BIOACCUMULATION OF TEXTILE WASTEWATER BY *CERATOPHYLLUM DEMERSUM*

Submitted By
Farzana Kousar
183FBAS/MSCES-F10

Supervised by
Dr.shazia Erum

Spring 2012

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Faculty of Basic and Applied Sciences
International Islamic University Islamabad

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A project report submitted to Department of Environmental Sciences, International Islamic University, Islamabad as a partial fulfillment of requirement for the award of the degree of MS.c Environmental Science

Department of Environmental Sciences
Faculty of Basic and Applied Sciences
International Islamic University Islamabad

International Islamic University Islamabad
Faculty of Basic and Applied Sciences
Department of Environmental Sciences

Dated: 5/7/2012

FINAL APPROVAL

It is certified that we have evaluated the project report "Bioaccumulation of Textile Wastewater with *Ceratophyllum Demersum*" submitted by Miss Frazana Kousar 183 FBAS/MSCES-F10 and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the Ms.c Degree in Environmental Sciences.

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DEDICATION

Dedicated to Prophet Pace Be Upon Him and to

My Parents & Teachers Whose

Prayers, Guidance & Support enabled us to

Complete this Task

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 5/7/12

Farzana

Farzana Kousar

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ACKNOWLEDGEMENT

ALL Praises to Almighty Allah, the omnipotent, the most compassionate and His prophet Muhammad (P.B.U.H) The Most prefect and among and ever born on the surface of the earth, who is forever of guidance and knowledge for humanity as a whole.

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Farzana Kousar

LIST OF ABBREVIATIONS

NARC	National Agriculture Research Center
EC	Electrical Conductivity
DO	Dissolve Oxygen
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
TDS	Total Dissolve Solids
Cl	Chlorides
ppm	Part per million
EPA	Environmental Protection Agency
CDF	Cation Diffusion Facilitator
PEPA	Pakistan Environmental Protection Act
NEQS	National Environmental Quality Standards
APH	American Public Health
GCMS	Gas chromatography–mass spectrometry
HPLC	High performance liquid chromatography
JICA	Japanese International Cooperation Agency
FEPA	Federal Environmental Protection Agency

LIST OF SYMBOLS

mg	Milli gram
µs	Micro Siemens
SO_4	Sulphates
Zn	zinc
Cu	copper
Cr	Chromium
$K_2Cr_2O_7$	Potassium dichromate
HNO_3	Nitric acid

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SUMMARY

PROJECT SUMMARY

During the present study sampling of wastewater was done from Faisalabad, as it covers most of the textile sector of Pakistan. Initially the pollution load in the textile wastewater was examined. As we know that Textile industry consume large amount of chemicals, dyes and water for processing of textile material and they discharge their wastewater into the environment without pretreatment. Results of present study showed that the pH (11.37), COD (533mg/l), BOD (246mg/l), SO_4 (4833.3mg/l), Cr (1.012mg/l) of the effluent was found above the permissible limits according to NEQS, 2000. However Cl, TDS, EC, Cu, Zn and temperature was observed under their permissible limits. During the present study, *Ceratophyllum demersum* was selected for reducing the pollution load from textile wastewater and it was observed that most of pollutants reduce to up to their permissible limits, except the SO_4 content. However it can be reduce by increasing the retention period of that water with *Ceratophyllum demersum.*

INTRODUCTION

Introduction

Environmental pollution is the contamination of the physical and biological components of the earth/atmosphere system to such an extent that normal environmental processes are adversely affected (Kemp, 1998). Environmental pollution had been a fact of life for many centuries but it became a real problem since the start of the industrial revolution. Adverse effects of industrial pollution are becoming a challenge for scientists and environmentalists around the globe. Different types of pollution generated by industries. Such as air pollution, water pollution, noise pollution, thermal pollution, radioactive pollution etc. But industrial revolution cause major impact on water resources. Worldwide Industrial sector is the major sector that use water resource in two way's in first hand it has high consumption rate in production process and on the other hand it drain out untreated waste water in natural water resources. Most of industries are situated near the water resources. Effluent coming from these industries deteriorates our fresh water resources because industries dispose their untreated water in the fresh water bodies.

Among entire industrial sector textile sector is at the top of water consumption. It not only uses the water in production process but it produces huge quantity of waste water. That waste water discharged directly in to local environment without any treatment. In developing as well as underdeveloped countries, the industrial effluents are released directly or indirectly into natural water resources, mostly without proper treatment, thus posing a serious threat to the environment (Altug and Balkis, 2009). Textile industry use excessive chemicals and it is the number one water polluter (after agriculture). Textile industries have shown a significant increase in the use of synthetic complex organic dyes as the colouring material. The annual world production of textiles is about 30 million tones requiring 700,000 tonnes of different dyes which causes considerable environmental pollution problems (Zollinger, 1987). Almost all dyes, specially chemicals and finishing chemicals are applied to textiles in water baths. Most fabric preparation steps, including desizing, scouring, dyeing, bleaching and mercerizing, use water. And each one of these steps must be followed by a thorough washing of the fabric to remove all chemicals used in that step before moving on to the next step. The water used is usually returned to ecosystem without treatment means that the wastewater which is returned to our streams contains all chemicals used during production of textile material (*oecotextiles.wordpress.com*). Dyes which are used in textile industries are highly colored and visible in water at very low concentration, even as 1 ppm. Dye bath effluents contain heavy metals, ammonia, alkaloid salts, toxic solids and large amounts of pigments many of which are toxic. About 40 percent of globally used colorants contain organically bound chlorine, a known carcinogen. That colored waste water degrades the aesthetic beauty of nature one side and on the other hand due to its high persistence, and chemically and photolytically high stability in nature it become harm for all the forms of life. They have the carcinogenic properties and have the ability to absorb the light that enters in water in this way it disturbed the microbial growth and metabolic activity. That Effluent contain chemicals such as formaldehyde, chlorine, carbonates, nitrates, sulphates, heavy metals (such as lead, chromium and zinc) and others. It also has high temperature, TDS, pH, COD and BOD levels which are significant causes of environmental degradation and human illnesses. Heavy metals cause health problems even at very low concentration. This above mention waste water is extremely damaging for aquatic life, human health and crops.

Industrial sector of Pakistan comprises of small, medium and large units with blend of old and new technologies. The major cities where industries are present are: Lahore, Karachi, Faisalabad, Multan, Peshawar, Gujranwala, Sialkot, and Gujarat. The major industries are following. Leather industry, textile industry, Dyes and pigment industry, and Paper and pulp industry. Very few of them are equipped with satisfactory operating treatment facility set up, there is common trend that industries dispose of their untreated effluents through open and covered routes into the water ways which degrade water quality (Farid, 2003). Hence these industrial effluents are the most potential source of water and soil pollution. These all industries are using large quantity of water during their production process. In Pakistan only 1 % of waste water is treated before being discharge directly in rivers and drains (*www.environment.gov.pk*). Among the Asian countries, Pakistan is at the top of list who will face sever water crisis in coming decades. There is a severe water shortage looming in Pakistan. The United Nations has placed Pakistan among the 'water hotspots' of Asia-Pacific region, saying that the country is facing major threats of increasing water scarcity, high water utilization, deteriorating water quality and climate change risk (*www.dawn.com*).In light of above discussion, there is need to protect the water resources from industrial as well as from other sources of pollution. In that context Environmental Protection Agency (EPA) has taken different initiatives to control the environmental pollution due to untreated wastewaters emissions. National Environmental Quality standards (NEQS) were developed under the Pakistan Environmental Protection Act PEPA 1997. Industries were required to comply with these standards. NEQS were developed for municipal and liquid industrial effluents, industrial gaseous emissions and motor vehicle exhausts and noise (Table1. 1)

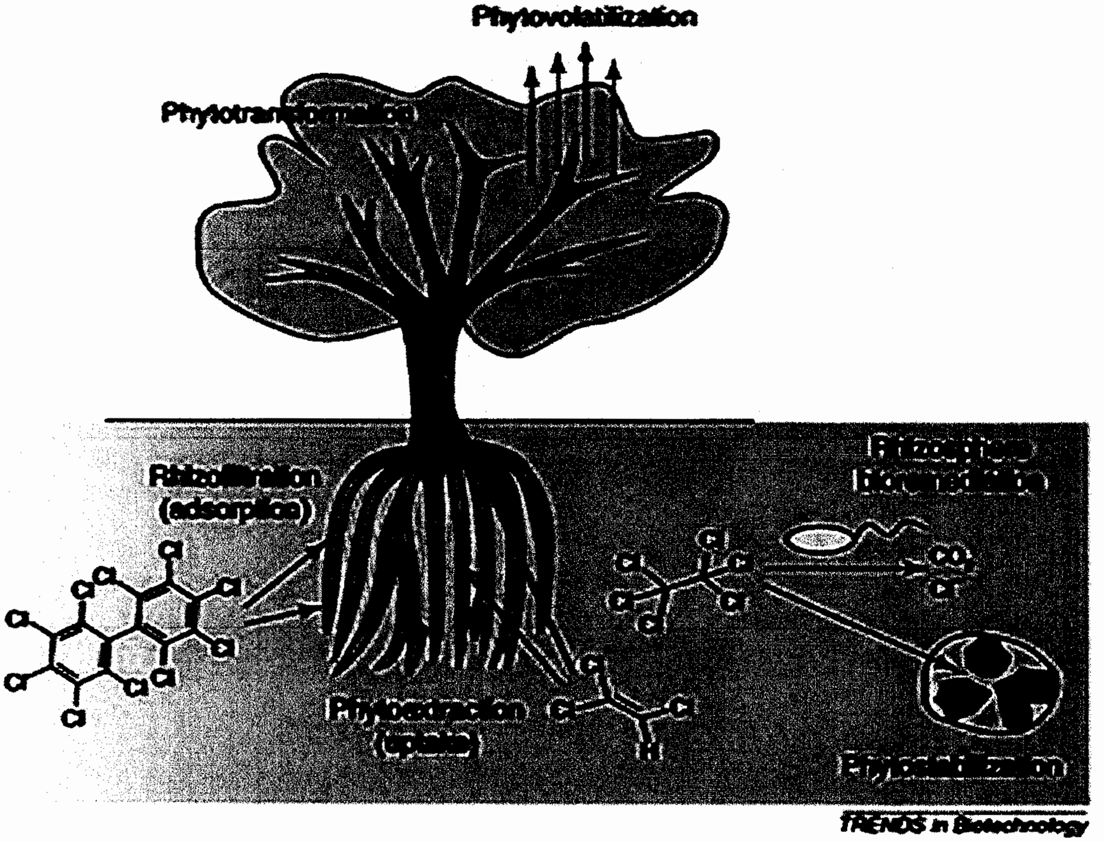
Table 1.1National Environmental Quality Standards

S. No	Parameter	Revised Standards (2000)			
	Water Quality Parameters	Existing Standards	Into Inland Waters	Into Sewage Treatment	Into Sea
1	Temperature or Temperature Increase	40 °C	≤3°C	≤3°C	≤3°C
2	PH value	6-10	6-9	6-9	6-9
3	Biocherhical Oxygen Demand (BOD),	80	80	250	80*
4	Chemical Oxygen Demand (COD)	150	150	400	400
5	Total suspended solid (TTS)	150	200	400	200
6	Total dissolved solids (TDS)	3500	3500	3500	3500
7	Grease and Oil	10	10	10	10
8	Phenolic compounds.	0.1	0.1	0.3	0.3
9	Chloride (as Cl)	1000	100	100	
10	Fluoride	20	10	10	10

