aquatic life which could be the consequence of respiration (Kelly and Linda, 1997).

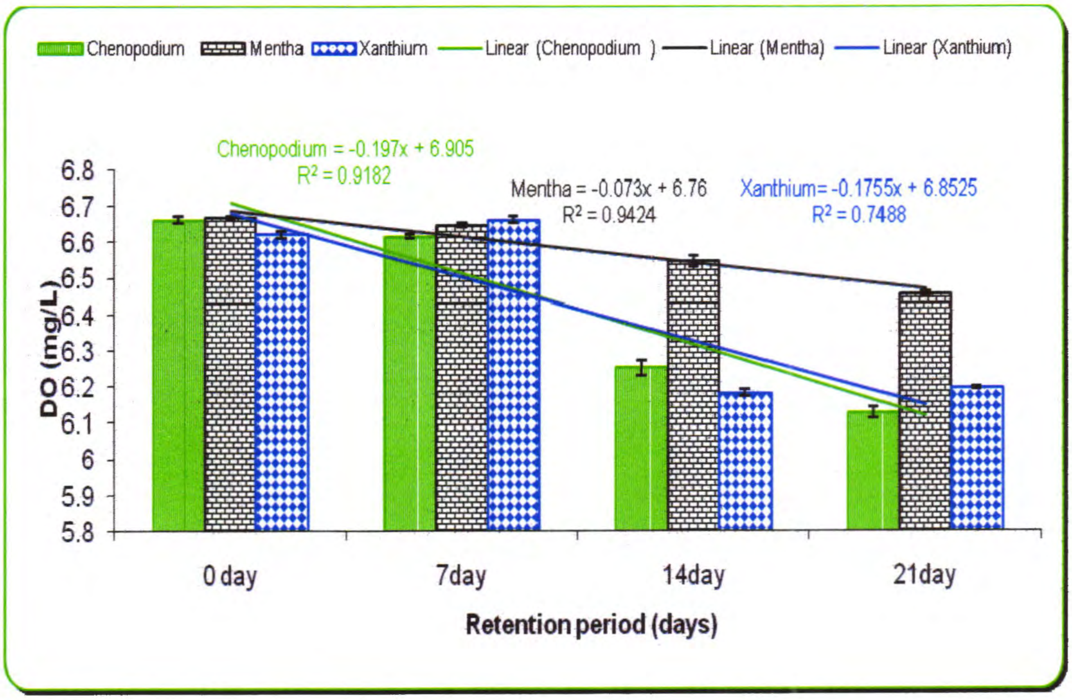

Figure 4.2. Effect of invasive plants on DO of the effluent

4.3.3 ELECTRICAL CONDUCTIVITY

In the present study, electrical conductivity (EC) of textile effluent was recorded before and after phytoremediation treatment. Initially EC was 665 mS/cm which was above the permissible limit of EPA (200 mS/cm) The high level of EC in textile effluent was due to the utilization of inorganic salt, such as sodium chloride or sodium sulphate, for dye transfer to and penetration into the fiber, and inorganic alkali, such as sodium bicarbonate, sodium carbonate or sodium hydroxide, for dye-fiber reaction (Awais Khatri, 2011). Electrical conductivity can also be influenced by the presence of fine sediment (fenn, 1987). For example, EC has been observed to increase after filtering for suspended sediment, possibly due to desorption of ions held on sediment surfaces (collins 1977; smart 1992).

After the treatment via *Chenopodium ambiosoides* had highest capacity of reducing salts (275 mS/cm) than *Mentha piperita* (511 mS/cm) and *Xanthium strumarium* (433mS/cm).

