TEMPORAL STUDY OF VARIATIONS IN RAW AND BIO-REMEDIATED WASTEWATER OF SHEHZAD TOWN, ISLAMABAD

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2. water reuse

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Submitted in partial fulfillment of the requirements for the

Master of Philosophy in discipline Environmental Science

At the faculty of Basic and Applied Sciences

International Islamic University,

Islamabad

Supervisor

Dr.Tahira Sultana

January, 2012

IN THE NAME OF ALLAH,

MOST BENEFICIENT,

THE MOST MERCIFUL

To my

Parents & Brothers

For their care, support and believe

(Acceptance by the Viva Voce Committee)

Title of the Thesis: Temporal Study of Variations in Raw and Bio-remediated Wastewater of Shehzad Town, Islamabad.

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Registration No: 83/FBAS/MSES-04

Accepted by the Faculty of Basic and Applied Science, Department of Environmental Science, INTERNATIONAN ISLAMIC UNIVERSITY, ISLAMABAD, in partial fulfillment of the requirements for the Master of Philosophy in Environmental Science

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02,01,2012

DECLARATION

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

Date 02- 01-2012

Atiya

Atiya Iram

FORWARDING SHEET

The thesis entitled "Temporal study of variations in raw and bio-remediated wastewater of Shehzad Town, Islamabad" submitted by Atiya Iram in partial fulfillment of MS in Environmental Science has been completed under my guidance and supervision. I am satisfied with the quality of student's research work and allow her to submit this thesis, for further processes per IIU rules and regulations.

Dr. Tahira Sultana

ABSTRACT

Use of untreated sewage water for irrigation practices is one of the most important concerns that have received attention at regional, local and global levels because of toxicological importance in ecosystems and impact on human health. Present study investigated temporal variations in quality of untreated and bio-remediated wastewater of Shehzad Town in relation with consumption of different chemical products at household level. Out of thirty one physico-chemical/microbial parameters investigated, mean concentrations of BOD (148 ppm), COD (206.8 ppm), EC (1826.5µS/cm), TDS (653.2 ppm), Nitrate (13.2 ppm), Sulphate (74.4 ppm), oil & grease (684.6 ppm), fecal coliform (466.8 MPN/100ml) and total coliform (753.7 MPN/100 ml) were above NEQ'S. All trace and heavy metals were found within NEQ'S range. After bioremediation process, mean concentrations of chemicals were significantly reduced i.e. BOD; 64.8 %, COD; 71.4 %, Nitrate; 90.2 %, Sulphate; 24.4 %, oil & grease; 16.1 %, total coliform; 52.8 % and fecal coliform; 52.07 %. Determined concentrations of parameters in winter raw wastewater were relatively higher than same samples collected in spring season with similar trends found in bio-remediated water. Multivariate analysis techniques were employed for source identification. Principle component analysis (PCA) showed 77.4 % of total variance among four factors among which factor 1 was due to heavy metals coming from domestic chemicals showing 40.2 % variance, factor 2 was due to BOD and K^+ showing 17.7 % variance, factor 3 explaining biological contaminants showing 12.5 % variance and factor 4 explaining Na^+ with 7 % variance. The results were in accordance with Cluster Analysis (CA) and correlation matrix. The study allowed drawing new information from the data sets as sources of pollution in the environment, seasonal behavior of chemical contents and time trends to get better information about the wastewater quality and design of monitoring network for effective management of wastewater.

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Any worth in this work is because of persons mentioned above and all faults are mine.

Atiya Iram

2011

Acronyms

%	Percent
Kg	Kilogram
ppm	Parts per million
mg/l	Milligrams per liter
°C	Degree Celsius
:	Ratio
°F	Degree Fahrenheit
APHA	American Public Health Association
Bicarb.	Bicarbonate
BioR	Bio-remediated
BOD_5	5- day Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CA	Cluster Analysis
Ca^{2+}	Calcium
Cd^{2+}	Cadmium
Cl^-	Chloride
Cr^{3+}	Chromium
Cu^{2+}	Copper
DO	Dissolved Oxygen
DDBSA	Do-Decyl Benzene Sulfonic Acid
EC	Electric Conductivity
EM	Effective Microorganisms
EPA	Environmental Protection Agency
FA	Factor Analysis
FAAS	Flame Atomic absorption Spectroscopy
Fe^{3+}	Iron
HACA	Hierarchical Cluster analysis
Hard.	Hardness
K^+	Potassium
L	Liter

Mg^{2+}	Magnesium
Mn^{2+}	Manganese
Na^{+}	Sodium
NARC	National Agricultural Research Center
NIB	National Institute of Bioremediation
Ni^{2+}	Nickel
NO_3^-	Nitrate
PARC	Pakistan Agricultural Research Council
PCA	Principle Component Analysis
Pb^{2+}	Lead
Salin.	Salinity
SPSS	Survey Package for Social Scientists
SO_4^{-2}	Sulphate
TDS	Total Dissolved Solids
Temp.	Temperature
Zn^{2+}	Zinc

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Acronyms

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Chapter 1

INTRODUCTION

1.0 INTRODUCTION

Wastewater reclamation and reuse is a common practice throughout the world especially for irrigation (Scott *et al.*, 2004). Due to scarcity of available freshwater, reuse of wastewater gives an excellent option for conservation and sustainable use of this renewable resource (Asano *et al.*, 2007). It is reported that about 80 to 90 percent of water supply for domestic, commercial and industrial use is returned back to environment as wastewater (Tchobanoglous & Schroeder, 1985; Asano *et al.*, 2007). Wastewater is combination of black-water (feces, urine and associated sludge) and grey-water (kitchen and bathing) along with waters from business premises, small-scale industries, institutions, storm water and urban runoff (Vander Hoek, 2004; Okonko *et al.*, 2007).

According to UNESCO, 80 % of the wastewater in developing countries does not receive any treatment and about only around 10 % of all wastewater in developing countries receives treatment (Carr *et al.*, 2004a; UNESCO 2009). These global estimates are supported by smaller scale studies like a nationwide survey in Pakistan that found that less than 2 % of the cities with populations of at least 10,000 inhabitants had any wastewater treatment facilities at all (IWMI 2003). The amount of wastewater that is disposed of untreated every day is already massive and this amount is increasing with population growth. There are various other reasons for a growing pressure on the existing sources of freshwater such as industrial development, changing dietary patterns, rising incomes and climate change (UNDP 2006, Bates *et al.*, 2008, PachaurI & Reisinger, 2008, Mcintyre *et al.*, 2009).

Water pollution is more complex than air pollution because almost anything can be spilled or dumped into water including kitchen sink (Anderson, 2004). Contaminants such as hydrocarbons, heavy metals and pesticides have been known to have direct toxic effects when released into the aquatic environment (Forstner *et al.*, 1998; Fleeger *et al.*, 2003). These practices threaten both human health and environment at downstream and more importantly reduce the effective availability of Pakistan's already short water supplies (Sial *et al.*, 2005).

Sewage effluent users are mainly interested in its visual benefits, like increased crop production, low cost water source, efficient way of effluent disposal, source of nutrients, organic matter and so many others (Scott, 2000; Butt *et al., 2005*). Sewage wastewater comprises a complex mixture of organic and inorganic matter, containing macro- and micro-organisms, thus is an excellent vehicle for their dissemination. It serves as an efficient carrier of human pathogen along with heavy metals that are hazardous to the environment and human health (Butt *et al., 2005*). So it is not suitable for irrigation (Furedy *et al.,* 1999; Zarsky & Hunter, 1999; Murtaza *et al.,* 2003; Ghafoor *et al.,* 2004).

As wastewater contains human excreta and hazardous chemicals from household and other sources, if untreated wastewater is used for irrigation, it poses serious risks to public health, not only for farm workers and related communities but also to crop consumers (Feachem *et al.,* 1983; Scheierling *et al.,* 2010). Large quantities of nutrients released in water through sewage wastewater may result in nutrient enrichment, dissolved oxygen depletion, bioaccumulation of organic and inorganic compounds, and alteration of trophic interactions among both aquatic flora and fauna (Danulat *et al.,* 2002; Russo, 2002).

Soil and groundwater pollution are the ultimate consequences of using untreated wastewater in agriculture (Scheierling *et al.,* 2010). Soil pollution can occur as a result of increased rates of salinization and water logging, through wastewater irrigation (Tanji and Kielen, 2002). Long term use of untreated sewage effluents for irrigation results in soil contamination to such an extent that it becomes toxic to plants (Quinn & Syers, 1978; Hemkes *et al.,* 1980; Rattan *et al.* 2002; Madyiwa *et al.,* 2002; Yadar *et al.,* 2002; Angin *et al.,* 2004). Moreover penetration of wastewater into soil can cause environmental degradation as well as river pollution (Kaynezhad and Ebrahimi, 1997). Crop yields may reduce if physicochemical quality of wastewater is not properly monitored. For example too saline wastewater or wastewater with high concentration of heavy metals, nitrogen and sodium inhibit plant growth by reducing plants ability to absorb nutrients (Ayers & Westcot, 1985). Chemical pollutants like nitrates can travel to greater distances and can be a source of groundwater pollution in vicinity of wastewater treatment plants (Rhoads *et al.,* 1992; Hillel, 2000). Its high level can cause methemoglobinemia or "blue baby" disease in rural areas (Mccasland *et al.,* 1985; USEPA 2004).

Heavy metals such as copper, lead, zinc and cadmium are carried in storm water runoff, and can bio-accumulate in aquatic systems because they cannot be broken down into less toxic forms. Sources of heavy metals are including car brake pads, building siding and roofs, tires, and atmospheric deposition (Davis *et al.,* 2001). Longterm irrigation with contaminated sewage effluents from urban sources resulted in the accumulation of heavy metals in the plough layer of agricultural soils in India and Pakistan (Singh and Verloo, 1996; Tirmizi *et al.,* 1996; Brar and Arora, 1997).

Sewage water contains 'anthropogenic' compounds including pharmaceuticals, hormones and endocrine disruptors, antimicrobials and antibiotics, and personal care products, causing long-term health effects (Bhandari *et al.,* 2009). Anthropogenic activities have led to increased metal pollution in the environment (Zhou *et al.,* 2008; Krishna *et al.,* 2009). With improvements in the standards of living, metals usage and its discharge as waste or by products is expected to increase alarmingly. Soil acts as a sink for all substances including metals and routes them to underground water, plants and animals consequently (Murtaza *et al.,* 2010). It is reported that the principal chemical risks to human health are resulting from the consumption of wastewater-irrigated foods. Chang also found that inorganic and organic pollutants present in wastewater are transferred in the environment by food chain (i.e., wastewater → soil → plants → humans) from the consumption of grains, vegetables, root/tuber crops, and fruits (Chang *et al.,* 2002).

Sewage water contains substantial amount of potentially harmful substances including soluble salts and heavy metals like Fe^{3+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} etc. (O'Riordan *et al.,*1983). Long term exposure to these metals can result in bioaccumulation and cause endocrine disruption (Nessim and Riad 2003; Papagiannis *et al.,* 2004; Brian 2005; Maffucci *et al.,* 2005; Nguyen *et al.,* 2005). High concentration of copper results in metabolic and gastrointestinal disorders, damages the liver and brain of sufferers of Wilson's disease and becomes toxic to aquatic life (Brewer 2000; Paris-Palacios *et al.,* 2000; Roberts and Schilsky 2003; WHO 2004). Cobalt is also lethal in higher concentration (Norberg and Molin 1983; Marr *et al.,* 1998). Cadmium is toxic to humans, aquatic life and wildlife (Canton and Slooff 1982; Leffel *et al.,* 2003; Barbier *et al.,* 2005). In higher concentration, nickel is also toxic and carcinogenic to humans (Denkhaus and Salnikow 2002; Kasprzak *et al.,* 2003; WHO 2004). Lead is cytotoxic, neurotoxic and a possible human carcinogen—Group B2 (Mameli *et al.,* 2001; WHO, 2004). It also affects mental development among infants and young children (WHO, 2004). Zinc is toxic in higher concentration and affects cognitive functions (Chen and Liao, 2004; Flinn *et al.,* 2005). Iron is carcinogenic and toxic in higher concentration (Deugnier and Turlin 2001; Papanikolaou and Pantopoulos 2005).

The risks concerning reclaimed wastewater reuse can be classified into biological and chemical risks (Salgot *et al.*, 2005). One of the significant risks is diseases transmission due to pathogens (Mara *et al.*, 1989; Blumenthal and Peasy, 2002). Due to cost and complexity of analyzing actual microbial pathogens in wastewater, professionals have relied on traditional fecal indicators to predict pathogenic level (Pancarbo 1999; Jnen *et al.*, 2000; Rompre *et al.*, 2002; Aksu & Vur Ala 2004). Coliform bacteria are the principle indicators and are the major source of diarrhea, fever and other complications (Fatoki *et al.*, 2001; Zamxaka *et al.*, 2004). A high contamination of fecal counts indicates deficiency in adequate treatment facility of sewage (Mathew *et al.*, 2000).

There are many physical and chemical parameters that can be determined in relation to wastewater reuse. Physico-chemical and microbiological analysis of surface waters are important in assessing the impact of domestic and industrial activities on water bodies. Seasonal changes affect water quality by both, natural and anthropogenic processes, such as temperature, precipitation and hydrological condition (Zhang *et al.*, 2008). Physical parameters such as pH, Total Dissolved Solids (TDS) and conductivity of the water have a major influence on biochemical reactions that occur within the water (Zamxaka *et al.*, 2004). pH, conductivity and TDS have a major influence on bacterial population growth. pH values ranging from 3 to 10.5 could favor both indicator and pathogenic microorganism growth (Zamxaka *et al.*, 2004). Temperature and salinity affect the capacity of the water to hold dissolved oxygen, so increases in temperature and conductivity will affect dissolved oxygen levels and therefore species diversity as well. Irrigation with sewage water increases soil electrical conductivity (EC) and decreases soil pH (Narwal et al. 1993).

Even if Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are reduced indicating reduced levels of organic matter and biological activity, use of this wastewater may pose environmental and health hazards. It may contain pathogens, toxic elements and compounds (Akram *et al.*, 2004). The impact of phosphorus and nitrogen on water quality is of particular concern, because nutrients in runoff can cause eutrophication where algal blooms grow excessively and deplete dissolved oxygen levels and increase turbidity. This results in poor water quality and low biodiversity. Nutrients in wastewater can be contributed by fertilizers, atmospheric deposition, soil erosion, animal wastes and detergents (Heish *et al.*, 2007).

Lipids (characterized as oils, greases, fats, and long-chain fatty acids) are one of the important organic components of wastewater (Quemeneur and Marty Y; 1994). Their amount in municipal wastewater is approximately 30-40 % of the total COD (Chipasa and Medrzycka, 2006). Lipids are one of the most important components of vegetable oil, many synthetic compounds and emulsions, and they are mostly found in pharmaceutical and cosmetic industrial effluents. Oil wastes either of petroleum or vegetable origin are considered as serious type of hazardous pollutants in aquatic environments, due to their high level of toxicity to the aquatic organisms (Mendiola *et al.* 1998). Biological treatment has been found to be the most efficient method for removing fat, oil and grease by degrading them into miscible molecules.

Wastewater contains its unique quality and characteristics upon which type of treatment is suggested (Onkonko *et al.*, 2007). Domestic effluents require various treatment processes like physical, chemical and biological processes but biological is preferably adopted (Aririatu *et al.*, 1995, 1999). The conventional cleanup processes for heavy metal contaminated soils and waters are expensive and only useful in small areas (Moffat, 1995), researchers have considered necessary new cost effective technologies that include the use of microorganisms, biomass and living plants (Cervantes *et al.*, 2001; Rehman and Shakoori 2001; Ebbs and Kochian 1998; Haq and Shakoori, 1998).

Bioremediation is a method which uses microorganisms, microbial processes and biological processes to degrade environmental contaminants (Atlas 1991). Phytoremediation is a type of bioremediation that uses plants to degrade, extract and immobilize contaminants such as metals, explosives, oil, excess nutrients, and pathogens from soil and water (EPA, 2000). Bioremediation has been identified as a more cost effective, natural, and publicly acceptable method of removing environmental contaminants than most chemical and physical methods (Arthur *et al.*, 2005). Many researchers reported the potential of utilizing vetiver to decontaminate heavy metals from wastewater (Kong *et al.*, 2003; Roongtankiat *et al.*, 2007) Microbial metal removal has acknowledged much attention in the last years due to potential use of microorganisms for cleaning metal polluted water (Ledin, 2000).

Effective Microorganisms (EM) is a mixture of groups of organisms that are coexisting anaerobic and aerobic beneficial microorganisms for humans, animals and the natural environment. The main species involved in EM are: Lactic acid bacteria, Photosynthetic bacteria, yeasts, Actinomycetes and fermenting fungi (Szymanski and Patterson, 2003). The basic purpose of EM is the restoration of healthy ecosystem in both soil and water by using mixed cultures of beneficial and naturally-occurring microorganism. Therefore, the EM has great potential in creating an environment most suitable for the existence, propagation, and prosperity of life (Higa & Parr, 1994). So wastewater characterization is very important when designing a wastewater treatment plant.

Pakistan is an agricultural country having irrigated agriculture as the largest consumer of fresh water resource. With increased competition for water by rapidly intensifying urban populations and industry, the use of urban wastewater in agriculture is receiving improved interests (Salgot *et al.*, 2004). A nation-wide wastewater assessment showed that total water supply is 4.6 x 10^6 m^3/day, and about 36 % of wastewater is used for irrigating an area of 32,500 hectares. 26 % of the country's domestic vegetable production was cultivated with wastewater (IWMI 2003, Ensink et al. 2004).It has also been estimated that 64 % of total wastewater of Pakistan is disposed off either into rivers or into the Arabian Sea. Similarly 400,000 m^3/day wastewater is additionally added to canals (Ensink *et al.*, 2004).

Planned and regulated use of wastewater is required for this valuable resource to use whilst at the same time avoiding risks to human health, crop productivity and the environment (WHO, 1973; WHO 1989; Pescod 1992; USEPA 1992). National Agricultural Research Center has taken this inititiative and established National Institute of Bioremediation (NIB). NIB established Bioremediation Gardens on pilot scale for the treatment of wastewater coming from NARC Hostels and found the treated water falls under NEQS range. Furthermore to take advantage on larger scale NIB has Bioremediation Orchards for research activities and its application on national level. Bioremediation Orchid is capable of treating about 0.6 million gallons of wastewater from Shehzad Town daily which is sufficient to irrigate 550 acre land through high efficiency irrigation system at an interval of 20 days.