11	Cyanide	2	1.0	1.0	1.0
12	An-ionic detergents	20	20	20	20
13	Sulphate (SO_4)	600	600	1000	
14	Sulphide $(S)_2$	1.0	1.0	1.0	1.0
15	Ammonia (NH_3)	40	40	40	40
16	Pesticide (3)	0.15	0.15	0.15	0.15
17	Cadmium	0.1	0.1	0.1	0.1
18	Chromium trivalent and hexavalent	1.0	1.0	1.0	1.0
19	Copper	1.0	1.0	1.0	1.0
20	Lead	0.5	0.5	0.5	0.5
21	Mercury	0.01	0.01	0.01	0.01
22	Selenium	0.5	0.5	0.5	0.5
23	Nickel	1.0	1.0	1.0	1.0
24	Silver	1.0	1.0	1.0	1.0
25	Total Toxic metals	2.0	2.0	2.0	2.0
26	Zinc	5.0	5.0	5.0	5.0
27	Arsenic	1.0	1.0	1.0	1.0
28	Barium	1.5	1.5	1.5	1.5
29	Iron	2.0	8.0	8.0	8.0
30	Manganese	1.5	1.5	1.5	1.5
31	Boron	6.0	6.0	6.0	6.0
32	Chlorine	1.0	1.0	1.0	1.0

Section 11 of PEPA 1997 deals with Prohibition of Certain Discharges or Emissions according to that section that 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. The Federal Government levy a pollution charge on any person who contravenes or fails to comply with the provisions of that act. For the compliance with NEQS there is needed to treat the waste water before its disposal and on the other hand Treatment of wastewater is become more important due to diminishing water resources, increasing wastewater disposal costs, and stricter discharge regulations that have lowered permissible contaminant levels in waste streams (Metcalf and Eddy, 1979*)*. So for the conservation of water first we need to treat that waste water and make it use full for irrigation and other beneficial purposes. Wastewater treatment methods are broadly classified into physical, chemical and biological processes (Metcalf and Eddy, 1979; Matsumoto *et al.,* 1992).

Chemical process of treatment includes precipitation; reduction, artificial membranes, and ion-exchange. Chemical precipitation is widely used technology for the removal of heavy metals from water. That process involves the transformation of dissolved contaminants into insoluble solids, thereby facilitating the contaminant's subsequent removal from the liquid phase by physical methods, such as clarification and filtration (NEESA, 1993). Reverse Osmosis is a membrane process that acts as a molecular filter to remove over 99% of all dissolved minerals. In this process, water passes through the membrane while the dissolved and particulate matter is left behind. The process is very effective for removal of ionic species from solution. The resulting concentrated by-product solutions make eventual recovery of metals more feasible. (Akpor and Muchie, 2010) Ion exchange is a reversible chemical reaction where in an ion from water or wastewater solution is exchanged for a similarly charged ion attached to an immobile solid particle. These solid ion exchange particles are either naturally occurring as inorganic zeolites or synthetically produced organic resins. It is a process that is very similar to biosorption whereby the latter is known to actually function predominantly on the basis of ion exchange. Ion exchange uses mainly hydrocarbon-derived polymeric resins (Vaca *et al.,* 2001; Volesky, 2003). Although all these technologies are very effective in waste water treatment but, all these methods are expensive and may generate a huge amount of waste, which leads to disposal problems. These methods require high amount of energy for proper functioning. With respect to environment they are not environmental friendly. So there is need of treatment technology which is cost effective and friendly to environment. Recently, it has been advocated that biological treatment of waste water is as cheap and eco-friendly technique not just for heavy metals removal but also for various recalcitrant pollutants Such as polychlorinated biphenyls (Smith *et al.,* 2007). Bioremediation has been described as "a treatability technology that uses biological activity to reduce the concentration or toxicity of a pollutant. It commonly uses processes by which microorganisms transform or degrade chemicals in the environment (King *et al.,* 1998). Bioremediation of waste water uses naturally occurring bacteria, fungi or plants to degrade or detoxify the hazardous substance. Degradation by fungi or bacteria is known as microbial degradation method in which microorganisms or microbial processes are used to detoxify and degradation of environmental contaminants. In fungal biodegradation mostly White-rot fungi are used. These organisms are able to degrade lignin, the structural polymer found in woody plants (Barr and Aust, 1994). Use of bacteria offers a cheaper and environment friendlier alternative for colour removal in textile effluents (Olukanni *et al.,* 2006). These microorganisms are may be indigenous to a contaminated site or they may be isolated from elsewhere to the effected site or water. These microorganisms transform the contaminated compounds through reaction that takes place as part of their metabolic processes. When micro organisms are imported to contaminated site to enhance degradation the process is known as bioaugmentation. Remediation by the plants is known as phytoremediation. It may be defined as the using of green plants to remove, destroy or sequester hazardous substances from environment. Phytoremediation can provide a cost-effective, long lasting aesthetic solution for remediation of contaminated sites (Ma *et al.,* 2001). Phytoremediation is a combination of two words phyto, which means plant, and remediation, which means to remedy. Phytoremediation is actually a general term for several ways in which plants can be used to clean up contaminated soils and water. Plants may break down or degrade organic pollutants, or remove and stabilize metal contaminants. Different methods of phytoremediation such as Phytoaccumulation in that method plant take up contaminant by roots and accumulate them in its different parts. In phytostabilization plant take the precipitation of metals by roots exudates and their mobilization is take place. Rhizofiltration is the process in which plant remove the toxic compound from water by filtration which done by root mass and pollutants remain absorbed or adsorbed in roots. These above three methods are used for metals. And for the degradation of organic matter these terms are used. Phytodegradation is process in which plant up take and subsequently degrade the organic matter. Another method is known as Phytovolatilization in that process plant up take metal ions as well as organic compounds, these compounds are subsequently modified in volatile organics which are evaporated by plant's leaves. Rhizodegrdation is a process in which plant remove the organic compound from water or soil by roots.



TRENDS in Biotechnology

Fig. 1.2 Decontamination through various phytoremediation approaches

Although bioremediation is an ecologically sound and sustainable reclamation strategy for bringing polluted sites into productive use but is still in experimental stage therefore it needs a lot of attention and scientific scrutiny (Govindwar and Kagalkar, 2010). A few studies are reported on removal of textile dyes and other contaminations by using plants. So there is need to do more research in that field. In today's environment of growing scarcity and completion of water, the incompatible claims for more and more irrigation water are imposing pressure on available water resources (Bastiaanssen *et al.,* 2002). To overcome that shortage most of countries use treated wastewater in arid regions not only as irrigation water but also as a liquid fertilizer. In that context, Ceratophyllum demersum is used in present study for the remediation of waste water to obtain safe irrigation water.

1.1 Objectives of the study

(i) Estimation of the pollution load in the collected sample.

(ii) Reduction of pollution level in textile waste water through *Ceratophyllum demersum*

LITERATURE REVIEW

Review of Literature

2.1 Water pollution

Any physical, biological or chemical change in water quality that adversely affects living organisms or makes the water unsuitable for desired use is called as water pollution. Water pollution is broad term that includes contamination of different water bodies such as lakes, rivers, oceans, and groundwater. Water pollution is caused by pollutants, mostly in form of different chemicals that are discharged either directly or indirectly into the water bodies without the adequate treatment to remove their harmful effects. Water pollution is not only creating ecological problem but also associative with the health problems. It disturbed the balance of aquatic ecosystems, economic development and social prosperity (Arsovski *et al.*, 1991). Poor water quality is responsible for the death of an estimated 5 million children in the developing countries (Holgate, 2000). The problem is further aggravated by rapidly increasing population which results in poor water-quality management (Huang and Xia, 2001). In Pakistan, 30% of all diseases and 40% of all deaths are due to poor water quality (Global Water Partnership, 200). There are different sources of water pollution but most important are following agricultural, domestic, and industrial. In the 2000 National Water Quality Inventory conducted by the Environmental Protection Agency (EPA), agricultural activity was identified as a source of pollution for 48% of stream and river water, and for 41% of Lake Water. Pesticides are present in surface and underground water bodies, far from the sites of pesticide application (Voltz *et al.*, 2007). The use of nitrogen in fertilizers can be a problem in areas where agriculture is become intensified. People dump their garbage into streams, lakes, rivers, and seas, thus making water bodies the final resting place of cans, bottles, plastics, and other household products and cleaning products which synthetic detergents and washing powders contain phosphates, which are used to soften the water among other things. Chemical in washing powders affect the health of all forms of life in the water (Harter, 2003). Industrialization has greatly increased throughout the world.

According to Mushala (1993), population growth rate exerts pressure on land resources for increased food production which contributes to environmental degradation. Most of polluted water from industries drains out in natural water resources without treatment. Which effect health and quality of soils and vegetation (Carter, 1985).

According to previous studies poor quality water causes health hazard and death of human being, aquatic life and also disturbs the production of different crops (Ashraf *et al*, 2010). In fact, the effects of water pollution are said to be the leading cause of death for humans across the globe, moreover, water pollution affects our oceans, lakes, rivers, and drinking water, making it a widespread and global concern (Scipeeps, 2009). In Pakistan industrial and municipal effluents are basic cause of pollution in canals and rivers. The untreated industrial and municipal wastes have created multiple environmental hazards for mankind, irrigation, drinking and sustenance of aquatic life.

2.2 Textile Waste Water

Among the entire industrial sector textile processing industry, consumed large quantity of water consumed and the variety of chemicals used in the process. Liquid wastes from various stages of the operation contain substantial pollution loads in terms of organic matter and suspended material such as fibers and grease. This wastewater is discharged untreated or partially treated, and causes serious environmental impacts on natural water bodies and land in the surrounding area. According to a joint report published by the Pakistan Environmental Protection Agency (PEPA) and Japanese International Cooperation Agency (JICA) 2005, 9000 million gallons of wastewater having 20,000 tons of BOD (Biological Oxygen Demand) loading are daily discharged into water bodies from the industrial sector into natural streams, canals, rivers and the sea. The World Bank estimates that 17 to 20 percent of industrial water pollution comes from textile dyeing and treatment. They have also identified 72 toxic chemicals in water solely from textile dyeing, 30 of which cannot be removed easily. The regulatory system framework for implementation of environmental policy in Pakistan evolved over a period of time. Colour in water can be recognized even at very low amounts (less than 1 ppm) of dye which is highly undesirable. The thin layer of dyes formed over the surface of water which decreases the amount of dissolved oxygen. It also reduces photosynthetic activities due to reduced light penetration which badly affects the aquatic flora and fauna. In addition to aesthetic damages to sites, dyes are also toxic and carcinogenic. Untreated water near the point of disposal, create foul smell and bad odour (Kullkarni, 1997). High levels of pollutants mainly organic matter in river water cause an increase in biological oxygen demand chemical oxygen demand, total dissolved solids, total suspended solids and make water unsuitable for drinking, irrigation (Hari *et al.*, 1994) or any other use. So it is essential to treat the waste water before disposal and reuse that treated water. Reuse of treated wastewater in aquaculture/agriculture practices is encouraged to minimize demand on freshwater resources. A major concern for reuse of wastewater is the bioaccumulation of hazardous wastes especially heavy metals and pesticides in food chain (Teisseire and Guy, 2000).

