# Effect of Anthropogenic Activities on Water Quality and Fish Fauna of Nullah Aik and Nullah Palkhu - Tributaries of River Chenab, Pakistan

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# Certificate of Approval

This thesis, "**Effect of Anthropogenic Activities on Water Quality and Fish Fauna of Nullah Aik and Nullah Palkhu - Tributaries of River Chenab, Pakistan**", is hereby approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY in the field of Environmental Biology, Department of Plant Sciences, Faculty of Biological Sciences.

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To

# My wife

**for her endless support,**

**encouragement and ardour**

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# List of Abbreviation

ASL - Above Sea Level
Ca - Calcium
CA - Cluster Analysis
CCA - Canonical Correspondence Analysis
Cd - Cadmium
CEC - Commission for Environmental Council
$Cl^-$ - Chloride
COD - Chemical Oxygen Demand
CPC - Cleaner Production Centre
Cr - Chromium
Cu - Copper
D - Simpson Diversity Index
DFA - Discriminant Function Analysis
Dis - Dissolved Form
DO - Dissolved Oxygen
E - Species Evenness
EM - Effective Micro-Organism
EBL - Environmental Biology Laboratory
EC - Electrical Conductivity
EPA - Environmental Protection Agency
ETPI - Environmental Technology Programme For Industry
EU - European Union
FA - Factor Analysis
FDA - Food and Drug Administration
Fe - Iron
FSAAS - Fast Sequential Atomic Absorption Spectrophotometer
GPS - Global Positioning System
H´ - Shannon Diversity Index
HACA - Hierarchical Algorithmical Cluster Analysis
$HCLO_4$ - Perchloric Acid
$HNO_3$ - Nitric Acid
IBI - Index of Biological Integrity
IR - Impaired Region
K - Potassium

LC50 - Lethal Concentration to Kill 50% Population of an Organism
LIR - Less Impaired Region
LOEC - Lowest Observed Effect Concentration
MAF - Million Acre Feet
m - meter
m/sec - Meter per Second
Mg - Magnesium
mg/L - milligram per Litre
MRL - Maralla Ravi
Na - Sodium
NaCl - Sodium Chloride
Ni - Nickel
NMDS - Non-Metric Multidimensional Scaling
$NO_3^-$ - Nitrate
Pb - Lead
PCRWR - Pakistan Council for Research of Water Resources
$PO_4^{3-}$ - Phosphate
RUR - Relatively Unimpaired Region
S - Species Richness
$S^{2-}$ - Sulphide
Sal - Salinity
St. Vel. - Stream Velocity (Flow)
St.Dep - Stream Depth
St.Wd - Stream Width
Sus - Suspended Form
TDS - Total Dissolved Solids
Temp. - Temperature
T-Hard. - Total Hardness
Turb. - Turbidity
UC - Upper Chenab
USEPA - United State Environmental Protection Agency
WHO - World Health Organization
Zn - Zinc
µg/g - microgram per gram
µg/L - microgram per Litr

# Effect of Anthropogenic Activities on Water Quality and Fish Fauna of Nullah Aik and Nullah Palkhu - Tributaries of River Chenab, Pakistan

# Abstract

Nullah Aik and Nullah Palkhu are major tributaries of River Chenab in Pakistan and important water resources in district Sialkot. These streams receive industrial effluents, municipal sewage from Sialkot City, which degrade the water quality and disrupt the ecological integrity. Present study was designed to highlight the effects of human activities on water quality and fish fauna of these streams. For this purpose, water samples and fish samples were collected at 18 sampling sites on seasonal basis from September 2004 to July 2006. Water samples were analyzed for 38 parameters. Hierarchical Agglomerative Cluster Analysis (HACA) identified three different classes of sites: relatively unimpaired, impaired and less impaired regions on the basis of variations in water quality parameters. Discriminant Function Analysis (DFA) identified 11 water quality parameters viz; stream flow, stream depth, DO, COD, TDS, $NO_3^-$, $PO_4^{3-}$, Pb(dis), Cr(dis), Mg(sus), and Ni(sus), which showed significant spatial variations, whereas, major seasonal variations were observed in stream flow, temperature, EC, salinity, total hardness, Na(dis), K(dis), Ca(dis), Mg(dis), Fe(dis), Cd(dis), Cu(dis) and Na(sus). Factor Analysis (FA) identified the six sources of contamination such as municipal waste, industrial effluents, tanneries effluents, agricultural, urban runoff and parent rock material. COD, TDS, Fe (dis), Pb (dis), Cd (dis), Cr (dis) and Ni (dis) were found to be higher than the permissible limits. Seven heavy metals (Fe, Pb, Cd, Cr, Ni, Cu and Zn) were analysed in different organs (liver, gills, kidneys and muscles) of eight fish species. Significant variations in heavy metals accumulation were observed in organs of studied fish species. The concentration of Pb and Cr was recorded significantly between fishes captured from different sampling zones, whereas, Fe, Cd, Cr and Cu in fishes varied significantly between post monsoon and pre monsoon. The muscles of *Channa punctata, Labeo rohita, Cirrhinus reba, Puntius sophore and Wallago attu* captured from downstream of Nullah Aik and Nullah Palkhu exceeded the international permissible limits of Pb, Cd and Cr. A total of 24 fish species belonging to 12 families were recorded from Nullah Aik and Nullah Palkhu. Highest diversity indices were calculated at upstream of Nullah and downstream of Nullah Aik. Fish assemblage at upstream of Nullah Aik was stable, whereas, other stream segments showed seasonal variations. CCA identified the three groups of fishes viz., sensitive species, ubiquitous species and tolerant species, which were grouped on the basis of related to stream flow, stream depth, DO, COD, salinity, turbidity, $NO_3^-$ and heavy metals. Biological Integrity index

(IBI) was developed for the assessment of stream ecosystem degradation. A total of 12 metrics were calculated on the basis of taxonomic richness, habitat preference, trophic guild, stress tolerance and origin of species to develop stepped and continuous IBI criteria. HACA segregated the sites based on species abundance into three groups viz., reference, moderately impaired and impaired groups. Non-Metric Multidimensional Scaling (NMDS) was applied to identify underlying ecological gradient to highlight the habitat degradation. Sites located in upstream of Nullah Aik showed higher IBI scores, which dropped to its lowest in downstream sites near Sialkot city, which gradually improve far downstream. Spatial variability in IBI values is related as a function of surface water quality degradation. The results indicate that water quality and fish fauna of these streams are facing severe degradation due to unwise anthropogenic in the catchment area. The findings of present study are alarming and highlight that there is an urgent need to protect the natural streams from further degradation. These results can be helpful for future management of other polluted streams and small rivers in the same eco-region.

**Chapter 1**

# General Introduction

Water is a primary driving force for major physical, chemical and biological changes all over the world (Brady & Weil, 1996; Boyd, 2000). Oceans and seas contain approximately 97%, whereas, freshwater resources consist of 3% of the entire water reserve of the earth (Wilson & Carpenter, 1999). About 68.7% of freshwater is locked up in glaciers and icecaps on poles, 30.1% in ground water, 0.3% in surface water bodies and 0.9% in other forms (Gleick, 1996). It is not only main component of biosphere but also a major part of the living organisms (Jackson *et al.,* 2001b; Pandey*,* 2006). Life cannot be sustained more than few days without water, even inadequate supply of water change the pattern of distribution of organisms as well as human being (WHO, 2005).

Freshwater is a limited resource, which is essential not only for survival of living organisms but also for human activities such as agriculture, industry and domestic needs (Bartram & Balance, 1996). The history of freshwater resource utilization is as old as human civilizations (Gleick *et al.,* 2002). Water has also played a vital role in the evolution of human civilizations. Human social cultural evolution started in those areas, where adequate quantity and quality of freshwater was available. Most of the ancient human civilizations established around the freshwater resources such as rivers (Gupta *et al.,* 2006)*.* Early civilizations like Mesopotamian, Egyptian, Chinese and Indo-Gangatic civilizations developed around rivers to fulfill the needs of water for domestic, agriculture and irrigation. Several rivers such as Euphrates, Tigris, Nile, Yangtze, Indus and Ganges have been the lifelines for ancient civilizations (Wichelns & Oster, 2006).

Freshwater resources can be classified into three major categories such as lotic (rivers and streams), lentic (lakes and ponds) and ground water (aquifer). Rivers and streams are characterized by uni-directional flow with relatively high velocity > 0.1 m/sec (Meybeck *et al.,* 1989; Shreshtha & Kazama, 2007). Pristine rivers and streams exhibit stable aquatic ecosystem, which are rapidly degrading due to over exploitation to fulfil human demands (Hinrichsen *et al.,* 1998). Freshwater as a resource has never been an issue of concern until recent population explosion that has caused an immense pressure on water resources (Fischer *et al.,* 1997), which became worst with the

advent of industrial revolution and rapid expansion of urban areas (Douglas *et al.,* 2002). Rapid Industrialization, extensive urbanization, intensive agriculture practices and burning of fossil fuel are amongst anthropogenic activities, which have increased rapidly and have been considered important for changing the natural condition of an aquatic ecosystem (Dale *et al.,* 2005; Grimm *et al.*, 2000). The adverse effect of human activities have resulted in degradation of stream and riverine ecosystems (Schleiger, 2000), which ultimately alter the water quality and structure and function of aquatic biota (Stoddard *et al.,* 2006). Streams and rivers are facing number of environmental problems throughout the world largely associated with anthropogenic activities in their catchment areas (Young *et al.,* 2004). The pollution of freshwater resources is a matter of concern all over the world, especially in developing and poor countries. However, developed countries have adapted various technologies for the protection of water ways from pollution. High intensity of aquatic pollution is more critical in developing countries (Bozzetti & Schulz, 2004), where effluents are indiscriminately discharged directly and/or indirectly into rivers and streams without considering environmental protective measures (Pandey, 2006). Most of the developing countries do not have infrastructure to control water pollution and enforce water quality standards (Hinrichsen *et al.,* 1998).

Natural as well as anthropogenic factors determine the water quality of an aquatic ecosystem, which is essential for biological communities and maintain the human demands (Scott *et al.,* 2002; Schoonover *et al.,* 2005; Pandey, 2006; Singh *et al.,* 2007). Information of surface water quality is an inevitable component for the assessment of pollution and long-term environmental impacts assessment of human activities in a particular area. Generally, pristine aquatic systems exhibit less variation in water quality parameters in comparison to those of polluted aquatic ecosystems. Any significant human activity in the catchment area can produce huge volume of pollutants such as heavy metals, organic pollutants, nutrients, salts and other synthetic compounds (Miller, 2002), which alter the water quality and consequently disintegrate the ecological integrity of lotic ecosystems (Lytle & Peckarsky, 2001; Brown *et al.,* 2005).

Downstream ecology of streams and rivers moving across cities and industrial areas are facing severe degradation; higher losses in biotic integrity and many of these fresh water resources have become unsafe for human consumption (Gafny *et al.,* 2000; Lima-Junior *et al.,* 2006). Among lotic system, streams are more at risk to

anthropogenic activities in comparison to rivers because most of the streams experience extreme fluctuation and discharge as compared to river (Paul & Meyer, 2001). Streams traversing from urban area are more vulnerable to human activities and display irregular discharge, altered water chemistry, disturbed and disrupted food chains (Brown *et al.,* 2005).

Spatial and temporal variations in water quality of streams are shaped by natural as well as human activities in the catchment area. The effects of pollutants are mostly high near the source, however; it becomes diluted as water traverses the distance (Qadir *et al.,* 2008). Streams affected from industrial activities and urbanization display more prominent spatial variations. Many authors have highlighted the deleterious effects of natural and anthropogenic factors on water quality on spatial and temporal basis worldwide (Simeonov *el al.,* 2000; Alberto *et al.,* 2001; Fernández-Turie *et al.,* 2003; Anazawa *et al.,* 2004; Kotti *et al.,* 2005; Singh *et al.,* 2004; Singh *et al.,* 2005a; Zeng & Rasmussen, 2005; Qadir *et al.,* 2008). Natural processes such as rainfall, weathering of parent rock material, erosion, surface runoff, sediment transport, changes in stream hydrology and flow influence water quality on seasonal basis. Anthropogenic factors include a range of activities that can degrade the water quality, depending upon the intensity and duration of contribution from point and non-point sources (Tarvainen *et al.,* 1997). A point source is a point at which pollutants are discharged directly into surface water at a particular point and can easily be recognized (Fang *et al.,* 2005) such as industrial effluents and domestic sewage discharged into streams or rivers without prior treatment (Connell *et al.,* 1999). Generally, more inlets of point sources are located in urban and industrialized areas, compared to those in non-urban areas (Fitzpatrick *et al.,* 1995). Point sources contribution become very high, where people and authorities don't follow the environmental rules and regulations; such situations exist mainly in poor and developing countries (Maillarda & Santos, 2008).