1.1 Significance of the Study

Present study is planned to investigate temporal variations in important chemical/microbial constituents of domestic waste water to analyze, improve and interpret seasonal changes in bio-remediation potential of plants/microbes in close connection with nature of sewage using indigenous resources. Sewage from urban localities significantly varies in composition associated with changes in the social setup, utilization of diverse chemical products and is also tied to general response of the community to improving lifestyles. This study will help in identification of underlying changes in sewage quality coupled with seasonal variations of known quality and will also abridge the gaps for always applying novel plants/microbes to same sewage for bioremediation purposes.

1.2 Objectives

The main objectives can be stated as follows,

- To determine the amount of pollutants in the wastewater and tracing out their possible sources.
- To determine temporal variations in sewage water quality
- Comparison of untreated wastewater with bio-remediated water

Chapter 2

2.0 MATERIALS AND METHODS

This chapter contains information about all the materials and methods which are opted in order to evaluate variations in sewage water quality on temporal scale and to examine the treatment efficiency of sewage wastewater through bio-remediation. Selected physico-chemical, microbial and trace and heavy metal analysis was performed for the purpose. This study was carried out in National Institute of Bioremediation (NIB) at National Agricultural Research Center (NARC). All experimental analysis was done at Water Quality Lab NIB.

2.1 Chemicals and Reagents

Barium Chloride Dehydrate (99 %), Sodium Carbonate (99 %), Potassium Hydroxide (85 %), Sodium Hydroxide (99 %), Ammonium Chloride (99.8 %), Sodium Chloride (99.5 %) were purchased by Merck Steinhiem, Germany. Potassium Chromate (99 %), Phenolphthalein (98 %), Hydrochloric Acid (95 %) and *n*- Hexane (95 %) were purchased by Sigma Germany. Sulphuric Acid (98 %) was of Fluka Germany where as Zinc Sulphate (99.5 %) was supplied by Anal R Spain. All the glassware with standard quick fit joints was used throughout the work after washing with detergents, rinsing with acetone and drying in an oven at 120 °C. The glassware (Witeg, Germany Ltd.) which was utilized during the research work includes beakers, funnels, flasks, seperatory funnels, volumetric cylinders, pippet, burette and glass rods.

2.2 Apparatus and Equipment

Cyber Scan Series PCD 650 was provided by EUTECH Spain. TDS Tester 11 and DO multimeter Sension 156 were supplied by Hach Germany. Atomic Absorption spectrophotometer (Analyst 800) and UV-Visible Spectrophotometer (Model No. C6180437) was provided by Perkin Elmer Germany. Digital weighing machine was provided by AE ADAM China whereas Aquatron A4S BSI testing 1986 provided by ASchle Canada for water distillation and purification.

2.3 Sample preparation

2.3.1 Standard solution preparation

Sodium thiosulphate (0.025N) was prepared by dissolving 24.8 g of $Na_2S_2O_3.5H_2O$ in boiled distilled water adding 0.4 g NaOH to it and diluted it up to 1 liter. This was the stock solution. Diluted 250 ml of stock solution to 1 liter to prepare 0.025 N solution. Potassium Dichromate (0.25 N) solution was prepared by dissolving 12.25 g $K_2Cr_2O_7$

in one liter. Potassium Dichromate (0.025 N) was prepared by diluting ten times 0.25 N $K_2Cr_2O_7$. Sodium Hydroxide (0.05 N) was prepared by dissolving 40 g of NaOH in 1 liter. That was 1.0 N stock solution. Then took 50 ml from that stock solution and diluted that up to 1 liter to make 0.05 N NaOH solution. Phenolphthalein indicator was prepared by dissolving 0.5 g phenolphthalein in 50 ml of ethanol + 50 ml of distilled water and mixed it with 0.05 N NaOH drop wise until solution became faintly pink. Methyl Orange indicator was prepared by dissolving 0.5 g of Methyl Orange and diluted it up to 100 ml distilled water. For preparation of 0.1 N HCl, 8.33 ml of concentrated IN HCl was dissolved in 100 ml of distilled water and further diluted it 10 times. Silver Nitrate (0.02N) was prepared by dissolving 3.4 g $AgNO_3$ in 1 liter distilled water.

2.3.2 Environmental sample preparation

Wastewater samples from inlet and bio-remediated point were collected and taken to the laboratory for selected phsico-chemical and microbial analysis and prepared according to methods used for analysis as discussed in detail in following sections.

2.4 Study Area

The study area comprised of Shehzad Town and Bioremediation Orchard Site, NIB NARC Islamabad, lying within geographical coordinates 33°39'-33°40' N Latitude and 73°06'-73°08 E Longitude, Elevation 507 m or 1666 feet and Area 1.6 Km, is situated 7.5 Km away, located in Zone IV of Federal Capital Islamabad (Fig.1)

2.4.1 Shehzad Town

It is peri urban community bounded by two regional roads Murree Road and Islamabad Expressway which connect the area to surrounding cities of Islamabad and Rawalpindi. The study area is one of the main sources of vegetable supply for Islamabad and carries strong potential for subsurface aquifer recharge (JICA, 1998).

The study area is characterized by a sub-humid climate. As it is located more than 1000 miles from Arabian Sea, it experiences wide variations in seasonal temperatures and precipitation. The monthly mean air temperature ranges from 9 to 32.5°C with an annual average of 21.96 °C. The lowest ever temperature recorded is -4 °C in the month of January, while the highest is 48 °C in the month of June.

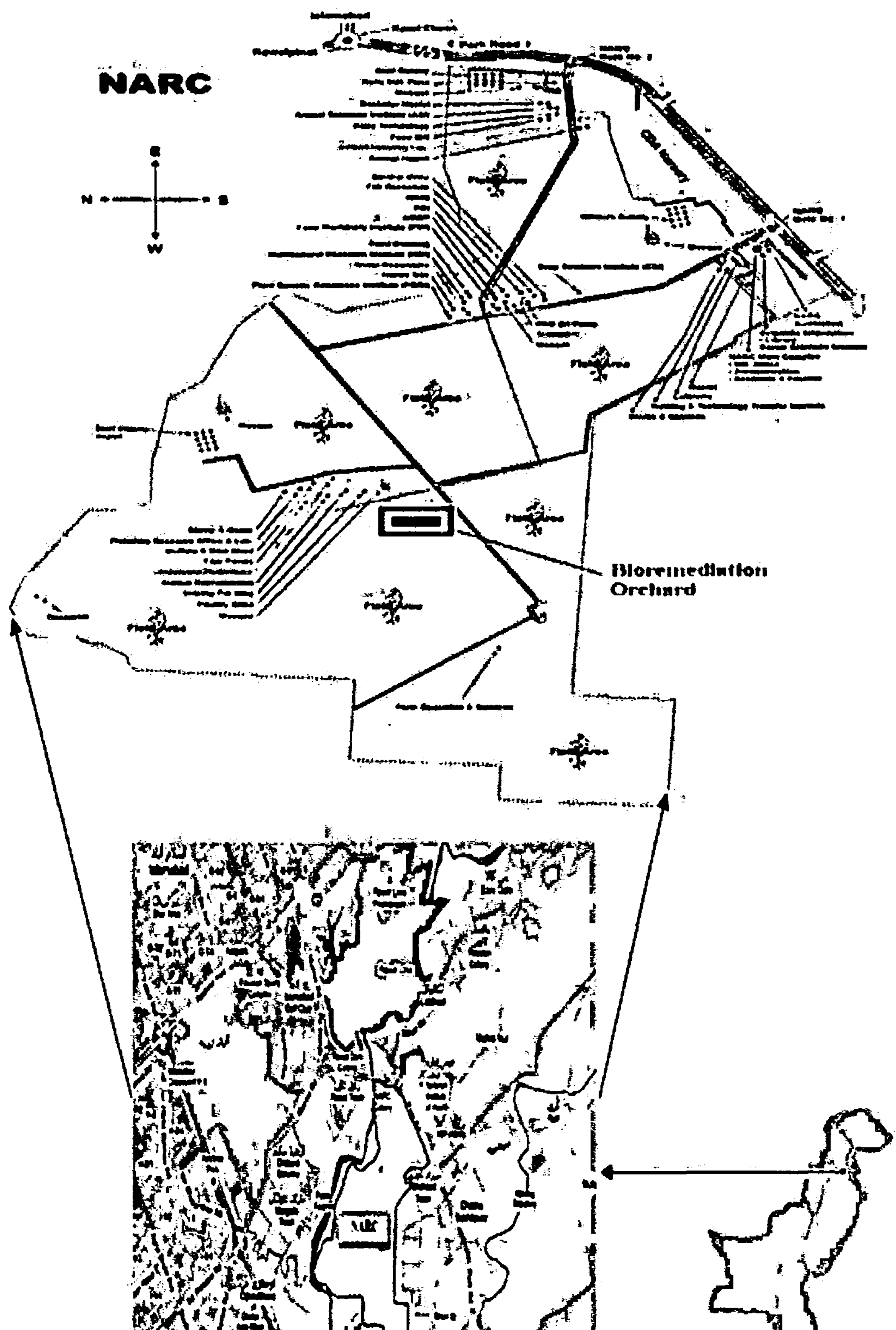


Figure 1. Study area map

Relative humidity in Islamabad ranges from 19 to 54 % recorded in the month of May and August respectively. The annual precipitation ranges from 1000 mm to 1200 mm (UNEPA, 1998). The highest precipitation occurs in the month of July and August followed by an almost dry spell. This dry spell is broken by slight rain showers from January to March. The study area is strongly influenced by pre-monsoon (April-June), monsoon (July-Mid September), post-monsoon (Mid September-November) and winter (December-February). Almost 90 % of the annual precipitation occurs in the monsoon season (Survey of Pakistan, 1999).

2.4.2 Bioremediation Orchard Site NARC

Bioremediation Orchard Site is spread over an area of 7 acre. It is a combination of constructed wetlands and six bioremediation ponds of varying size having specific plants and microbes in every pond for degradation and elimination of pollutants and toxic compounds in waste water of Shehzad Town (Fig. 2). At some distance from Inlet point of wastewater, two detention ponds have been constructed for settlement of sewage sludge and for preliminary phyto-treatment by using Duckweed, Water Lettuce and Water Hyacinth.

2.4.2(A) Constructed wetland

Approximately after 1 Km from Detention ponds, wastewater entered into Sub Surface Flow Constructed Wetland. Constructed Wetland was divided into six cells on top. Different plants like *Typha latifolia, Cyprus papyrus, Vitiveira zizaniodes, Pharagmites australis, Scripus acutus, Hydrocotyle verticillata* etc were introduced along with layers of gravel, brick pieces, sand, coal, crush etc. These aquatic plants have high efficiency in removal of chemical and microbial contaminants.

2.4.2(B) Bioremediation Ponds

There were two types of plants present at site i.e. floating; *Water hyacinth,* water lettuce, lesser duckweed, fat duckweed and submerged; *Typha Latifolia*, water cress, vetiver grass, bulrush, phragmites, umbrella plants, papyrus sedge, flat sedge, couch panicum, giant cane and common reed.

Effective Microorganisms (EM) which is mixture of different strains of microorganisms, is introduced in septic tanks, P1, P2, P3 and P4 for bioremediation purposes and approximately 300L EM had been used for 4 months duration. This amount was sufficient for the treatment of daily available wastewater entering to treatment plant in combination with plants capable of phyto-remediation.

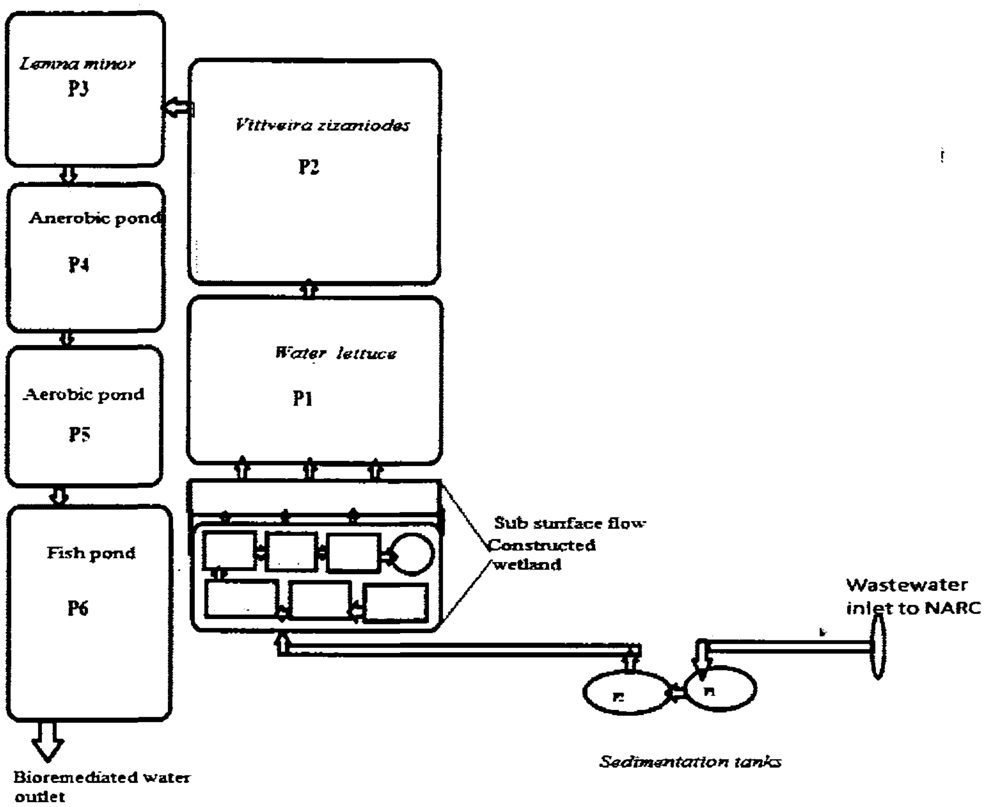


Figure 2. Schematic diagram of Bioremediation Orchard Site NARC

Wastewater entered to Pond 1 which is adjacent to constructed wetland. It comprises of *Pistia stratiotes* and water lettuce. The main purpose was to reduce Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), heavy metals and other hazardous chemicals. Water then enters to Pond 2, filled by *Pistia stratiotes/Lemna minor* for treatment of inorganic contaminants. Then it entered into Pond 3 in which *Vitiveira zizaniodes* contributed in reduction of Cd^{2+}, Cu^{2+}, Fe^{3+}, Pb^{2+} and Mn^{2+} along with BOD and COD.

In Pond 4 *Eichhornia crassipes* contributed in heavy metal uptake and reduction of chlorides, nitrates and phosphates. In Pond 5 *Hydrocotyle verticillata* performed reduction in total dissolved solids (TDS). At the end clean water entered to Pond 6 which is fish hatchery. At the outlet pond of P6 water is available for irrigation and aquaculture.

2.5 Study Design and Sampling Strategy

Samples of wastewater were collected from two points at the Bioremediation Orchid Site, NARC Islamabad;

- Raw (Inlet point to NARC)
- Bio-remediated (Outlet point from Bioremediation Site)

Weekly composite samples were collected for a period of 4 months from 13 Dec 2010 to 15 April 2011. One liter plastic and glass bottles with hard plastic screw caps were used for sample collection. Before sample collection all sample bottles were thoroughly washed with metal free detergent, soaked with 10 % HNO_3 and rinsed with distilled water. During sampling clean and powder-free rubber gloves were used. Sampling was done by dipping each sample bottle approximately 20–30 cm below the water surface, opening and allowing the bottle to fill up and covering with cap under water without trapping any air bubble. After collection, the samples were placed in walk-in cooler boxes with ice chests while being transported to the laboratory and kept at about 4°C until analyzed (APHA 1999). Samples for microbial analysis were processed within 7 hours of collection, while samples for nutrients and metals analysis were frozen and acidified with nitric acid until analysis.

In each sampling station, two different samples were taken in triplicate for chemical and microbial analysis respectively. All samples were filtered through Ash less filter Paper except the ones used for microbial analysis. Therefore, a total of 6 water samples were collected resulting in a total of 96 samples on both sampling stations. At each sampling point 3 separate samples were collected for chemical analysis with exception of BOD and microbial analysis.

The samples were analyzed for 31 physical, chemical and microbial parameters, namely temperature, pH, Electrical Conductivity (EC), salinity, Total Dissolved Solids (TDS), Dissolved Oxygen (DO), 5-days BOD, COD, carbonates (CO_3^{-2}), bicarbonates (HCO_3^{-1}), free CO_2, chloride (Cl^-), nitrate (NO_3^-), sulfate (SO_4^{-2}), oil and grease, sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), cadmium (Cd^{2+}), chromium (Cr^{3+}), iron (Fe^{3+}), manganese (Mn^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), zinc (Zn^{2+}), nickel (Ni^{2+}), total coliform, faecal coliform and E.*Coli*.

2.6 Preliminary Survey indicating chemicals commonly used at household level

Before experimental analysis baseline household data was collected through survey questionnaire in the month of September 2010. It included questions on domestic chemicals of daily usage (type of product, quantity used per month) and Socio-economic variables i.e. literacy, type of house construction, income level and car ownership). Sanitation related characteristics were source of drinking water supply, size of water tank, filling of water tank (reflecting good availability of water) and some information about their social activities like car washing at home, cloth washing schedule reflecting their behavior towards water usage (Annex. 1).

2.7 Analysis of selected physical parameters

The pH and temperature of the wastewater samples were measured in field with a portable pH meter and a mercury thermometer respectively. EC and salinity were measured by EUTECH Cyber Scan Series. TDS of wastewater samples was determined by TDS Tester 11, DO was measured with Hach DO/Multimeter Sension DO meter.

2.8 Analysis of selected chemical parameters

The water samples were analyzed for chloride, nitrate, sulphates, carbonates, bicarbonates, free CO_2, oil and grease and trace & heavy metals using standard methods for the examination of water (APHA, 1998 & 2000). The samples were pre-concentrated by evaporating acidified water samples slowly by gentle boiling on a hot plate (Vernon and Wani, 1993). The concentrations of metals (Pb^{2+}, Cd^{2+}, Zn^{2+}, Fe^{3+}, Mn^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+} and Cu^{2+}) in the water samples were determined with a Flame Atomic Absorption Spectrophotometer (FAAS) as discussed in detail in following sections.