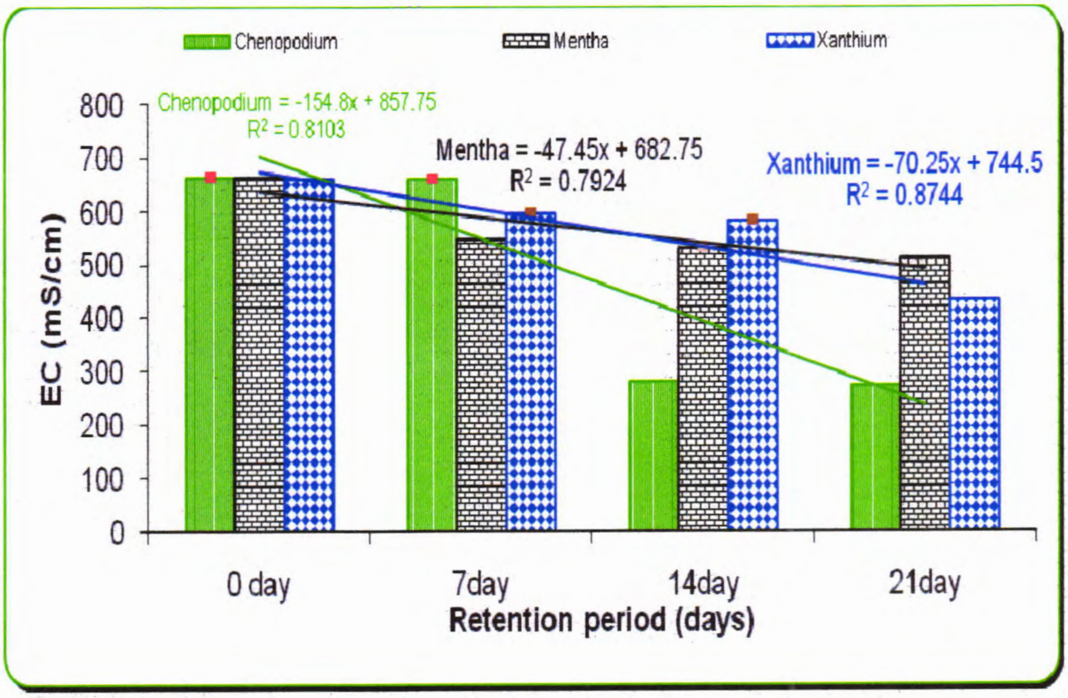

Figure 4.3. Effect of invasive plants on EC of the effluent

4.3.4 TURBIDITY

The turbidity of sample waste water measured by the turbidity meter was 24 NTU.Textile effluents contains sequestering agents, surface active agents and inorganic salts that are the main cause of the turbidity of water (Sudpathom & Phalakornkule, 2011).Higher turbidity increases water temperatures because suspended particles absorb more heat. This, in turn, reduces the concentration of dissolved oxygen (DO) because warm water holds less DO than cold. Higher turbidity also reduces the amount of light penetrating the water, which reduces photosynthesis.

Chenopodium ambiosoides, Mentha piperita and *Xanthium strumarium* reduced the level of pollutants to 0.915 NTU, 1.44 NTU and 4.71 NTU, respectively.

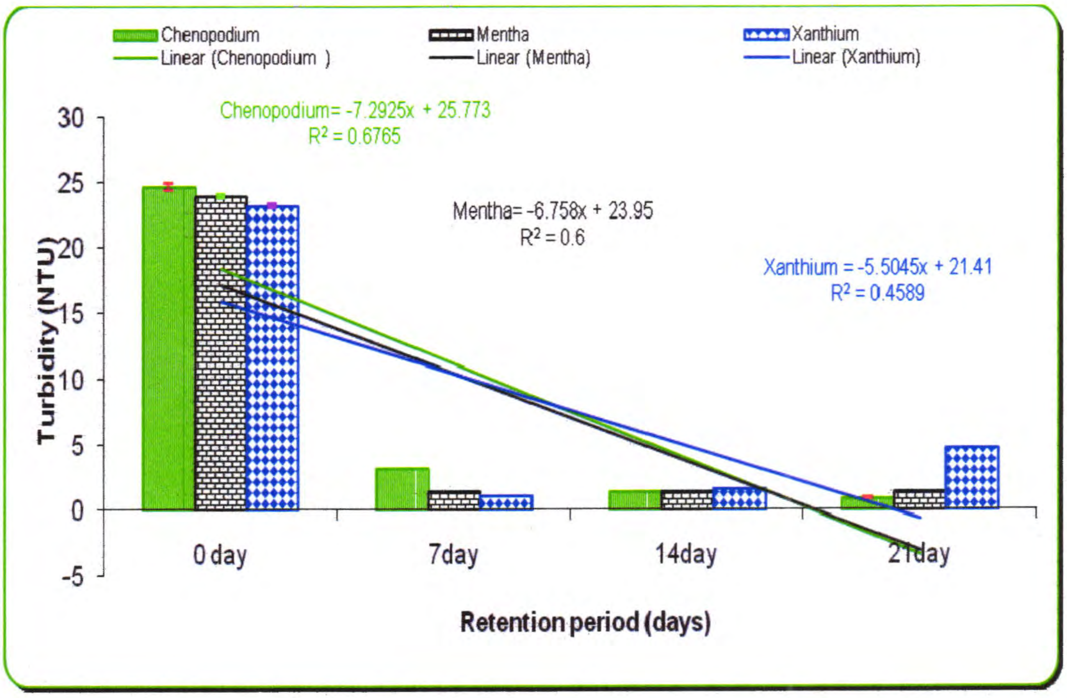

Figure 4.4. Effect of invasive plants on turbidity of the effluent

4.3.5 TOTAL DISSOLVED SOLIDS

Total dissolved solids (TDS) in the textile waste sample were 469.5 mg/l.TDS detected could be attributed to the high color from the various dyestuffs being used in the textile mills and they may be major sources of the heavy metals. The settle able and suspended solids are high and this will affect the operation and sizing of treatment units. Solids concentration is another important characteristic of wastewater (Lee and Lin, 1999).