Table 2.1 Types of common dyes used in textile and their characteristics

Dye Class	**Description**	**Typical Pollutants Associated with Various Dyes**
Reactive Dyes	water-soluble, anionic compounds; largest dye class	Color, salts, alkali, unfixed dye, surfactants, defoamer, diluents and finish.
Acid Dyes	water-soluble anionic compounds	Color, organic acids and unfixed dyes
Direct Dyes	water-soluble, anionic compounds; can be applied directly to cellulosic's without mordents (or metals like chromium and copper)	Color, salt, unfixed dye, cationic fixing agents, surfactant, defoamer leveling and retarding agents.
Vat Dyes	oldest dyes; more chemically complex; water-insoluble	Color, alkali, oxidizing agents and reducing agents
Sulfur Dyes	organic compounds containing sulfur or sodium sulfide	Color, alkali, oxidizing agent, reducing agent and unfixed dye
Basic Dyes	water-soluble, applied in weakly acidic dye baths; very bright dyes	N/A
Disperse Dyes	not water-soluble	Color, organic acids, leveling agents, phosphates, defoamers, lubricants, dispersants; delustrants.

Chemical treatment relies upon the chemical interactions of the contaminants which are needed to remove from water, and the application of chemicals that either aid for the separation of contaminants from water, or assist in the destruction or neutralization of harmful effects associated with contaminants. Chemical treatment methods are applied both as stand-alone technologies and as an integral part of the treatment process with physical methods (Ranganathan *et al.*, 2007).Chemical and physical methods are highly expansive and with regard to sustainable approach these are not environmental friendly. Some of their byproducts shows harm full impacts on environment. It is found that biological wastewater treatment possesses high degree of efficiency with minimum running cost (5 to 6 times less) compared to other methods. In Bioremediation pollution control methodology applied in biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. The general approaches for bioremediation include the enhancement of natural biodegradation, by native organisms such as *Trichoderma reesei, white rot fungi Phanerochaete chrysosporium,* and plants like *water hyacinth, pennywort, waterlettuce, C.demersum, waterferns etc* (Atlas and Unterman, 1999).

2.3 Phytoremediation

Phytoremediation is a green technology that uses plant systems for remediation and restoration of the contaminated sites. Plants have inbuilt enzymatic machinery capable of degrading complex structures and can be used for cleaning of the contaminated sites. Different enzymes in plants phytodegarde the toxic pollutants in less toxic such as Dehalogenase enzyme use in dehalogenation of chlorinated solvents, Nitroreductase used for reduction of nitro groups and removal of nitrogen from ring structures. Wide range of genes involved in transport of trace elements and heavy metals in plant tissues during remediation process. such as heavy metal ATPases (HMAs) , the natural resistance associated macrophase proteins (Nramps) , the cation diffusion facilitator (CDF), and the cation anti-porters. Complexation of toxic metal ions by peptides or proteins also helps in detoxification process. Plants make two types of peptide metal binding ligands: Metallothioneins (MT's) and Phytochelatins (PC's) (Goldsbrough, 2002). Among the various plants species group, aquatic macrophytes attain greatest interest in the field of phytoremediation. Aquatic plants have ability for up-take various nutrients from waste water was examined by Dings, (1978), Rai and Datta, (1978). Most common aquatic macrophytes used in wastewater treatment are *water hyacinth, pennywort, waterlettuce, C.demersum, waterferns* and *duckweeds.* Phytoremediation is an ecologically sound and sustainable reclamation strategy for bringing polluted sites into productive use but is still in experimental stage. Therefore it needs a lot of attention and scientific scrutiny (Govindwar and Kagalkar, 2010).That treatment system for wastewater is the need of developing countries, because it is cheaper in construction and a little skill is required to operate it. This technology has proved to be an effective, efficient, pollution free and low cost technology. Heavy/trace metal removal by aquatic plants was studied by (Guilizzoni 1991). Use of aquatic plant *Ceratophyllum demersum* for water treatment reported in various studies. (Foroughi, 2011). Another study shows that aquatic plants play an important role in the uptake, storage, and recycling of metals. The uptake of metals depends on the life form of the macrophytes (floating, free floating, well rooted, or rootless). The free-floating species *(Eichhornia, Lemna, Pistia)* absorb elements through the roots/leaves, whereas the rootless species *Ceratophyllum demersum* absorbs mainly through the finally divided leaves (Chandra and Kulshreshtha, 2004).

2.4 *CERETOPHYLLUM DEMERSUM*

Ceratophyllum demersum is known as *Coontail*, or *hornwort* belongs to the family *Ceratophyllaceae.* It has number of worldwide known species such as *Ceratophyllum australeand, C.demersum, C.echinatum, C.llerenae, C.muricatum, C.oryzetorum, C.platyacanthum, C.submersum,* and *C.tanaiticum* (Donald, 1985). Basically it is a rootless submerged floating aquatic plant. It does not produce roots, instead it absorbs all the nutrients it requires from the surrounding water. If it is growing near the lake bottom, it will form modified leaves, which it uses to anchor to the sediment. However, it can float free in the water column and sometimes forms dense mats just below the surface (Keskinkan.*et al.*, 2004). Its fan shaped dichotomous leaves divided into many narrow segments. Each leaf has several small teeth on the midrib. These tiny teeth give the plant a rough feel. The size of plant is up to 1 meter long. Inter node of stem is 1 to 3cm long and 4 to 9 leaves are arranged in a whorl. During winter the tips of branches may become much shortened, thickened and break off. These structures act as buds and grow in to a new plant (Patel and Kanungo, 2010). This species is native to a wide area of North America, from where it has spread to other parts of the world. Habitats include quiet inlets of lakes, ponds, rivers with slow-moving currents, marshes, and springs. Generally, *Coontail* is typically found in bodies of water with muddy bottoms, although it also occurs where the water bottom contains some sand or rocky material. Sometimes *Coontail* is cultivated as an aquarium plant. *Ceratophyllum demersum*, is the best "accumulator", with high potential for phytoremediation in ecosystem.

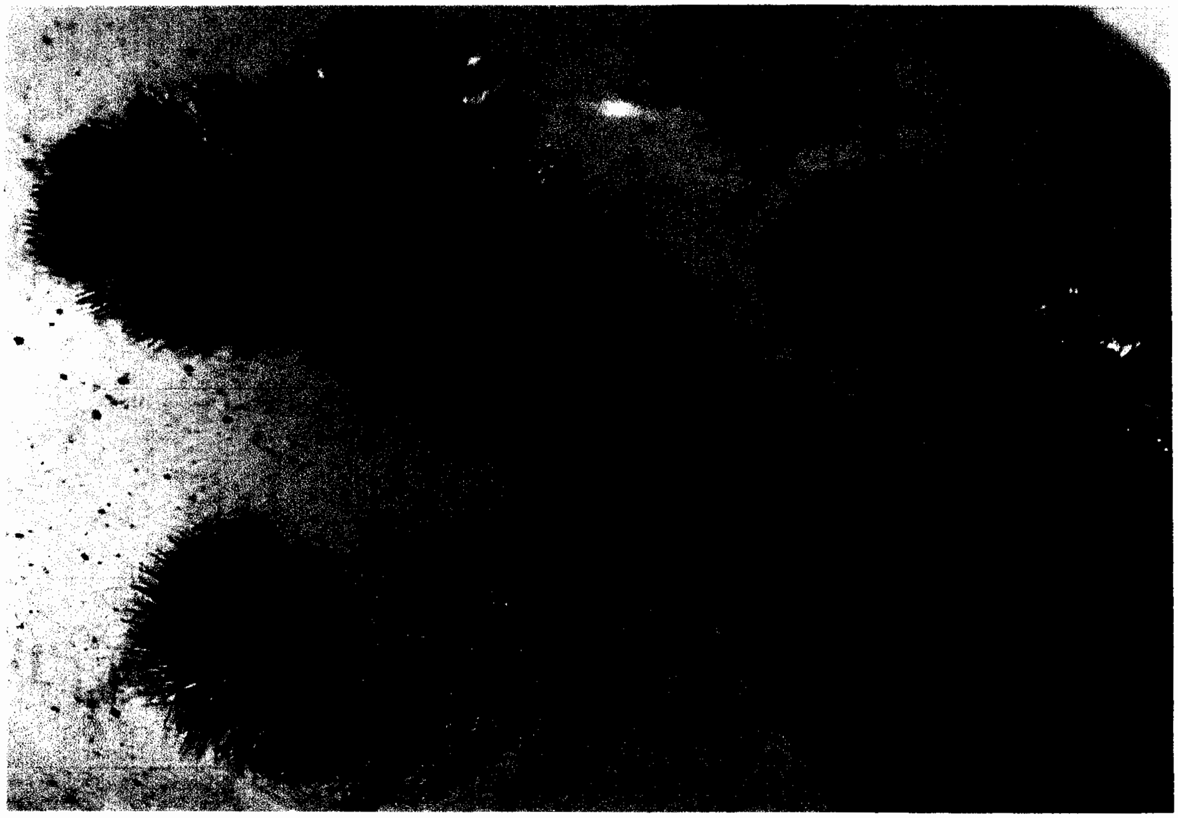


Fig.2.4 Coontail *Ceratophyllum demersum*

2.5 Hyperaccumulation of metals

Previous studies showed that some aquatic plants are hyperaccumulaters of metals in waste water. The term "hyperaccumulator" describes a number of plants have the ability to grow on metalliferous soils, water and to accumulate extraordinarily high amounts of heavy metals in the aerial organs, without suffering phytotoxic effects. Three basic hallmarks distinguish hyperaccumulators from related non-hyperaccumulating taxa: a strongly enhanced rate of heavy metals uptake, a faster root-to-shoot translocation and a greater ability to detoxify and sequester heavy metals in leaves. An interesting breakthrough that has emerged from comparative physiological and molecular analyses of hyperaccumulators and related non-hyperaccumulators is that most key steps of hyperaccumulation rely on different regulation and expression of genes found in plants. In particular, in hyperaccumulators the constitutive over expression of genes encoding transmembrane transporters play an important role in metal uptake, translocation and finally, sequestration in cell walls (Manuela *et al.* 2010). Liddle (1982) conducted experiments to check the uptake of Arsanic by *Ceratophyllum demersum*. He found that plants grown in arsenic solutions with <0.1 mg/L reached at equilibrium in one or two days. Results of his study showed that hornwort (*Ceratophyllum demersum*) fit for hyperaccumulation criteria.

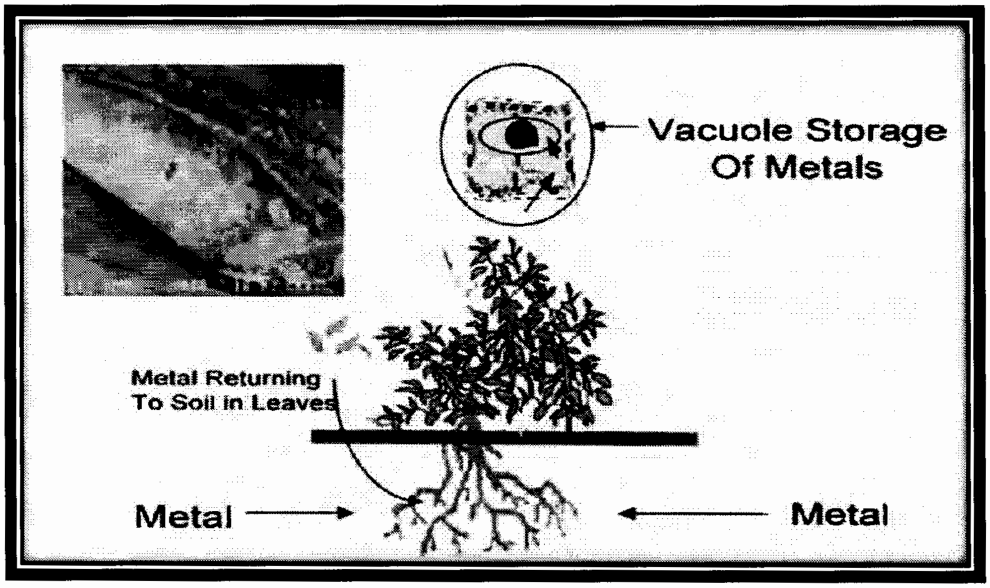


Fig.2.5 Accumulation of metals by plants

Material and Method

3.1 Experimental Area

Sample was collected from Arshad Textile Dying Mills Ltd located in the vicinity of Faisalabad. This is a textile manufacturing company that uses synthetic complex organic dyes as coloring material. The analytical work was carried out at the National Agricultural Research Center (NARC) Islamabad Pakistan and Federal Environmental Protection Agency (FEPA), also known as CLEAN Laboratory, located at H-8/2 Islamabad. Both the National Institute of Bioremediation Department of NARC and FEPA comprise of standardized laboratories, where chemical analysis was conducted during April 2012.