2.8.1 Chemical Oxygen Demand (COD)

COD was determined by Reactor digestion method. Two ml of the wastewater sample was taken in the COD vial. The solution turned orange yellow. Distilled water was used for the preparation of blank. Vial was capped and shaken 2-3 times holding from the cap. Then vials were inserted into the DRB200 preheated reactor at 150 °C for two hours. After the particular time, instrument was turned off and the vials were placed in the test tube rack until their temperature decreased to 120 °C. These vials were shaken again 2-3 times and cooled down up to room temperature. COD was determined at 620 nm using HACH Spectrophotometer (Model: DR 5000).

2.8.2 Biochemical Oxygen Demand (BOD)

BOD was determined by dilution method. Two BOD bottles (300 ml) were used for each sample. Therefore total 4 BOD bottles were used for two samples, in which 2 were used as control while remaining 2 were used as experimental bottles. Dilution water was prepared by adding 1ml of each Phosphate buffer solution (pH - 7.2), Magnesium Sulphate (22.5 g $MgSO_4 . 7 H_2O$ in 1 L), Calcium Chloride (27.5 g $CaCl_2$ in 1 L) and $FeCl_3$ (0.25 g $FeCl_3 .6 H_2O$ in 1L) in 1L of distilled water and aerated for 15 minutes using aeration pump (Hach). In every BOD bottle 300 ml of dilution water and 3 ml of sample was added. Dissolved oxygen of experimental bottles were measured simultaneously using DO meter (Hach) while remaining two control bottles were incubated for 5 days at 20 °C and the DO was measured again (Method 5210B: APHA 2005).

BOD was calculated by using following formula;

$$BOD\ ppm = DO_{final} - DO_{initial}$$

2.8.3 Oil and Grease

Oil and grease was determined by solvent solvent extraction. 1 liter wastewater sample was taken in a conical flask and its pH was maintained at 2 using concentrated HCl (1 M). Then it was mixed in a separating funnel with 30 ml of n-hexane. After thorough mixing the water was drained off and any solids and remaining water were separated from the solvent by filtering it through anhydrous Na_2SO_4 on preweighted filter paper. Dried filter paper was weighed and quantity of oil and grease was determined (APHA, 1998).

Oil and grease was calculated by using following formula:

$$Oil\ and\ Grease\ (ppm) = W1 - W2 * 100/ V$$

Where

W1: final weight of filter paper

W : initial weight of filter paper

V : volume of sample taken

2.8.4 Chloride (Cl^-)

Chlorides in wastewater samples were determined by taking 20 ml of sample in a conical flask followed by titration against standard solution of Silver Nitrate (0.02 N) using Potassium Chromate (K_2CrO_4) as indicator. Chloride concentration was measured by using following formula;

$$Cl^- (ppm) = \frac{volume\ of\ AgNO_3 \times N \times 35.5}{volume\ of\ sample} \times 1000$$

Where, N= Normality of $AgNO_3$

2.8.5 Bicarbonate (HCO_3^-) and Carbonate (CO_3^{-2})

pH of the sample greater than 8.3 showed a possibility for presence of carbonates. For this purpose 10 ml sample was titrated against 0.01N HCl until pink color turned to colorless. For bicarbonates 10 ml of sample was taken in a conical flask and added 2 drops of mixed indicator (Bromocerol green and Methyl red 2:1). The sample turned green. Titrated it with 0.02 N HCl until solution turned pink (APHA, 1998).

2.8.6 Hardness

The total hardness of wastewater samples was calculated by using following expression (APHA, 1992).

$$Hardness = (Ca^{+2} \times 2.496) + (Mg^{+2} \times 4.118)$$

Concentrations of Ca^{2+} and Mg^{2+} were calculated as discussed in section 2.8.10.

2.8.7 Sulphate (SO_4^{-2})

Sulphates in the sample were measured by Barium Chloride method. 10 ml of sample was taken in a conical flask and 2 ml of Sulphate buffer (pH= 4.5) and a pinch of $BaCl_2$ was added to it. Contents in conical flask were shaken vigorously and allowed to stay for 5 minutes. SO_4^{-2} concentration was measured at 420 nm using spectrophotometer. Standard curve was prepared in range 0 – 40 ppm with R^2 = 0.99 (Fig. 3). Distilled water was run as blank.

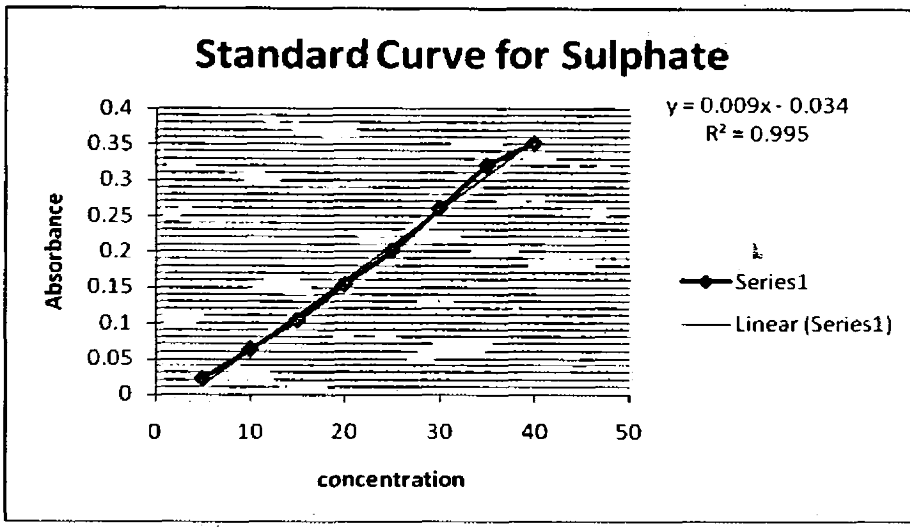


Figure 3. Standard calibration curve for Sulphate

2.8.8 Nitrate (NO_3^-)

Nitrates were measured by spectro-photometric method. 10 ml sample was taken in a conical flask and 0.2 ml of 1 N HCl was added to it. It turned to orange yellow. Standards of different nitrate concentrations and samples were run at 220 nm using spectrophotometer. Ten point calibration curve was prepared in range with $R^2 = 0.99$ (Fig. 4). Distilled water was run as blank.

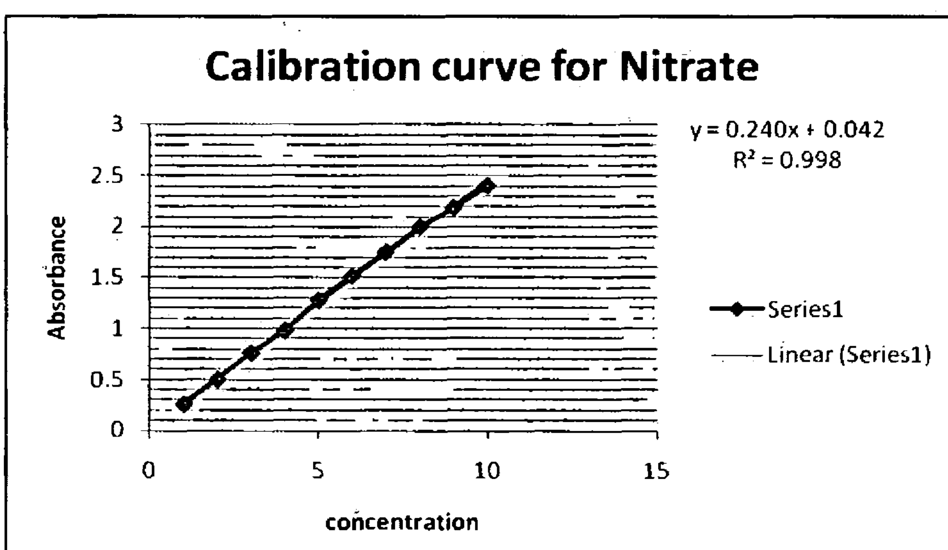


Figure 4. Standard calibration curve for Nitrate

2.8.9 Free CO_2

Free CO_2 was determined by using acid-base titration method. 10 ml sample was titrated against NaOH (0.5 N) using phenolphthalein as indicator and end point was identified by the development of the pink color. Procedure was repeated thrice to get an average value from burette. Free CO_2 was calculated by using following formula;

$$mg\ CO_2/L = A * N * 4400 / ml\ sample$$

Where
A = mL of NaOH
N = Normality of NaOH.

2.8.10 Metals

250 ml of wastewater sample were filtered by using Ash-less Filter Paper (Whatman No. 42) and its pH was adjusted at 2 to determine dissolved metal contents using FAAS. Suspended metals collected over the filter paper were also determined by FAAS after digestion of the filter paper cuttings in the microwave accelerated reaction system at 180 °C for 15 minutes. For filter paper digestion, 13.5 ml HNO_3 (0.02 N) and 4.5 ml HCl (0.1 N) in combination were used with optimal concentrations. Total metal content was determined by adding suspended and dissolved metal content found in the effluent samples. A total of 12 metals namely Cr^{3+}, Cu^{2+}, Fe^{3+}, Na^{+}, Ni^{2+}, Pb^{2+}, K^{+}, Mn^{2+}, Ca^{2+}, Cd^{2+}, Mg^{2+} and Zn^{2+} were determined in the wastewater samples (USEPA Method: 3005A).

2.9 Analysis of selected Microbial parameters

Wastewater samples were analyzed for Fecal Coliform (Method 9221 E; APHA 2000), Total Coliform (Method 9221 B; APHA 2000) and *Escheria. Coli.* (Method 9221F;APHA 2000). MPN (Most Probable Number) technique and filtration technique was adopted for analysis. For presumptive test Lauryl tryptose sulphate broth was used. Incubation was carried out at 35 °C for coliforms and 44 °C for thermo tolerant faecal coliforms. MacConkey agar was used to isolate the pathogens. Identification of colonies was done by biochemical tests.

2.10 Calculation of Treatment Efficiency

The bioremediation treatment efficiency of each parameter was calculated by following formula:

$$Treatment\ Efficiency\ \% = \frac{Concentration\ (Raw) - Concentration\ (BioR)}{Concentration\ (Raw)} * 100$$

2.11 Statistical Analysis

Descriptive statistics was employed to present the main findings of the study, and all statistical analyses were carried out using Excel 2007 (Microsoft, 2007) and STATISTICA version 7.0 (StatSoft, Inc., USA, 2001) software. Hierarchical cluster analysis (HACA) was used to assess the similarity or dissimilarity in wastewater in terms of water quality parameters. Squared Euclidean was applied as a distance matrix while Ward's method as a linkage method. HACA was performed on the mean values of all water quality parameters.

Principal component analysis (PCA) provided information for the most meaningful parameters describing the whole dataset interpretation, data reduction, and to summarize the statistical correlation among constituents in the water with minimum loss of original information (Kazi *et al.,* 2009). Factor analysis (FA) based on principal component analysis (FA/PCA) was employed to interpret the underlying dataset and identify possible sources of contamination through a reduced new set of orthogonal variables called principal components (PCs) which provided information in decreasing order of importance (*Qadir et al.*, 2008). Factor analysis revealed the underlying structure of the dataset by means of distance between variables in a multidimensional space. In this study, FA/PCA was applied on raw data separately for temporal analysis. In temporal analysis seasons (winter and spring) and two sites groups (raw and bio-remediated group) identified using Hierarchal Cluster Analysis (HACA) were used as independent variables. Correlation coefficient matrix using Pearson's moment was used to identify interrelationship between total metal contents and other parameters and to support results obtained from FA/PCA.

Chapter 3

3.0 RESULTS AND DISCUSSION

3.1 Preliminary survey indicating chemicals commonly used at household level in Shehzad Town

There were 400 houses and 20 streets reported in the area. The total population of the area was approximately 1200 with average family size of 6 members per house. The survey showed that there were 7 schools, 5 mosques, 9 beauty parlors, 6 clinics/hospitals and 6 markets present in the area. There were 77.7 % double storeyed houses present in the area while only 22.3 % single storeyed. It was found that 77.8 % people of the area were non govt. employed/ businessmen while 22.2 % were working in govt. sector. Among them 61.1 % were having monthly income level ≥ 75,000 Rs. while 22.2 % were having 75,000 - 50,000 Rs. and 16.6 % were having 50,000 - 25,000 Rs. There were 48.8 % people of the area using CDA filtration plant water for drinking purposes while 44.4 % were using boring/ground water. A total of 89 % people of the area were using ground water for washing, bathing and activities other than drinking. The average tank size per house in 53.3 % houses of the area was ≥ 500 gallons while 27.7 % houses were having tank size of ≤ 250 gallons. 53.3 % people of the area filled their water tank twice daily while only 27.7 % were filling once daily. There were 83.7 % people of the area had cars for their personal use while 11.1 % were having motor bikes. However 48.9 % of the people were washing their vehicles at home daily and 51.1 % were washing weekly at home. Similarly 65.5 % people of the area were washing their clothes after a week while 32.2 % were engaged in washing activities on daily basis.

To assess the type and amount of contaminants at household level, information regarding quantity and quality of different products like ghee & oil, soap, shampoo, toothpaste, detergents, utensil washers and toilet cleaners was collected in the area (Table 3.1). It showed that in case of shampoo 60 % of the people were using Head & Shoulders 500 ml per house, 28.8 % using Sunsilk 500 ml and 22.2 % using Pantene 300 ml per house. This really affected the sewage water quality as products like Head & Shoulders contain active Zinc ingredient, Pantene contains Magnesium Nitrate and Magnesium Chloride while Sunsilk contains Zinc Sulphate as an ingredient during its manufacturing. Similarly 58.8 % people of the area were using Dettol soap 690 g per house, 28.8 % using Lux soap 575g and 23.3 % people of the area using other products as soap 575 g/house. The active ingredient in Dettol soap is Benzalkonium Chloride which is highly toxic to fish. For washing purposes, 48.8 % of the people were using 1000 g Surf Excel and 24.4 % using 1000 g Ariel per month. Similarly quantities of dish washers and toilet cleaners are given in table below. As it is clear from the table that 85.5 % of the area were using 900 g Lemon Max as dish washer per month, it contain Caustic soda (50 % sodium hydroxide), Dodecylbenzene sulfonic acid (DDBSA), Trycol, Nonylphenol, Sodium ethoxylated alcohol sulfate and citric acid. These all are included in class of surfactants which are entirely biodegradable in the atmosphere. It has reported to be advantageous in bioremediation technology.

Table 1. Preliminary survey indicating chemicals commonly used at household level

Sr. No	Category	Item	People using Product (%)	Quantity used per month per House
1	Shampoo	Head & Shoulders	60	500 ml
		Sunsilk	28.8	500 ml
		Pantene	22.2	300 ml
2	Soaps	Dettol	58.8	690g
		Lux	28.8	575g
		Others	23.3	575g
3	Detergents	Surf excel	48.8	1000g
		Ariel	24.4	1000g
		Others	26.6	1500g
4	Dish Washers	Lemon max	85.5	900g
		Vim	22.2	900g
		Others	3.3	250g
5	Toilet Cleaners	Acid	61	300ml
		Harpic	33.3	250ml
		Others	5.5	Undefined*
6	Oil & Ghee	Dalda	54.4	12Kg
		Seasons Canola	36.6	8 liter
		Others	8.8	10kg

Undefined* include miscellaneous (acid, soap, washing powder)

3.2 Temporal Variations in selected physical parameters

3.2.1 Temperature (°C)

Temperature measurement of water is significant because it affects the amount of dissolved oxygen in the water. The amount of oxygen that will dissolve in water increases as temperature decreases. During study period temperature increased from 12 °C to 30 °C with 20.5 mean and 5.23 standard deviation influencing other parameters significantly (Table 3). These values were within range of NEQS (40 °C). It showed strong negative correlation with DO (-0.94) and nitrate (-0.77) while positive correlation with hardness (0.71) of untreated sewage water (Annex. 2).

Cluster analysis (CA) showed that it forms group with most of the metals including Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ca^{2+} and Mg^{2+} along with TDS and Hardness (Fig. 5) . PCA/FA showed that it has 0.87 value in Factor 1(household chemicals) showing possible sources of contamination of other parameters like DO, TDS, Hardness, Fe^{3+}, Cr3+, Mn^{2+}, Zn^{2+}, Cd^{2+}, Ca^{2+} and Mg^{2+} within same factor (Table 3). The results of PCA/FA were in close relation with CA.

Microbial growth usually doubles for every 10 °C rise in temperature (Thibault, G.T and Elliott, N.W. 1979). Raising temperature also decreases adsorption of ions, which makes more organic material available for microorganisms to degrade (JRB Associates, Inc. 1984). Highest temperature (30 °C) was recorded at end of March. High temperatures were often accompanied with heavy rainfall. The combined effect of elevated temperatures and heavy rains could explain the high coliform counts observed in this period.

Table 2. Statistical summary of physical parameters of raw and bio-remediated wastewater

Variable	Sample type	Mean	Minimum	Maximum	Std. Dev.	St. Error	*P**-value
T (°C)	Raw	20.56	12	30	5.23	1.31	**
	Bio R	20.56	12	30	5.23	1.31	
pH	Raw	8.03	7.4	8.43	0.27	0.07	***0***
	Bio R	7.48	6.4	8.4	0.41	0.1	
EC	Raw	1826.56	1077	3300	671.57	167.89	***0.034***
(µS/cm)	Bio R	1369.63	862	2420	478.91	119.73	
DO (ppm)	Raw	5.1	3.2	7.2	1.17	0.29	0.82
	Bio R	5.2	3.3	7.5	1.35	0.34	
Sal. (ppm)	Raw	0.58	0.2	1.1	0.24	0.06	***0.01***
	Bio R	0.38	0.1	0.6	0.14	0.03	
Hard.(ppm)	Raw	625.73	393.8	757.6	107	26.75	***0***
	Bio R	515.59	382.3	610.4	75.49	18.87	
TDS (ppm)	Raw	653.24	158.6	1245	259.86	64.96	0.21
	Bio R	546.11	116.3	820	194.81	48.7	

* Bold and italicized values are ≤ 0.05

** Temp. is not included in t-test because of no variations between samples

3.2.2 pH

pH is a measurement of the acidic or basic quality of water. The measured pH of the samples was slightly alkaline with mean value of 8.03 and 7.48 at raw and bio-remediated point respectively (Table 2). The values were within range of NEQS for irrigation (6 - 10) and were unaffected by seasonal variations (Fig. 6a). pH values ranging from

Table 3. The factor loadings obtained from a PCA carried out on the raw wastewater dataset.