The highest reduction of pollutants (TDS) was recorded by *Chenopodium ambiosoides* (175.95 mg/l) as compared to *Mentha piperita* (205mg/l) and Xanthium strumarium (277mg/l).

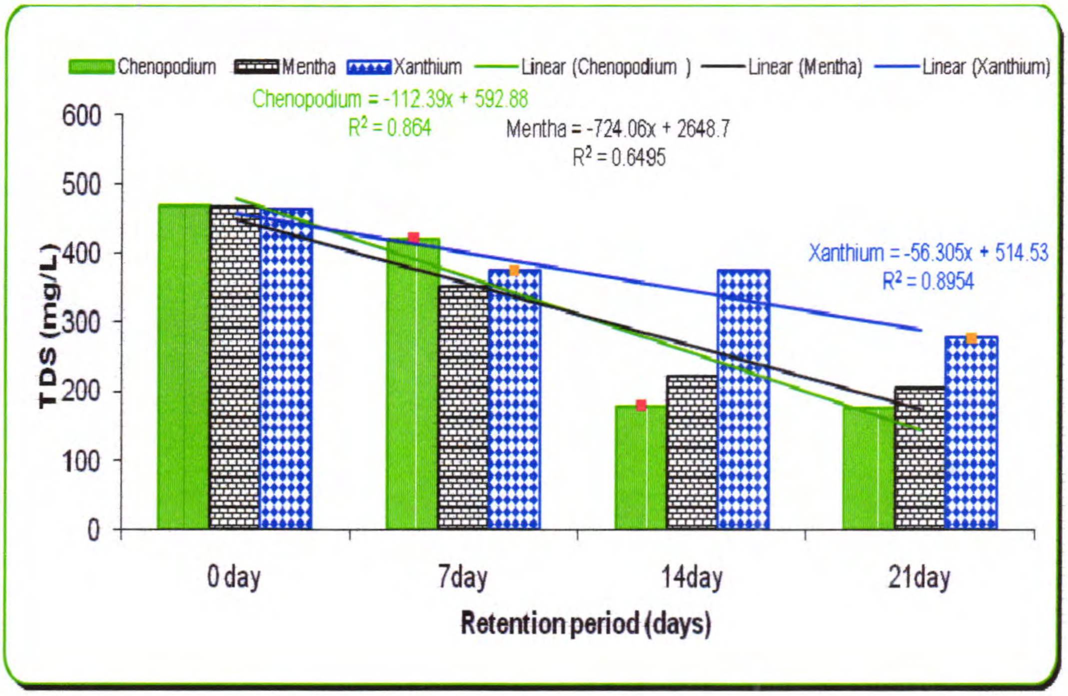

Figure 4.5. Effect of invasive plants on TDS of the effluent

4.3. 6. CARBONATES AND BICARBONATES

The concentration of bicarbonates was recorded as 1134.6 mg/l, this high range was due to the chemical (sodium bicarbonate) used in various steps of cloth process (Hussain et al; 2004).

Chenopodium ambiosoides reduced 32.2% of the bicarbonates from the textile waste water (781.6 mg/l). The remaining two plants *Mentha piperita* and *Xanthium strumarium* also reduced bicarbonate content to (mg/l) and (mg/l), respectively.

After the chemical analysis of carbonates and bicarbonates recorded, carbonates was not present at all in the textile effluent might be due to the conversion of carbonates into bicarbonates in the presence of excessive water molecules.