Non-point sources pour pollutants in streams from multiple locations or sites such as after rainfall through surface runoff from urban and agricultural areas (Choi & Blood, 1999; Kyriakeas & Watzin, 2006). The gaseous and suspended particulate matter from burning of fossil fuels and industrial emissions can get deposited on soil surface or on urban structures. During rainfall, these atmospheric deposits erode or dissolve into rainwater and enter into streams via multiple inlets (Davis *et al.,* 2001). Fertilizers, pesticides and soil improving agents are used to increase the crop

production. After heavy irrigation or rainfall, these additives get dissolved and leach down to ground water or moves to streams/rivers with surface runoff after soil saturation (Tsihrintzis & Hamid, 1997). The concentration of pollutants in surface run off varies from one place to another e.g. surface runoff from industrial area possesses high concentration as compared to un-inhabited areas (Sanger *et al.,* 1999). Non-point sources are usually scattered or diffused making the source identification a difficult task.

Metals are one of the important contaminant group responsible for deterioration of surface water quality, which either originate naturally from parent rock material as a result of weathering or contributed from anthropogenic process (Selin & Selin, 2006). Alkali metals (Na, K) and alkaline earth metals (Ca, Mg) are found abundantly in earth's crust, whereas, heavy metals are present in trace amount. Heavy metals such as Fe, Cr, Ni, Cu and Zn are essential in living organisms because of their structural and functional roles in various physiological processes (Wepener *et al.,* 2001), whereas, non-essential metals have no known role in metabolic functions of the organisms and are toxic even in trace amount. Essential heavy metals are required in trace quantities by organisms and if their concentration exceeds the threshold level become toxic (Wright & Welbourn, 2002). Toxic effects of heavy metal vary according to their position in food chain. At higher trophic levels, their effect of heavy metals become more conspicuous among aquatic organisms (Devlin, 2006; Rasmussen *et al.,* 2008).

In an aquatic ecosystem, metals are present either in dissolved form or bind with suspended particulate matter. Dissolved heavy metals are bio-available and highly toxic to aquatic organisms, whereas, metals in particulate matter are comparatively stable and less toxic (Morrison *et al.,* 1990). It is difficult to estimate the biological availability of metals; however, the dissolved content of metals gives a general estimate of available metals (Charlesworth & Lees, 1999). Particulate metals settle down in the form of sediments in stream bed may build up to high concentrations with the passage of time (Colman & Sanzolone, 1992). Availability of heavy metals depends upon several factors such as pH, organic matter, electrical conductivity, salinity and total hardness (Wright & Welbourn, 2002). Other factors such as turbidity, flow rates and size can also influence the availability of metals and metal load in surface water (Caruso *et al.,* 2003).

Heavy metal pollution is a long term and irreversible process that affects the productivity of aquatic ecosystem, may lead to complete loss of species and biological communities and disrupt the structural and functional integrity (Majagi *et al.,* 2007). Heavy metals in aquatic medium are harmful for aquatic biota even at very low concentration (Schüürmann & Markert, 1998). Aquatic organisms can absorb heavy metals from their surrounding aquatic environment and accumulate in their bodies. This process may accelerate if the concentrations become elevated in aquatic environment. The absorbed heavy metals in organisms can bind with cellular components (nucleic acids and proteins) and interfere with metabolic processes (Zhang & Casey, 1996) that leads to genotoxic, neurotoxic, mutagenic and teratogenic effects (Lachance *et al.,* 1999; Das, 2007). Heavy metals disrupt the physiology and histology of aquatic organisms that may leads to death (Sehgal & Saxena, 1986). There are several physical and chemical factors, which affect the bioavailability of heavy metals (Smolders *et al.,* 2004), which are transferred from aquatic medium to bodies via ingestion of food and suspended particulate matter while metal ion exchange through gills and skin (Nussey *et al.,* 2000). The level of heavy metal accumulation depend on metal type, distance from the metal source, season, type of species and trophic level (Asuquo *et al.,* 2004; Terra *et al.,* 2008). Accumulation of heavy metals varies from species to species (Wright & Welbourn, 2002).

The loss of biological communities in any stream results due to deterioration of water quality. Aquatic organisms are good biological indicators of water pollution in a river or stream. Biological indicators are sensitive to any change in physical and chemical change in stream water, which directly or indirectly influence the biological communities. Ecological health of an aquatic ecosystem can be analysed through biological indicators on the basis of their presence or absence, relative abundance, community structure and function (Karr *et al.,* 1986; Landres *et al.,* 1988). Aquatic organisms such as algae, invertebrates, fishes and amphibians are important organisms for understanding the impacts of human activities on stream ecosystem.

Among aquatic organisms, fishes are good indicators of pollution stress and have wide range of tolerance. Fishes respond to change in physical, chemical and biological conditions of aquatic ecosystem caused by human activities (Plafkin *et al.,* 1989). The distribution of stream fishes is influenced by different local physical characteristics, as well as regional interactions (Smith & Kraft, 2005). Fishes are sensitive to any type of human disturbance such as inflow of industrial effluents,

municipal waste, stream discharge, habitat alteration and fragmentation (Pont *et al.,* 2006). Sensitive species indicates the stream health in a better way in comparison to tolerant species. However, tolerant fish species are adapted to unfavorable conditions created by natural or anthropogenic factors and can recover rapidly as the stress reduces. Natural factors viz; drought and flood conditions influence the structure and function of fish communities. Flood increases diversity in fish assemblages in irregular streams by connecting isolated pools and creating favourable conditions for the fish movement barriers (Taylor & Warren, 2001). Anthropogenic activities strongly influence the distribution, migration, colonization, and re-colonization of fishes (Magalhães *et al.,* 2002). In addition to water quality, physical characteristics of stream also influence the distribution and pattern of fish assemblage (Ghosh & Ponniah, 2001). Most of the temperate streams exhibit well marked spatial and temporal variations in physical characteristics of water quality that also determines the fish assemblage (Arunachalam, 2000). Unwise human activities in catchment area have significant impact on species composition within ecological communities which results in disappearance of native fish species and appearance of exotic species (Karr *et al.,* 1986; Lyons *et al.,* 2000). Change in species composition in a stream converts the complex and stable ecosystem into fragile one and disrupts the functional ecological integrity of an aquatic ecosystem (Lyons *et al.,* 2000).

Index of Biological Integrity (IBI) is an important index to determine the stream health on the basis of information from various tiers of aquatic ecosystem such as individual organism, population, community and ecosystem levels and express health status of any aquatic ecosystem into an index (Karr *et al.,* 1986). This index is used to measure the intensity of anthropogenic impacts on natural environmental processes inside stream ecosystem and to take the managerial decision about restoration activities (Yeom & Adams, 2007). Different aquatic organisms have been introduced as candidate organisms for the biological assessment of streams and rivers but fishes and other invertebrates are frequently used as model organisms. Several authors in different parts of the world used fish as organism to determine the health conditions of stream and rivers (Ganasan & Hughes, 1998; Kleynhans, 1999; Wang *et al*. 2003; Toham & Teugels 1999; Bozzetti & Schulz, 2004; Breine *et al.,* 2004; Joy & Death, 2004; Yeom & Adams, 2007).

IBI is formulated on the basis of various biological aspects such as species diversity, stress tolerance, habitat preferences, feeding behaviours and origin of

species (Ganasan & Hughes, 1998). IBI has been applied successfully on streams and small rivers in various geographical areas all over the world since last two decades. IBI provide overall information about fish species at community level in stream ecosystem.

The importance of protection, restoration and management of aquatic resources has been realized all over the world (Nakamura *et al*., 2005). Indiscriminate human activities have destroyed the ecological and biological integrity of aquatic resources and make unfit for resource utilization in many parts of the world (Karr & Chu, 1999). Therefore, the protection and management of aquatic resources must be ensured for sustainable agricultural, domestic, recreational and industrial uses. The knowledge about biological integrity and impacts of anthropogenic activities in the catchment area of urban streams provides an understanding for protection, conservation and restoration of stream ecosystem. Unwise and indiscriminate industrialization, urbanization and intensive agriculture in developing countries are putting the aquatic resources under threat of degradation. Pakistan is also among those developing countries, where aquatic resources are facing the severe degradation.

## Problem Statement

Pakistan exhibits diverse climatic conditions and is enriched with plenty of freshwater resources (Khan, 1991). Rivers, canals, streams and dams, which are the major surface water resources of Pakistan and lifeline of its irrigation system, currently irrigates over 36 million hectares of land (Alam & Naqvi, 2003). About 7.8 million hectors of freshwater in Pakistan has been estimated, including 3.1 million hectares of rivers and streams (Naik, 1985). Pakistan is among those few countries, which have well developed irrigation system for irrigating its vast planes (Punjab and Sindh). Rivers and streams are flowing down from Himalayas and Karakoram to Indus plains provide base for the world largest irrigation system, which plays a vital role in agriculture and energy generation (Alam & Naqvi, 2003).

Pakistan is struggling to develop its industrial sector along with agriculture to fulfil demands for local population. Indiscriminate industrialization process and rapid urbanization has created several environmental problems related to water, air and soil resources. Untreated industrial effluents and raw municipal sewage are continuously discharged into streams and rivers. Water quality of rivers and stream in Pakistan is

generally poor and rapidly getting deteriorated due to pollution from industrial, municipal, and agricultural sources (UNIDO, 2000). Pakistan is amongest water stressed countries facing water scarcity due to human population pressures (World Bank, 2005) and have shortage of over 40MAF (Million Acre feet) of water that will increase over 151MAF by year 2025 (Mirjat & Chandio, 2001). This scarcity of water needs protection and improvement of existing freshwater resources, otherwise, it will face acute shortage of water for irrigation, domestic and industrial use. This is the right time to recognize the intensity of problem and to take concrete steps towards its sustainable utilization and protection of water resources.

About 80% of industrial and urban growth is restricted to major cities viz; Karachi, Lahore, Faisalabad, Hyderabad, Multan, Sialkot, Gujranwala, Rawalpindi, Peshawar and Kasur (Aftab *et al.*, 2000). Away from the cities, in rural areas, agriculture land is under an indiscriminate use of fertilizers and pesticides that makes their way to rivers and streams through surface runoff. The intensity of human stress is continuously amplifying with an increase in human population, which was 33.7 million in 1950, reached to 140 million in 2000 with an increase of 8 million persons per annum (Qureshi, 2002). Urban settlements occupy about 1% of total land area; contribute 48 % of the Gross National Productivity (GNP) and more than 80% of industrial manufacturing (Khan, 1996). The urban centres of Pakistan are developing rapidly, which are putting the natural water ways under stress.

Sialkot is the 9th largest city of Pakistan situated in north east of Punjab. It is an important industrial city known worldwide for its leather garments, surgical instruments and sports good production (Ghani, 2002). This city is in the grip of pollution problem caused by industries, which are posing threat to the local environment (Mehdi, 2005). These environmental issues have been emerged with advent of industrial revolution since last three decades (Qadir *et al.,* 2008). Exports of tanned leather from Sialkot have been increased as a consequence of more stringent environmental controls curtailing the process in western countries. At present, about 264 tanneries are scattered within and outside the city along with hundreds of other industrial units. According to an estimate, tanneries located at Sialkot are producing about 9400 $m^3$/ day of heavily polluted tannery effluent (EM Research Organization, 2002). Industrial and municipal area generates huge volume of effluents and sewage, which causes irreparable loss to local environment. Nullah Aik and Nullah Palkhu the

important surface water resources of Sialkot more vulnerable to deleterious effects of pollutants in effluents and sewage without treatment from industries and municipality.

Nullah Aik and Nullah Palkhu, two main natural tributaries of River Chenab pass through the Sialkot. More than 2.5 million people are residing in the catchment of Nullah Aik and Nullah Palkhu. In the past, these streams were an important water resource for drinking, domestic use and irrigation purposes. Land around these streams is alluvium deposits resulting from regular floods and is under cultivation since pre historic times. Presently, these streams are receiving huge volume of effluents, raw sewage and solid waste that influence physical, chemical and biological characteristics of these streams. Major industrial activities are concentrated in and around the urban areas and along the major roads/highways. A major share of effluents is being contributed by tanneries, which produce a huge volume of liquid waste containing about 130 chemicals, municipal sewage (Khan & Mahmood, 2007). High flux of effluents containing toxic chemical including heavy metals drains directly or indirectly into Nullah Aik and Nullah Palkhu (Mohtadullah *et al.,* 1992; Mehdi, 2005). Effluents from tanneries contain high chemical oxygen demand, sulfides, chlorides, metals and salt content (Bajza & Vrcek, 2001). Among these notorious chemicals, heavy metals such as Cr and its salts have been reported to contaminate the stream water and aquifer (Bhalli & Khan (2006). Sialkot city generate 1503gallons per day of waste water, which directly or indirectly discharged into Nullah Aik and Nullah Palkhu (Randhawa, 2002). The average effluents production of each tannery in Sialkot district is 547- 814 $m^3$/day (ETPI, 1998) with a total volume of effluents as 11000$m^3$ (Dawn Daily, 2006). Sialkot city generate 19million $m^3$ waste water per annum (WWF, 2007), which is drained into natural streams, ponds and croplands. A complex mixture of industrial effluents, municipal sewage and surface running water discharged directly or indirectly into drains (Plate 1.1a) and open discharge to fields (Plate 1.1b). Most of the drains fall into the local sewerage system, which ultimately finds their way into the Nullah Aik and Nullah Palkhu and finally fall in River Chenab.