Variables	Factor 1*	Factor 2**	Factor 3***	Factor 4****
Temp	***-0.90***			
pH		0.48	-0.43	
EC		-0.61	-0.36	0.51
DO	***0.94***			
Salin	-0.40		-0.52	-0.47
Hard	***-0.87***			
TDS	-0.66	-0.44		
BOD		***0.90***		
COD				
Bicarb		0.50		-0.62
Chloride	-0.51		0.61	
Nitrate	***0.91***			
Sulph			0.56	
Oil	0.57		-0.44	
Free.CO_2			0.48	
Na^+				***0.77***
K^+		***0.85***		
Ca^{2+}	***-0.74***			
Mg^{2+}	***-0.91***			
Cu^{2+}		-0.62		
Fe^{3+}	***-0.95***			
Cr^{3+}	***-0.95***			
Ni^{2+}	-0.61			
Mn^{2+}	***-0.81***		-	
Zn^{2+}	***-0.81***		-0.41	
Pb^{2+}	***0.78***			
Cd^{2+}	***-0.71***			0.57
F.Coli			***-0.83***	
T.Coli			***-0.82***	
Expl.Var (%)	40.2	10.5	8.4	3.6
Prp.Totl	0.39	0.13	0.12	0.09

Loadings with above 0.7 in bold and italicized and those less than 0.4 are omitted

* Household chemicals

** BOD and K^+

*** Biological parameters

**** Na^+

3 to 10.5 could favor both indicator and pathogenic micro-organism growth (Kunte, 1998). These values were also falling within range (6 – 9) for fisheries and aquatic life (Chapman, 1996). pH, temperature and turbidity have a major influence on bacterial population growth (Nübel *et al.,* 1999; Byamukama *et al.,* 2000, Goni-Urriza *et al.,* 2000, Nishiguchi, 2000). Samples with low pH (6.4 - 7.4) attributed to the discharge of water into these sources by the agricultural and domestic activities.

CA showed that pH was forming group with BOD, bicarbonates, K^+ and Salinity and this was further confirmed by PCA/FA results (Table 3 and Fig. 5). Although pH has no direct effect on human health but all the biochemical reactions are susceptible to variation of pH (Gupta *et al.,* 2009). The use of fertilizers and detergents can increase pH. Extreme values of pH can cause problems for aquatic fauna. For example, fish may develop skin irritations, ulcers and impaired gill functioning as a result of water that is too acidic. Death of most aquatic fauna may result from extremely acid or alkaline water.

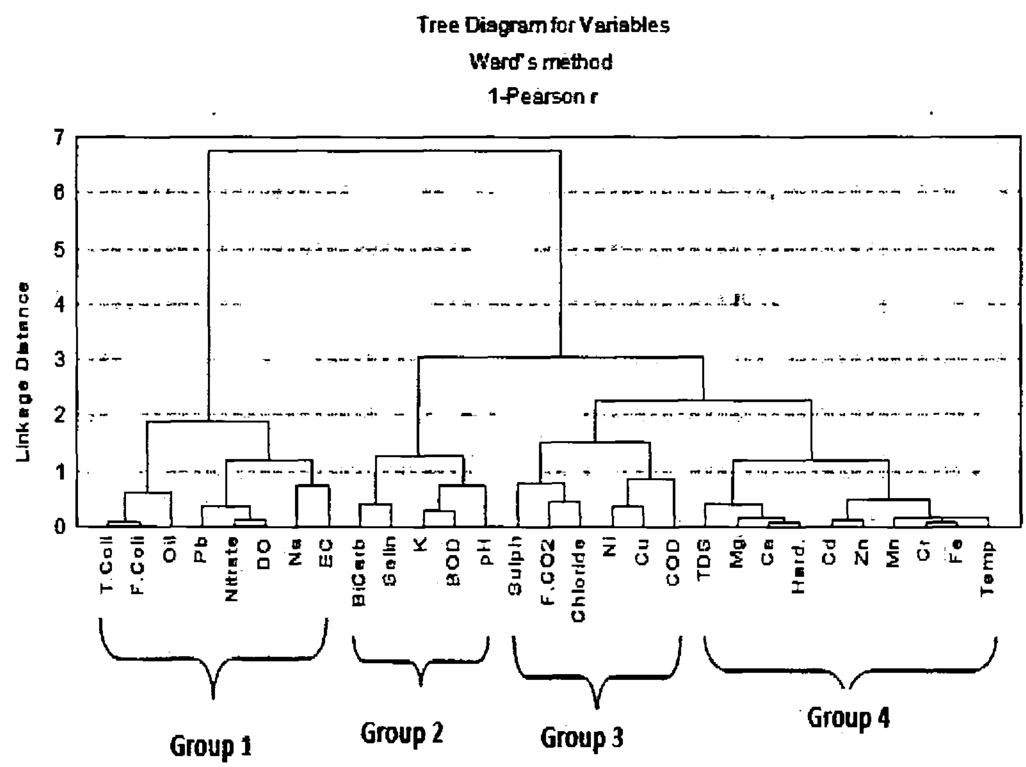


Figure 5. Clustering of Raw wastewater parameters (N=31) using Ward's method (Pearson r). Linkage Distance (Dlink / Dmax) * 100.

3.2.3 Electric Conductivity (EC)

EC is a measure of salt content of water in the form of ions (Laluraj and Gopinath, 2006), it depends on the ionic strength of the water and relates to the nature of various dissolved substances and the temperature at which the measurement is made. EC also provides a rapid and convenient means for estimating the concentration of electrolytes and gives information about all the dissolved minerals (Ullah *et al.*, 2009). It is an indirect measurement of dissolved solids, which is harmful for the aquatic organisms as the density of the water determines the flow of water into and out of an organism's cells (USEPA, 1997).

The mean values of electrical conductivity were (1826.5-1369.6 μS/cm) at raw and bio-remediated point respectively with 25 % mean treatment efficiency (Table 2). Mean untreated wastewater EC value (1826.5 μS/cm) was found higher than NEQS (1500 μS/cm) however after bio treatment it was reduced (Annex. 3). In winter season EC of the both, raw and bio-remediated wastewater samples was relatively higher than in spring (Fig. 6b). The highest value of EC was measured in December when Temperature was lower and sewage water was in less quantity and in stagnant position. The correlation matrix showed that EC is correlated with K^+, TDS and Bicarbonate (Annex. 2). CA showed that it was present in group 1 with microbial parameters and oil (Fig 5). The mean values were higher at raw point while they were significantly lower after bioremediation treatment (Fig. 6b).
EC of most fresh waters is normally in the range of 50 - 500 mS/cm. Its values are considerably higher than reported by in chemical investigation of ground water of Rawalpindi and Islamabad (Sajjad and Tahir 1998).

3.2.4 Total dissolved Solids (TDS)

TDS concentration ranged between 158.60 - 1245 ppm for raw point and 116.3 - 820 ppm for bio-remediated samples (Table 2). The measured concentrations were far lower than NEQ'S (Annex. 3). The mean concentration of TDS at both raw and bio-remediated point was higher during winter season and gradually decreased till spring (Fig. 6d). The mean treatment efficiency of TDS was found 16.4 %. TDS in relation with salinity and EC explain overall salt content of water.

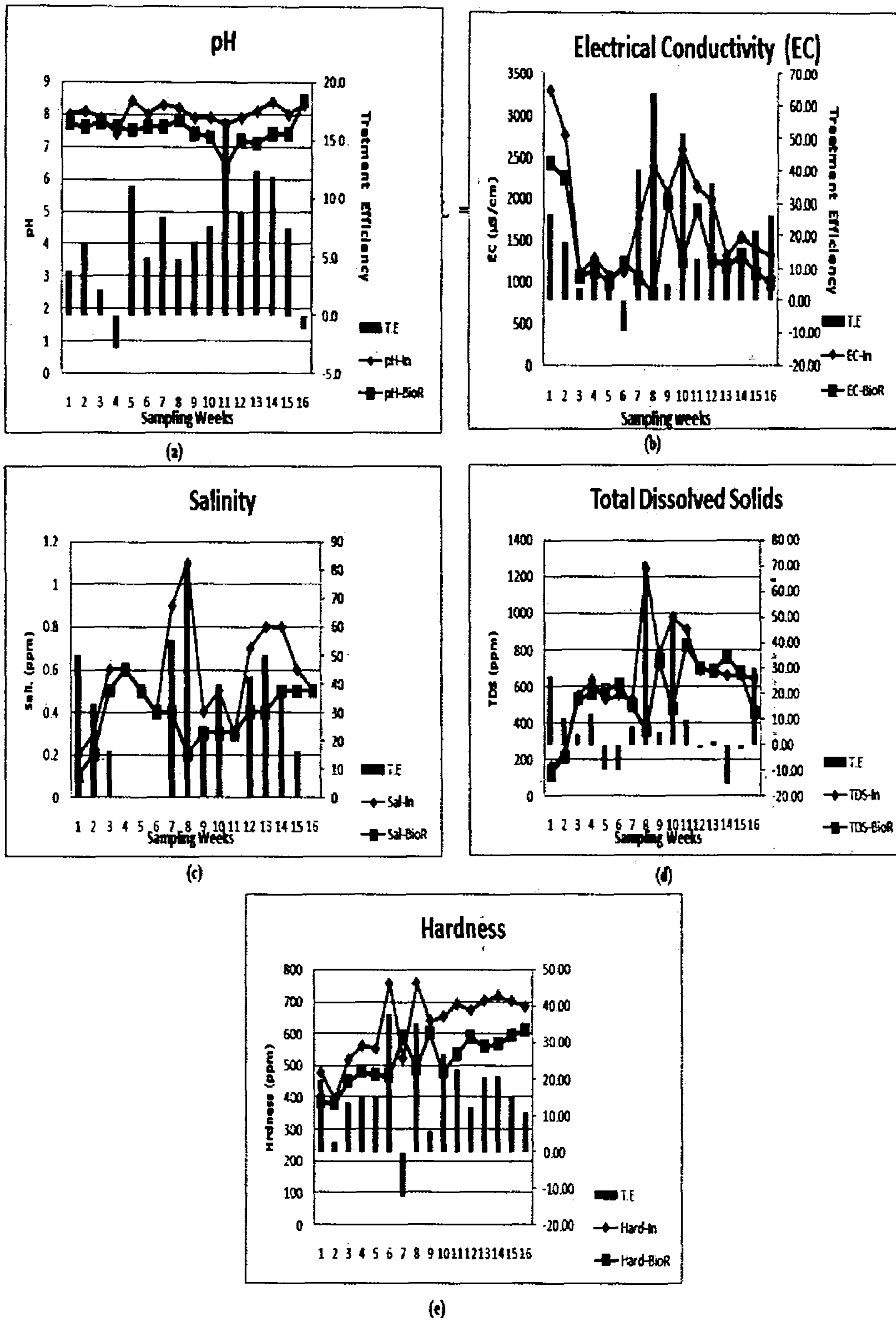


Figure 6. Temporal variations in physical parameters of Raw and Bio-remediated wastewater compared with treatment efficiency (a) pH, (b) EC, (c) salinity, (d) TDS and (e) hardness

TDS are more important measurements to be considered when examining water quality. TDS comprises of inorganic salts and small amounts of organic matter that are dissolved in water (Ali, 2009). It determines the suitability of water for agriculture uses. Temporal variability in TDS and DO can be related to flow regime, seasonal effects, and anthropogenic impacts (Voutsa *et al.*, 2001). The sweetness of water with TDS level less than 600 ppm is generally considered to be good whereas water with TDS greater than 1200 ppm becomes increasingly indigestible (McCutheon *et al.*, 1983).

3.2.5 Salinity

Salinity is a measure of the dissolved salts in the water. The mean value of salinity at raw point was 0.58 ppm whereas after bioremediation, it was reduced to 0.38 ppm (Table 2) which was falling within NEQS range for irrigation (Annex. 3). The correlation matrix showed that it was correlated with TDS (r = 0.52), Bicarbonates (r = 0.59) and Pb^{2+} (r = -0.59) (Annex. 2). CA showed that it was present in group 2 with BOD, K^+ and pH (Fig. 5).

Continuous use of wastewater irrigation caused the problem of salinity. It is a matter of common observation that the wastewater carries undesirable contaminates which travel into plant tissues and ultimately end up in human veins (S. Mahmood and A. Maqbool, 2006). Salty water conducts electricity more readily than purer water. Sources of salinity include urban and rural run-off containing salt, fertilizers and organic matter. Land use issues related to high levels of salinity include clearing of vegetation and the resultant rise in the water table, excessive irrigation, groundwater seepage and runoff containing dissolved solids from industry, sewage, and agriculture and storm water.

3.2.6 Hardness

The results indicated that at raw point, wastewater was slightly hard with range of 393.8 ppm to 663.45 ppm (Table 2). However at bio-remediated point the values tend to fall from 512.6 to 382.3 ppm. The mean treatment efficiency was found to be 17.6 %. Correlation matrix showed that it is correlated with TDS, nitrates, chlorides, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+} (Annex. 2). PCA showed that hardness (-0.87) was present in factor1 with most of trace and heavy metals verifying the results of correlation matrix and CA (Table 3). The measured concentrations of hardness in raw and bio-remediated point in winter were lower than spring (Fig. 6).

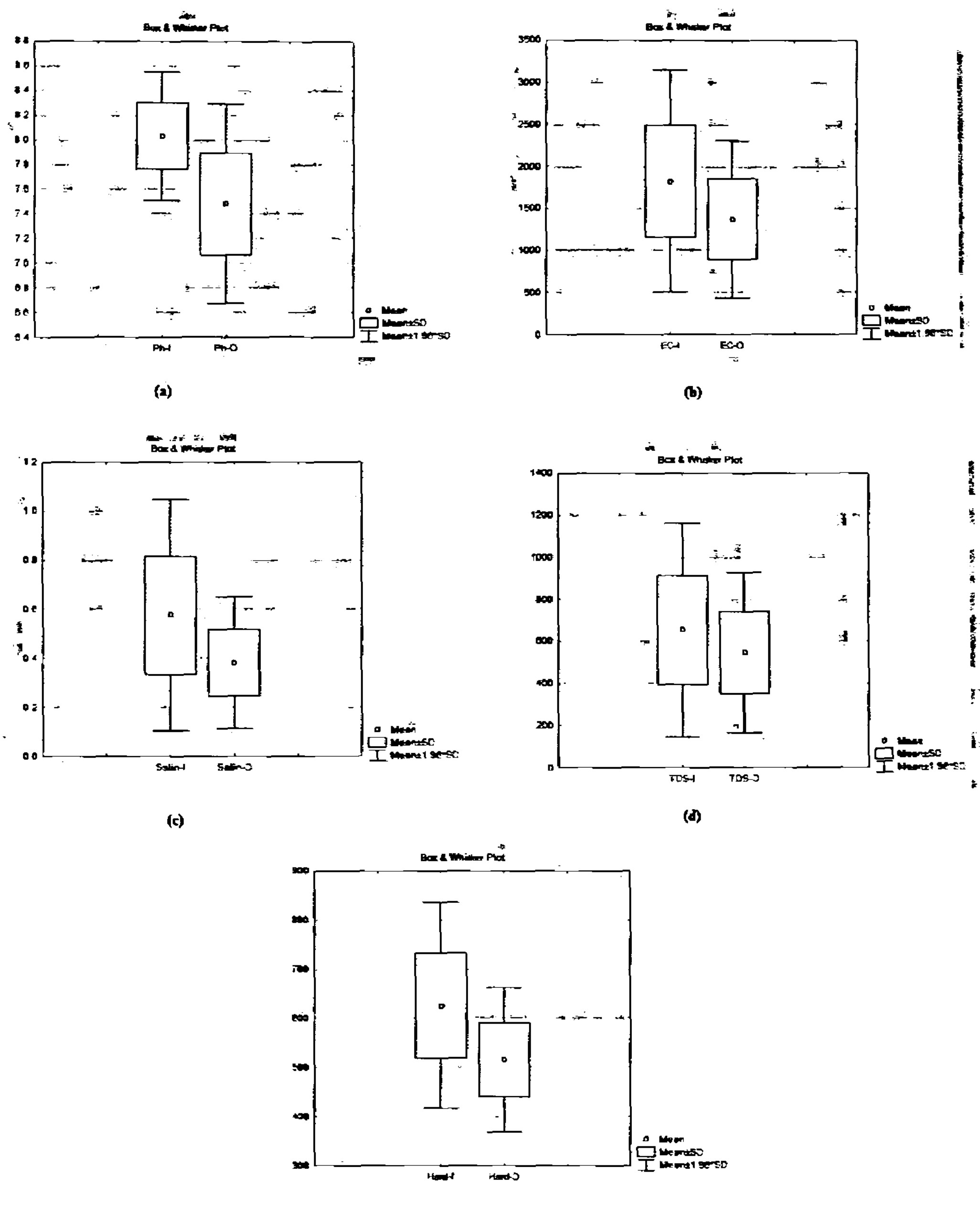


Figure 7. Box and whisker plots (a) pH, (b) EC, (c) TDS, (d) salinity and (d) hardness for raw and bio-remediated wastewater samples during study period (Dec-April). Median is represented by the horizontal line in the box; the box represents the 25th to 75th percentile range; whiskers indicate data values within 1.5 times the inter quartile range, and outliers are data values greater than 1.5 times the inter quartile range

The strong correlation-ship between these parameters attributed to changes in land use namely deforestation, disruption in internal sources of hardness and alkalinity, climatic factor or industrialization. Hardness is the traditional measure of the capacity of water to react with soap, hard water requiring considerably more soap to produce lather. It is not caused by a single substance but by a variety of dissolved polyvalent metallic ions, predominantly Ca^{2+} and Mg^{2+}, although other cations likes Ba^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} also contribute. The high concentration of total hardness in water samples may be due to dissolution of polyvalent metallic ions from sedimentary rocks, seepage and run off from soil.