3.2 Sampling Strategy

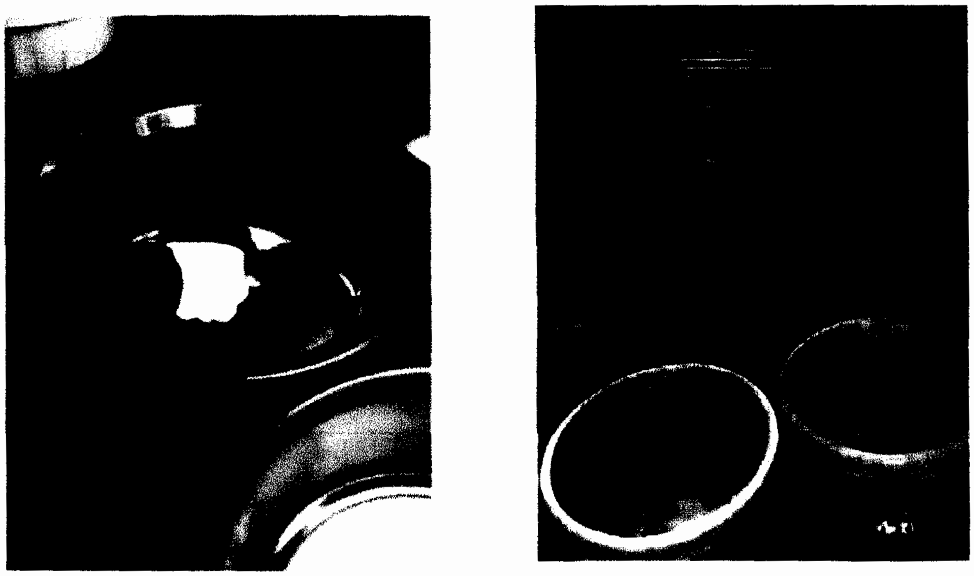
Sample of textile effluent was collected during the month of April 2012. The textile effluent was collected by grab method. Polyethylene containers were used for storage purpose. Before collection, the containers were properly cleaned with non ionic detergents, followed by thorough washing with tap water and then rinsing with de-ionized water. The samples were stored at 4°C.

3.3 Plant used in the Experiment

Aquatic plants have been found to play a significant role in removing chemicals from polluted water (Boyod, 1970 and Steward, 1970). Many aquatic plants found in tropical and subtropical fresh water ecosystem sued in remediation of effluent. *Ceratophyllum demersum* is one of them which is successfully employed in waste water treatment. This is an aquatic plant that grows throughout the year in fresh water habitat (Reddy and Busk -1994).

3.4 Sampling Design

The selected plant species Ceratophyllum demersum was chosen for the research study. Nearly equal weight (100g) of the plant was taken for experimental design. The plants were thoroughly washed with distilled water to remove soil particles attached with the leaves and other parts of the plant. An average length and width of plant parts were generally taken for each experiment. The sample was taken in triplicate and the plants were placed in three circular tubs, each having five liters of textile waste water. The fourth tub was kept for control purpose .The experimental and control tubs were kept in the natural condition (outdoor) of NARC, and the plants were introduced in the tubs for 15 days.



(a) (b)

Fig 3.4 (a), (b) Sampling design of study

3.5 Physico-chemical Analysis

Detailed physico-chemical tests were carried out for the textile waste at the beginning of the experiment to determine the pH, electrical conductivity, dissolved oxygen, total dissolved solids, chlorides, sulphates, carbonates, bicarbonates, COD and BOD, copper, chromium and zinc etc. The physico-chemical tests were in conformity with the (APH 1998) method, where as the Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and metals tests were conducted with EPA method.

3.5.1 pH

The pH of textile effluent was measured with the help of pH meter (model WA. 2015). For measurement each time the pH meter was adjusted to neutral, using the buffers of pH 4.0, 9.0, and 10.0 respectively.

3.5.2 Electrical Conductivity

The Electrical Conductivity of textile effluent was measured with the help of EC meter (model WA. 2015) and reading was recorded in micro Siemen (µS). The EC meter was calibrated with 0.01M potassium chloride (KCL) solution (Method 2510 BAPHA, 1998).

3.5.3Total Dissolved Solids

Total Dissolve Solids' were measured with the help of TDS meter (model WA. 2015). TDS meter was calibrated with 0.01M potassium chloride (KCL) solution (Method 2510 BAPHA, 1998).

3.5.4 Dissolve Oxygen

Dissolve Oxygen was measured with the help of DO meter (model WA. 2015). DO meter was calibrated with 0.01M potassium chloride (KCl) solution (Method 2510 BAPHA, 1998).

3.5.5 Chlorides

The concentration of chlorides was determined by Argentometric method (Method 4500-Cl- BAPHA, 1998). Sample of (20ml) was taken in conical flask and 2-3 drops of indicator potassium chromate (K_2CrO_4) was added. The solution was titrated against standard silver nitrate solution ($AgNO_3$) till the achievement of end point (i.e. change of colour from greenish yellow to reddish brown).

3.5.6 Carbonates

The concentration of carbonates was determined by Argentometric method (Method 4500-Cl- BAPHA, 1998). Initially 10 ml sample was taken in conical flask and then 2-3 drops of phenolphthalein was added as an indicator. The solution was titrated against standard 0.02 normal HCL till the achievement of end point (i.e. change of colour from purple to colorless).

3.5.7 Bicarbonates

The concentration of Bicarbonates was determined by the Argentometric method. 10 ml of the sample was taken and methyl orange was used as indicator. The sample was titrated against standard 0.02 normal HCL till the achievement of end point (i.e. change of colour from orange to pink).

3.5.7 Sulphates

The concentration of sulphates was determined by turbidimetric method (Method 4500-SO_4^{-2} BAPHA 1998). A standard solution with SO_4^{-2} concentration was prepared in the range of 0 to 50mg/L. Solid 0.3 mg Barium Chloride ($BaCl_2$) was added with continous stirring. According to the reaction: $BaCl_2$+ $(SO4)^{2-}$= $BaSO_4$+2Cl^-, where $BaSO_4$ is white precipitate, and normally this reaction is used as a qualitative test for the presence of sulphate ion in the sample. Afterwards the solution was transferred to a cuvette and the absorption reading was recorded at 420 nm (UV-VIS Spectrophotometer) over a period of two minutes. The highest measured value was noted.

3.5.8 Nitrates

The concentration of nitrates was checked by turbidimetric method. A standard solution with NO_3^- concentration was prepared in the range of 0 to 10mg/L. 0.2 ml of Standard 0.1 Normal HCL was added to it while stirring. Afterwards the solution was transferred to a cuvette and the absorption reading at 220 nm (UV-VIS Spectrophotometer) was taken repeatedly over a period of two minutes. The highest measured value was noted.

3.5.9 Chemical Oxygen Demand

COD for the sample was determined by closed reflux method (EPA Method).The textile effluent (30ml) sample and concentrated potassium dichromate ($K_2Cr_2O_7$) (10ml) were placed in the flask with anti bump granules then 15 ml of sulphuric acid containing silver (AgH_2SO_4) was added carefully. 1g of mercuric sulphate was added to the flask. The mixture was then carefully boiled in draft chamber for three hours. The mixture was cooled down and 20 ml of distilled water was added to the mixture to stabilize the temperature. Ferrion was added as an indicator and the mixture was titrated with Ferrous Aluminum sulphate till the end point (i.e. till the colour is muddy brown indicating very high COD concentration).

3.5.10 Biological Oxygen Demand

BOD for the sample was determined by taking 2 ml sample and 5 ml each of the following four reagents: Calcium Chloride, Phosphate buffer, Ferrous Chlorate and Magnesium Sulphate in 2 sets of BOD bottles. One set is analyzed on the spot to measure the dissolved oxygen. The second bottle was kept in the incubator for five days (Method EPA). The result of BOD is obtained by subtracting the Final value of BOD from the initial value.

3.5.11 Detection of Metals

Digestion of sample for metals detection was done by taking 100 ml sample with 10 ml of nitric acid (HNO_3) added and then the mixture was boiled on hot plates till the volume reduced to 10 ml. The sample was then transferred into volumetric flask and made up to 100ml with 2% nitric acid.

3.5.12 Copper, zinc and chromium

Analysis of copper, zinc and chromium in textile waste water was observed by Atomic absorption spectrophotometer (Model No. Analyst 800- Perkin Elmer). Standard stock Solution of 0.2, 0.4 and 0.8 ppm was prepared for Cu, Zn and Cr analysis. In 1ml of metals (Cu, Zn and Cr) added in 2%nitric acid and make it to 100ml it became 10 ppm. Multiply and divide 0.2 by 10 to get 0.2ppm standard solution of Cu, Zn and Cr. Pour 0.2 ml of 10ppm solution of metals by digital pipette in 10ml flask and fill it with 2% nitric acid and put a lid on it. For 0.4 ppm standard solutions multiplies and divide 0.4 by 10. Pour 0.4 ml of 10ppm solution of metals (Cu, Zn and Cr) by digital pipette in 10ml flask and fill it with 2% nitric acid and put a lid on it. For 0.8 ppm standard multiply and divide 0.8 by 10. Pour 0.8 ml of 10ppm solution of metals by digital pipette in 10ml flask and fill it with 2% HNO_3 and put a lid on it. Put the nebulizer of the Atomic absorption spectrophotometer in the flask. It will show a curve on the computer get the reading from there.