No industrial effluents and sewage treatment plants have been established to treat the wastewater from multiple sources. Nullah Aik and Nullah Palkhu are gradually turning into industrial and municipal drains. Large volume of industrial and urban sewage is considered a major threat to aquatic life of the focussed streams. Stream biota especially, fishes are facing severe impacts of toxic pollutants. This

situation was more severe in close downstream sites of Nullah Aik and Nullah Palkhu, where fishes were completely vanished due to high load of toxic pollutants. As a result, fish population reported from upstream and downstream is spatially fragmented due to barrier of pollutants. Downstream segment of streams are experiencing loss of many native fish species. Extinction of sensitive species, introduction of exotic and tolerant species, reductions in fish diversity, increasing the risk of diseases and abnormalities in fishes are the negative impacts of elevated level of pollutants in stream ecosystem.

Fishing is common activity in upstream and downstream of Nullah Aik and Nullah Palkhu for human consumption. Several fish species (*Channa punctata, Wallago attu, Heteropneustes fossilis, Cirrhinus reba and Labeo rohita*) are consumed due to their palatable values. People, who consume contaminated fishes captured from downstream of Nullah Aik and Nullah Palkhu, are more vulnerable to toxic effects of pollutants. Domestic animals' bathing in polluted water of Nullah Aik and Nullah Palkhu is also common practice, which may cause health problems in domestic animals (Plate 1.2a). In addition, along the banks of streams hundreds of pumps are used to suck the polluted water for irrigation purposes in surrounding cropland putting the agriculture and soil resources at risk (Plate 1.2b).

Conservation of freshwater fauna of Nullah Aik and Nullah Palkhu is a challenge for environmental managers due to its rapidly increasing human population, urbanization, agricultural and industrial activities in their catchment area (Qadir *et al.,* 2008). Human stress on streams has created an alarming situation, which needs an immediate attention.  Indiscriminate discharge of industrial effluents and municipal sewage deteriorates the water quality and displace and/or vanish the ichthyo-fauna. Unfortunately, no comprehensive scientific work, to date, is available about the water quality, sources of pollutants and their impacts on distribution of fishes. There is an urgent need to initiate concrete efforts to assess the impacts of human activities on water quality and fish fauna of these streams.

Present research project was designed, keeping in view environmental problems of Nullah Aik and Nullah Palkhu. For this purpose, a reliable and statistically sound data was required that highlight the effect of the anthropogenic activities on water quality and fish fauna in Nullah Aik and Nullah Palkhu. This is the first attempt to uncover the environmental problems of these streams caused due to anthropogenic activities in Pakistan. This research presents useful information about

status of water quality and fish fauna and will highlight the various anthropogenic threats responsible for degradation of stream ecology. These results will also provide the information about the impacts of pollutants on fish distribution, which can be used in decision making for re-introduction of extinct native fish species. The results of IBI will be quite useful for ecological stream health and measure indirect intensity of human activities in the catchment area, which will be useful for restoration and management of these aquatic resources. This study will be the guideline for sustainable watershed management, wise urbanization, environment friendly industrialization and agro-ecosystems not only in the catchment area of Nullah Aik and Nullah Palkhu but also for other streams in the same eco-region.

## Objectives

Following main objectives have been focused:

- to highlight the spatial and temporal variations in water quality of Nullah Aik and Nullah Palkhu, identification of water quality parameters and contamination sources, which bring variations in water quality.
- to assess heavy metals (Fe, Pb, Cd, Cr, Ni, Cu and Zn) accumulation patterns in different organs of fish species on spatial and temporal scales.
- to study the effects of environmental variables on diversity and spatio-temporal distribution of ichthyofauna.
- to develop an index of biological integrity for the quantification of stream habitat degradation of fish dwelling in Nullah Aik and Palkhu.

Plate 1.1a    Point source discharging effluents containing toxic effluent in Nullah Aik.



Plate 1.1b    Open discharge of untreated effluents of tanneries in fields near Sambrial, Sialkot

Plate 1.2a      Buffalo bathing in Nullah Aik Near Wazirabad



Plate 1.2b      Foam dunes developed during irrigation with untreated polluted water from Nullah Palkhu near Chitti Sheikhan, Sialkot.

## Structure of Thesis

The main objectives of present thesis was to study the impacts of anthropogenic activities on water quality and ichthyo-fauna of Nullah Aik and Palkhu stream system and to achieve these objectives, the research thesis is divided into seven chapters; each one will be focusing on specific objectives in details.

**First Chapter** describes the general introduction and background information that reviews the role of anthropogenic factors deteriorating the water quality and affecting the fish fauna. This chapter also describe the environmental problems in study area and presents research objectives.

**Second Chapter** provides description of the study area in relation to topography, climate, geology, history, drainage pattern, land use, human population and other anthropogenic activities. This chapter also describes the sampling strategy for collection, transportation, preservation and analysis of stream water and fish samples.

**Third Chapter** highlights the spatio-temporal variations in surface water quality of Nullah Aik and Palkhu, identification of important variables responsible for variations and their source of origin. Comparison of stream water quality with regional and international standard has also been discussed.

**Fourth Chapter** describes heavy metal accumulation in different organs of selected fishes on spatial and temporal basis. The results of heavy metal accumulation in muscles of fishes were compared with international permissible limits of heavy metals for human consumption.

**Fifth Chapter** describes the fish diversity, feeding guilds, spatio-temporal distribution pattern of fish species in relation with environmental factors.

**Sixth Chapter** explains the stream fish assemblage at community level with the application of index of biological integrity (IBI) to assess the impacts of anthropogenic activities.

**Seventh Chapter** concludes the findings of the research and provides guidelines for restoration and management of Nullah Aik and Palkhu stream system. Finally, this chapter also identifies the areas of future research programs.

**Chapter 2**

# Materials and Methods

## Study Area

Present study focuses on Nullah Aik (32°63′N- 74°99′E and 32°45′N-74°69′E) and Nullah Palkhu (32°69′N- 74°99′E and 32°37′N- 74°02′E) tributaries of the River Chenab (Fig. 2.1) that originate from Lesser Himalayas in the Jammu and Kashmir, at an altitude 530m and 290m, respectively. Nullah Aik and Nullah Palkhu drain about 1,875km$^2$ catchments area and travel a total distance of about 131.6km and 98km with an average annual discharge of 315 and 288Cs per second, respectively (Fig 2.2). Sialkot is the main city along with several towns such as Sambrial, Bhopalwala, Ugoki, Jathekay, Sodhra, Begowala and Wazirabad located in the catchment area. These streams traverse through the city of Sialkot from east to west and converge at downstream near Wazirabad city in district Gujranwala before falling in Chenab River.

The catchment area of studied streams experience four distinct seasons viz., summer (pre monsoon; April to mid-June), rainy season (monsoon; mid-June to mid-September), autumn (post monsoon; mid-September to November), winter (December to February) and a short spring (March). Climate is hot and humid during summer and cold during winter. June is the hottest month of the year with maximum daily temperature soaring to 40$^o$C and above (Fig. 2.3a; Appendix 2.1). The temperature during winter may usually drop to 4$^o$C but occasionally may even decline to freezing point during the month of January. The mean annual rainfall in the catchment is about 950mm of which maximum precipitation (~80%) occurs during the monsoon season (Fig. 2.3b). During frequent rainfall in monsoon season, rain water flows into streams through surface runoff and cause flooding which usually have devastating effects on crops and human settlements (Plate 2.1a & b). Most of the floods result in deposition of new alluvial soil in catchment area.

The catchment area of Nullah Aik and Nullah Palkhu is the part of upper *Rachna Doab* (Region between River Chenab and Ravi). The great plain of Punjab started from the upper parts of *Rachna Doab* with an average slope (0.37 m/km), which gradually decrease from North East to South West (Jehangir *et al*., 2002).
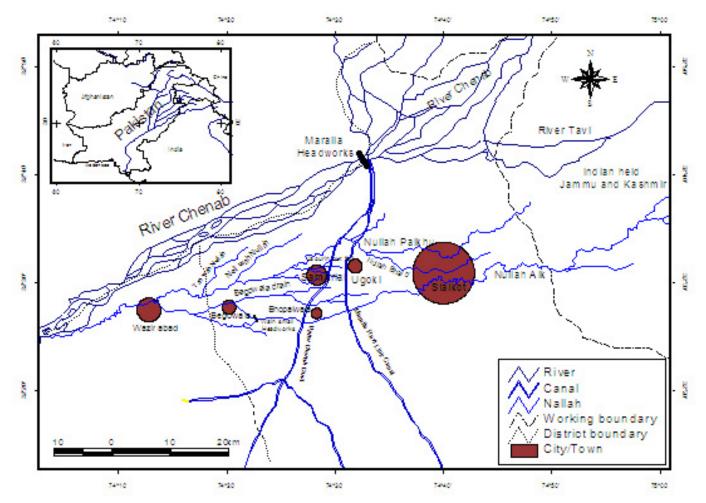
Fig. 2.1        Map of study area showing the location of Nullah Aik and Nullah Palkhu- tributaries of River Chenab.
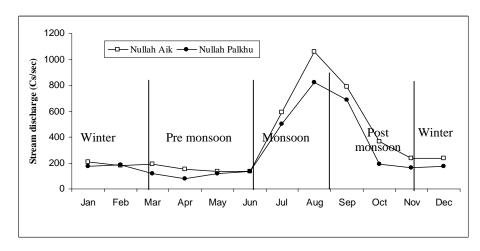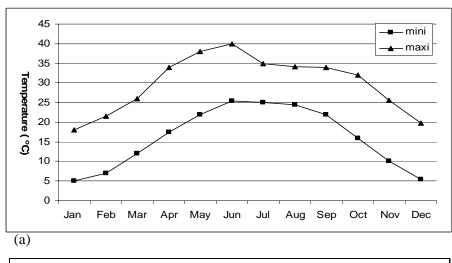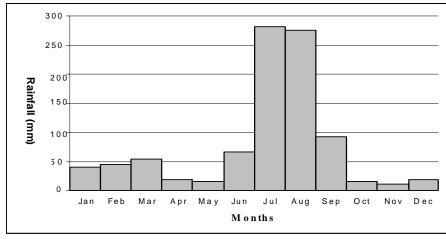
Fig. 2.2 Average stream discharge of Nullah Aik and Nullah Palkhu (source; Anonymous, 2007)



(a)



(b)

Fig. 2.3   (a) Mean monthly minimum, maximum temperature (°C)
            (b) Mean monthly rainfall (mm) in the catchment of Nullah Aik
                and Nullah Palkhu Sialkot

Plate 2.1a   Devastating flood during monsoon season, 2006 in lower catchment area due to over flow of Nullah Aik (Near Kotli Maralan).



Plate 2.1b   Devastating flood during monsoon season, 2006 in lower catchment area due to over flow of Nullah Palkhu (Near Kotli Khokhran).



Plate 2.2   Aik crossing over the Maralla Ravi Link canal located near Sahibkay (Site 5). (arrows indicate the direction of flow)

The upper part of Punjab plain originated during late Pleistocene alluvial deposition from Lesser Himalayas, which is approximately 200meter thick (Greenman *et al*., 1967). The soil is predominantly alluvial of varying textural classes such as clay and silt loam while sandy loam and sandy clay loam also prevail in the region. The soil types in upper catchment area of Sialkot are mainly *maira* (light reddish- yellow loam) and sandy loam in relict areas. In lower catchment, two types of soil i.e., *rohi* (predominant in clay) and *maira* (loamy soil) are common.