3.3 Temporal variations in selected chemical parameters of Raw and Bio-remediated wastewater.

3.3.1 Dissolved Oxygen (DO)

The mean values of DO at raw point were 0.58 ppm with minimum 3.2 ppm and maximum 7.2 ppm. Whereas at bio-remediated point, its mean value was 5.2 ppm (Table 4). Its concentration in both raw and bio-remediated point was found higher in winter and was gradually decreased (Fig. 8a). The fact is also verified by correlation matrix (-0.9) because the solubility of oxygen in water decreases with increasing temperature; BOD, COD and nitrogen and phosphorous compounds are also anti-correlated with dissolved oxygen as organic matter is partially oxidized by oxygen, whilst nutrients are responsible for eutrophication of freshwater, thus causing a further increase in organic matter concentration and, hence, in oxygen demand. It also showed strong correlation with hardness (r = -0.75), TDS (r = -0.55), NO_3^- (r = 0.88), Ca^{2+} (r = -0.61), Mg^{2+} (r = -0.83), Fe^{3+} (r = -0.93), Cr^{3+} (r = -0.97), Mn^{2+} (r = -0.86), Zn^{2+} (r = -0.81), Pb^{2+} (r = 0.71) and Cd^{2+} (r = 0.70) (Annex. 2).

The inverse relationship between Temp. and DO is a natural process, because warm water easily becomes saturated with oxygen and thus can hold less DO (Wu *et al.*, 2009b). The amount of oxygen in water shows its overall health. That is, if oxygen levels are high, the pollution levels in the water are low. Conversely, if oxygen levels are low, there is a high oxygen demand ultimately water is not of optimal health. Sewage discharges in water bodies change its environmental conditions (Xu, 1989).

Table 4. Statistical summary of selected chemical parameters of raw and bio-remediated wastewater

Variable	Sample type	Mean	Minimum	Maximum	Std. Dev.	St. Error	P*-value
DO (ppm)	Raw	5.1	3.2	7.2	1.17	0.29	0.82
	Bio R	5.2	3.3	7.5	1.35	0.34	
BOD (ppm)	Raw	148	46	292	69.3	17.33	*0*
	Bio R	52.06	20	90	19.14	4.79	
COD (ppm)	Raw	206.81	100	352	62.91	15.73	*0*
	Bio R	58.19	12	109	26.46	6.62	
Bicarb. (ppm)	Raw	548.44	490	610	33.25	8.31	*0*
	Bio R	460.56	300	640	108.57	27.14	
Chloride (ppm)	Raw	68.84	52.8	88.8	11.18	2.8	*0.04*
	Bio R	58.83	24	84	14.95	3.74	
Nitrate (ppm)	Raw	2.93	0.42	8.3	2.89	0.72	*0.04*
	Bio R	1.29	0.25	3.7	0.83	0.21	
Sulphate (ppm)	Raw	74.46	39.6	121	21.71	5.43	*0.01*
	Bio R	56.26	31.2	76.6	13.45	3.36	
OIL (ppm)	Raw	684.66	256.6	980	224.05	56.01	0.08
	Bio R	538.79	94.4	824	238.96	59.74	
Free CO_2 (ppm)	Raw	29.81	5	100	21.34	5.33	*0.02*
	Bio R	15.63	0	30	8.76	2.19	

How much DO an aquatic organism needs depends upon its species, physical state, water temperature, pollutants present, and lot more. Consequently, it's impossible to accurately predict minimum DO levels for specific fish and aquatic animals. For example, at 5 °C (41 °F), trout fish use about 5.06 mg of oxygen per hour; at 25 °C (77 °F), they may need five or six times that amount. Fish are cold-blooded animals, so they use more oxygen at higher temperatures when their metabolic rate increases. Numerous scientific studies suggest that 4 - 5 ppm of DO is the minimum amount that will support a large, diverse fish population. The DO level in good fishing waters generally averages about 9.0 ppm.

3.3.2 Biological Oxygen Demand (BOD_5)

The mean values of 5 day BOD test were 152 ppm at raw point with maximum 292 ppm and minimum 46 ppm where as at bio-remediated point, its mean was 52 ppm with 90 ppm maximum and 20 ppm minimum (Table 4). These values were decreased significantly from end of January to end of February (Fig. 8). These values were falling within range of NEQS (Annex. 3). The mean bio-remediation treatment efficiency was found to be 64.8 %.

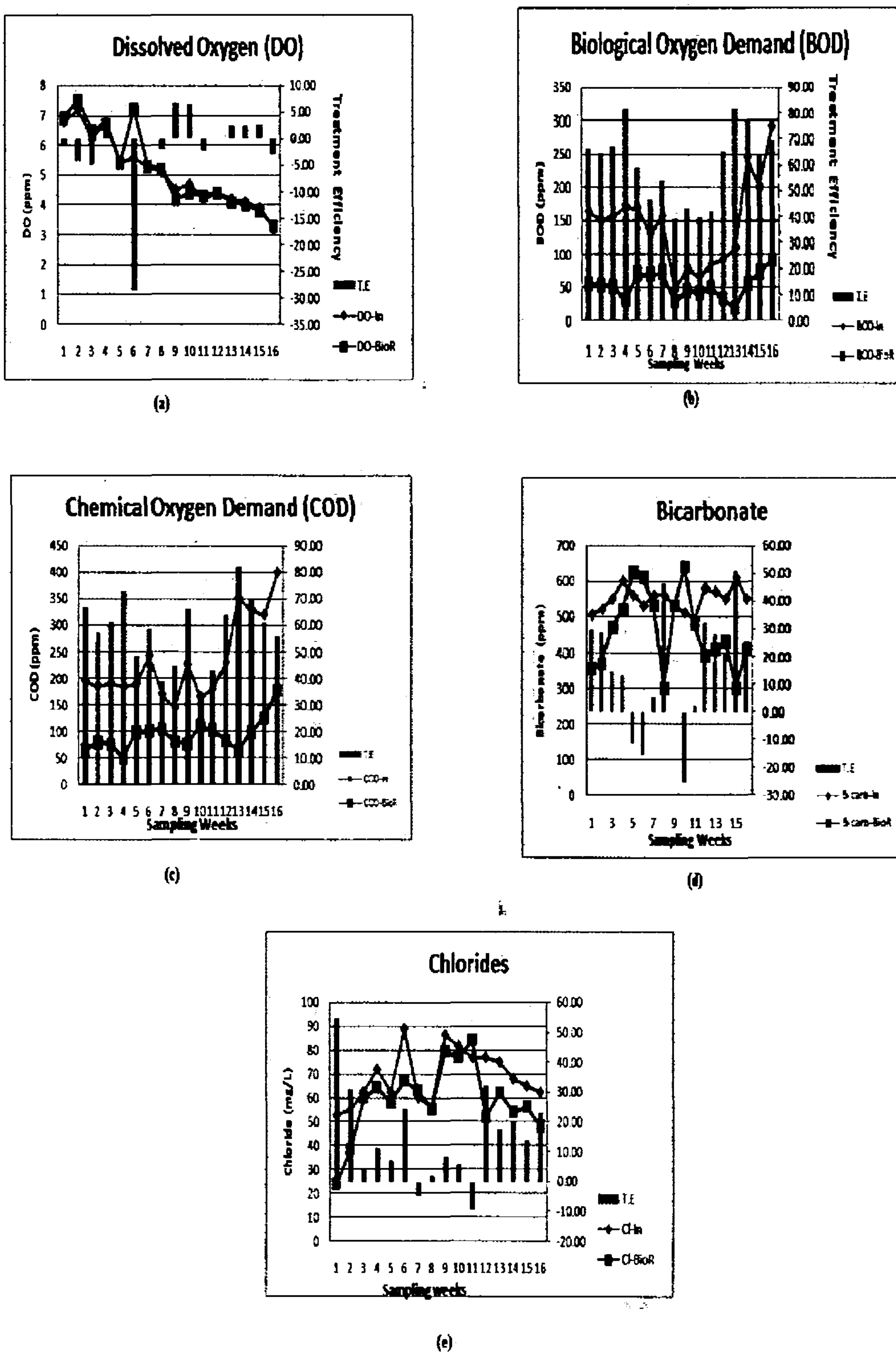


Figure 8. Temporal variations in selected chemical parameters of raw and bio-remediated wastewater: (a) DO, (b) BOD, (c) COD, (d) HCO_3^- and (e) Cl^-

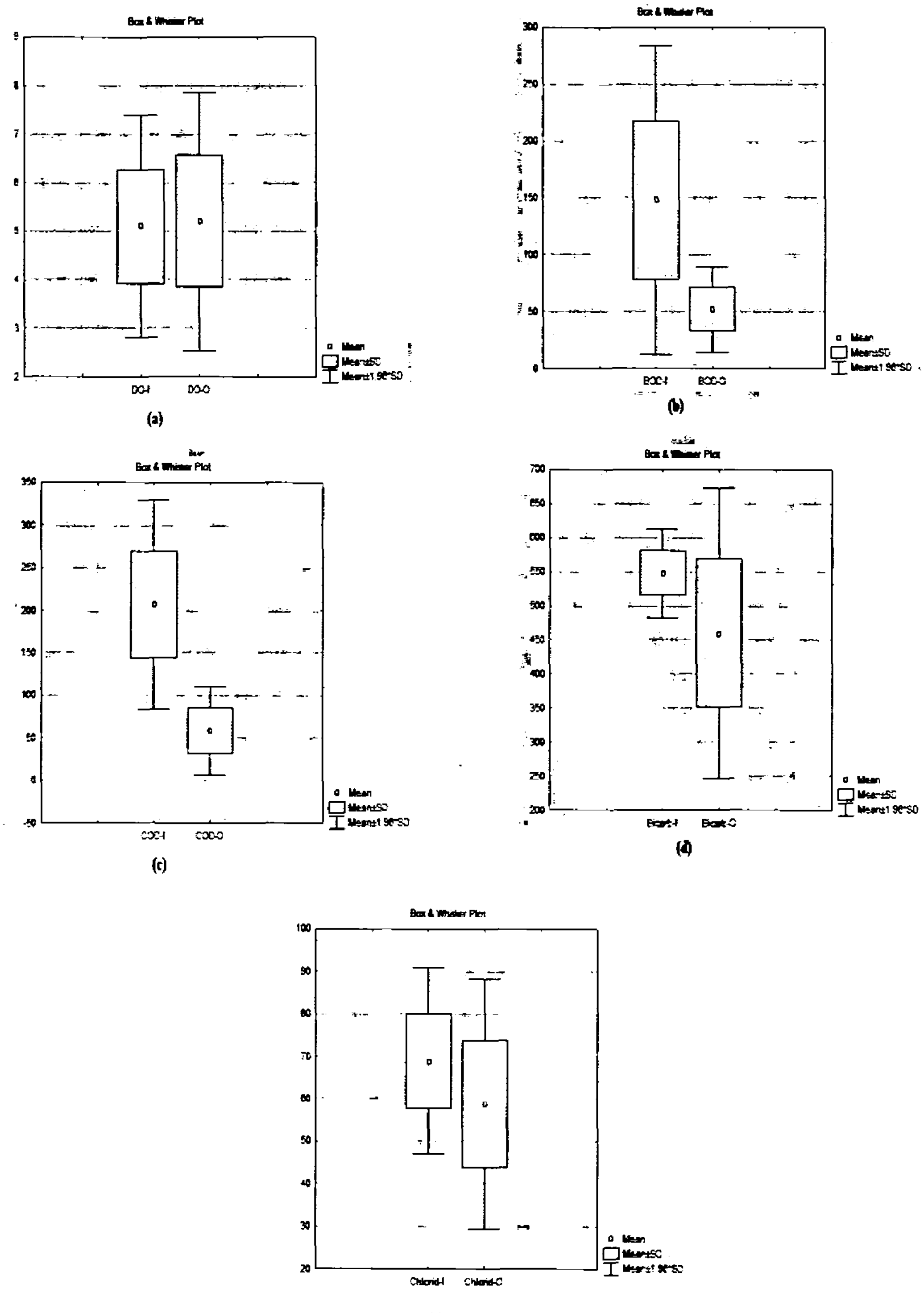


Figure 9. Box and whisker plots; (a) DO, (b) BOD, (c) COD, (d) HCO_3^- and (e) Cl^-

BOD was positively correlated with K^+ (r = 0.74), COD (r = 0.64) and negatively correlated with Cu^{2+} (r = -0.54) as shown in correlation matrix (Annex. 2). While PCA also showed BOD and K^+ in same factor explaining 10.5 % total variance (Table 3). CA showed that it is forming a group with K^+, salinity, bicarbonate and pH (Fig. 5).

It was reported that 10 - 20 ppm BOD is optimum range for aquaculture and fisheries (Chattophadyay *et al.,* 1988). Less than 20 ppm BOD is main requirement for aquaculture practices (Fosberg *et al.,* 1996). It was also reported that the mean concentration of BOD_5 in autumn and summer was higher than in spring and winter, suggesting a high load of dissolved organic matter was added from land-based resources, such as domestic wastewater, agricultural-related activities and industrial effluents (Wu *et al.,* 2009a).

3.3.3 Chemical Oxygen Demand (COD)

COD is the measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and water. It gives valuable information about pollution potential of sewage effluent in the aquatic systems.

COD values varied from 100 ppm to 352 ppm with 206.8 mean at raw point where as its values varied from 12 ppm to 109 ppm with 58.19 mean at bio-remediated point (Fig. 8c). The mean bioremediation treatment efficiency was found to be 71.7 % with highest about 80 % found in the end of March when Temp. had been significantly increased and was increasing efficiency along with other factors.

COD values of raw and bio-remediated point were fairly low in winter but found to be continuously increasing with Temp. till spring (Fig 9c). COD showed positive correlation with K^+ (r = 0.66), Cr^{3+} (r = 0.52) and Mn^{2+} (r = 0.66) (Annex. 2). CA showed that COD was present in Group 3 with Cr^{3+}, Ni^{2+} and Cl^- (Fig. 5).

3.3.4 Bicarbonate (HCO_3^-)

The Bicarbonate values ranged from 490 - 610 ppm at raw point with average value of 548.4 ppm. At bio-remediated Point, the average value of bicarbonates was found 460.5 ppm with 300 - 640 ppm range. The mean treatment efficiency was 16. 2 %. The values of bicarbonate were

T-test results indicated that both points were significantly varied from each other (p = 0.0) (Table 3). Correlation matrix showed that they were negatively correlated with sulphates (r = -0.59) (Annex.2). CA showed that they were falling in Group 2 with salinity, BOD, K^+ and pH (Fig.5).

3.3.5 Chloride (Cl^-)

The mean Cl^- concentration at raw point was 68.84 ppm with 52.80 ppm minimum and 88.8 ppm maximum where as at bio-remediated point, the values were tremendously reduced with 58.83 ppm mean and 24 and 84 ppm minimum and maximum respectively (Table 4). The treatment efficiency was found to be 14.5 %. No specific behavior of increase or decrease was found during study period (Fig. 8e). Measured concentrations were far greater than those reported in surface water of Rawal Lake in pre and post monsoon (Malik and Nadeem 2011). These values were present within range of NEQS (Annex. 2). CA showed that Cl^- was falling within Group 3 with Ni^{2+}, Cu^{2+}, Free CO_2 and COD. Correlation Matrix showed that it was correlated with Free CO_2 (r = 0.57), Ca^{2+} (r = 0.55) and Ni^{2+} (r = 0.51).

Presence of Cl^- in surface waters is mainly due to atmospheric deposition, weathering of sedimentary rock, from sewage effluents, agricultural and road runoff. It is an indicator of possible fecal contamination and a measure of the extent of dispersion of sewage discharge in water bodies (Chapman, 1996). In addition, activities related to urban and/or residential development, and atmospheric deposition can be significant sources of nutrients and other chemicals that impair water quality (Carpenter *et al.*, 1998).

3.3.6 Nitrate (NO_3^-)

The average concentration of NO_3^- ranged from 4.2 ppm and 13.2 ppm for raw wastewater to 2.8 ppm and 8.7 ppm for bio-remediated water during the study period (Fig. 10a). The treatment efficiency was found to be 90.2 % for nitrates. Average concentration of raw and bio-remediated waste water samples were found higher in both seasons. These temporal changes might be due to the fact that the nitrate loading is usually highest in winter and spring. Therefore, soil-water recharge in autumn and winter causes N-mineralization to be increased when soil is drying, followed by a re-wetting period (Peter *et al.*, 1997).

Nitrate may be from septic tanks, livestock wastes, fertilizers, municipal landfills and nonpoint sources of pollution, such as runoff from agriculture areas (Kotti *et al.*, 2005). It also gets into waterways from lawn fertilizer run-off, leaking septic tanks , manure from farm livestock, animal wastes (including fish and birds), and discharges from car exhausts. CA showed that nitrate was present within group 1 with biological parameters and oil (Fig. 5). PCA/FA showed that it was falling in Factor 1(explaining 40.2 % variance) with most of the heavy metals (Table 3).

In addition, the risk of NO_3^- leaching is particularly high after the harvest, if plant uptake is low, but N-release as mineralization continues Moreover, denitrification and leaching causes most 'N' loss from a catchment. For example, aerobic conditions created by ploughing enable ammonification and subsequent nitrification that results in NO_3^- releases from organic compounds in soils (Peter *et al.*, 1997).

3.3.7 Sulphate (SO_4^{-2})

The mean concentration for sulphates in raw wastewater varied from 39.6 ppm in March to 121 ppm in the start of January and showing an overall decrease (Fig. 10b). However in bio-remediated water, the mean concentration of sulphate varied from 31.2 ppm to 76.6 ppm. The treatment efficiency was found to be 24.4 %.

These values were far greater than measured pre and post monsoon values for Rawal Lake (Malik and Nadeem 2011). These values are falling within range of NEQS for municipal and industrial effluents (Annex. 2). Those did not show any significant correlation with other parameters. CA showed that they were falling within Group 3 with chloride, Ni^{2+}, Cu^{2+} and COD (Fig. 5). t-test showed a significant variance between two sampling sites (p = 0.01). High concentrations of phosphates are indicative of organic pollution which is largely responsible for eutrophic conditions.

Sulphates exist in nearly all natural waters, the concentrations varying according to the nature of the terrain through which they flow. They are often derived from the sulphides of heavy metals (Fe^{3+}, Ni^{2+}, Cu^{2+} and Pb^{2+}).

Iron sulphides are present in sedimentary rocks from which they can be oxidized to sulphate in humid climates; the latter may then leach into water bodies so that ground waters are often excessively high in sulphates. As Mg^{2+} and Na^+ are present in many waters their combination with sulphate will have an enhanced laxative effect of greater or lesser magnitude depending on concentration.