RESULTS &DISCUSSION

RESULTS AND DISCUSSION

In the present study estimation of contamination load in the textile waste water was measured by the standard procedures (APH, 1998). Results indicate that pollution load was above the permissible limits that might be due to excessive use of chemicals used in textile processing. Chemical and dyes used in textile processing (Table 2.3). 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). Above mentioned chemical, dyes and salt might be the reason of high levels of pollutants in textile waste water which analyzed in present study. Phytoremediation strategy was adopted for the reduction of pollutant level. Details are given below

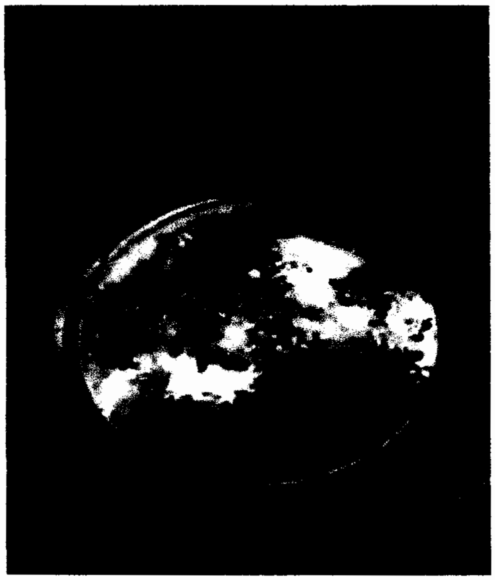


Fig.4 Treatment of effluent with *Ceratophyllum demersum*

4.1 HYDROGEN ION CONCENTRATION (pH)

In the present study the textile waste water which was collected from Arshd Dyeing Textile Ltd., Faisalabad was analyzed for hydrogen ion concentration with the help of pH meter. Initial observation showed that the pH of waste water was 11.37.which was above the recommended limits (6-9) by government of Pakistan in National Environmental Quality standards (2000).Value of pH violate the section 11 of PEPA 1997. The higher pH values may be due to the different chemicals that are being used during the processing of textile material like caustic soda for mercerizing cotton yarn, bleaching and dyeing. Different types of pollutants associated with dyes such as salt, alkali and color etc. (Table 2.1). Different processes such as de-sizing and scouring, done before bleaching might also be responsible for the basic nature of the waste water. Chemicals like soda ash and hydrogen peroxide used for scouring tends to increase pH (Ogunlaja and Aemere, 2009). Moreover it is reported from the previous studies that high values of carbonate and bicarbonate contribute to the total alkalinity of the sample (Balakrishnan and Karruppusamy, 2005). During the present research work highly alkaline pH 11.37 decreased to 7.9 with the help of *ceratophyllum demersum* with in fifteen days of retention period. As shown in (Fig 4.1). Decrease in pH might be due to absorption of pollutant by plant. A previous study showed that *C.demersum* is very efficient in dye removal and dye concentrations of the effluent reduced from 11.0 mg/L to 0.65 mg/L (Keskinkan and lugal, 2007).As it is shown in (Table 2.1) that the most of dyes are responsible for alkalinity in textile waste water. So the dye removal ability of *C.demersum* is another reason of pH reduction from higher values of alkalinity to lower values.

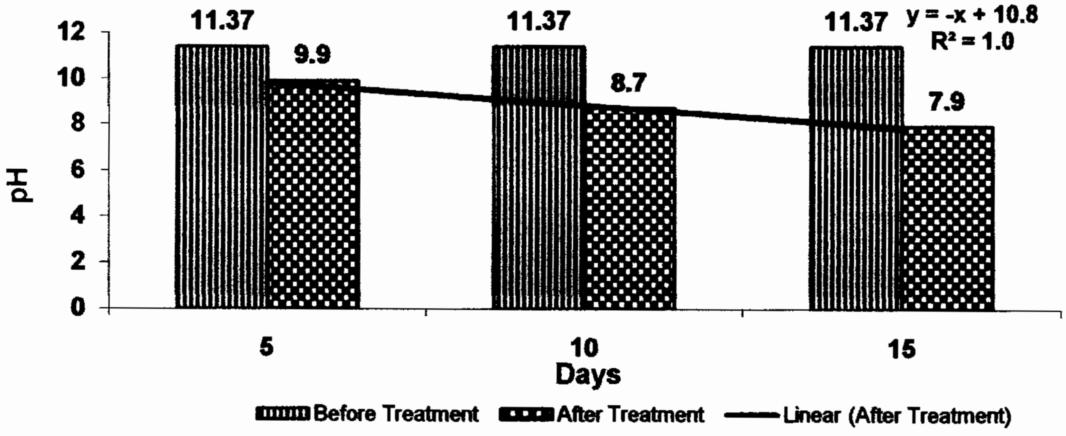


Fig.4.1 Reduction in pH during the 15days of phytoremediation treatment

4.2 TEMPERATURE

Temperature is very important factor for phytoremedation process. Temperature regulates the growth of plant, solubility of gases and salts in water (Pateland and Kanungo, 2010).During the present research study highest temperature was observed 39.2°C and lowest temperature was 35.9°C. The temperature of waste water was within limits (less then 40°C) during research time period as shown in (Table 1.1). Highest temperature was recorded at day one. According to (Iqbal *et al.*, 2011) The textile waste effluents have high temperature because in several steps of the dyeing operation, hot rinse waters of up to 90°C was used. Studies showed that temperature of waste water is commonly high because of addition of warm water from industrial activities (Sankpa and Naikwade, 2012). Reduction in temperature achieved through *Ceratophyllum demersum* might be due to the decrease in light penetration in the waste water by aquatic plant (Olga and Alenka, 1989). Plants have a strong influence on the temperature within an aquatic environment and they decrease evaporation rate from the water's surface. Temperature of air and radiant energy from the sun has greatest influence on water temperature (Picard *et al.*, 2005). As well as, the aquatic vegetation declines penetration of solar heat in water (Al-Kenzawi, 2007).That might be the cause of temperature reduction of effluent during the present study.

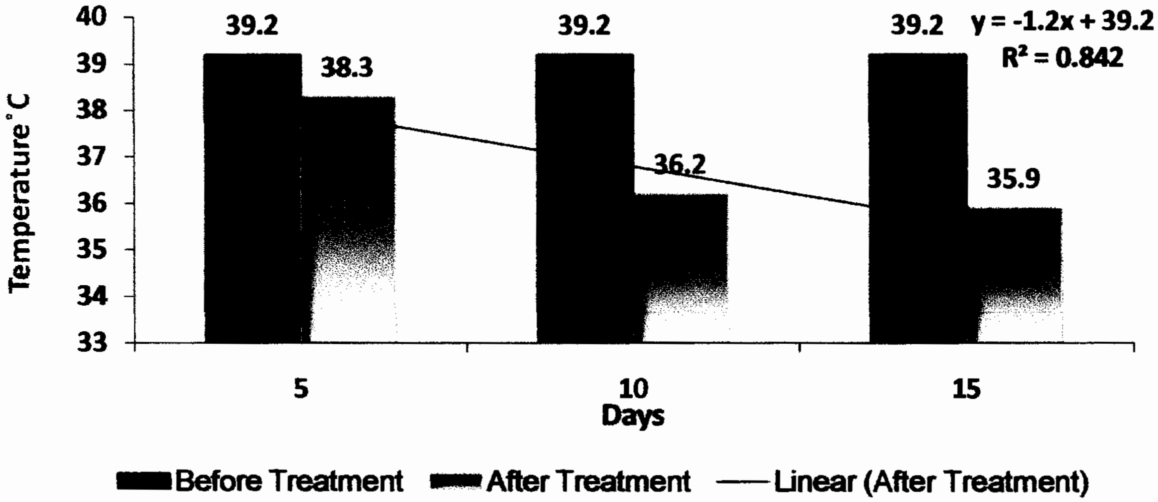


Fig. 4.2 Reduction in temperature during the 15days of phytoremediation treatment

4.3 ELECTRICAL CONDUCTIVITY (EC)

Electrical conductivity is the measure of total concentration of dissolved salts in water. When salts dissolve in water, they give off electrically charged ions that conduct electricity. The more ions in the water, the greater the electrical conductivity it has.

It is measured in Micro Siemens (µs). EC is an important factor because conductivity is directly proportional to the concentration of dissolved solids in the effluents. In the present study, electrical conductivity of textile effluents was recorded before and after phytoremediation treatment. Results showed that there is a significant decrease in the EC from 42770(µs) to 21200(µs) after 15 days of remediation with *C.demersum*. Before phytoremediation treatment the higher electrical conductivity value of the effluent might be due to the presence of chemicals as cations and anions in the waste water. The higher conductivity alters the chelating properties of water bodies and creates an imbalance of free metal availability for flora and fauna (Akan *et al.*, 2008). Higher EC values indicate the presence of high contents of dissolved salts in water (Abdullah and Mustafa, 1999). Electrical Conductivity measurements are useful for the management of temporal variations in total dissolved solids and major ions (Chapman, 1996). It was reported by Moorhead and Reddy, (1988) that 48% reduction in EC value done within 22 days of remediation with *Ceratophyllum demersum*.

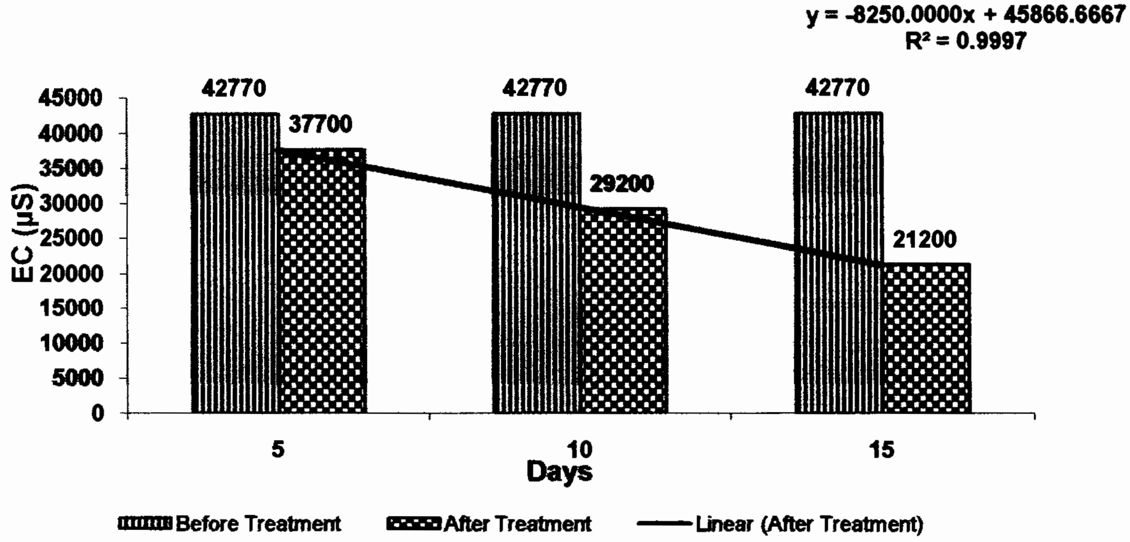


Fig.4.3 Reduction in EC value during the 15 days of phytoremediation treatment

4.4 TOTAL DISSOLVED SOLIDS (TDS)

Total dissolved solids are due to the presence of carbonates, bicarbonates, chlorides, sulphates, phosphates, nitrates, nitrogen, calcium, sodium, potassium and iron. Initial concentration of TDS in textile waste water was 2138mg/l but it reduced to 1096mg/l during the present study after the phytoremediation via *ceretophyllum demesum* (Fig.4). EC and TDS were found closely related hence, exhibited same trend of variation in value. Permissible limit of total dissolved solids is 3500 mg/L according to NEQS. Present study shows that TDS are within limit as purposed by NEQS. Reduction in TDS during remediation process may be due to up take of trace element and metals by plant as shown in (Fig 4.4).