The resource utilization of Nullah Aik and Nullah Palkhu started 5000 years ago in Vedic Era, when Raja *Sáliváhan* founded the Sialkot named as *Sakala* (Population and Censes organization, 2000). Raja *Sáliváhan* and his son (Raja *Rasálu*) invited the people from various parts of Northern India to come and settle in Sialkot (http://en.wikipedia.org). They also promoted the irrigation system around the Sialkot city based on water resources of Nullah Aik. During Sikh regime (1800- 1849), many small channels (*Khands*) were constructed on both side of Nullah Aik for irrigation purposes (Fig. 2.5). Among these *Khands,* one of the major *Khand* (abandoned canal) was constructed to irrigate the lands of Sikhs around the Daska city (Fig. 2.5). It remained functional till the construction of Maralla Ravi Link (MRL) canal in 1955. In British era (1857-1947), a small headwork was constructed on Nullah Aik at Wain to divert the water of Nullah Aik for irrigation purposes in vicinity of Begowala Town, which was destroyed during heavy floods in 1954. After signing of Indus water treaty between Pakistan and India, riverine water irrigation system was expanded and local (*Khand*) irrigation system become vanished due to construction of MRL canals.

Nullah Aik receives water from springs in eastern parts of Jammu district and from heavy rains in its upper catchment area. Its maximum discharge level is approximately 35,000Cs per second at upstream which gradually reduces as the Nullah progresses towards downstream (Anonymous, 2007). Its maximum capacity to retain the water in downstream at the crossing of MRL canal is approximately 5,000Cs per second (Plate 2.2) and at the Wain small headworks can retain approximately 2000Cs per second. Nullah Aik exhibited maximum width in upper catchment area that gradually narrows down in lower catchment area. During monsoon season, heavy rains occur and rainwater drains into Nullah Aik and Nullah Palkhu. Sometimes these streams cannot retain the rainwater that ultimately flow out and spread over the vast area (Fig. 2.5). Floodwater brings large quantity of silt that

deposits new layers of soil every year. This unique attribute is utilized by local inhabitants in the downstream of Nullah Aik for irrigation through small distributaries (*Khands)*. At present only single irrigation channel is active that originates from small headworks at Wain*,* which further divides into sub channels that irrigate the croplands around the Begowala town. The surplus water from these sub-channels falls into Begowala drain which finally descends into Nullah Aik.

In contrast to Nullah Aik, discharge capacity of Nullah Palkhu increases as the stream moves from upstream towards downstream. At upstream, it has limited discharge capacity and collects rain water from its upper catchment area. Sometimes, in early summer season, it become dry and water inside the stream can be seen as isolated ditches and pools. At downstream after crossing UC (Upper Chenab) canal it receives water from many *Saim* Nullahs (Jourian and Roras out falls) and freshwater streams (Neil Wah and Tannai Wah) resulting high discharge level. Before falling into River Chenab, it joins Nullah Aik near Wazirabad.

According to Population and Censes Organization (2000) estimated population density of catchment area is 903 persons/ $km^2$ and one of the populous areas of Pakistan. Most of the land in catchment area is under intensive cultivation. Major crops grown in the area are wheat and rice. However, fodder crops and vegetables are also cultivated to meet the local demands. According to an estimate, there are about 1,000,000 domestic animals including cattle, buffaloes, horses, donkeys, sheep and goats in the rural areas (Population and Censes Organization, 2000). Application of fertilizers, pesticides and soil improving agents is a common practice in the catchment area. These synthetic chemicals make their way to streams after heavy rainfall or irrigation. Three kinds of irrigation sources are common in the area which are tube well irrigation (water from aquifer), stream water irrigation by pumping (hundreds of pumps have been established along the sides of streams) and diversion of water into small distributaries (this type of irrigation is restricted to the surroundings of Begowala town).

(a)

Surface runoff

**Nullah Aik**

(b)

Overflow of water
during flood

**Nullah Aik**

(c)

Surface run off

**Nullah Palkhu**

(d)

Flooding in lower
catchment

**Nullah Palkhu**
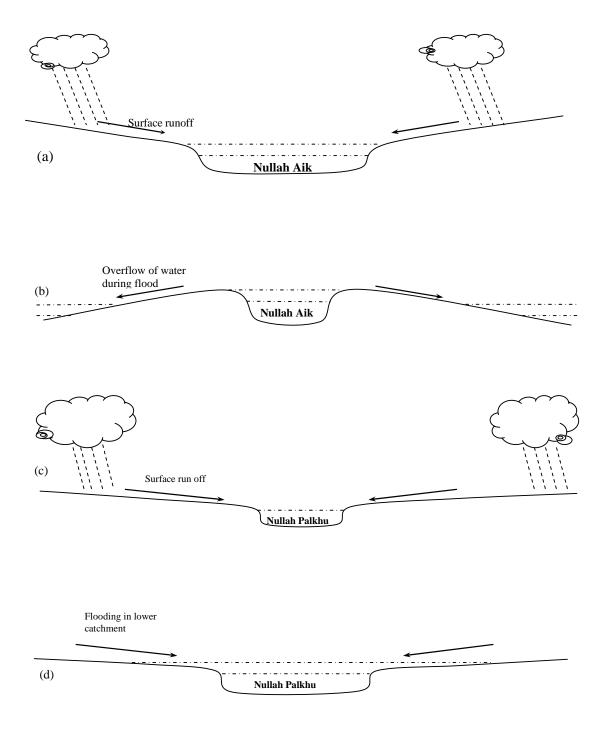
Fig. 2.4        Drainage pattern in catchment area of Nullah Aik and Nullah Palkhu
                (a) Upstream of Nullah Aik
                (b) Downstream of Nullah Aik
                (c) Upstream of Nullah Palkhu
                (d) Downstream of Nullah Palkhu.

Begowala channel **(Active)**

River Chenab

Nullah Palkhu

Sialkot City

Nullah Aik

Small head works at Wain

Small channels locally called *KHANDS* (abandoned)

Main Aik Canal **(abandoned)**
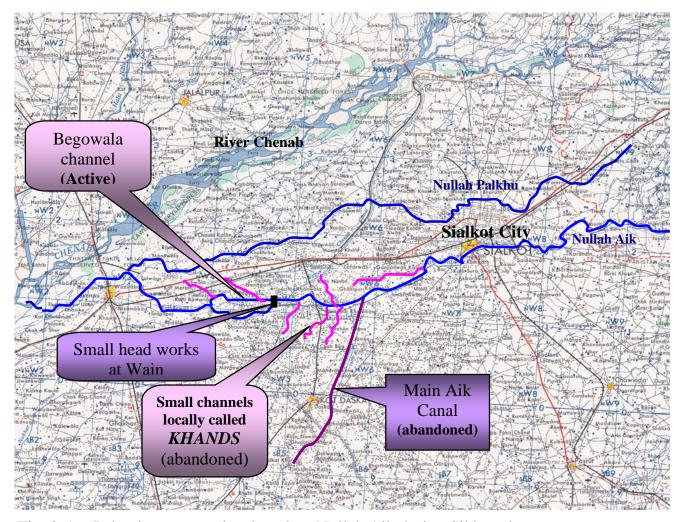
Fig. 2.5    Irrigation system developed on Nullah Aik during Sikh regime

**Anthropogenic Activities and Sources of Pollutants**

The catchment area of the streams is densely populated and house a significant number of industrial units in urban areas, whereas, rural areas are intensively used for agricultural purposes (Fig. 2.6). Major industrial activities are concentrated in and around the Sialkot city and along the roads. The industry of Sialkot consisted of small and medium units that are scattered throughout the city. The major industries consist of leather, sports goods and surgical instruments manufacturing. A total of 3,229 industrial units are present in Sialkot, comprising of 264 tanneries, 220 surgical instruments producing factories and 900 sports goods manufacturing units (Qadir *et al.,* 2008). Among these industrial units, tanneries are the major contributors in the production of effluents and sludge. Effluents of tanneries are also loaded with different types of salts (containing metals such as chromium, sodium and calcium), suspended solid matter and organic solvents. Most of the industries discharge their effluents without any treatment into drains that directly or indirectly fall into Nullah Aik and Nullah Palkhu.

According to an estimate of Cleaner Production Centre (CPC), Sialkot, the optimum level of leather production is about 297tons/ day with a daily production of 9388m$^3$of tanneries effluents. The average effluents production of each tannery in Sialkot district is 547- 814 m$^3$/day (ETPI, 1998) with a total volume of effluents as 11000m$^3$ (Dawn daily, 2006). Surgical instruments manufacturing units produce acid containing effluents which contain dissolved metals (used in electroplating) etc. However, amount of effluents produced is very low as compared to the tanneries. Surgical instruments producing units, sports goods production units are the least contributors in effluent production.

Municipal sewage is another source of pollution of surface water. There is no municipal waste treatment plant in Sialkot city and untreated raw sewage, organic and inorganic pollutants along with high quantities of suspended organic matter as well as animal and human excreta, which are discharged directly/ indirectly into streams. Sialkot city generate 19million m$^3$ waste water per annum (WWF, 2007), which is drained into natural streams, ponds and croplands.
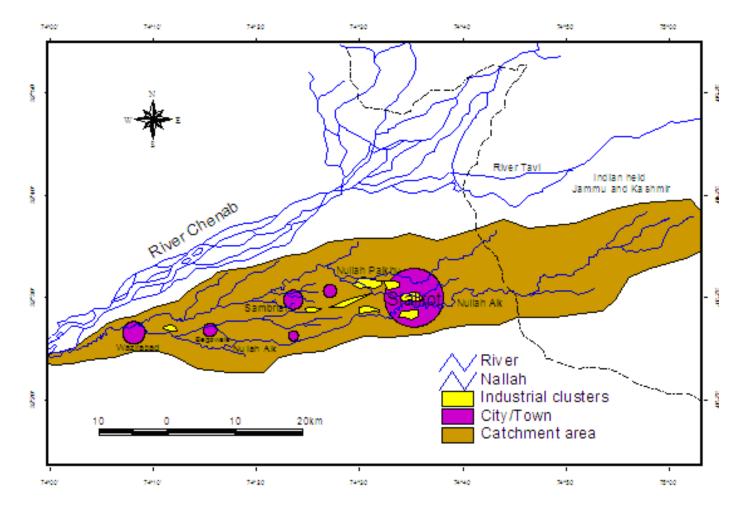
Fig. . 2.6    Activities in the catchment areaof Nullah Aik and Nullah Palkhu

## Sampling Methodology and Study Design

### Selection of Sampling Sites

Nullah Aik and Nullah Palkhu stream system was selected on the basis of similarities in topography, surface geology, relief and anthropogenic activities. A preliminary survey of Nullah Aik and Nullah Palkhu was conducted during the months of June and July, 2004 to extract information related to human impacts on water quality and aquatic life. During the preliminary surveys, each site was marked using a GPS (Global Positioning System, Garmin) for future sampling visits (Appendix 2.2). The selection criteria for each site was based upon anthropogenic activities in catchment area, variations in habitat, presence or absence of fish species and accessibility of the sites. After preliminary survey, a total of 18 sites were selected for water and fish sampling. Each stream consisted of nine sites and among these nine, two sites were located in upstream of Sialkot city, whereas, seven sites in downstream (Fig. 2.7; Plate 2.3). The sampling was started early in the morning usually commencing from 5:00am to 12:00pm. Every day, three sites were taken into account and whole sampling schedule lasted for one week. Before water and fish sampling, a detailed weather forecast for study area was studied to avoid the rainy days. All samplings were done during sunny days when the stream flow was at normal level. From each site, water and fish samples were collected on seasonal basis from September, 2004 to July, 2006. Water sampling was carried out from post monsoon (September - October), winter (December - January), pre monsoon (April - May) and monsoon (July), whereas, fish sampling during pre monsoon and post monsoon season, respectively.

Brief description of each site is given below.

### Nullah Aik

*Site 1* (Umranwali, 74° 39′ 45″ E, 32° 30′ 21″ W and 247m ASL) was located on working boundary (undecided international boundary) between Pakistan and Indian held Jammu & Kashmir, the point, where Nullah Aik enters in Pakistan (Plate. 2.3). This site was situated on upstream of Nullah Aik and least disturbed with relatively good water quality. No point source of pollution was identified, however, agricultural runoff and atmospheric disposition from Indian held Jammu district can be considered as a non-point source of pollution.
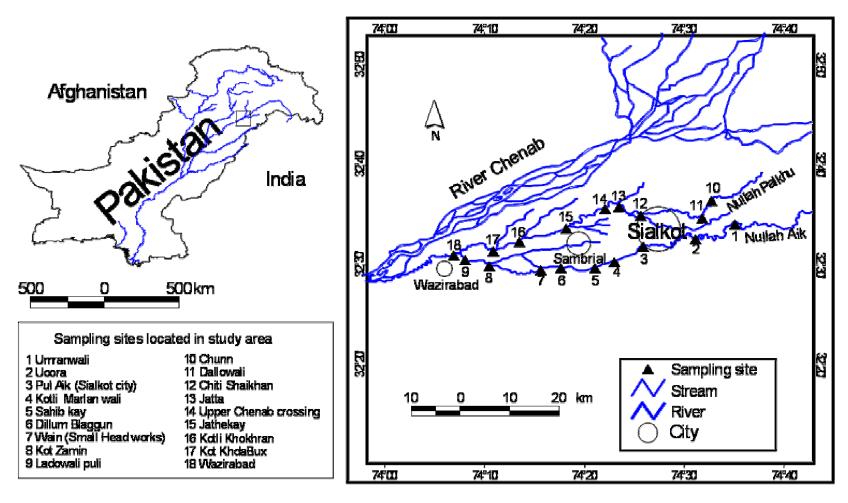
Fig. 2.7        Map of study area showing sampling sites located on Nullah Aik and Nullah Palkhu; tributaries of River Chenab, Pakistan.