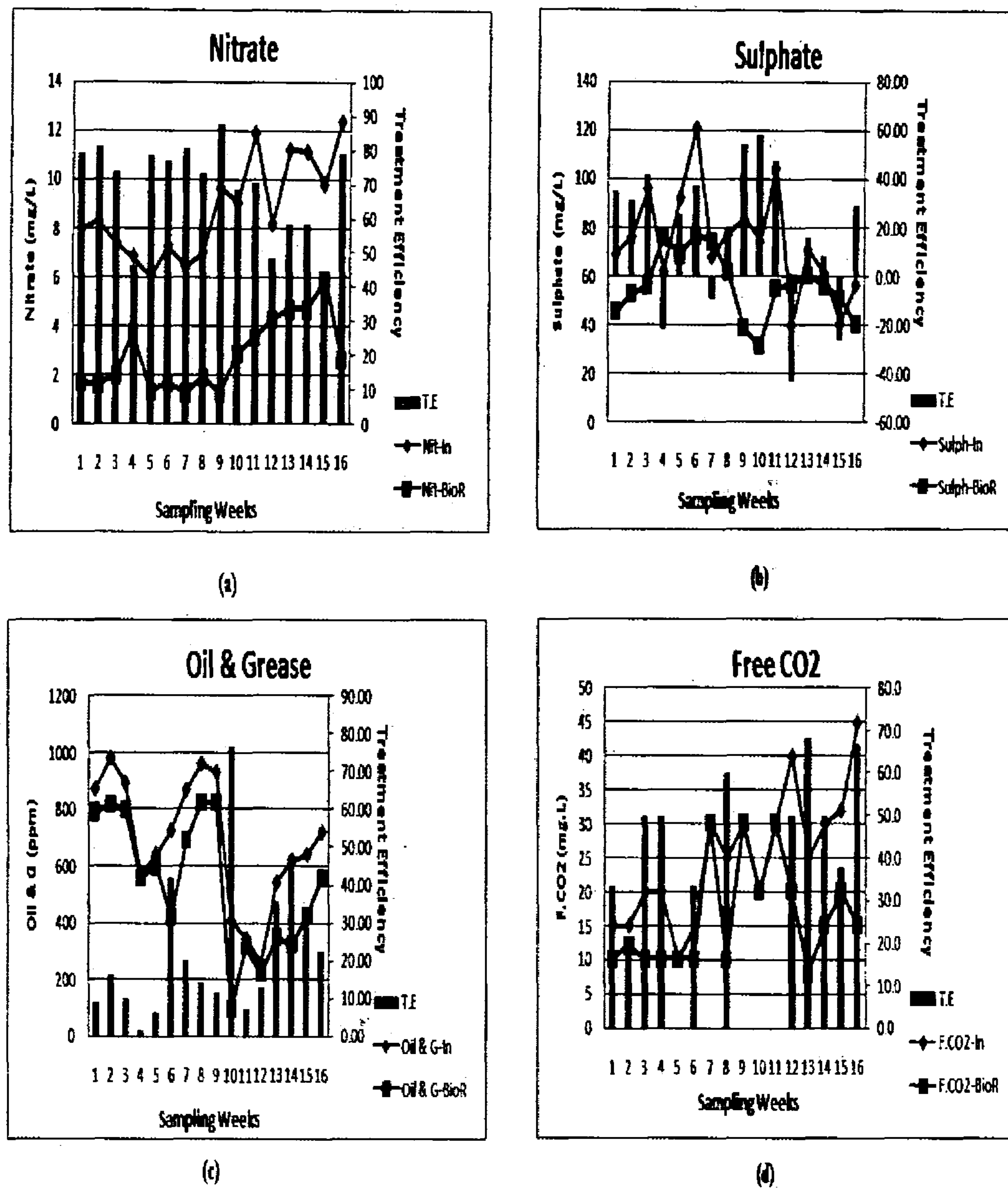


Figure 10. Temporal variations in chemical parameters of raw and bio-remediated wastewater (a) NO_3^-, (b) SO_4^{-2}, (c) Oil & Grease and (d) Free CO_2

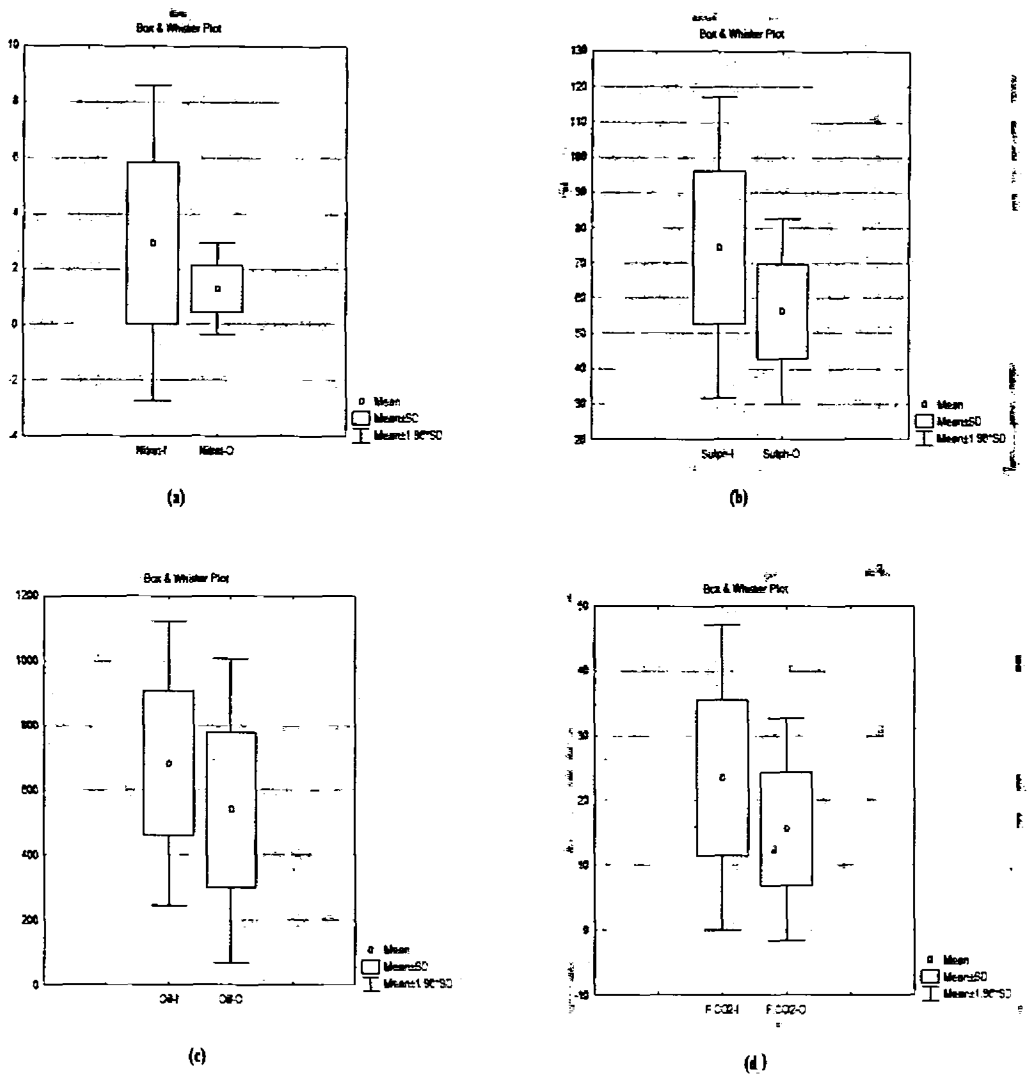


Figure 11. Box and Whisker plot for selected chemical parameters of raw and bio-remediated wastewater (a) NO_3^-, (b) SO_4^{-2}, (c) Oil and grease and (d) Free CO2

3.3.8 Oil and Grease

The mean concentration of oil and grease varied from 256.6 ppm minimum to 980 ppm maximum at raw point with 684.6 ppm mean (Fig. 10). While at bio-remediated point, its mean value was found 538.79 ppm with 94.4 ppm minimum and 824 ppm maximum (Table 4). The treatment efficiency was found 16.1 %. These values are far greater then mentioned in NEQS for municipal and industrial effluents (Annex. 2). Correlation matrix showed that oil and grease were negatively related with Cu^{2+} (r = -0.64), Fe^{3+} (r = -0.51), Cr^{3+} (r = -0.55) and positively correlated with Ni^{2+} (r = 0.66) and fecal coliforms (r = 0.6) (Annex. 3). CA showed that oil and Grease was falling in Group 1 with NO_3^-, Pb^{2+}, DO and Fecal coliforms (Fig. 5).

3.3.9 Free CO_2

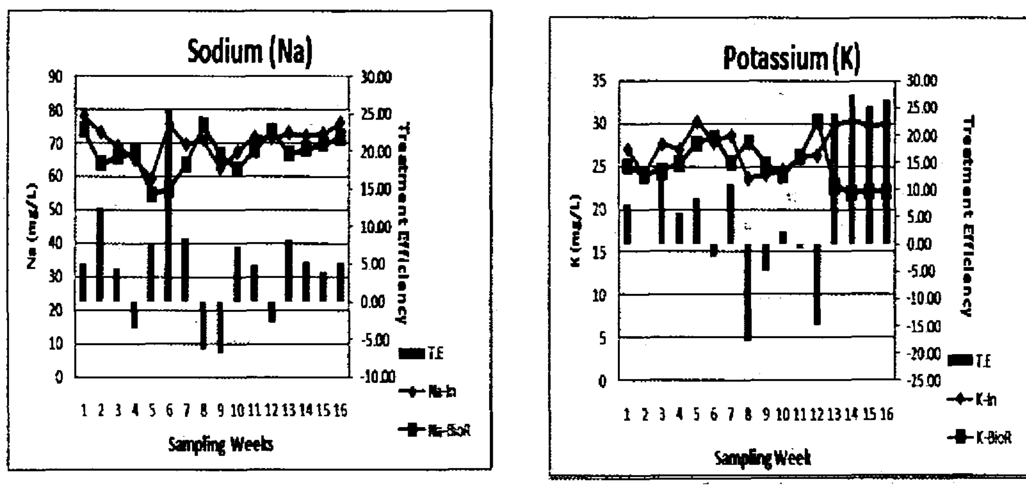
The mean value of Free CO_2 varied from 5 ppm minimum to 27.5 ppm maximum with 29.8 mean at raw point where as at bio-remediated point it was found between 0.00 ppm minimum to 30 ppm maximum with 15.6 mean (Table 4). The higher value at bio-remediated point attributed to microbial processes occurring in wastewater (Fig. 10). The treatment efficiency was found to be 47.6 %. Correlation Matrix showed that it was positively correlated with Mg^{2+} ($r = 0.56$) (Annex. 2). CA showed that it was falling in Group 3 with SO_4^{-2}, Cl^- and COD (Fig. 5).

CO_2 is an odorless, colorless gas produced during respiration. When CO_2 levels are high and oxygen levels are low, fish have trouble respiring and their problems become worse with rise in Temp. When several days of heavy cloud cover occur, plant's ability to photosynthesize is reduced. When that happens in a pond containing lots of plant life, fish can be hurt in two ways: by low dissolved oxygen and by high carbon dioxide levels (USEPA 1963, 1972). CO_2 quickly combines in water to form carbonic acid, a weak acid. The presence of carbonic acid in waterways may be good or bad depending on the water's pH condition. If the water is alkaline (8 - 14), the carbonic acid will act to neutralize it. But if the water is already quite acidic (0 - 7), the carbonic acid will only make things worse by making it even more acidic.

3.3.10 Temporal variations of metals in Raw and Bio-remediated water

Trace metals have been reported as common pollutants, which are widely distributed in the environment, can accumulate in aquatic environments and cause toxic effects on aquatic life and increase health risks of drinking water (Marian, 1991; O'Neil, 1993). These chemicals are at very low concentrations in the natural environment; however, the level of these metals in the environment has increased tremendously in the past decades as a result of inputs from human activities.

Total metal concentration in raw wastewater was in the order: $Ca^{2+} \geq Na^+ \geq Mg^{2+} \geq K^+ \geq Pb^{2+} \geq Fe^{3+} \geq Mn^{2+} \geq Ni^{2+} \geq Zn^{2+} \geq Cr3+ \geq Cd^{2+} \geq Cu^{2+}$, whereas in the bio-remediated water, the trend was slightly different: $Ca^{2+} \geq Na^+ \geq Mg^{2+} \geq K^+ \geq Pb^{2+} \geq Fe^{3+} \geq Ni^{2+} \geq Mn^{2+} \geq Zn^{2+} \geq Cr^{3+} \geq Cu^{2+} \geq Cd^{2+}$. Greater total mean concentration of metals such as K^+, Mn^{2+}, Zn^{2+}, Ni^{2+}, Mg^{2+} and Ca^{2+} were measured in raw wastewater, whereas their concentration significantly reduced in Bio-remediated water i.e. K^+ ($p = 0.02$), Mn^{2+} ($p = 0.0$), Zn^{2+} ($p = 0.0$), Ni^{2+} ($p = 0.0$), Mg^{2+} ($p = 0.02$) and Ca^{2+} ($p = 0.0$) (Table 5). Mean concentration of metals such as Cd^{2+}, Pb^{2+}, Cr^{3+}, Fe^{3+}, Cu^{2+} and Na^+ showed less variation as compared with other metals.



(a) (b)

Figure 12. Temporal variations in alkali metals (a) Na^+ and (b) K^+

The results indicated that most of the metals showed positive correlation: Ca^{2+} with Mg^{2+} ($r = 0.82$), Fe^{3+} ($r = 0.61$), Cr^{3+} ($r = 0.59$), and Pb^{2+} ($r = -0.52$); Mg^{2+} with Fe^{3+} ($r = 0.82$), Cr^{3+} ($r = 0.80$), Mn^{2+} ($r = 0.59$), Zn^{2+} ($r = 0.73$), Pb^{2+} ($r = -0.73$) and Cd^{2+} ($r = 0.61$); Cu^{2+} with Ni^{2+} ($r = 0.63$); Fe^{3+} with Cr^{3+} ($r = 0.94$), Ni^{2+} ($r = 0.55$), Mn^{2+} ($r = 0.88$), Zn^{2+} ($r = 0.83$), Pb^{2+} ($r = -0.72$) and Cd^{2+} ($r = 0.80$); Ni^{2+} with Cd^{2+} ($r = 0.51$); Mn^{2+} with Zn^{2+} ($r = 0.75$), Pb^{2+} ($r = -0.61$) and Cd^{2+} ($r = 0.73$); Zn^{2+} with Pb^{2+} ($r = -0.52$) and Cd^{2+} ($r = 0.87$). Na^+, K^+, Cd^{2+} and Pb^{2+} show no correlation with metals (Annex. 3)

Some of the metals also showed significant association with other physico-chemical parameters and nutrients (K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} with Temperature; K^+ with EC; Mg^{2+}, Cu^{2+}, Fe^{3+}, Cr^{3+}, Mn^{2+} Zn^{2+}, Pb^{2+}, Cd^{2+} with DO; Pb^{2+} with Salinity; Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} with Hardness; Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+} , Pb^{2+} with TDS; Mg^{2+} with BOD; Ca^{2+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+} , Cd^{2+} with COD; Ca^{2+}, Fe^{3+}, Ni^{2+} with Chloride; Ca^{2+}, Mg^{2+}, Cu^{2+},

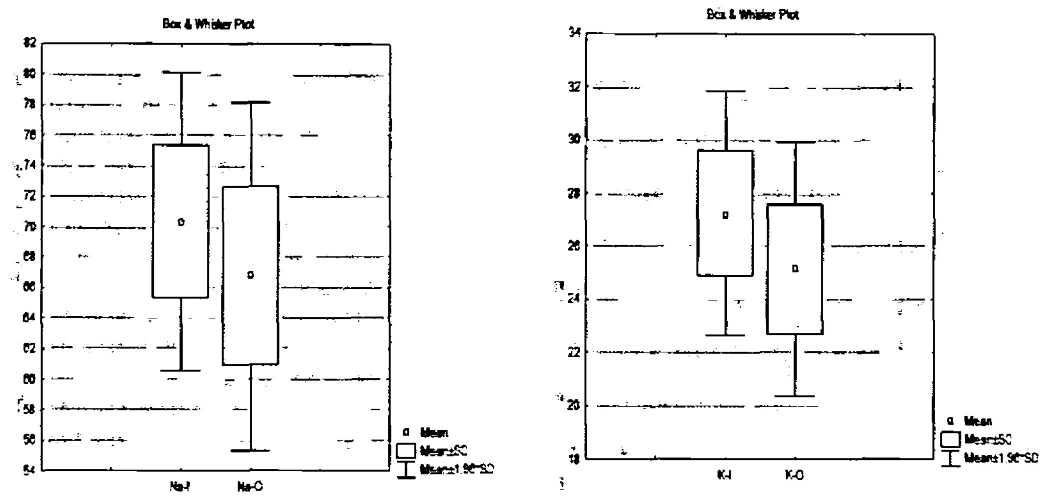
Fe^{3+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} with Nitrate; Cu^{2+}, Fe^{3+}, Cr^{3+}, Ni^{2+} with Oil & Grease and Mg^{2+} with Free CO_2 (Annex 3).

Table 5. Statistical summary of selected metals and raw and bio-remediated wastewater

Metals (ppm)	Sample type	Mean	Median	Mini.	Max.	Std.Dev.	St. Error	*p*- Value
Na^{+}	Raw	70.36	71.5	59.2	78	4.98	1.25	0.07
	Bio R	66.78	67.15	54.7	75.4	5.82	5.82	
K^{+}	Raw	27.25	27.3	23.6	30.2	2.35	0.59	***0.02***
	Bio R	25.13	25.1	21.9	30.2	2.44	2.44	
Ca^{2+}	Raw	169.79	173.63	120.5	199.38	22.82	5.7	***0***
	Bio R	140.11	140.35	112	160.7	16.14	16.14	
Mg^{2+}	Raw	50.03	53.4	22.62	64.76	12.96	3.24	***0.02***
	Bio R	40.3	42.5	19.95	54.8	9.72	9.72	
Cu^{2+}	Raw	0.02	0.02	0.01	0.02	0	0	0.1
	Bio R	0.02	0.02	0.01	0.02	0	0	
Fe^{3+}	Raw	0.21	0.23	0.13	0.27	0.05	0.01	0.16
	Bio R	0.19	0.19	0.12	0.28	0.05	0.05	
Cr^{3+}	Raw	0.04	0.05	0.01	0.06	0.02	0.01	0.59
	Bio R	0.05	0.04	0.01	0.09	0.03	0.03	
Ni^{2+}	Raw	0.12	0.1	0.09	0.22	0.04	0.01	***0***
	Bio R	0.07	0.09	0.01	0.11	0.03	0.03	
Mn^{2+}	Raw	0.17	0.17	0.09	0.27	0.08	0.02	***0***
	Bio R	0.1	0.1	0.05	0.21	0.03	0.03	
Zn^{2+}	Raw	0.1	0.11	0.06	0.12	0.02	0	***0***
	Bio R	0.07	0.08	0.03	0.1	0.03	0.03	
Pb^{2+}	Raw	0.1	0.09	0.08	0.17	0.02	0.01	0.43
	Bio R	0.09	0.09	0.07	0.16	0.02	0.02	
Cd^{2+}	Raw	0.08	0.09	0.05	0.1	0.01	0	0.32
	Bio R	0.07	0.08	0	0.1	0.03	0.03	

The existence of correlation between these parameters helps to improve understanding of the nature of metals and their speciation and to identify their sources in the aquatic environment (Qadir *et al.*, 2008).