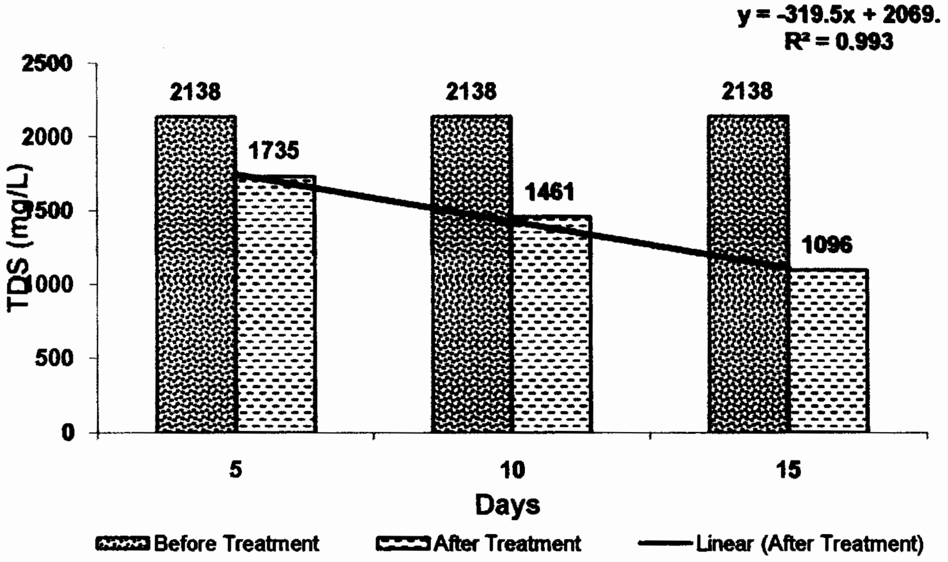


Fig. 4.4 Reduction in TDS during 15 days of phytoremediation treatment

4.5 CHLORIDE

The concentration of chlorides was determined by Argentometric method. The chloride content before and after treatment with *Ceratophyllum demersum* was viewed within ranging from 900mg/L to 160mg/L (Fig.5). 82% reduction was observed in chloride content after remediation. Percentage was calculated by given formula

$$\frac{(\text{Initial value-final value}) \times 100}{\text{Initial value}}$$

Obtained results were within limits according to NEQS. According to Hajrasuliha, (1979) Chloride tends to accumulate in tissues, particularly leaves, of some plants. Its accumulation in plants is closely related to chloride concentration in the external solution and the genotype of plant. Accumulation of chloride in leaves by plant might be reason of chlorides reduction in textile effluent in present study.

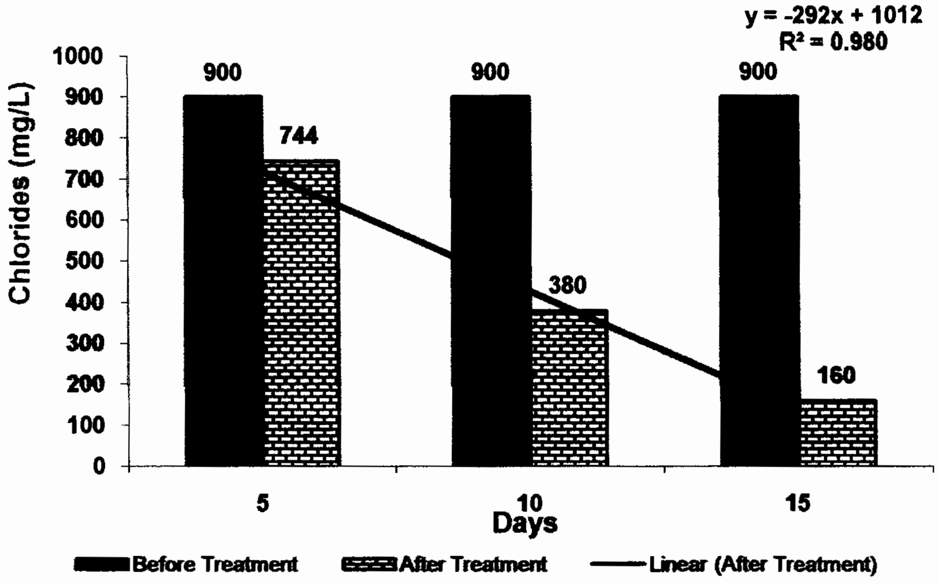


Fig.4.5 Reduction in chloride during the 15 days of phytoremediation treatment

4.6 CARBONATES AND BI-CARBONATES

Argentometric method was used for the bi carbonates estimation. Concentration of bi-carbonates before treatment was observed as 7500mg/l and after treatment with *C.demersum* value had reduced to 4503mg/l. 39% reduction of bi-carbonates was observed after 15 days of phytoremediation. It is reported in literature that high values of carbonate and bicarbonate contribute to the total alkalinity of the sample (Balakrishnan and Karruppusamy, 2005). In the present study bi-carbonate content in textile effluent was 7500mg/l. That may be the reason of extreme alkaline pH of effluent. During the treatment of waste water with *C.demersum* reduction in pH value indicate the concentration of carbonates and bi-carbonates was also reduced.

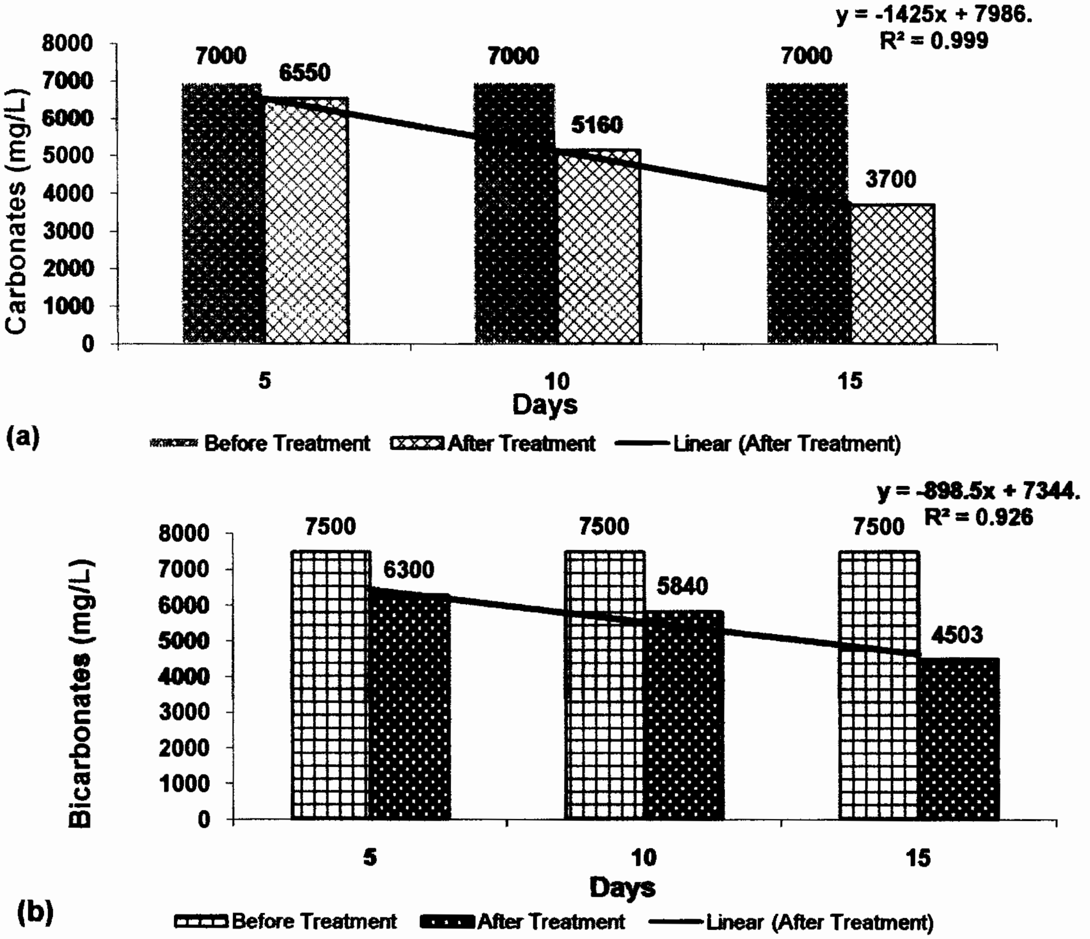


Fig. 4.6(a), (b) Reduction in carbonates and bi-carbonates during the 15 days of phytoremediation treatment

4.7 SULPHATES

Initial value of sulphates was observed 4833.3 mg/l that was extremely higher than the permissible limits (600mg/l) according to NEQS. That concentration violates the Pakistan Environmental Protection Act (PEPA 1997). Previous studies showed that sulphates (SO_4) through industrial discharge such as tanneries, pulp mills and textile mills were considered toxic to the plants and animals. In humans, small concentrations of SO_4 cause a temporary laxative effect. However, doses of several thousand units cause long-term illness effects. Problems caused by sulphates are most frequently related to their ability to form strong acids which change the pH (Tüfekci *et al.*, 2007). Observation in present study showed that the concentration of sulphates in textile waste water was very high. After the 15 days of remediation with *C.demersum* it was reduced to 2111mg/l. Almost 56% sulphate reduction was done by the plant. That might be due to ability of aquatic plants to absorb different types of pollutants and accumulated them in their tissues (Mkandawire and Dudel 2007). Although this value also higher then recommended value but if time period of the treatment increased then it is proposed that may be more reduction can be possible.

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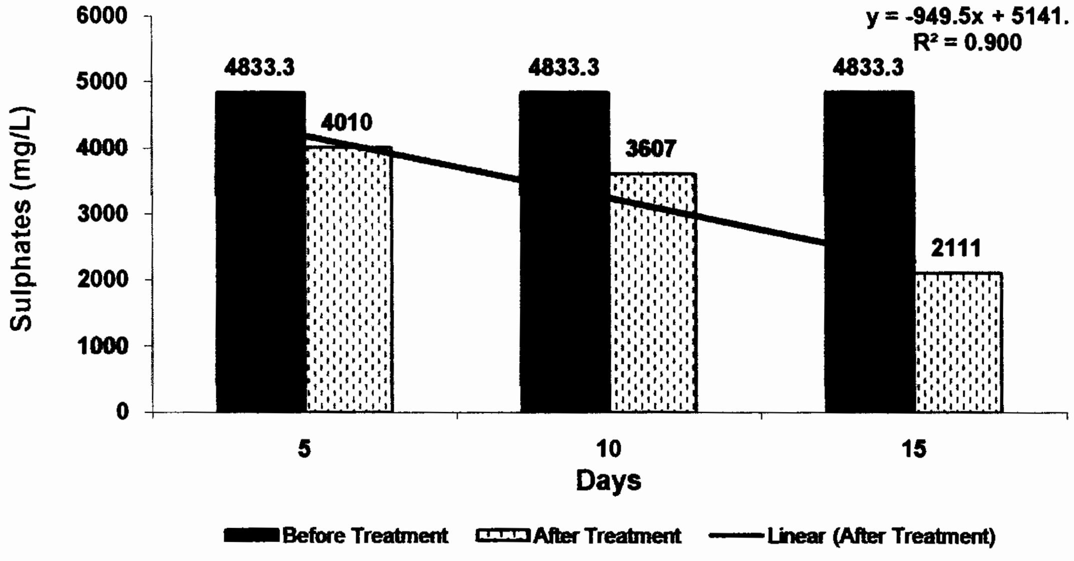

Fig. 4.7 Reduction in sulphates during the 15 days of phytoremediation treatment

4.8 CHEMICAL OXYGEN DEMAND (COD)

COD for the sample was determined by closed reflux method (EPA Method).The measured COD in waste water at first day without treatment was 533mg/l this value of COD is higher them permissible limit (400mg/l) according to NEQS. After the 15 days of remediation *C.demersum* COD was reduced to 139.9mg/l. results showed that COD comes with acceptable limits. Similar observations for COD was studied for domestic and industrial waste (Chavan and Wagh, 2005).Another study showed that aquatic plants can be used for design calculations regarding expected removal of pollutants by aquatic plants and they can decrease COD and increased dissolved oxygen (Tripathi and Shukla, 1991;Zimels *et al.*, 2009).