***Site 2*** (Uoora, 74° 36′ 55″ E, 32° 29′ 30″W and 245m ASL) was selected at upstream of Nullah Aik near a village Uoora (Plate 2.3). This site was represented by relatively good water quality and inhabited by healthy aquatic life. Number of water pumps has been installed on stream banks to pump the water for irrigation. No major point source was found, however, it does receive some contaminants from agricultural runoff along with atmospheric deposition.

***Site3*** (Pul Aik, Sialkot city, 74° 31′ 50″ E, 32° 30′ 39″ W and 242m ASL) was located in Sialkot city (Plate 2.3) and receives effluents from small industries, tanneries, municipals waste, urban surface runoff and solid waste from Sialkot city.

***Site 4*** (Kotli Marlanwali, 74° 29′ 05″ E, 32° 27′ 26″ W and 237m ASL) was situated approximately seven km downstream of Sialkot city (Plate 2.3). The stretch between site 3 and site 4 receives industrial and municipal wastes from Sialkot city.

***Site 5*** (Sahibkay, 74° 25′ 03″ E, 32° 25′ 58″ W and 233m ASL) was located on Nullah Aik just after crossing the MRL canal (Plate 2.3). The water of Nullah Aik flows on upper side from east to west, whereas, the MRL canal water passes through a siphon underneath the Nullah Aik, which flows from north to south (Plate 2.2). The stream does not receive any substantial amount of contamination from a significant point source but agricultural activities and atmospheric deposition in the surrounding areas may contribute contamination of surface water.

***Site 6*** (*Dillum Blaggun* 74° 21′ 22″ E, 32° 25′ 48″W and 230m ASL) was located about 24km downstream of Sialkot (Plate 2.3) and site receives domestic sewage from Dillum Blaggun and Bhopalwala town. The major activity in its catchment area was agricultural farming.

***Site 7*** (*Wain small head works* 74° 18′ 03″ E, 32° 25′ 39″ W and 227m ASL) was situated near Wain village (Plate 2.3). Small headworks have been constructed to divert the water of Nullah Aik to irrigate fields around the Begowala town. Highest stream depth was recorded at this site. Stream flow is regulated by small headworks at downstream of Wain and remains low except monsoon season. Main sources of surface water contamination at this site are mainly from non-point sources.

*Site 8* (*Kot Zamin* 74° 11′ 55″ E  32° 26′ 09″ W and 222m ASL) receives effluents from Begowala drain that bring effluents from tanneries zone and Sambrial city after traversing a distance of about 20km (Plate 2.3). This site was selected to highlight the possible impacts of Begowala drain on the stream water quality of Nullah Aik.

*Site 9* (*Ladowali Puli* 74° 09′ 95″ E 32° 27′ 02″ W and 219m ASL) was located near Wazirabad city (Plate 2.3) and is the last site on Nullah Aik before it falls into Nullah Palkhu. The site receives effluents from tanneries located along Sialkot-Wazirabd road and municipal sewage from eastern part of Wazirabad city and surface runoff from agricultural and urban area.

**Nullah Palkhu**

*Site 10* (*Chun* 74° 37′ 54″ E 32° 33′ 34″ W and 250m ASL) was situated near the origin of stream located near working boundary between Pakistan and Indian held Kashmir on upstream of Nullah Palkhu (Plate 2.3). Agriculture is the main activity in the upstream catchment area. Stream flow at this site is strongly influenced by rainfall, which is high in monsoon season and low even dry in early summer season, however, water remains in ditches and pools.

*Site 11* (*Dolluwali* 74° 36′ 14″ E 32° 32′ 22″ W and 247m ASL) was situated at upstream of Dolluwali and Sialkot Cantonment (Plate 2.3) and experiences occasional summer drought. Main source of pollution at this site is the agricultural activities.

*Site 12* (*Chitti Sheikhan* 74° 30′ 43″ E 32° 31′ 45″ W and 238m ASL) was located at downstream of Sialkot Cantonment (Plate 2.3) and receives tannery effluents, municipal waste from northern parts of Sialkot and its surrounding areas.

*Site 13* (*Jatta* 74° 27′ 24″ E, 32° 32′ 30″ W and 232 ASL) was situated at downstream of Sialkot (Plate 2.3) and strongly influenced by Nullah Bhed, which passes through the center of Sialkot city and brings heavy load of pollutants from Sialkot City.

*Site 14* (*UC canal crossing* 74° 25′ 20″ E, 32° 32′ 38″ W and 229m ASL) was located on Nullah Palkhu after crossing the Upper Chenab canal (Plate 2.3). The water of Nullah Palkhu passes under the UC canal through a siphon. No visible point source of

pollutant discharge was found.

***Site 15*** (*Jathekay* 74° 19′ 47″ E, 32° 32′ 29″ W and 227m ASL) was located at the junction of Nullah Palkhu and Jaurian out fall near Jathekay (Plate 2.3). Land in the periphery of UC and MRL canal become waterlogged due to seepage from canals. Jaurian out fall collects water from waterlogged land and drains into Nullah Palkhu, which may dilutes the contaminants concentration and improve the water quality. Farmers along each side of the stream pump stream water to irrigate their crops.

***Site 16*** (*Kotli Khokhran* 74° 16′ 40″ E, 32° 29′ 03″ W and 224m ASL) was situated downstream of Nullah Palkhu (Plate 2.3) after joining Nullah Neil Wah, which further dilute the concentration of contaminants in stream water. Agriculture was the major human activity in its catchment area.
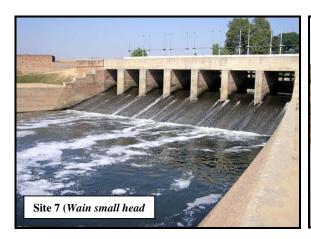
***Site 17*** (*Kot Khuda Bux* 74° 12′ 30″ E, 32° 27′ 45″ W and 221m ASL) was located in the lower catchment area of Nullah Palkhu (Plate 2.3) and receives domestic sewage from Sodrah town and surface runoff from agricultural fields.

***Site 18*** (*Wazirabad* 74° 07′ 08″ E, 32° 27′ 06″ W and 217m ASL) was located on Nullah Palkhu after joining Nullah Aik (Plate 2.3) and collects effluents and municipal wastes from Wazirabad city, known for cutlery production industry.

Site 1 (*Umanwali*)

Site 2 (*Uoora*)

Site 3 (*Pul Aik Sialkot*)

Site 4 *(Kotli Marlanwali)*

Site 5 (*Sahikay*)

Site 6 (*Bhopalwala*)

*Continued...*

Plate 2.3    Pictorial representation of sampling sites located on Nullah Aik and Nullah Palkhu.

Site 7 (*Wain small head*)


Site 8 (*Kot Zamin*)


Site 9 (*Ladowali Puli*)


Site 10 (*Chun*)


Site 11 (*Dolluwali*)


Site 12 (*Chitti Sheikhan*)

*Continued...*
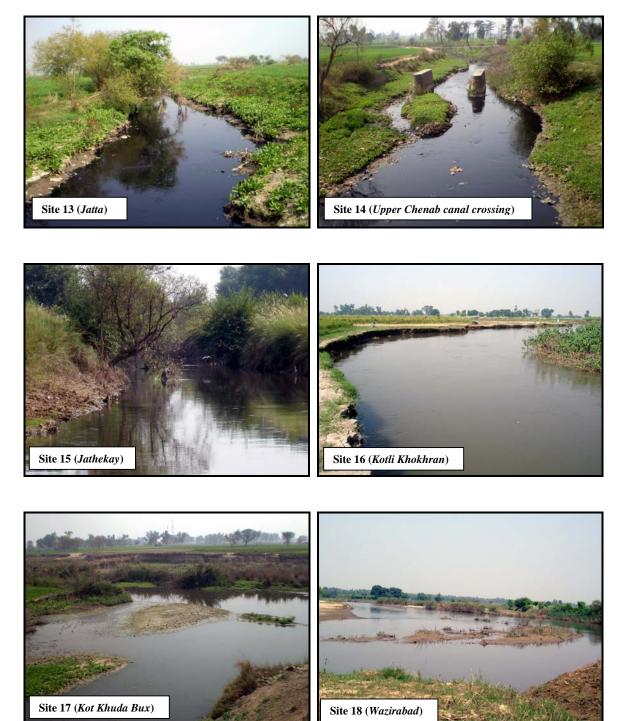
Plate 2.3    Pictorial representation of sampling sites located on Nullah Aik and Nullah Palkhu.

**Water Sampling and Laboratory Analysis**

Water sampling was carried out with the help of self-designed sampler which was consisted of long plastic rod of adjustable length and angle. One end consisted of two circles of stainless steel covered by rubber coating in which glass container was fixed to get water sample from desired depth of stream water. Glass container was washed with nitric acid and rinsed with deionised water at every site. Water samples were taken 30cm below the water surface, within 100m range of a sampling site. Sampling container was drained into sampling bottles up to the mouth without trapping any bubble. From each site, three sub-samples of stream water were collected, which were combined to obtain composite samples during sampling. The water samples were stored in plastic bottles (pre cleaned with $HNO_3$) and were tightly sealed to avoid leakage during transportation. All water samples were preserved in ice boxes and transported to laboratory according to prescribed standard method (APHA, 1998). All samples were transported to the laboratory of Cleaner Production Center (CPC), Sialkot and stored in freezer to cease any probable physico-chemical changes. All analyses on water except metal were performed in CPC laboratory. Water samples were separated out for the dissolved metal contents and particulate matter. The water samples for dissolved metals were acidified with few drops of $HNO_3$ to make it remain available in dissolved form during storage.

Stream width, depth and flow dissolved oxygen (DO), pH, Electrical conductivity (EC), Salinity and Total Dissolved Solid (TDS) of the water samples were measured in field at each site.

**Stream Width** was measured at four different points within sampling site. The average distance between two points was 25m along the longitudinal direction of stream. The mean stream width was calculated by averaging the readings of four points (Fig. 2.8a) and expressed in meters (Arunachalam, 2000 and Bhat, 2004).

**Stream Depth** was measured at three points; one was taken at centre of stream, while two measurements were taken at 1/3 distance from each bank of the stream. These three measurements were taken in same transect that fall across the stream (Fig. 2.8c; Arunachalam, 2000; Bhat, 2004).

**Stream Velocity** was measured at the point where stream flow was fastest. To measure stream velocity two bamboo sticks were fixed at stream bed at the distance of 10 meter from each other. A cork was dropped at the point towards upstream and

collected after passing through second point (Fig. 2.8b). The time taken by cork to travel the distance between two poles was measured by stopwatch (Bhat, 2004). Stream velocity was converted into meter per second (m/ sec).

**Water pH** was determined using portable pH meter (Thermo Orien 240A) with accuracy of $\pm$ 0.01. pH meter was calibrated between neutral and alkaline medium with calibration buffer solutions of pH 7 and pH10 every day before field study.

**Dissolved Oxygen (DO) and Temperature** were measured by a portable meter (970 DO2 Meter IP65 Jenway Essex, UK) with an accuracy of $\pm$ 0.5mg/L and $\pm$ 0.1°C, respectively.

Water quality parameters viz., total hardness, turbidity, chemical oxygen demand (COD), nitrates ($NO_3^-$), phosphates ($PO_4^{3-}$), chlorides ($Cl^-$), sulfides ($S^{2-}$) and metals (dissolved and particulate matter) such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu) and zinc (Zn) were determined in laboratory (Table 2.1).