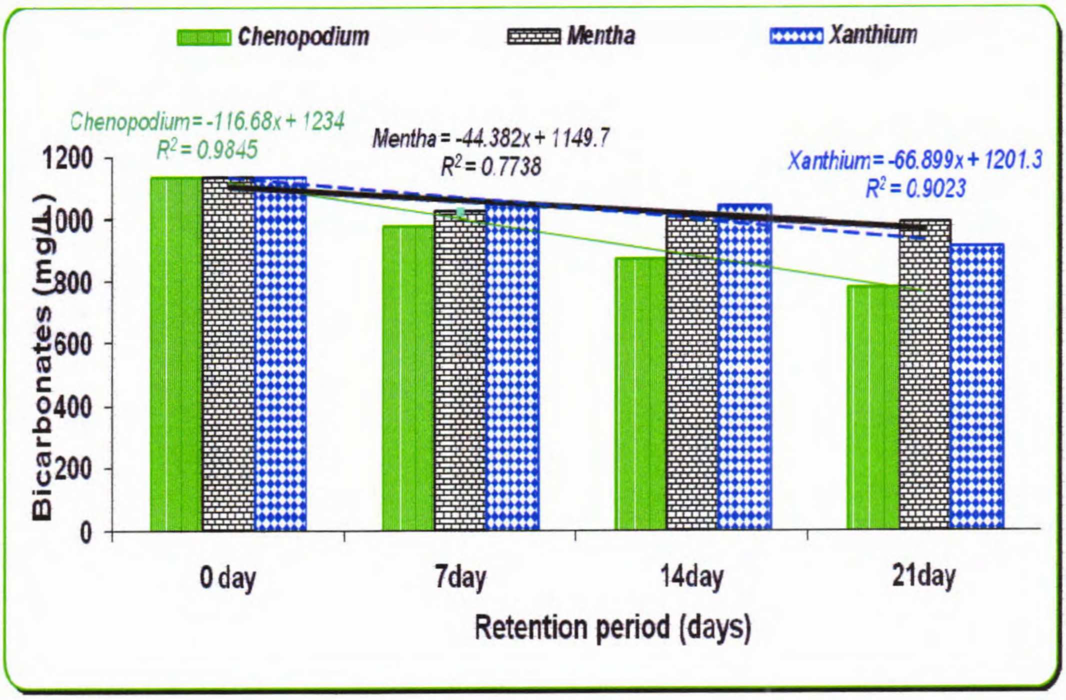

Figure 4.6. Effect of invasive plants on bicarbonates of the effluent

4.3.7 CHLORIDES

The concentration of chlorides was determined by Titrometric method was 4040592 mg/l infringing the National Environmental Quality Standards (1000 mg/l). In majority of the industries, the main source of chlorides in the effluent is the use of Lime or Sodium Hydroxide for the neutralization of acidic effluents (Sagar *et al.*, 2011).

The chloride content was abridged during the 21 day treatment of textile waste water with the plants i.e. *Chenopodium ambiosoides* (1620527 mg/l), *Mentha piperita* (2117618 mg/l) and *Xanthium strumarium* (1811838 mg/l). Hajrasuliha (1979) ,Zalesny J.A et al., (2007) reported that chlorides tend to accumulate in tissues, particularly leaves of the plants and chloride accumulation in plants is closely related to Cl^- concentration in the external solution and the genotype.