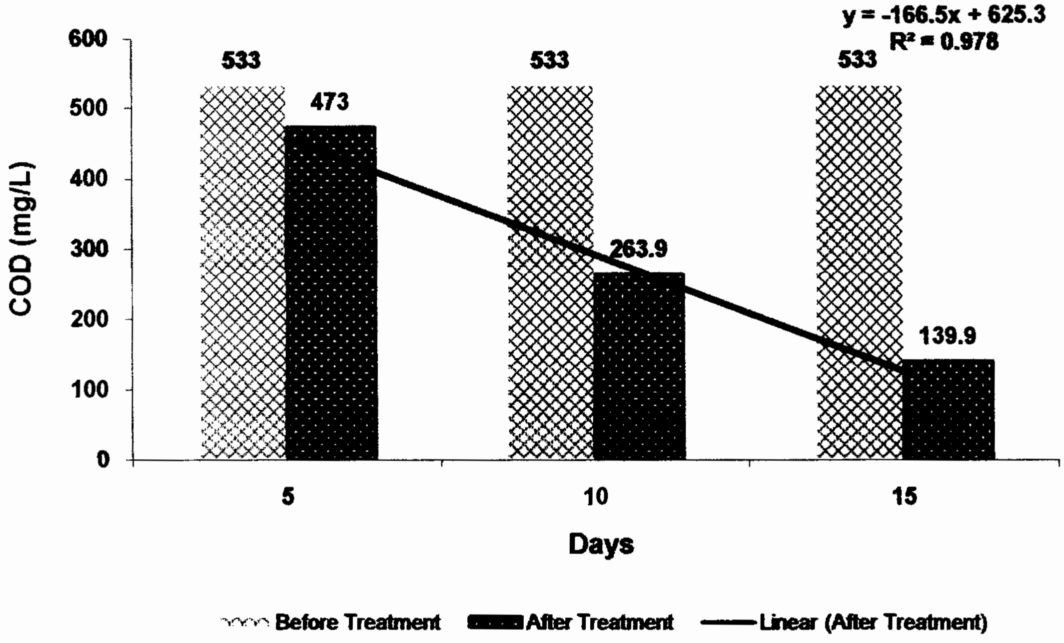

Fig.4.8 Reduction in COD during the 15 days of phytoremediation treatment

4.9 BIOLOGICAL OXYGEN DEMAND (BOD)

BOD is measured by standard method (EPA Method).It is a parameter which is widely used to determine the pollution load of waste water. It is the amount of organic matter in the water and the amount of oxygen required by the micro organisms to stabilize the biologically decomposable organic matter in wastes under aerobic conditions (Bhalli and Khan, 2006).In the present study BOD of textile effluent was observed 246mg/L. Observed value was higher than recommended standards as given by NEQS. After remediation BOD value reduced to 108.1mg/l within fifteen days. Final concentration of BOD was within limits. Total 56%reduction of BOD was done by *C.demersum.* The reduction in pH favored microbial action to degrade BOD and COD in the wastewater (Mahmood *et al.*, 2005).

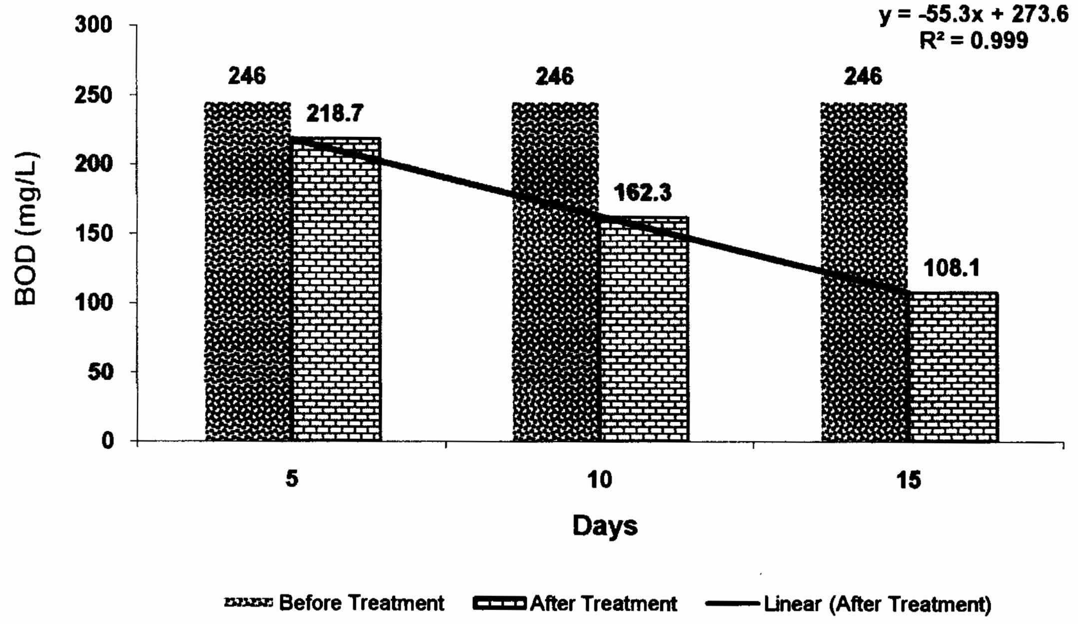

Fig.4.9 Reduction in BOD during the 15 days of phytoremediation treatment

4.10 DISSOLVE OXYGEN (DO)

In the present study the textile effluent was analyzed for the presence of dissolved oxygen with the help of DO meter. Results showed that initially it was 37mg/l. After data recording textile effluent was subjected for treatment via phytoremediation. Observations showed that DO increased from 37 to 41mg/l only within 15 days of treatment. Increase in DO after treatment indicated by reduction of BOD and COD in the wastewater. According to (Reddy, 1981), the presence of plants in wastewater can deplete dissolved CO_2 during the period of high photosynthetic activity. This photosynthetic activity increases the dissolved oxygen of water, thus creating aerobic conditions in wastewater which favor the aerobic bacterial activity to reduce the BOD and COD.

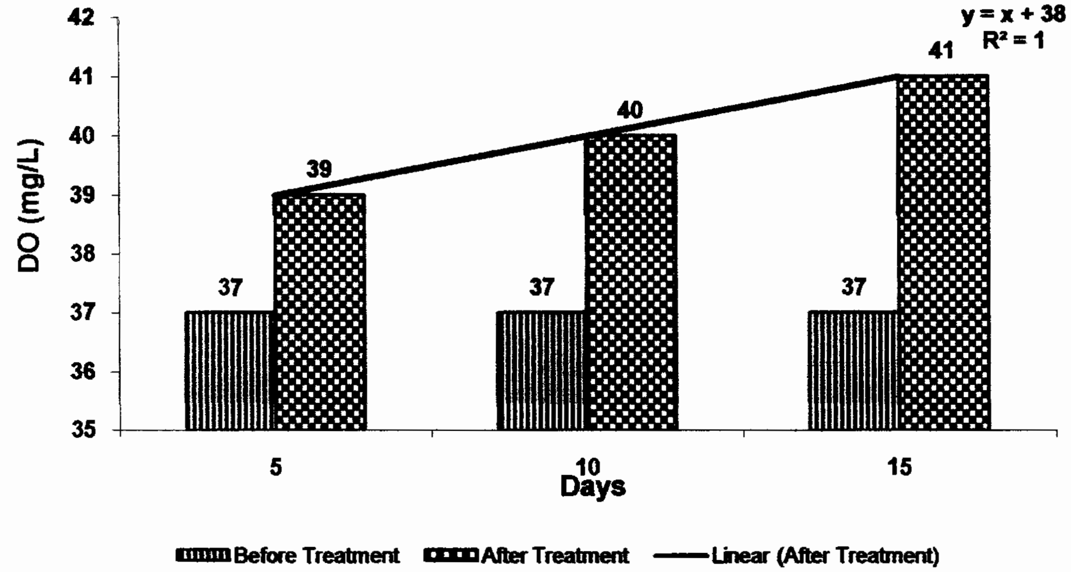

Fig.4.10 Increase in DO during the 15 days of phytoremediation treatment

4.11 NITRATES

The concentration of nitrates was determined by turbid metric method. Initial value of nitrate was 3.26 mg/l and after fifteen days of remediation with *C.demersum* it reduced to 0.205mg/l.Previous studies show that nitrate is not stable as it is readily absorbed by plants and microorganisms and immobilized as part of their protein (Patterson,1994). Unrooted submerged vegetation such as *C. demersum* requires nutrient uptake from the water (Mjelde and Faafeng, 1997). Might be due to the instability of nitrates *C.demersum* shows an effective uptake of nitrate from effluent to fulfill its nutrient requirement.

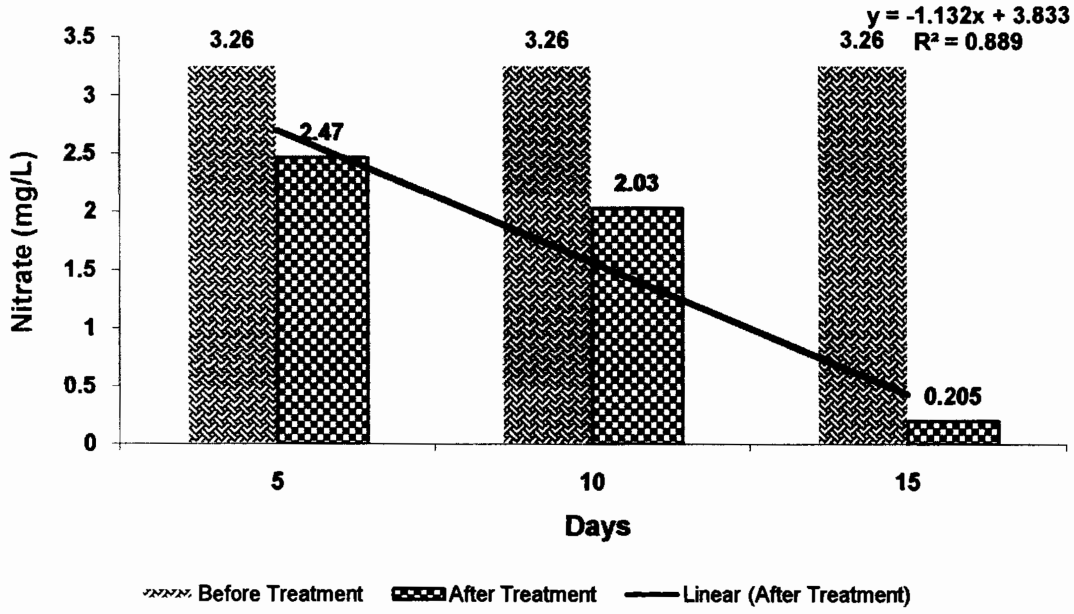

Fig.4.11 Reduction in nitrates during the 15 days of phytoremediation treatment

4.12 COPPER (Cu)

Digestion method was used for the metals by atomic absorption spectrophotometer. 0.138mg/l was the initial concentration of Cu in waste water and after treatment it was reduced to 0.11mg/l. Only 19% reduction was done by *ceratophyllum*. According to Oliveria *et al.*, (2007), the presence of heavy metals in industrial and urban wastewater is one of the main causes of water and soil pollution. Accumulation of these elements in waste water depends on a number of local factors such as the type of industries in the region, lifestyle and awareness of the impact to the environment by careless disposal of water (Nordberg *et al.*, 2007).