**Chemical Oxygen Demand (COD)** was expressed in mg/L COD and was determined using Hach spectrometric methodology as described by Boyles (1997).
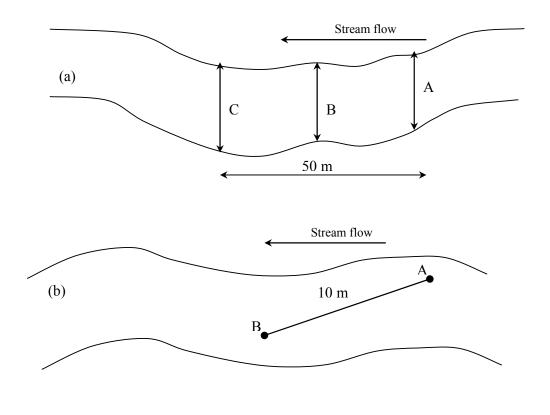
**Electrical Conductivity (EC), Total Dissolved Solids (TDS) and Salinity** were measured by using Hi 8033 Hanna conductivity meter (Hungry), which automatically compensates the temperature differences. The meter was calibrated with KCl before analysis. EC, TDS and salinity were measured in $\mu$S/cm, and mg/L of $CaCO_3$, respectively.

**Total Hardness** is expressed as mg/L of $CaCO_3$. Water hardness was calculated as amount of dissolved calcium and magnesium in water (APHA, 1992) by using the equation:

$$\text{Hardness mg/L} = (Ca \times 2.497) + (Mg \times 4.118)$$

**Turbidity** was measured in nephelometric turbidity units (NTUs) using Turbiditimeter (Model Hach 2100 A).

**Nitrate ($NO_3^-$) and Orthophospate ($PO_4^{3-}$)** were determined spectrophotometrically by using the phenol-disulphonic acid and ammonium molybdenum method following (Allen *et al.*, 1974; Garg *et al*., 2000).
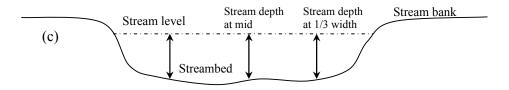
Fig. 2.8      Strategy to measure (a) stream width, (b) Stream flow and (c) Stream depth measured at a sampling site

Table 2.1    Parameters, abbreviations, units and analytical methods as measured during 2004- 2005 for water quality assessment of Nullah Aik and Nullah Palkhu.

| Variables | Abbreviations | Units | Analytical Methods |
|---|---|---|---|
| Stream width | St.Wd | m | Bhat,2004 |
| Stream velocity (flow) | St. vel. | m/sec | Bhat,2004 |
| Stream depth | St.dep | m | Bhat,2004 |
| Temperature | Temp | ºC | Thermometer inside DO meter |
| pH | pH | pH | pH-meter |
| Dissolved oxygen | DO | mg/L | DO meter |
| Chemical oxygen demand | COD | mg/L | Spectrophotometric |
| Electrical conductivity | EC | $\mu$S/cm | E.C. meter |
| Total dissolved solids | TDS | mg/L | TDS meter |
| Salinity | Sal | mg/L of $CaCO_3$ | Titrimetric |
| Total hardness | T-Hard | mg/L | Ca and Mg based |
| Turbidity | Turb | NTU | Turbiditimeter |
| Nitrates | $NO_3^-$, | mg/L | Spectrophotometric |
| Phosphates | $PO_4^{3-}$ | mg/L | Spectrophotometric |
| Chlorides | $Cl^-$ | mg/L | Titrimetric |
| Sulphides | $S^{2-}$ | mg/L | Spectrophotometric |
| Sodium * | Na | mg/L | FS AAS |
| Potassium* | K | mg/L | FS AAS |
| Calcium* | Ca | mg/L | FS AAS |
| Magnesium* | Mg | mg/L | FS AAS |
| Iron* | Fe | mg/L | FS AAS |
| Lead* | Pb | mg/L | FS AAS |
| Cadmium* | Cd | mg/L | FS AAS |
| Chromium * | Cr | mg/L | FS AAS |
| Nickel* | Ni | mg/L | FS AAS |
| Copper* | Cu | mg/L | FS AAS |
| Zinc* | Zn | mg/L | FS AAS |

*metals in dissolved form (mg/L) and suspended particulate matter (mg/L)

**Chloride ($Cl^-$)** were measured in water samples by titration with standard solution of silver nitrate with sulphuric acid using potassium chromate as an indicator in mg/L. $Cl^-$ concentration was calculated by the following equation (Garg *et al*., 2000).

$$Cl^- \ (mg/L) = \frac{\text{volume of AgNO3 x N x 35.5}}{\text{volume of sample}} \ x \ 100$$

Where N stands for normality of $H_2SO_4$

**Sulfides ($S^{2-}$)** were measured by Methylene Blue method (Hach method # 8131) adopted for the estimation of $S^{2-}$ using Hach DR5000 spectrophotometer as described by Hach Company (2005).

**Dissolved Metal Contents** were measured by taking 250 ml of stream water samples filtered through Whatman filter paper (No. 45) in the laboratory (APHA, 1998). The

filtrate was acidified with 1-2 ml of ultra pure $HNO_3$ to drop its pH below 2 and preserved at 4 C for dissolved metal analyses (De Carlo *et al.,* 2004).

**Metal Contents in Suspended Particulate Matter**

Suspended particulate matter was separated from 250ml water sample on filter paper (Whatman filter paper No.45). These filter papers were oven dried and weighted before separation of suspended sediment from water samples. After separation of Suspended particulate matter filters were oven dried (Herman *et al*., 2000). Suspended particulate matter was digested according to the method of USEPA (1990). Oven dried suspended particulate matter was transfered to 100ml Kjaldahl flask and digested in 10 ml of $HNO_3$ and $HClO_4$ with ratio of 5:1, boiled for 20 - 30 minutes until a transparent digest was appeared. Digests were filtered and volume of obtained aliquot was raised up to 50ml in volumetric flask.

All prepared samples for dissolved, and suspended particulate matter were subjected to metal analysis. Concentration of eleven metals (Na, K, Ca, Mg, Fe, Pb, Cd, Cr, Ni, Cu and Zn) in prepared samples was determined by using flame atomic absorption spectrophotometric technique. Fast Sequential Atomic Absorption Spectrophotometer (FSAAS Varian AA-240) was used to determine metal concentrations. A set of standards consisting of a series of standard that varies from lowest concentration to highest concentration were prepared to obtain a calibration curve (Hayes & Buckney, 1998). A blank was also prepared by repeating the same process. The concentration of blank was subtracted from concentration of samples to obtain a final concentration values (Vazquez *et al.,* 1999). Three replicates of each sample were prepared to obtain the precision in metal analyses and 5 - 10% standard deviation was observed during analysis of metal concentration. For quality control and to ensure the accuracy of running analysis BCR-714 reference materials (Waste water influent) was used obtained from European Commission, DG JRC, Institute for Reference Materials and Measurements (IRMM) Reference Materials Unit, Belgium. About 5 - 15% variations in results of reference material for heavy metals were observed after repeating it for six times. The measured and certified values of reference material are given in Table 2.2.

Table 2.2      Mean ± standard deviation of measured values in comparison with
               certified values of the reference material.

| Metal (mg/L) | Certified values | Measured |
|:---:|:---:|:---:|
| **BCR-714 Wastewater (influent)** | | |
| Fe | 1.03 ± 0.11 | 0.988 ± 0.023 |
| Pb | 0.145 ± 0.011 | 0.162 ± 0. 020 |
| Cd | 0.019 ± 0.016 | 0.017± 0.005 |
| Cr | 0.123 ± 0.010 | 0.131 ± 0.045 |
| Ni | 0.108 ± 0.015 | 0.119 ± 0.013 |
| Cu | 0.309 ±0.023 | 0.324 ± 0.098 |
| Zn | 1.00 ± 0.010 | 0.984± 0. 031 |

## Fish Sampling

Fish samples were collected from each site using variety of fishing nets of varying mesh sizes as suggested by Bhat (2003). Seine net (8 m × 1.5 m with mesh size of 1cm), cast nets (circumference 15 m with 1.5 cm mesh size) and dragnets (7m x 1m with mesh size of 1.5cm) were used within 100 meters reach of each sampling site. Cast and dragnets were used to sample fish in water with depth less than 1.5 m, while seine net was used where depth of water was more than 1.5 meter. The intensity of sampling for each site was standardized as 20 efforts for cast net, three hours duration for seine net and 10 trials for drag net covering the stream within 100m of stream along longitudinal gradient (Bhat, 2003). All sampling sites were intensively sampled in an effort to capture as many fish species as possible in proportion to their abundance (Matthews, 1985). Captured fish specimens were transferred immediately into stream water filled tubs place on stream bank to reduce the chances of fish mortality. Minimum of four persons were the member of sampling crew, who handle netting process. The whole process of sampling was done very carefully with fishing nets traversing the distance from downstream to upstream. During collection of fishes, the length and weight of each fish was measured (Appendix 2.3a & b). Maximum five specimens of each species were packed, labelled in separate polythene bags, placed in icebox and transported to laboratory within 12 hours for the purpose of identification and further chemical analysis (Van Aardt & Erdmann, 2004). The remaining specimens were released back into stream water at the same point from where fish was captured (Ganasan & Hughes, 1998). Released fishes were only native, whereas, exotic fishes were not returned back to the stream. The juvenile fishes, which have tail length less than 20mm, were excluded because of inadequacy in their capturing

(Helms *et al.,* 2005). Taxonomic identification and classification was done on the basis of morphometric characteristics up to the species level. Fish species were identified following regional keys (Mirza & Bhatti, 1993; Mirza, 2003; Talwar & Jhingran, 1991; http://www.fishbase.org).

**Metal Analysis in Fish Organs**

Out of 24 fish species (details are given in chapter 5), only eight abundant fish species (*Channa punctata, Cirrhinus reba, Labeo rohita, Heteropneustes fossilis, Mystus cavasius, Oreochromis niloticus, Puntius sophore* and *Wallago attu*) were selected for heavy metal analysis indifferent organs. A total of seventy five and seventy nine fish specimens were dissected during post monsoon and pre monsoon, respectively.

Each specimen was dissected in laboratory to obtain different organs (Liver, kidney, gills and muscle) with corrosion resistant stainless steel knives. These knives were washed after dissection of each specimen with a detergent solution, rinsed in 50% $HNO_3$ and finally by distilled water. First of all, gills were obtained from both sides then homogenized to form a composite sample. After removal operculum, epidermal and subcutaneous tissues were carefully removed to obtain sample of muscle. To ensure uniformity, all samples were taken from dorso-lateral muscles of the right side (Fréry *et al.,* 2001). After opening the body cavity with a scalpel, liver was identified in the presence of surrounding organs and the connective tissue around the liver was removed to separate the liver along with gall bladder. Finally kidneys were located and separated from connective tissues. Each dissected organs was placed in polythene bag and provided with necessary information about identification code, date, locality, season, species name and type of tissue. Polythene bags containing different organs were stored at $-20°C$ before digestion to avoid contamination (Mormede & Davies, 2001).

One gram of wet fish tissue (liver, gills, kidneys and muscles) of different species was digested (Zyadah & Chouikhi, 1999) by using 5ml of $HNO_3$ and 1 ml $HClO_4$ in volumetric flask. Digestion of fish tissues was carried out on a hotplate (200 to 250°C) until digest of fish organs converted into colourless liquid and volume of the digest raised up to 50ml (Nussey *et al.*, 2000). Each digest was filtered through 0.42μm filter paper, transferred to polyethylene bottle and sealed to avoid contamination and evaporation. Before heavy metal analyses, digested samples were

stored at 4°C. The prepared samples were analyzed for heavy metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn using Fast Sequential Atomic Absorption Spectrometer (Varian Spectra AA-240). Metal concentration in fish tissues was determined in microgram/gram (μg/g). Concentration of each metal was further confirmed using external standard method (Bajc *et al.,* 2005). The precision and accuracy of procedure was evaluated by analyzing two standard reference materials (cod muscle BCR 422 and bovine liver BCR 185R) obtained from European Commission, DG JRC, Institute for Reference Materials and Measurements (IRMM) Reference Materials Unit, Belgium. The level of metal accuracy was checked for heavy metal as 83 - 96% (Table 2.3).

Table 2.3    Comparison of measured and certified values (Mean ± standard deviation) of certified reference materials of during metal analysis

| *Metals* | *Bovine Kidney BCR 186R* | | *Cod Muscle BCR 422* | |
| --- | --- | --- | --- | --- |
| (μg/g) | *Measured* | *Certified* | *Measured* | *Certified* |
| Fe | 217± 18 | 229 ± 10 | 5.21 ± 0.45 | 5.46 ± 0.30 |
| Pb | 314 ± 22 | 306 ± 11 | 0.07 ± 0.03 | 0.085 ± 0.015 |
| Cd | 2814 ± 234 | 2710 ± 150 | 1.24 ± 0.10 | 1.05 ± 0.07 |
| Cr | 71 - 115 | 58 -142 | - | - |
| Ni | 402 ± 39 | 420 | - | - |
| Cu | 27.4 ± 4.4 | 31.9 ± 0.4 | - | - |

**Chapter 3**

# Spatial and Temporal Variations in Water Quality of Nullah Aik and Nullah Palkhu

## Introduction

Surface water quality of lotic ecosystems is influenced by natural processes (erosion of parent rock material and precipitation) as well as anthropogenic activities such as industrial, municipal and agricultural runoff (Singh *et al.,* 2005a; Shrestha & Kazama, 2007). The magnitude of this problem become further intensified due to indiscriminate growth in industries, expansion of population and degradation of natural aquatic systems (Ghosh & McBean, 1998). Streams and rivers characterized by unidirectional water flow (Gafny *et al.,* 2000) are often treated as easy dumping sites and act as sink for industrial and municipal waste (Singh *et al.,* 2007), which makes them highly vulnerable (Korfali & Davies, 2003). Inflow of hazardous pollutants such as heavy metals and other organic pollutants into streams and rivers can be related to two main major sources (Honnen, *et al.,* 2001; Gallo *et al.,* 2006). Point sources (industrial and municipal effluents) are released at specific points throughout the year and non-point sources (atmospheric deposition, surface runoff from urban and agricultural areas) are highly influenced by seasonal changes (Sundaray *et al.,* 2006).