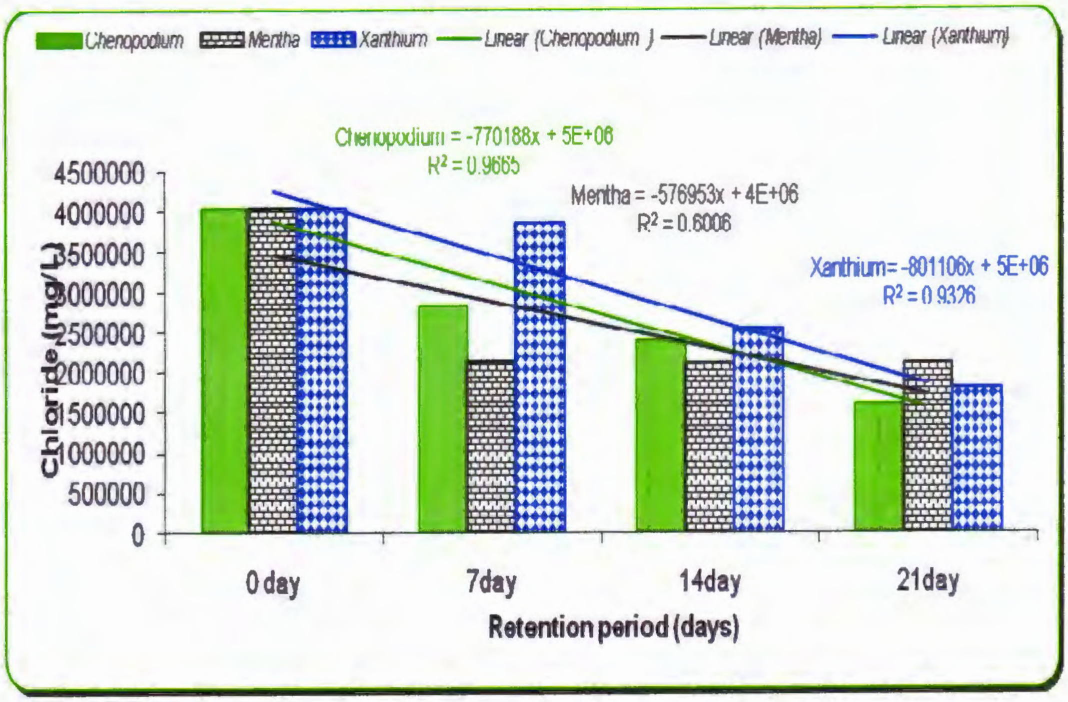

Figure 4.7. Effect of invasive plants on chlorides of the effluent

4.3.8 HEAVY METALS (COPPER, CHROMIUM AND CADMIUM)

Analysis of heavy metals like copper, chromium and cadmium in textile waste water was done by atomic absorption spectrometric method. Copper, chromium and cadmium content in the sample was 0.009 mg/l, 0.081 mg/l and 0.048 mg/l, respectively. Metal availability and bioaccumulation is governed by several environmental factors, viz. chemical speciation of the metal, pH, organic chelators, presence of other metals and anions, ionic strength, temperature, salinity, light intensity, oxygen level and other prevailing electrochemical functions (Kara, 2005).

Chenopodium ambiosoides and *Xanthium strumarium* reduced copper concentrations to 0.0002 mg/l and 0.01 mg/l. *Mentha piperita* exhibited different results. In first two week it reduced vital concentration of Cu but unexpectedly in last week amount of Cu increased; it might be due to the wilting and dying of plant because of harsh weather conditions (38-42°C). The dead parts added small quantities of Cu back to the effluent.

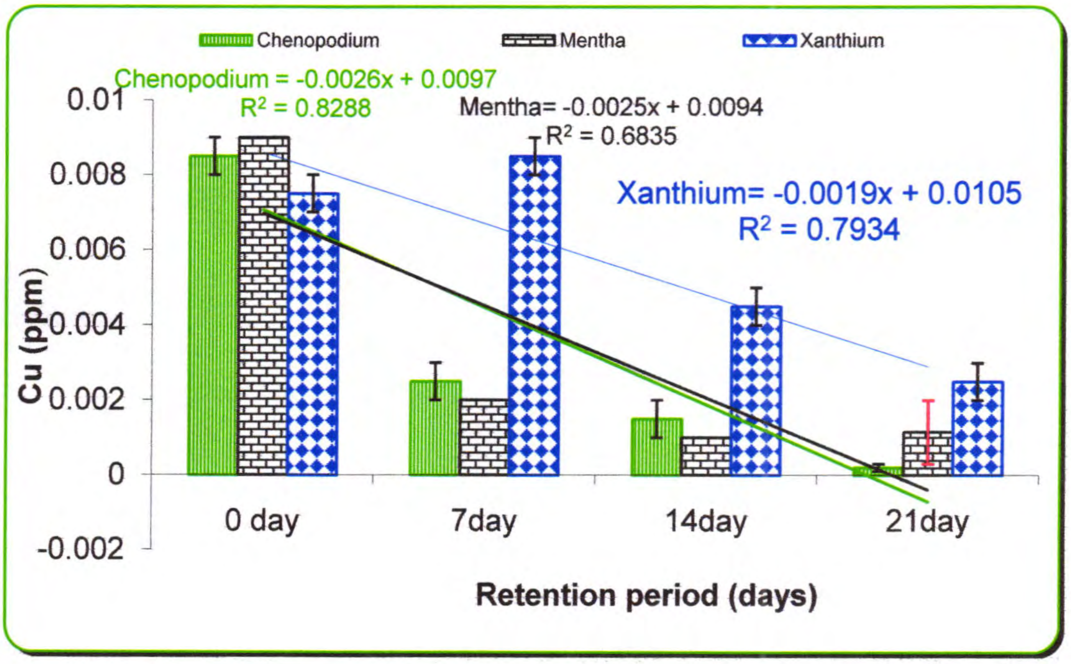

Figure 4.8. Effect of invasive plants on Cu of the effluent

Effluents released from the textile industries contain various organic dyestuffs, chrome dyes and chemicals during various operations and produce a large quantity of solid and liquid waste containing hexavalent chromium (Deepali, 2011).

The Cr level from effluent reduced by *Chenopodium ambiosoides, Xanthium strumarium and Mentha piprita* were 0.0119ppm, 0.0125ppm and 0.0418ppm respectively.