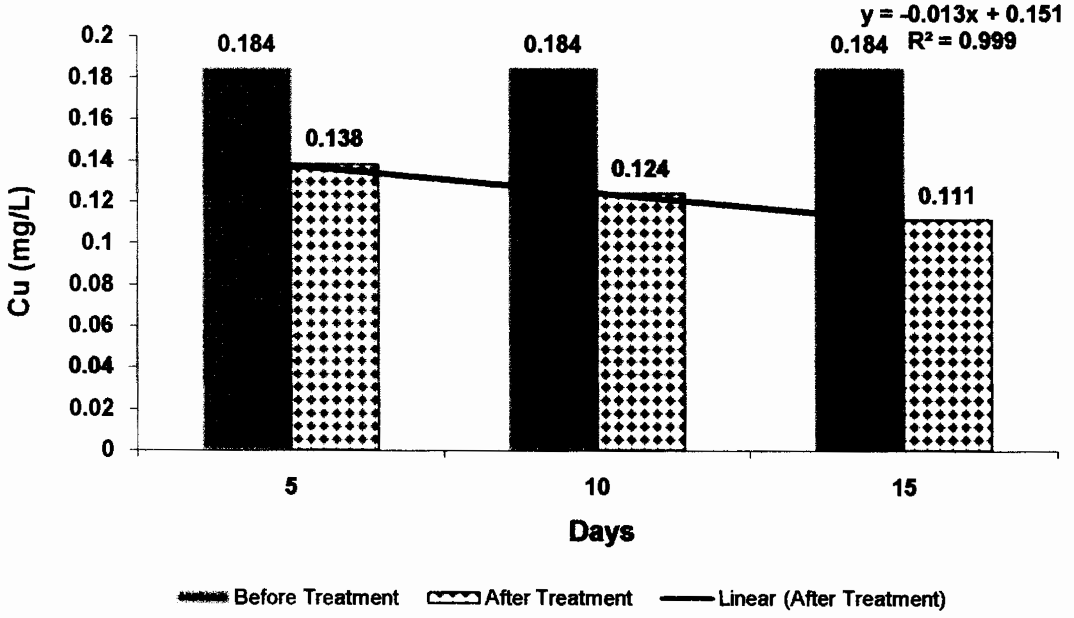

Fig.4.12 Reduction in Cu during the 15 days of phytoremediation treatment

4.13 CHROMIUM (Cr)

Most of Chromium in textile waste water comes from dyeing process. Previous studies show that it was used for the first time in the production of corrosion resistant steel (stainless steel) and coatings. Subsequently, chromium was widely deployed in various industries; namely electroplating, dyes and pigments, textiles, photography, and wood processing. It occurs in several oxidation states, ranging from Cr^{2+} to Cr^{6+} with trivalent and hexavalent states being the most stable and common in terrestrial environments (Zayed *et al.*, 1998). In present study concentration of Cr was determine by a standard method (APH 1998). Maximum value of chromium was 1.012mg/l and minimum value after remediation was observed as 0.453mg/L. Overall 55% reduction was done by *C.demersum* after fifteen days. Initial value of Cr violates the PEPA (1997) it was above the set by NEQS. After remediation with *C.demersum* value of Cr become within limits. Previous studies shows that aquatic vascular plants play an important role in the uptake, storage, and recycling of metals. The uptake of metals depend on the life form of the macrophytes (floating, free floating, well rooted, or rootless).The free-floating species *(Eichhornia, Lemna, Pistia)* absorb elements through the roots/leaves, whereas the rootless species *Ceratophyllum demersum* absorbs mainly through the finally divided leaves. Submerged species showed higher chromium accumulation than floating and emergent ones (Sinha *et al.*, 2002). It is very dangerous metal cause skin problems, convulsions, kidney and liver damage. It also generates all types of genetic effects in the intact cells and in the mammals (Khe'rici-Bousnoubra *et al.*, 2009). It has also been reported that intensive exposure to Cr compounds may lead to lung cancer in man (Jordao *et al.*, 2002).

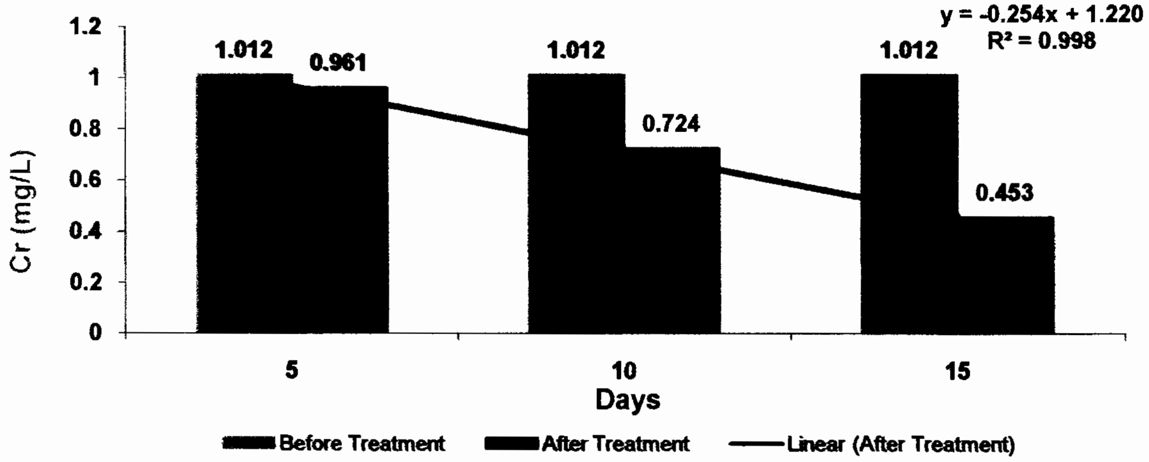

Fig.4.13 Reduction in Cr during the 15 days of phytoremediation treatment

4.14 ZINC (Zn)

Concentration of zinc in textile affluent ranged from 0.46mg/l to 0.009mg/l. Values of Zn were within limits according to NEQS (Table 1).Total 98%of zinc reduction was done by *C.demersum* from waste water after fifteen days. It shows that aquatic submerged plant *C. demersum* could be an effective biosorbent for zinc. Batch adsorption studies showed that *C. demersum* would adsorb zinc, lead and copper (Keskinkan *et al.*, 2004).Small quantity of zinc is an essential element for the human beings and plants but higher concentration were toxic (Jordao *et al.*, 2002). Moreover, elevated intake of Zn can cause muscular pain and intestinal hemorrhage (Honda *et al.*, 1997).

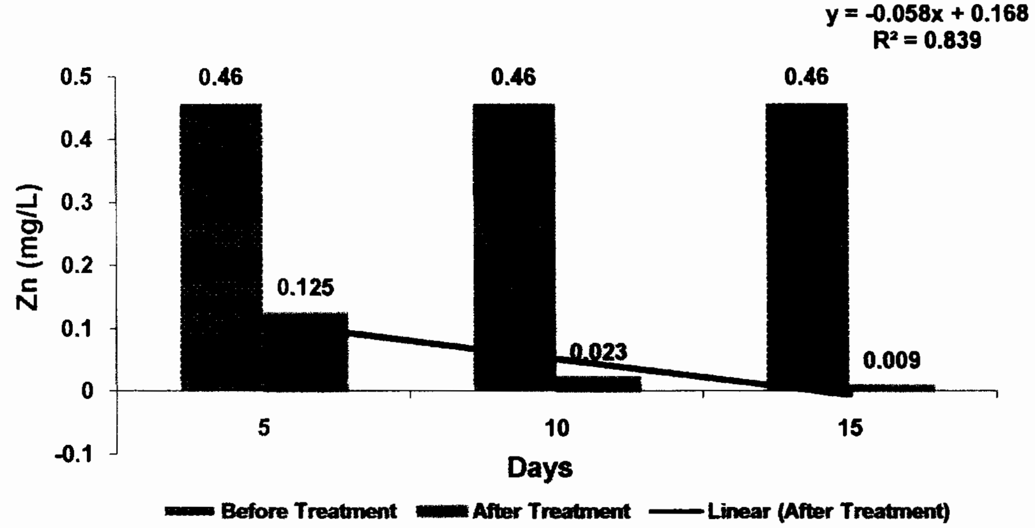

Fig.4.14 Reduction in Zn during the 15 days of phytoremediation treatment

CONCLUSIONS
&
RECOMMENDATIONS

CONCLUSION AND RECOMMENDATIONS

From the present study it is concluded that the values of pH, COD, BOD, Sulphates and chromium in the textile waste water was above the permissible limits according to NEQS and discharge of effluent violating the PEPA (1997). However, phytoremediation technology was found efficient via *Ceratophyllum demersum.* Its ability to tolerate high amount of metal levels was apparently associated with its ability to limit translocation of metals to shoot. It is evident from above that *Ceratophyllum demersum* revealed a significant reduction in all parameters and reduction in the pollution load can be enhance with the increase of retention period.

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

- Explore more plant species for the phytoremediation studies. That is commonly cultivated /grows in our natural climatic conditions.
- Search out their mechanism of degradation through HPLC, GCMS, NMR and mass spectrometry etc.
- Explore and extract the proteins /enzymes from plant species that could be utilize for treating the waste water having diversified pollutant and recalcitrant compounds.
- For effective phytoremediation the retention period of plant should be increase.

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ANNEXURE

STANDARD SOLUTIONS

Normal solutions

A normal solution is a solution that contains an equivalent weight of solute in one liter of solution. The mathematical expression is given in Eq. 1.

Eq. 1 $\text{Normality (N)} = \frac{\text{equivalents}}{\text{liters}}$

Or

$$\text{Normality (N)} = \frac{\text{Number of equivalents}}{\text{number of liters}}$$

.1 normal HCL

Preparation of 0.1 N solution
100 ml of 1.0N HCL1000 ml de ionized water.

or

Use the Stock bottle of 37% HCL.
Determination of molarity of 37% HCL V/V
37 ml of solute/100 ml of solution.
HCL - 37% v/v. Specific gravity: 1.19 g/ml
37ml/100 ml or 370 ml/1000 ml x 1.19 g/ml = 440.3 g/L

HCL Molecular weight = 36.5

Molarity

440.3 grams /36.5 grams = 12.06 M or ~12M

M1V1 = M2V2
(0.1) (1000) = (12) (x)

x = (0.1) (1000) / 12
x = 8.3 ml

Therefore add 8.3 ml of 37% HCL to 1000ml of de ionized water to create a 0.1N HCL solution.

0.02 normal HCL

For .02 normal HCL first make the .1 normal HCl.

M1V1 = M2V2
(0.1) (1000) = (12) (x)

x = (0.1) (1000) / 12
x = 8.3 ml

Therefore add 8.3 ml of 37% HCL to 1000ml of de ionized water to create a 0.1N HCL solution. Take the 200ml of .1 normal HCL and dissolve it in 1000 ml of de ionized water.

Silver nitrate (for Chloride test)

Dissolve the 2.39678g of silver in 1000 ml of de ionized water.0.041 normal silver nitrate is used for chloride test. Kept that solution in blind bottles because it effected by light.

Preparation of Sulphate buffer

For sulphate buffer solutions take the 30g of magnesium chloride, 5g sodium acetate, 1g of potassium nitrate and 20ml of acetic acid. Put all of them in the flask of 1000ml and fill it with the de ionized water. Through continues shaking dissolve them in de ionized water.

Preparation of 2%nitric acid

20ml of nitric acid put in the flask of 1000ml and 980 ml of de ionized water add in it. It is become 2%nitric acid.

Standard solution for metals

From the Stock solution of 1000 ppm we make the standards of 0.2, 0.4and 0 .8. First we make the standard solution of 10 ppm. For that 10x100/1000=1ml.after that 1ml/100ml=10ppm.this is known as stock B.

formula	standards
.2x10/10	.2
.4x10/10	.4
.8x10/10	.8

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