Industrial effluents and municipal wastes contain heavy metals, which are persistent in nature and cannot be removed or degraded by natural processes (Singh *et al.,* 2005b). In surface water heavy metals are present either in dissolved phase or in suspended particulate phase (Saeedi *et al*., 2004) which exchange continuously as water traverse the distance (Gallo *et al.,* 2006). Dissolved metals are available to aquatic organisms and determine the health of an ecosystem (Morrison *et al.,* 1984). Metals in suspended particulate phase are comparatively stable and less toxic (Morrison *et al.,* 1990).

Distribution of heavy metals in water and sediment is greatly influenced by various physico-chemical characteristics such as pH, organic matter and mineral compounds (Singh *et al.,* 2005b; Gallo *et al.,* 2006; Rothwell, *et al.,* 2006). After the introduction of heavy metals into aquatic ecosystem, heavy metals undergo the

process of various chemical processes such as dissolution, sedimentation and re-suspension along longitudinal gradient (Lee *et al.,* 2003). There are different physical or chemical factors that promote or reduce the adsorption process of dissolved contents of metal to suspended particulate matter (Dojlido & Best, 1993; Saeedi *et al.,* 2004). Heavy metals are mainly transported in association with suspended particulate matter, which settles down in streambed, when stream flow becomes slow with low turbulence. Sedimentation process of highly polluted streambed sediment becomes more pronounced especially where streams receive industrial and urban effluents (Tsai *et al.,* 2003). Stream suspended particulate matter consisted of many constituents such as clay, organic matter, metals oxides that scavenge the heavy metals from the environment (Rodrigues *et al.,* 2006). Heavy metals in sediments are not permanently fixed and can re-mobilize during flood period resulting changes in pH, organic matter and TDS and may become available in the particulate or dissolved form (Lee *et al.,* 2003; Kucuksezgin *et al.,* 2007). The distribution of heavy metals in particulate matter varies along longitudinal gradient of stream and between different seasons.

Variations in stream water originate from catchment area as a result of precipitation, surface run off, sub-surface inflow of water through aquifer and drainage pattern. Other in-stream processes that affect the availability of heavy metals is dilution of effluent by addition of water from associated streams, pumping of water for irrigation, sediments formation, and re-mobilisation from sediments (Qadir *et al.,* 2008). These processes exhibits seasonal and spatial variations and have a strong effect on the equilibrium of dissolved and suspended form of heavy metals in surface water (Singh *et al.,* 2005a). Distribution of heavy metals in running waters is generally evaluated from limited number of points on spatial and temporal basis (Temnerud & Bishop, 2005). Spatial and temporal variations of different heavy metals in streams are highly variable and regulated by complex processes such as stream discharge, natural and human activities (Simeonov *et al.*, 2003). Temporal variations in water quality are determined by precipitation, stream discharge level (Singh *et al.,* 2005a) and become more pronounced in streams of temperate region, where flow is regulated by rains (Shah *et al.*, 2007). Streams and rivers of Indo-gengatic plains are located in temperate zone, with distinct seasonal pattern of rainfall. A number of regional studies on the spatial and temporal variations in surface water of streams and rivers have been conducted. Singh *et al.* (2005a) reported the spatio-temporal variations and source

identification of contaminants in Gomuti River. Chakrapani (2005) highlighted spatial and temporal variations in upper part of river Ganga, India. Ansari *et al.* (1999) studied the impact of industrial effluents, domestic sewage and agricultural runoff on water quality from River Ganga. Qadir *et al.* (2008) highlighted spatio-temporal variations in water quality of Nullah Aik, which is an important tributary of River Chenab, Pakistan.

Nullah Aik and Nullah Palkhu major tributaries of River Chenab, Pakistan, receive untreated industrial and municipal discharge from different industrial units and urban settlements of district Sialkot. These contaminants are putting ecological integrity of these streams at risk and have been considered as major threat to degradation of aquatic ecosystem, which are ultimately turning into municipal drains (Qadir *et al.,* 2008). High load of pollutants into studied streams are severely altering the water quality and resulted degradation in its natural ecosystem. No previous data and scientific work is available on water and sediment quality of Aik-Palkhu stream system and no attempt has been made to assess the extent of contamination and characterize the potential impacts on streams ecology of Nullah Aik and Nullah Palkhu. There is dire need for comprehensive assessment to find out the trends and variations in the water quality of these streams and to address the consequences of present and future threats of contamination. It is also important that spatio-temporal monitoring of water quality of these streams should be done for future water resource management. A monitoring program was felt necessary to provide a representative and reliable spatial and temporal dataset of water quality for future restoration of stream ecology.

The main objectives of this chapter are

- to measure the spatial and seasonal trends of heavy metals in dissolved and suspended sediments of Aik- Palkhu stream system,
- to highlight the most influencing variables that contribute in spatio-temporal variations in water and suspended particulate matter and
- to identify the possible sources of heavy metals along with physico-chemical variables in surface water.

## Materials and Methods

### Sampling Strategy

Details of water sampling and analysis of 38 parameters of water quality are given in Chapter 2.

### Statistical Analyses

Basic Statistics analyses were performed by using Statistica 5.5 software (Stat Soft, Inc. 1995) and for graphical representation of water quality data Microsoft Excel 97 (Microsoft Corporation 1985) was used. Three multivariate techniques such as Hierarchical Algorithmical Cluster Analysis (HACA), Discriminant Function Analysis (DFA) and Principal Component Analysis based on Factor Analysis (PCA/FA) were used for the water quality assessment and interpretation of the results (Simeonov *et al.,* 2000; Wunderlin *et al.,* 2001; Singh *et al*., 2005b). These multivariate statistical techniques have been widely used in various studies for the explanation of spatio-temporal variations and interpretation of chemical/physical characteristics of water quality parameters (Simeonov *et al.,* 2000; Wunderlin *et al.,* 2001; Singh *et al.,* 2005b) in comparison to uni-variant techniques that usually fail to give adequate information on multivariate dataset (Santos-Roman *et al*., 2003).

The stream water quality data set was subjected to HACA to identify clusters of the sampling sites indicating their similarity based on water quality parameters (Table 2.1). For this purpose, 38 water quality parameters measured from 18 sites (38 variables $\times$ 18 cases) were subjected to HACA analysis. HACA was performed on the mean values of water quality parameters. Euclidean distances were chosen as a measure of linkage that uses analysis of variance to evaluate the distances between clusters, attempting to minimize the sum squares of any two clusters that can be formed at each step (Kent & Coker, 1992). To determine the association of metals and their possible sources in water quality data, eleven metals within dissolved and suspended particulate form were also subjected to HACA. Pearson correlation was used to confirm the results of HACA and to find association between different metals.

DFA was used to determine the water quality parameters that best discriminate between spatial groups identified by HACA. The basic idea underlying DFA is to determine whether groups differ with regard to mean of a variable, and then to use that variable to predict group membership. DFA was applied to water quality data set (18 sites x 38 variables) without any standardization to define the spatial and temporal

variations in these parameters. Standard, forward stepwise and backward stepwise modes of the DFA were applied to construct discriminant functions (DFs) by following by Wunderlin *et al.*, (2001) and Singh *et al.*, (2005a). DFs were calculated using Equation (I):

$$f\left(Gi\right) = ki + \sum_{j=1}^{n} w_{ij}\, p_{ij} \tag{I}$$

where $i$ is the number of groups ($G$), $k_i$ the constant inherent to each group, $n$ the number of parameters used to classify a set of data into a given group, $w_j$ the weight coefficient, assigned by DFA to a given parameter ($p_j$).

In DFA, the sites were taken as spatial grouping variable, whereas, seasons were considered as temporal grouping variables. These grouping variables (spatial and temporal) were used in the analysis as dependent variables, while the water quality parameters were considered independent variables. In forward stepwise mode, DFA variables were added step by step until no significant change occurs, whereas, in backward-stepwise mode variables were removed starting from least significant until significant change occurs.

Principal Component Analysis based on Factor Analysis (PCA/FA) to extract a lower dimensional linear structure from the water quality data set of three spatial groups viz; relatively unimpaired region (4 sites x 38variables), impaired region (4 sites x 38variables) and less impaired region (5 sites x 38variables) separately. The main purpose of this analysis was to reduce the contribution of less significant variables of the water quality parameters, which was achieved by rotating the axis defined by PCA to produce new groups of variables (varimax factors). PCA technique starts with the covariance matrix describing the dispersion of the original variables and extracting the eigenvalues and eigenvectors (Singh *et al.*, 2005a; Hill & Lewicki, 2006).

FA attempts to extract a lower dimensional linear structure from the data set and can be used for source identification. It further reduces the contribution of less significant variables obtained from PCA and the new group of variables known as varimax factors (VFs) is extracted through rotating the axis defined by PCA. In FA the basic concept is expressed in Equation (II) given by Singh *et al.* (2005a).

$$Z_{ji} = a_{f1}f_{1i} + a_{f2}f_{2i} + a_{f3}x_{3i} + \ldots + a_{fm}f_{mi} + e_{fi} \tag{II}$$

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## Results

### Stream Morphology

Basic descriptive statistics (range, mean and standard deviation) of stream morphological parameters measured at 18 sites located on Nullah Aik and Nullah Palkhu during 2004- 2006 are summarized in Table 3.1. Stream width ranged from 3.47 to 37.1m with an average of 13.12m. Maximum stream width (37.07m) was recorded from Uoora located at upstream of Nullah Aik. Stream width of Nullah Aik decreased from upstream to downstream, whereas, Nullah Palkhu exhibited an increasing trend from upstream to downstream (Fig. 3.1a). Average value of stream flow was 0.88 m/sec. A decreasing trend in stream width was observed in case of Nullah Aik from upstream to downstream sites, whereas, in contrary to Nullah Aik, Nullah Palkhu showed an increasing trend. Average depth of stream was measured as 1.54m; maximum depth (6.0m) of stream was recorded from Wain small headworks and minimum stream depth (0.49m) at Dallowali. Generally stream depth increases from upstream to downstream in both streams (Fig. 3.1a).

### Physiochemical Variables of Stream Water

Mean and range values (min. – max.) of 16 Physico-chemical parameters of water samples collected from different sites located on Nullah Aik and Nullah Palkhu are given in Table 3.1. Variations in stream water temperature were recorded that varied between 12 to 29$^{o}$C during the study period. Maximum temperature was recorded during monsoon season whereas minimum was recorded in winter season (Fig. 3.1b).

Most of the stream water samples were alkaline in nature with pH varied between 6.59 and 9.71. Maximum pH values were observed in upstream sites, which gradually decreased towards downstream sites (Fig. 3.1b). The level of DO in stream water was ranged between 0.1 to 10.9mg/L. The maximum values of DO were found in the sample collected from upstream site (Uoora) of Nullah Aik and minimum values at Chiti Sheikhan. The results showed decreasing trend of DO from upstream sites to mid-stream sites near Sialkot city and gradual improvement in far downstream sites (Fig. 3.1b). Average COD values varied between 4.82 and 1090 mg/L. The maximum values were recorded from downstream sites such as Chiti Sheikan and Pul Aik and minimum in upstream sites of both streams. Highest value of COD was

measured in those stream segments, which pass through urban areas of Sialkot (Fig. 3.1c). EC and TDS also showed distinct variations and exhibited similar trend like COD (Fig. 3.1c). Maximum values were recorded from Jatta situated at downstream of Nullah Palkhu. Salinity and total hardness showed an increase in concentration trend from upstream towards mid-stream sites and decrease in far downstream sites (Fig. 3.1c). Maximum turbidity (1526NTU) was recorded near Upper Chenab- Palkhu crossing and minimum (2.5 NTU) at Umeranwali at upstream of Nullah Aik (Fig. 3.1d). Highest concentrations of $Cl^-$ (2463mg/L) and $S^{2-}$ (3.412mg/L) were measured at Chiti Sheikhan and Jatta located at downstream of Nullah Palkhu and minimum $Cl^-$ (97.11mg/L) and $S^{2-}$ (0.169mg/L) at upstream sites of studied streams. Nullah Palkhu exhibited marked variations in $Cl^-$ and $S^{2-}$ as compared to Nullah Aik (Fig. 3.1e).