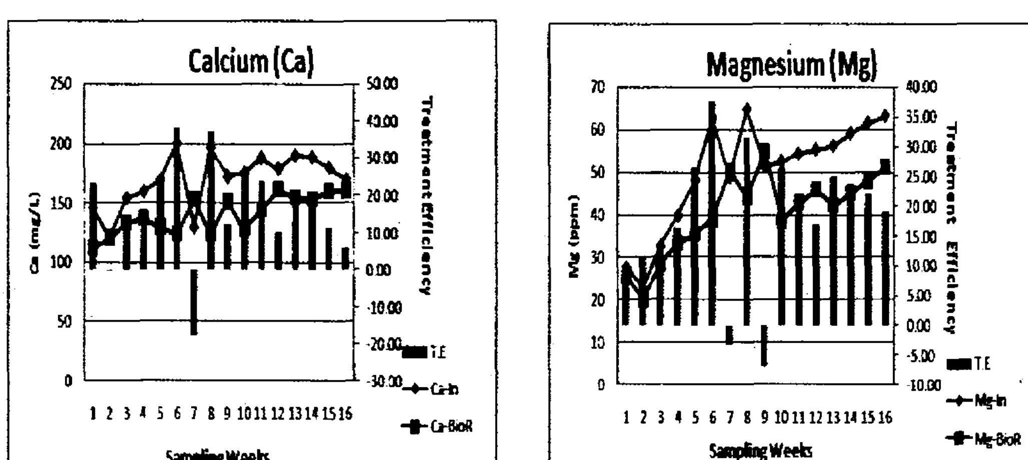
Amongst major elements, Ca^{2+} was dominant with concentrations varying from 120.5 ppm minimum to 199.38 ppm maximum in raw wastewater and from 112 ppm minimum to 160.7 ppm maximum in bio-remediated (Table 5). Ca^{2+} was related to Fe^{3+}, Mg^{2+}, Cr^{3+} and Pb^{2+} indicating a common source related to natural input from weathering of parent rock material. High Ca^{2+} concentration is generally associated with high Mg^{2+} contents (Fig.12 & 13).



(a) (b)

Figure 13. Box and Whisker plots for (a) Na^{+} and (b) K^{+}

Magnesium concentrations ranged from 22.62 ppm minimum to 64.76 ppm maximum in raw wastewater and from 19.95 ppm minimum to 54.8 ppm maximum in bio-remediated wastewater. Whereas Na^{+} concentrations varied between 59.2 ppm minimum to 78.00 ppm maximum in raw wastewater and whereas 54.7 ppm minimum to 75.4 ppm maximum bio-remediated wastewaters and were found as main nutrients for plant growth (Fig. 14).



(a) (b)

Figure 14. Temporal variations in alkaline earth metals of raw and bio-remediated wastwater (a) Ca^{2+} and (b) Mg^{2+}

Mg^{2+} was significantly associated with Zn^{2+}, indicating common sources related to effluents from urban areas. Main sources of Na^{+}, Mn^{2+}, and Fe^{3+} can presumably be attributed to local geological features. Measured mean concentrations of Fe^{3+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Cr^{3+}, Ni^{2+} and Zn^{2+} were within range of NEQS (Annex. 3).

Mean concentration of Zn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+}, Cr^{3+}, and Pb^{2+} and Mn^{2+} in raw and bio-remediated wastewater were within limits of NEQS (Annex. 3). Correlation analysis indicated that concentration of these metals in surface water is mainly controlled by anthropogenic inputs. Runoff water generally carries metals from municipal wastewater, and surface runoff from urban areas and agricultural land (Zacharias et al. 2002).

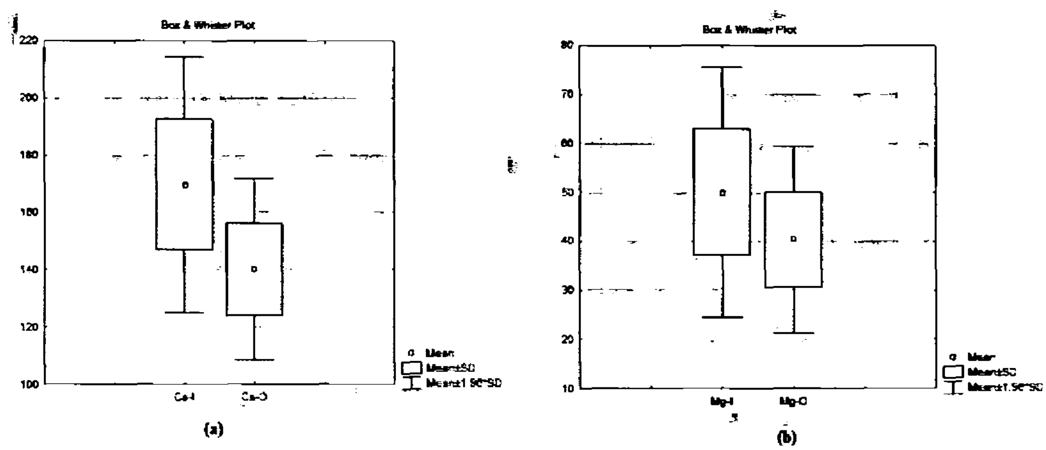


Figure 15. Box and Whisker plot for alkaline earth metals (a) Ca^{2+} and (b) Mg^{2+}

Pb^{2+} comes from sources such as metal manufacturing, sewage, paints, fertilizers, pesticides, and ashes (Qadir *et al.*, 2008). Pb^{2+} in aquatic environment may also come from automotive exhaust deposition and agricultural chemicals (Hanh *et al.*, 2010). This is probably the most efficient mechanism of anthropogenic Pb^{2+} contributions into the wastewater (Fig. 16).

Metal plating, electrical equipment, pesticides, paint additives, and wood preservatives are sources of copper. Cu^{2+} is also toxic to juvenile fish. Other toxicants that are associated with industrial effluent are mercury and silver.

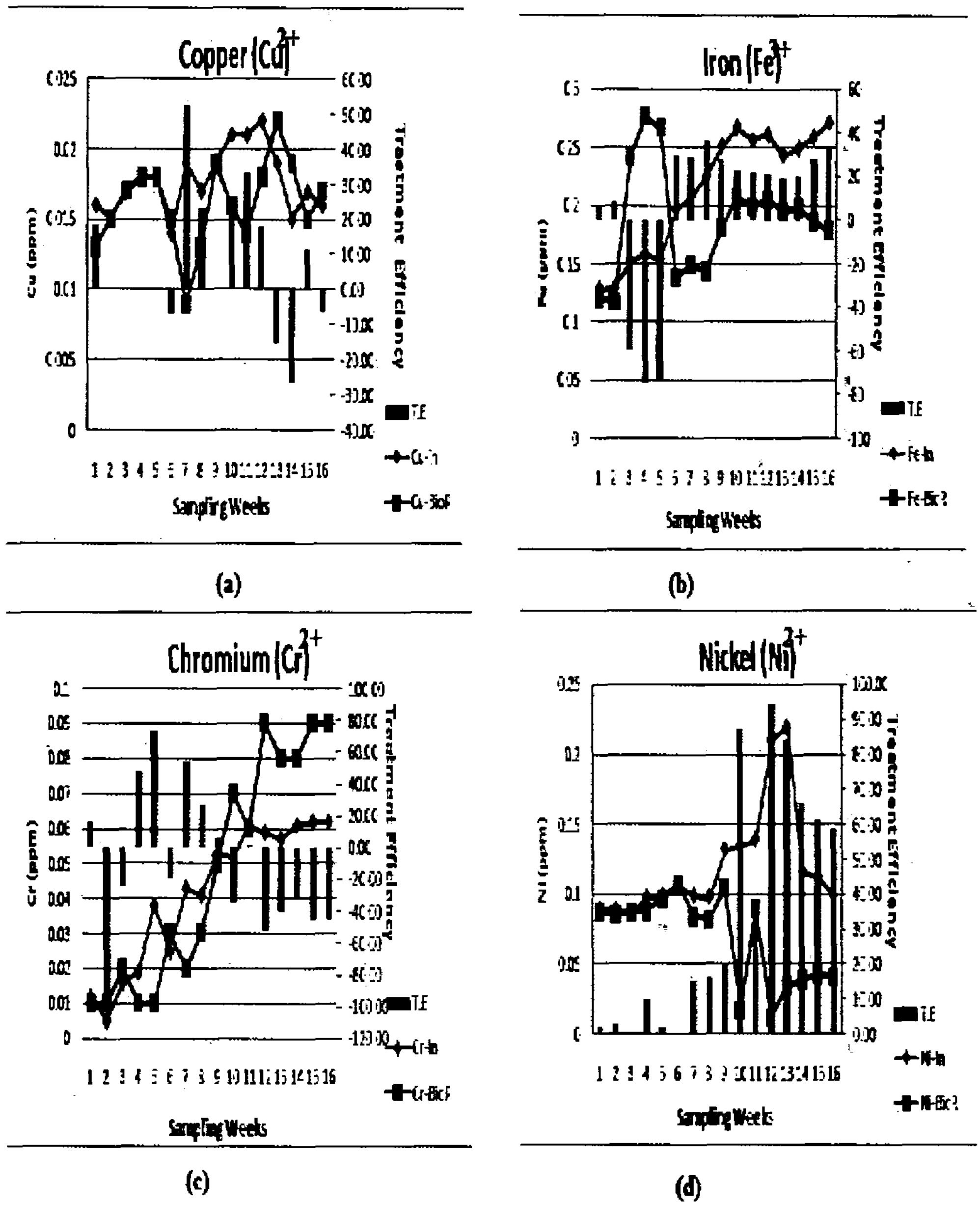


Figure 16. Temporal variations in heavy metals of raw and bio-remediated wastewater compared with treatment efficiency (a) Cu^{2+}, (b) Fe^{3+}, (c) Cr^{3+} and Ni^{2+}

Mercury and silver affect fish in ways similar to Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}. When fish are exposed to either of these at certain concentrations, gill tissues are damaged and death by asphyxiation can occur. Most of the detergents used contain trace amounts of heavy metals (Fig. 17).

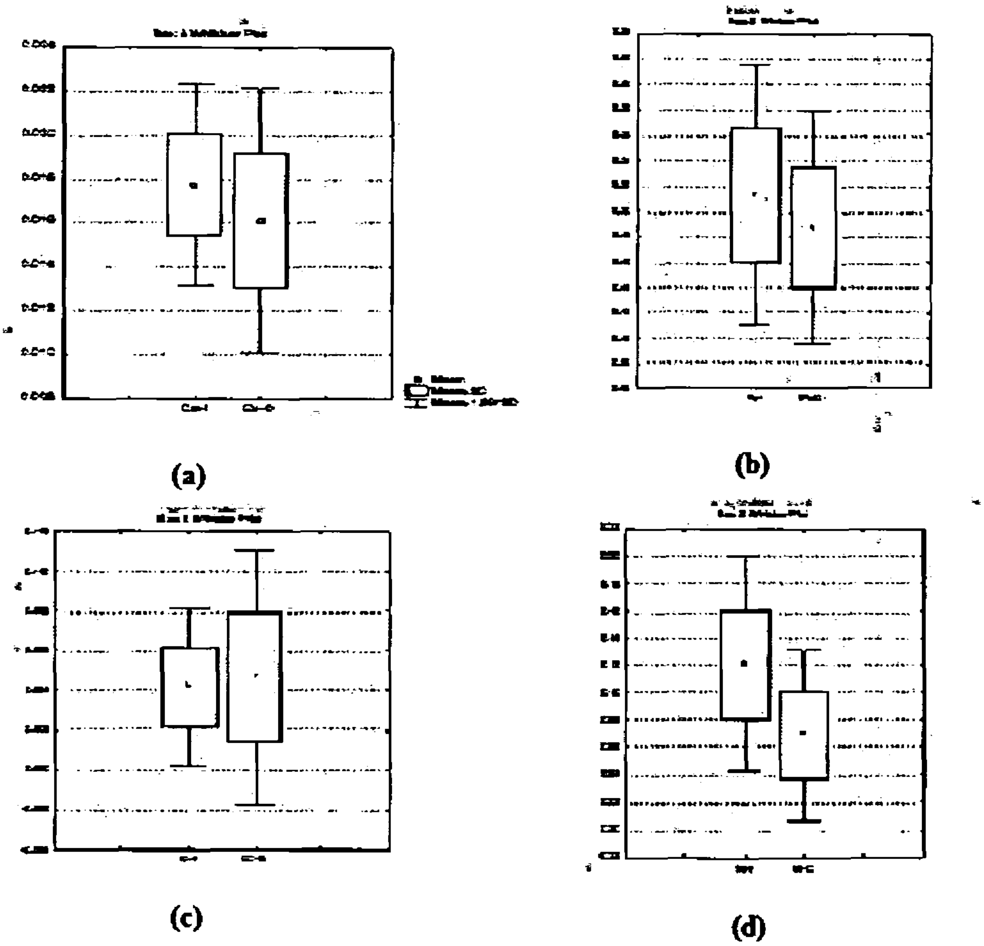


Figure 17. Box and Whisker plots for a) Cu^{2+}, (b) Fe^{3+}, (c) Cr^{3+} and (d) Ni^{2+}

Maximum concentration of Fe^{3+} was recorded in rainy season, because that has more solubility at acidic pH, due to this reason large quantities of iron are leached out from the neighboring soils through acidic water (acid mine drainage). It is also one of the most abundant elements of rocks and soils. The inter conversion of Fe^{3+} and Mn^{2+} both in their reduced and oxidized forms have a significant bearing on the water chemistry. With the intensity of precipitation of their hydroxides, phosphates may also get co precipitated. Excess sulphides can also be precipitated as ferrous sulphide. Water bodies generally have higher concentration of iron at the bottom sediments due to the prevailing reducing conditions.

Levels of Cu^{2+} in the raw wastewater and bio-remediated wastewater varied between 0.01 and 0.02 ppm respectively (Fig. 18). The background level and the WHO guideline for Cu^{2+} in domestic water supply is 1.00 ppm. The range obtained was lower than the set value, hence, adverse effects from domestic use are not expected as far as this parameter and the results obtained are concerned. The concentration of Zn^{2+} ranged between 0.064 ppm in raw wastewater and 0.12 ppm in bio-remediated wastewater. The concentrations obtained were far below the background level of 0.50 ppm (Annex. 3).

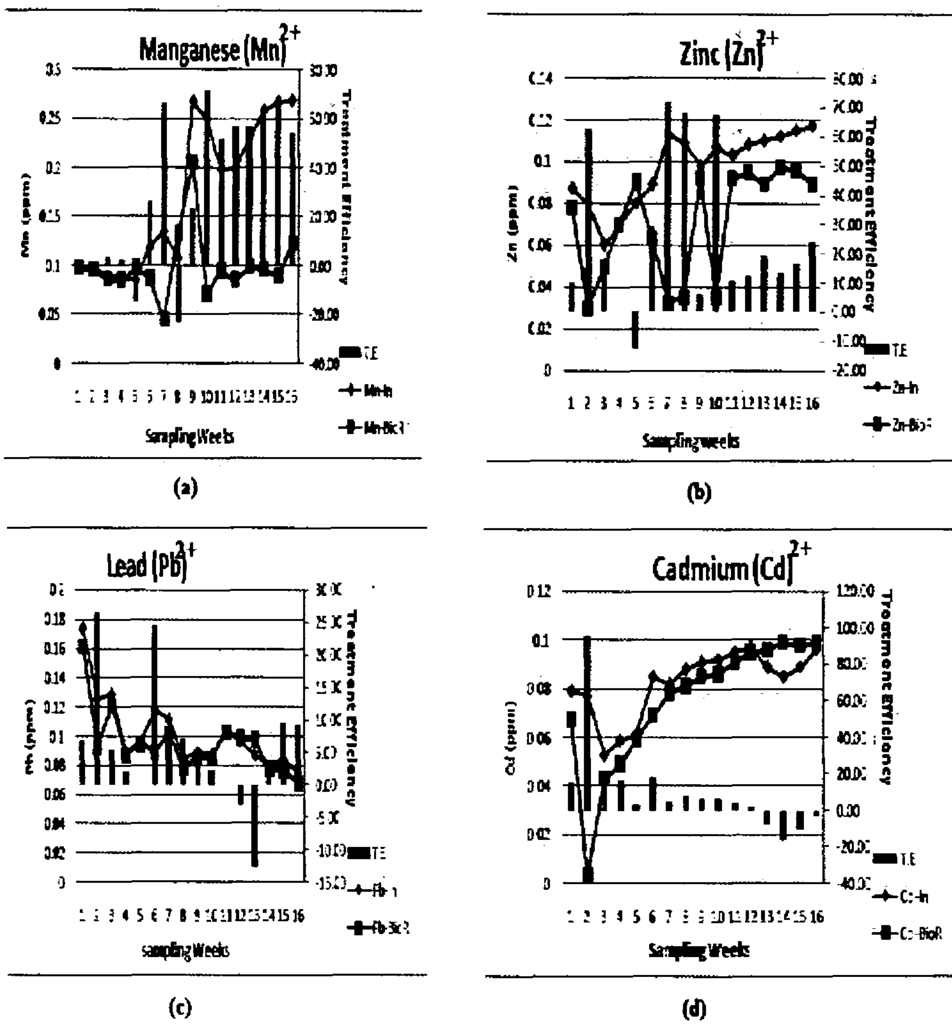


Figure 18. Temporal Variations in selected heavy metals of Raw and Bio-remediated wastewater compared with treatment efficiency; (a) Mn^{2+} and (b) Zn^{2+}, (c) Pb^{2+} and (d) Cd^{2+}.