The distribution of Cr between root and shoot in hyperaccumulator plants, however, indicated that the leaves also contained a much higher Cr concentration, suggesting better translocation of Cr from root to shoot for hyperaccumulator plants. Although the detailed mechanisms of Cr translocation is not understood, there are reports that Fe-deficient and P-deficient plants can better translocate Cr from roots to shoots (Cary et al., 1977a; Bonet et al., 1991). Plant species differ significantly in Cr uptake capacity and distribution within the plant (Grubinger et al., 1994; Soane and Saunder, 1959) depends upon rooting pattern, transpiration rate and metabolism of plants (Hossner et al., 1998).

Chenopodium ambiosoides, Mentha piperita and *Xanthium strumarium* reduced small amount of Cd from 0.048 ppm to 0.044ppm, 0.0431ppm and 0.0433ppm correspondingly.

The amount of Cd accumulated within the plant is limited by several factors, including:(1) Cd bioavailability within the rhizosphere, (2) rates of Cd transport into roots via either the apoplastic or symplistic pathways, (3) the proportion of Cd fixed within roots, as a Cd phytochelatin complex, and accumulated within the vacuole, and (4) rates of xylem loading and translocation of Cd (Hossner et al; 1998).

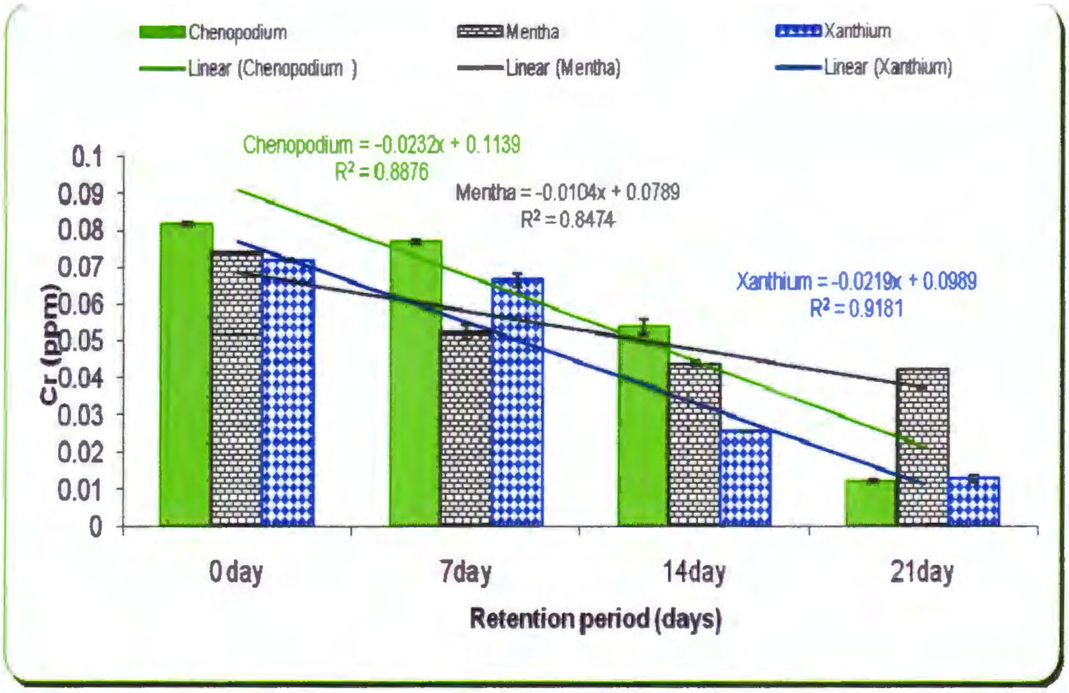

Figure 4.9. Effect of invasive plants on Cr of the effluent

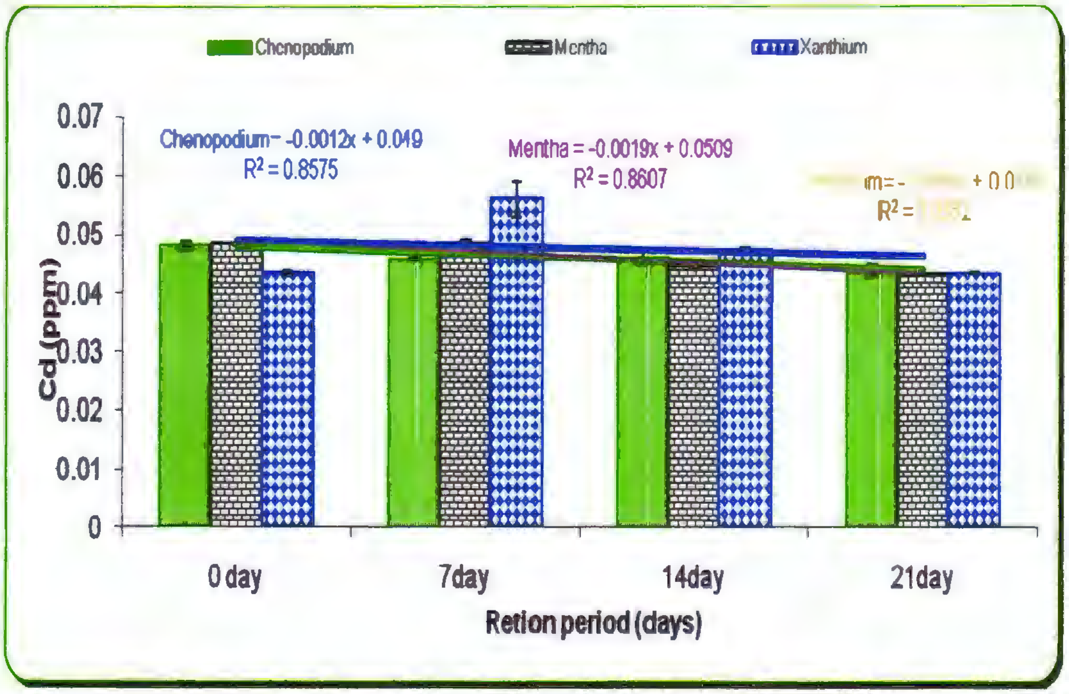

Figure 4.10. Effect of invasive plants on Cd of the effluent

sediment with PCA in two boreal lakes in southern Finland. Chemosphere, 35, p56-2645.

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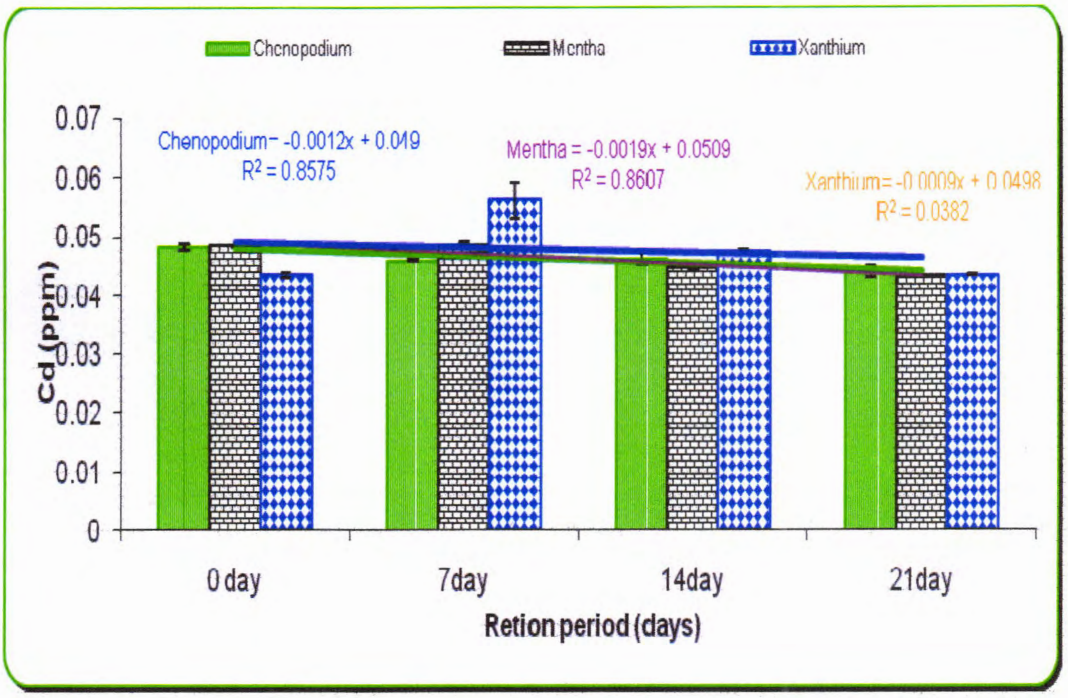

Figure 4.10. Effect of invasive plants on Cd of the effluent

CONCLUSION AND RECOMMENDATIONS

It is evident from the results that three plants *Chenopodium abriosoides, Mentha piperita* and *Xanthium stromarium* revealed a momentous potential of dropping the values of all the pollution parameters like pH, EC, TDS, Turbidity and the amount of chlorides, carbonates and bicarbonates from textile effluent. In three week interval pH value was slightly increased to reach the level of neutrality by three plants however, DO remain constant throughout the experiment. The magnificent results are shown by *Chenopodium abriosoides* in reducing EC, Turbidity and TDS values as compared to the other plants *Menthe piperita* and *Xanthium stromarium* also accumulate salts in their different body parts and showed positive correlation with time. Bicarbonate content reduced 32.2% *by Chenopodium abriosoides* and other two plants are having enough capacity to hyperaccumulate and absorb alkalies. Textile effluent contains greater amounts of chlorides which were reduced by plants enormously. Metal analysis by AAS shown that *Chenopodium ambiosoides* have the capacity to accumulate heavy metals like Cr and Cu, *Mentha piperita* and *Xanthium stromarium* also take part in reducing of Cu and Cr however lesser than *Chenopodium ambiosoides* .All three plants have not shown noteworthy results for Cadmium throughout the treatment period.