Cd^{2+} is widely used in industry and is often found in solution in industrial waste discharges. It replaces Zn^{2+} in the body, and longterm consumption of Cd^{2+} may lead to bodily disorders. It is toxic to both humans and fish and seems to be a cumulative toxicant. Small salmon fry have been killed from concentrations of 0.03 ppm. The mean concentration of Cd^{2+} in bio-remediated water was 0.07 ppm which may be lethal for fish.

Pb^{2+} sources are batteries, gasoline, paints, caulking, rubber, and plastics. It can cause a variety of neurological disorders. In children, it inhibits brain cell development. It also prevents the uptake of Fe^{3+}, so people ingesting Pb^{2+} often exhibit symptoms of anemia including pale skin, fatigue, irritability, and mile headaches.

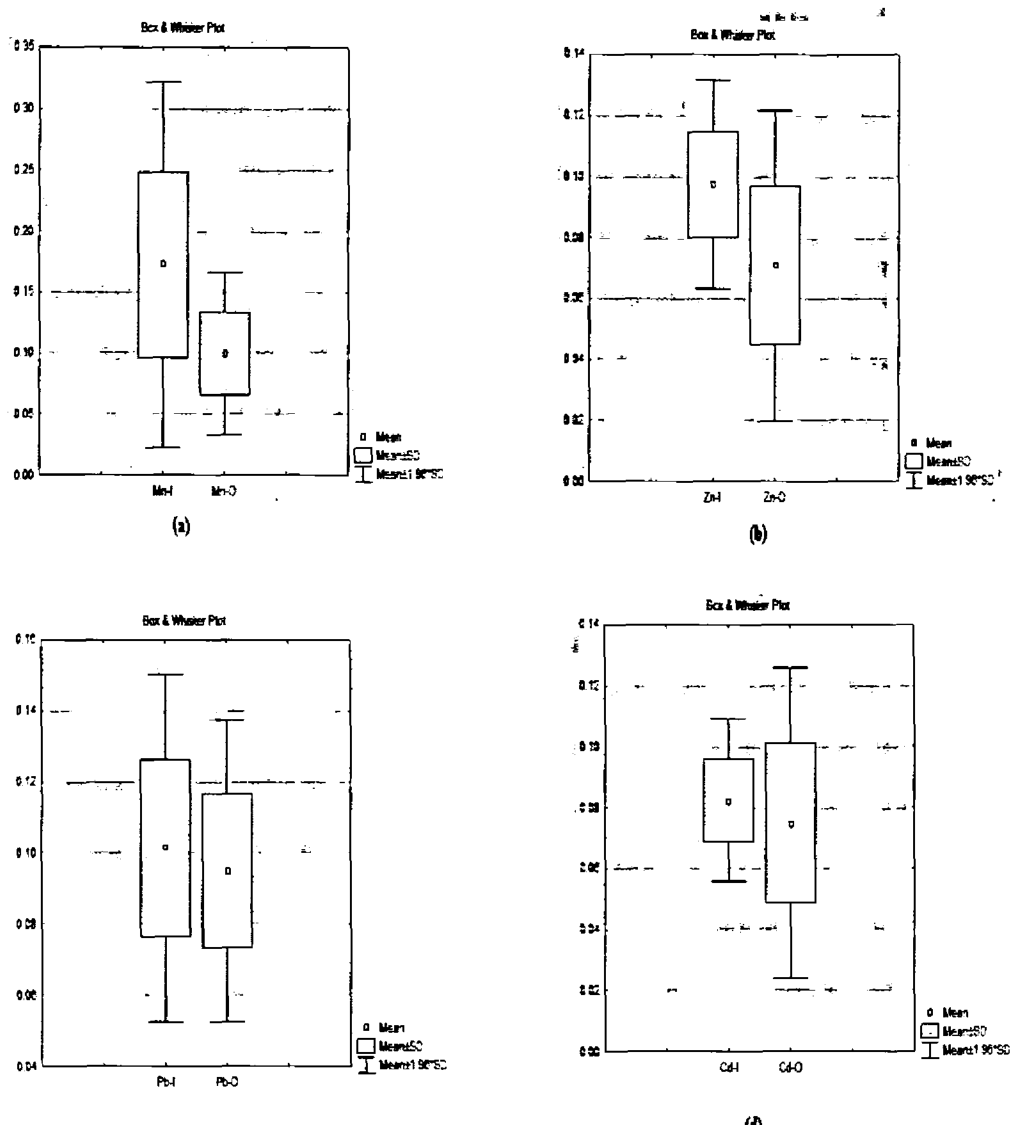


Figure 19. Box and whisker plots; (a) Mn^{2+} ,(b) Zn^{2+}, (c) Pb^{2+} and (d) Cd^{2+} for raw and bio-remediated wastewater samples

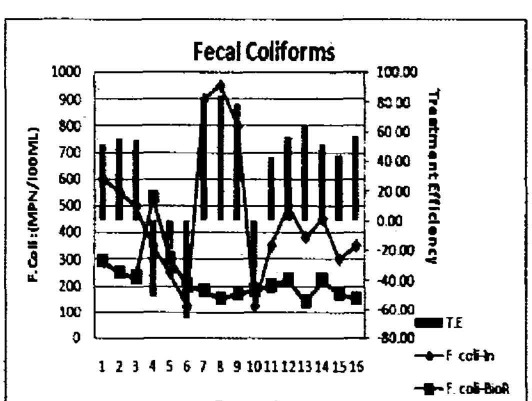
3.4 Temporal Variations in selected microbial parameters of raw and bio-remediated wastewater

The total coliforms ranged from 200 to 1500 MPN/100 ml at raw point while the fecal coliforms ranged from 120 to 950 MPN/100 ml. While at bio-remediated point, the total coliforms ranged from between 150 to 800 MPN/100 ml at raw point while the fecal coliforms ranged from 140 to 530 MPN/100 ml in bio-remediated wastewater (Table 6).

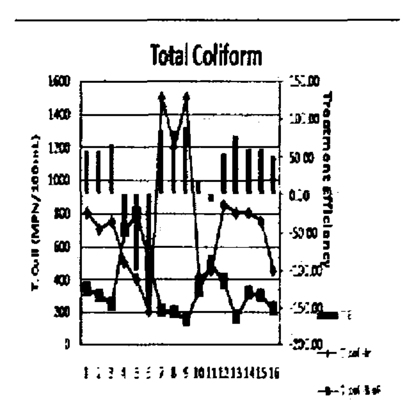
The mean treatment efficiency of fecal coliform was 52.07 % while mean treatment efficiency for total coliform was 52.8 %. Concentrations of both were significantly reduced after bio/phyto treatment process (Fig. 20 & 21). These were greatly affected by seasonal variations as microbial efficiency increase by increase in Temp. of wastewater. CA showed that total and fecal coliforms were present in group 1 with oil, Ec, Pb^{2+} and NO_3^- (Fig. 5). PCA/FA confirmed that these were third most important factor explaining 8.4 % total variations among all factors (Table 3).

Table 6. Statistical summary of selected microbial parameters of raw and bio-remediated wastewater

Variable	Sample type	Mean	Minimum	Maximum	Std. Dev.	St. Error	*P**-value
F. Coli (MPN/100 ml)	Raw	466.88	120	950	248.08	62.02	*0*
	Bio R	223.75	140	530	94.3	23.57	
T. Coli (MPN/100 ml)	Raw	753.75	200	1500	376.86	94.22	*0*
	Bio R	355.63	150	800	185.94	46.48	



(a)



(b)

Figure 20. Temporal variations in microbial parameters of raw and bio-remediated waste water (a) Fecal Coliform (b) Total Coliform

Total and fecal coliforms pollution were widespread, and the untreated sewage water samples were not suitable for irrigation without treatment as there is a possibility of contamination from vegetables and other crops eaten in their raw state.

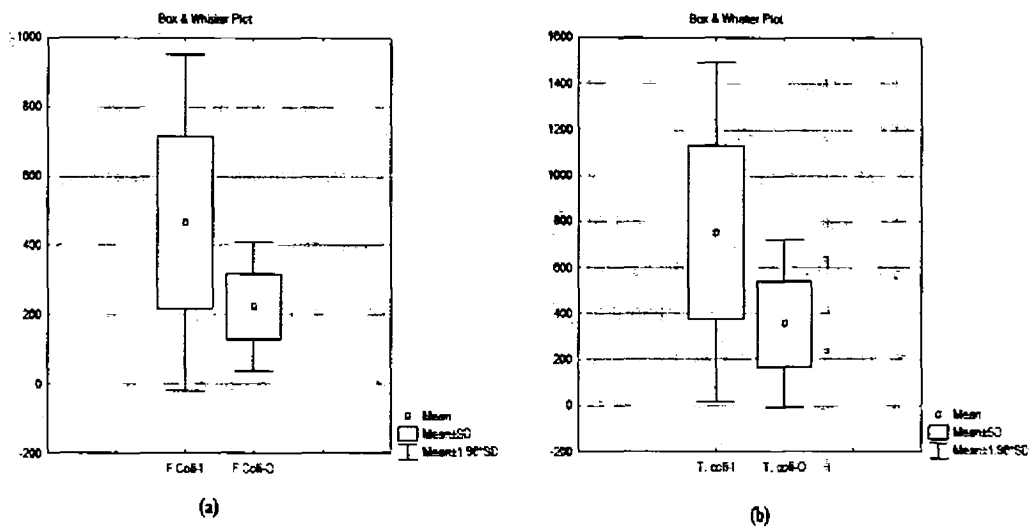


Figure 21. Box and whisker plots for (a) Fecal Colifom (b) Total Coliform

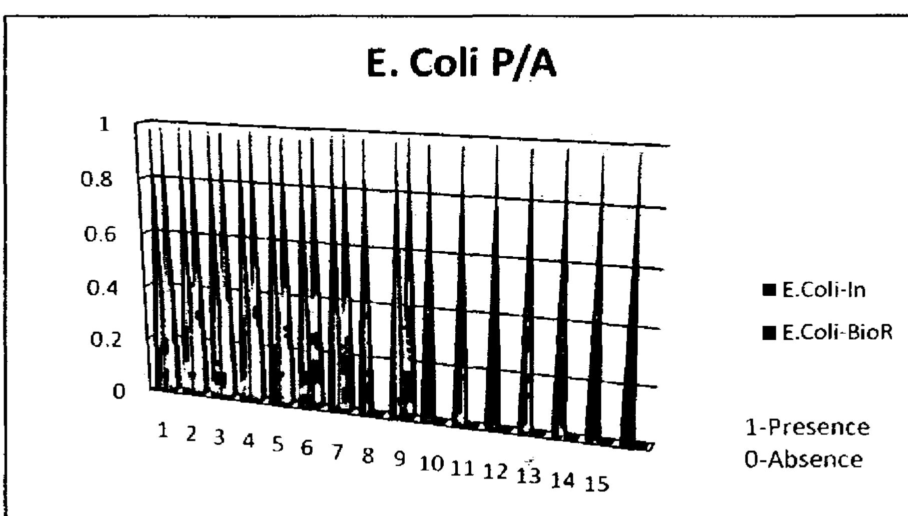


Figure 22. E. Coli presence/ absence in Raw and bio-remediated wastewater

E.coli was analyzed for presence/absence and it was found that in raw wastewater samples, E.coli was present throughout the study period. In bio-remediated wastewater samples E.coli was also present till first seven weeks and the found absent (Fig. 3.16c).

These results have indicated fecal pollution of the water sources, and imply that these water sources pose a serious health risk to consumers. The poor microbiological quality might be due to contamination caused by human activities and livestock. It is a common practice for people living along the river catchment to discharge their domestic and agricultural wastes as well as human excreta/wastes into rivers. In addition to use the river as a source of drinking water people use the source for bathing, washing of clothes and for recreational purposes such as swimming. Wild and domestic animals seeking drinking water can also contaminate the water through direct defecation and urination.

Chapter 4

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

Present study was conducted to evaluate temporal changes in selected parameters of raw and bio-remediated waste water during winter and spring seasons and was also conducted to measure treatment efficiency of bioremediation. Following are the important conclusions.

1. Studied variables were found strongly associated with changes in temperature, humidity, precipitation and other related environmental factors.
2. Determined concentrations of Dissolved Oxygen (DO) (5.1 ppm), Electric Conductivity (EC) (1826.5 µS/cm)[a], Nitrates (NO_3^-) (13.2 ppm)[b], Sulphates (SO_4^{-2}) (74.4 ppm)[c], Oil & grease (684.6 ppm)[d], fecal colifom (466.8 MPN/100 ml)[e] and total colifom (753.7 MPN/100 ml)[f] in winter raw wastewater samples were relatively higher than same samples collected in spring season with similar trends found in bio-remediated water season wise.
3. Alkali metals (Na^+; 70.36 - 66.78 ppm and K^+; 27.25 - 25.13 ppm), alkaline earth metals (Ca^{2+}; 169.7 - 140.1 ppm and Mg^{2+}; 50.03 - 40.3 ppm) and heavy metals concentrations (Cu^{2+}; 0.21 - 0.19, Fe^{3+} 0.4 - 0.5, Cr^{3+}; 0.12 - 0.07, Ni^{2+}; 0.17 - 0.1, Mn^{2+} 0.13 - 0.07, Zn^{2+}; 0.1 - 0.09, Pb^{2+}; 0.1 - 0.09 and Cd^{2+}; 0.08 - 0.07 ppm) in raw and bio-remediated water respectively were within NEQ's range for industrial and municipal effluents. However metals tend to bio-accumulate with the passage of time and increased expansion in urban/industrial developments in near future can amplify metals hazardous impact on soil, water, flora and fauna respectively.
4. The levels of Biochemical Oxygen Demand (BOD) (148 ppm), Chemical Oxygen Demand (COD) (206.8 ppm), EC [(a)], Total Dissolved Solids (TDS) (653.2 ppm), NO_3^- [(b)], SO_4^{-2} [(c)], Oil & Grease [(d)], and fecal coliform/total coliform [(e & f)] were far higher in raw wastewater and well above to NEQS. Such higher levels of fecal coliform/total coliforms and *E.Coli* pose serious health risks to farm workers and crop consumers. Suitable pH (mean; 8.03) and elevated TDS levels (mean; 625 ppm) in untreated wastewater favor excellent

growth of pathogens leading to risks of dysentery, cholera, typhoid and tuber closes among raw vegetable/fruit consumers.

5. Untreated discharge of wastewater to open grounds and fresh water channels can potentially result in soil and ground water pollution adding to environmental degradation. Bioremediation offers cost effective, eco-friendly solution to reduce chemical (DO; 5.2 ppm, BOD; 52.06 ppm, COD; 58.19 ppm, HCO_3^-; 460.5 ppm, Cl^-; 58.8 ppm, NO_3^-; 1.29, SO_4^{-2}; 56.2 ppm, oil & grease; 538.7 ppm and free CO_2 15.6 ppm), microbial (Total coliform; 355.6 MPN/100 ml, fecal coliform; 223.7 MPN/100 ml) contaminants in waste water through combined effect of micro-remediation and phyto-remediation.
6. The bio-remediated water can thus be safely applied for irrigation and aqua culture purposes reducing pressures on fresh water resources.

4.2 Recommendations

One only seemingly insignificant change that is required is a re-examination of negative attitudes towards wastewater use, which still seems to be prevalent among most planners, politicians and scientists. Instead of only thinking about it as a potential risk factor, domestic wastewater should also be considered as a potential resource.

- The results of this study suggest that domestic wastewater does require prior treatment before its use in agriculture because use of domestic wastewater for irrigation under controlled conditions is a better option than its disposal into open water bodies.
- Bioremediation is cost effective, environmental friendly technique to treat domestic and industrial wastewater, but it should be properly managed and monitored.
- Novel substrate materials in constructed wetlands are recommended for enhanced treatment efficiency.
- More aquatic plants must be used for phyto-treatment of heavy metals and contaminants

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ANNEXURES

Annexure 1

Preliminary survey indicating chemicals commonly used at household level in Shehzad Town

Date of interview........................

1. General information

Name of Respondent						
Relationship with household						
Employee	Govt.		Non Govt./ Business			
House #						
Sector/Colony/Muhallah						
Education						
Family members	Male		Female		Total	
Monthly income	≥50,000 Rs.		≤50,000 Rs.		≥25,000 Rs.	
House	Single portion		Double Portion			
Transportation	Car		Bike		Total	

2. Information regarding Water usage

Average Tank Size	≥ 500 Gal.		≤ 500 Gal.		Others (specify)	
Filling of Tank	Twice a day		Daily		Continuous	
Drinking water Source	Filtration Plant		Boring		Others (specify)	
Cloth washing	Daily		Weekly			

Car washing at home	Daily			Weekly		
Dish washing	Once daily		Twice daily		Thrice daily	

3. Information regarding domestic use of chemicals

Category	Item	Quantity used per month
Shampoo	Pantene	
	Head & shoulders	
	Sunsilk	
Soap	Dettol	
	Lux	
	Other	
Detergent	Arial	
	Surf Excel	
	Other	
Dish Washer	Lemon Max	
	Vim	
	Other	
Toilet Cleaner	Harpic	
	Acid	
	Others	
Oil/Ghee	Dalda	
	Season Canola	
	Other	
Cosmetics	Lipstick	
	Cold cream	

	Hair Gel	
Paint	ICI	
	Nippon	
	Others	
Toothpaste	Colgate	
	Pepsodent	
	Others	

4. **Are you satisfied with sewerage system in your area?**
   YES/NO

5. **How you conserve fresh water at home?**
   ____
   ____
   ____
   ____
   ____

Annexure 3

National Environmental Quality Standards for municipal and liquid industrial effluents in Pakistan (1995)

Parameter	Permissible limit (ppm)
Temperature (°C)	40
pH	6-10
BOD_5 at 20 °C	80
COD	150
Total Dissolved solids	3500
Oil & Grease	10
Chloride	1000
Sulphate	600
Cyanide	2
Floride	20
Sulphide	1
Ammonia	40
Pesticides, herbicides, fungicides and insecticides	0.15
Selenium	0.01
Barium	1.5
Cadmium	0.1
Chromium	1
Copper	1
Lead	0.5
Nickel	1
Zinc	5
Iron	2
Manganese	1.5
Chlorine	1
Boron	6