FUTURE PLANS

From the present study we found that plants have great potential of reducing the pollution load of the environment. Therefore following are the recommendations that should be follow for the treatment of waste water.

- Explore more plants species for phytoremediation studies which are commonly cultivated /grown in our natural climatic conditions
- Explore and conduct high resolution of microanalysis system of hyperaccumulator species (scanning and transmission electron microscopy with X-ray energy dispersive spectroscopy) to determine

the discrete sites of metal sequestration and bioaccumulation in specific plant organs, tissues, cells and organelles

- For effective phytoremediation the retention period of plant should be increased
- Extend investigation on Phytoremediation using different aquatic and invasive species and also other terrestrial species

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ANNEXURE

ANNEXURE

STANDARD SOLUTIONS

Normal solutions

A normal solution is that solution that contains equivalent weight of solute in one liter of solution. The mathematical expression is given below.

$$\text{Normality (N)} = \frac{\text{No of equivalents}}{\text{no of liters}}$$

Preparation of 0.1 normal HCl

Preparation of 0.1 N solution

100 ml of 1.0 N HCl

1000 ml of deionized water

Or

Use the stock bottle of 37% HCl v|v

37ml of solute|100 ml of solution

Specific gravity = 1.19 g|L

HCl – 37% v|v

Molecular weight of HCl = 36.5

Molarity

Molarity is the number of moles of solute dissolved in one liter of solution.

$$\text{Molarity} = \frac{\text{moles of solute}}{\text{liter of solution}}$$

440.3 grams| 36.5 grams = 12.06 M

M1V1 = M2V2

(0.1) (1000)| (12) (X)

X = (0.1) (1000) | 12

X = 8.3 ml

So add 8.3 ml of 37% HCl to 1000 ml of deionized water to create a 0.1N HCl solution.

Preparation of 0.02 normal HCl

For 0.02 N HCl first made 0.1N HCl.

M1V1= M2V2

(0.1) (1000) | (12) (x)

(x) = (0.1) (1000) | 12

X = 8.3 ml

Thus add 8.3 ml of 37% HCl to 1000ml of deionized water to create 0.1 N HCl solution. Take 200 ml of 0.1 normal HCl and dissolved it in 1000 ml of deionized water.

Silver nitrates (for Chloride test)

Dissolved 2.39678g of silver nitrate in 1000 ml of deionized water. Normally 0.041 normal silver nitrate is used for chloride test. Keep the solution in blind bottles because it is affected by light.

Preparation of 2% nitric acid

Take 200ml of nitric acid in 1000ml flask and add 980 ml of de ionized water in it and shake it well.

Sodium sulphate stalk solution

Take 0.1479 g Sodium sulphate in a flask and add 1000 ml distilled water in it. Then shake it well so that it dissolved in it.

Sulphate buffer formation

Take 30 g magnesium chloride, 5g sodium acetate, 1g potassium nitrate, 20 ml acetic acid in 1000ml flask. Then filled the remaining flask with distilled water and shake it well.

Phenoplthlinc prcparation

Take 0.5g Phenophthaline, ethanol 85 ml (95%) in 200 ml flask and add distilled water so that it becomes 100 ml.

Ferrous Ammonium sulphatc

Take 19.6 g of ferrous ammonium sulphate in distilled water and add 4 ml concentrated Sulphuric acid and allow it to cool. Then add distilled water in order to dilute it till to 200 ml.

Ferron indicator

Take 1.485 g of Phenonthroline momnohydrate and 0.695g of ferrous sulphate in a flask and dilute it with distilled water until it becomes 200ml.

Standard solution for mctals

From the stock solution of 1000 ppm we make the standard of 0.2, 0.4 and 0.8. First we make the standard solution of 10 ppm. For that 10 x 100 | 1000 = 1ml. After it we made stock B solution as 1ml | 100ml = 10ppm.

Formula	Standard solution
.2 x 10 | 10	0.2
.4 x 10 | 10	0.4
.8 x 10 | 10	0.8