## Nutrients in Stream Water

The maximum concentration of $NO_3^-$ (9.8mg/L) was recorded in water samples collected from Dallowali at upstream of Nullah Palkhu site and minimum in water samples taken from Wain small head works at downstream of Nullah Aik. The results indicated high concentration of $NO_3^-$ exhibited by the site located in agricultural area (Fig. 3.1f). In contrast, high concentration of $PO_4^{3-}$ was measured at those sites, which were located in industrial and urban areas (Fig. 3.1f).

## Dissolved Metals in Stream Water

Mean and range values of 11 dissolved metals in water samples are given in Table 3.2. The average concentrations of major metals such as Na, K, Mg, and Ca in stream water were higher to those of heavy metals. Maximum concentration of dissolved Na (203.9mg/L) was measured in water sampled collected from Jatta whereas, minimum (5.45mg/L) from Uoora at upstream of Nullah Aik. Concentration of dissolved Na increased from upstream to downstream of Nullah Aik whereas, highest concentration was observed in sites receiving effluents form tanneries which gradually decrease in far downstream (Fig. 3.1g).

Like Na, K exhibited similar spatial pattern. Nullah Aik showed lesser concentration of K in comparison with Nullah Palkhu (Fig. 3.1g). An increasing trend in concentration of dissolved Ca was observed from upstream to mid-stream and decreased in downstream of studied streams (Fig. 3.1h), whereas, dissolved Mg did not show marked variations in water quality of Nullah Aik, whereas, comparatively, significant variations were observed in Nullah Palkhu (Fig. 3.1h).

Sites located near downstream of Sialkot exhibited high concentration of dissolved Fe which gradually decreases in far downstream sites (Fig. 3.1i). Similarly, dissolved Pb concentration exhibited similar spatial pattern. High concentration of dissolved Pb was recorded at sites close to Sialkot city (Fig. 3.1i). High concentration of Cd (dissolved) was recorded at sites located far from human settlements (Fig. 3.1j). The spatial trend of Cr (dissolved) showed marked variations. Minimum concentration of Cr (dissolved) was found (0.001mg/L) at Umeranwali and maximum (1.146mg/L) at Jatta. High concentration was observed from sites, which receive effluents from tanneries such as Chitti Sheikhan, Jatta, Pul Aik and Wazirabad (Fig. 3.1j). Concentration of Ni in dissolved form increases from upstream sites towards downstream sites and highest concentration was found in water samples collected from far downstream sites (Fig. 3.1k). The highest concentration of Cu in dissolved form was observed at Wazirabad, whereas, lowest at sites downstream of Nullah Aik (Fig. 3.1k). Zn exhibited similar spatial pattern to that of Cr (Fig. 3.1k).

## Suspended Particulate Metals in Stream Water

Mean and range values of 11 metals in suspended particulate form in water samples are given in Table 3.3. Suspended particulate matter exhibited average Na concentration (20.658mg/L), which showed an increasing trend from upstream to downstream with exception of three sites viz; Kot Zamin, Kot Khudabux and Wazirabad, which exhibited lower concentrations (Fig. 3.1l). Maximum concentration of K (123 mg/L) was recorded at Upper Chenab – Palkhu crossing and minimum concentration (0.075mg/L) at Kot Zamin located at downstream of Nullah Aik. The concentration of K in suspended form did not showed spatial variations pattern in Nullah Aik, however, an increasing trend was observed from upstream to downstream of Nullah Palkhu (Fig. 3.1l). Ca (suspended particulate form) exhibited maximum concentration (101.836mg/L) at Jathekay, whereas, minimum (0.987mg/L) at Kot Zamin. In case Nullah Aik, Ca in suspended particulate form decreased from upstream to downstream site, whereas, its highest concentration was recorded at midstream of Nullah Palkhu, which decreases towards downstream. Highest concentration (106.97mg/L) of Mg was recorded at Jatta and minimum (3.163mg/L) at Kot Zamin. Like Ca in suspended particulate form, Mg (suspended particulate form) showed high concentration at sites situated on mid-stream of Nullah Palkhu (Fig. 3.1m).

Average concentration of Fe in suspended form varied between 0.759 - 71.054 (mg/L). High concentration of Fe in suspended particulate matter recorded at sites located in close vicinity of Sialkot city (Fig. 3.1m). Concentration of Pb in suspended particulate form increases from upstream to downstream sites (Fig. 3.1n). Cd in

suspended form varied from 0.001 to 0.832mg/L and sites with high concentration of Cd were found near downstream of streams (Fig. 3.1o).

The concentration of suspended Cr was varied from between 0.017 and 26.47 mg/L and higher concentration was recorded at sites close to Sialkot city (Fig. 3.1o), which receive effluents from tanneries. The concentration of Ni in suspended form was ranged from 0.001 to 4.235 mg/L. Ni in suspended particulate form exhibited marked differences in its spatial distribution (Fig. 3.1p). High concentration of suspended Ni was recorded from sites where stream receive effluents from municipal waste. Cu in suspended particulate form showed the lowest value 0.001 at Urooa and highest 3.201mg/L at Jatta. Maximum concentration of Cu in suspended form was observed near downstream sites on Nullah Palkhu. Less variations of suspended Cu were recorded in Nullah Aik, in comparison to Nullah Palkhu which showed marked fluctuations (Fig. 3.1p). Mean concentration of Zn in suspended form was 1.322mg/L. Zn in suspended particulate form showed higher concentration near downstream of Nullah Palkhu (Fig. 3.1p).

Concentrations of metals in dissolved and suspended particulate matter is depicted in Fig. 3.2(a – j). Higher concentrations of major metals were recorded in dissolved form as compared to heavy metals. Na and Ca showed higher concentration in dissolved form to those in suspended form, whereas, other metals (K and Mg) showed highest concentration in suspended particulate form. The general trend of alkali and alkaline earth metals in dissolved and suspended form was: Ca > Na > Mg > K and Mg > Ca > Na > K, respectively. Among heavy metals, highest concentration of Fe and lowest concentration of Cd was recorded in dissolved and suspended particulate form. The general trend of metals in dissolved and suspended particulate form was: Fe > Pb > Cr > Ni > Zn > Cu > Cd and Fe > Cr > Zn > Pb > Ni > Cu > Cd, respectively.

Table 3.1 Means ± S.D. and Ranges (min– max) of Physico-chemical parameters in stream water at various sampling sites located on Nullah Aik and Nullah Aik

| St # | | Stream width | Stream velocity | Stream depth | Temp | pH | DO | COD | E.C. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Means ± S.D | 25.46 ± 8.82 | 1.00 ± 0.61 | 1.10 ± 0.56 | 20.21 ± 5.73 | 8.26 ± 0.22 | 7.99 ± 1.69 | 24.3 ± 14.1 | 535.4 ± 98.3 |
| | Min - Max | 3.77 - 9.67 | 0.40 - 0.74 | 0.49 - 1.50 | 14 - 22 | 6.59 - 8.59 | 0.1 - 2.8 | 71.6 - 2886.0 | 301.0 - 1868.0 |
| 2 | Means ± S.D | 30.64 ± 3.14 | 0.91 ± 0.35 | 1.00 ± 0.22 | 21.33 ± 5.88 | 8.16 ± 0.22 | 7.86 ± 2.04 | 33.7 ± 36.5 | 524.9 ± 99.3 |
| | Min - Max | 4.39 - 9.66 | 0.35 - 1.21 | 1.10 - 1.80 | 14 - 30 | 7.13 - 8.64 | 0.23 - 2.01 | 54.2 - 1527.0 | 633.0 - 1693.0 |
| 3 | Mean ± S.D | 12.61 ± 1.73 | 1.00 ± 0.58 | 1.30 ± 0.15 | 21.88 ± 5.73 | 7.78 ± 0.31 | 2.03 ± 0.66 | 267.0 ± 327.6 | 753.5 ± 238.0 |
| | Min - Max | 9.84 - 13.80 | 0.46 - 1.76 | 1.58 - 2.93 | 12--29 | 7.23 - 8.31 | 0.79 - 4.98 | 12.4 - 140.4 | 545.0 - 926.0 |
| 4 | Mean ± S.D | 17.03 ± 1.64 | 1.00 ± 0.47 | 1.26 ± 0.12 | 22.33 ± 5.88 | 7.65 ± 0.51 | 2.24 ± 0.51 | 215.5 ± 243.2 | 711.8 ± 165.4 |
| | Min - Max | 23.73 - 29.07 | 0.40 - 1.98 | 0.60 - 2.10 | 11--29 | 7.89 - 8.67 | 5.25 - 10.58 | 4.8 - 59.0 | 411.0 - 31.0 |
| 5 | Means ± S.D | 11.18 ± 1.79 | 1.09 ± 0.50 | 1.26 ± 0.35 | 21.72 ± 5.87 | 7.88 ± 0.34 | 3.12 ± 0.85 | 202.6 ± 167.3 | 767.2 ± 170.2 |
| | Min - Max | 3.70 - 21.27 | 0.43 - 1.08 | 0.50 - 1.73 | 12--30 | 3.38 - 9.28 | 4.1 - 8.96 | 11.5 - 67.1 | 505.0 - 971.0 |
| 6 | Mean ± S.D | 12.10 ± 1.19 | 1.12 ± 0.47 | 1.11 ± 0.53 | 21.21 ± 5.74 | 7.72 ± 0.30 | 3.67 ± 1.19 | 62.0 ± 39.8 | 696.5 ± 125.3 |
| | Min - Maxi | 26.28 - 37.07 | 0.44 - 1.34 | 1.23 - 1.95 | 11--30 | 7.76 - 8.45 | 4.01 - 10.99 | 9.0 - 132.0 | 403.0 - 694.0 |
| 7 | Mean ± S.D | 11.72 ± 1.34 | 0.37 ± 0.72 | 3.89 ± 1.10 | 21.33 ± 5.87 | 7.93 ± 0.36 | 4.07 ± 1.30 | 57.8 ± 37.4 | 651.3 ± 139.3 |
| | Min - Max | 10.83 - 15.91 | 0.43 - 1.26 | 1.09 - 1.61 | 13 - 30 | 7.34 - 8.38 | 1.03 - 3.84 | 28.4 - 1090.0 | 523.0 - 304.0 |
| 8 | Means ± S.D | 16.27 ± 1.33 | 0.90 ± 0.72 | 1.74 ± 0.40 | 20.33 ± 6.67 | 7.56 ± 0.19 | 2.65 ± 1.08 | 191.3 ± 129.5 | 718.7 ± 74.8 |
| | Min - Max | 3.48 - 9.11 | 0.30 - 1.53 | 0.90 - 1.90 | 14 - 30 | 6.99 - 8.23 | 0.5 - 2.35 | 13.7 - 740.2 | 624.0 - 2531.0 |
| 9 | Means ± S.D | 19.43 ± 1.18 | 1.01 ± 0.65 | 1.72 ± 0.23 | 25.33 ± 3.67 | 7.91 ± 0.16 | 1.26 ± 0.87 | 52.1 ± 17.3 | 1051.7 ± 413.4 |
| | Min - Max | 4.87 - 12.88 | 0.38 - 0.86 | 0.63 - 1.08 | 11--29 | 7.56 - 8.5 | 4.31 - 7.73 | 11.3 - 201.6 | 525.0 - 926.0 |
| 10 | Means ±S.D | 13.71 ± 6.75 | 0.67 ± 0.20 | 0.80 ± 0.44 | 21.21 ± 5.73 | 7.79 ± 1.05 | 6.01 ± 1.35 | 33.4 ± 15.8 | 768.0 ± 136.8 |
| | Min - Max | 11.54 - 17.52 | 0.38 - 1.90 | 1.03 - 1.90 | 13 - 29 | 7.24 - 8.88 | 1.12 - 5.56 | 17.6 - 468.2 | 463.0 - 261.0 |
| 11 | Means ±S.D | 7.66 ± 2.78 | 0.60 ± 0.15 | 0.76 ± 0.17 | 21.33 ± 5.88 | 8.01 ± 0.28 | 6.20 ± 0.98 | 58.4 ± 53.4 | 744.3 ± 134.4 |
| | Min - Max | 19.40 - 21.37 | 0.41 - 1.65 | 1.13 - 1.50 | 12 - 30 | 7.32 - 7.86 | 1.26 - 4.32 | 18.5 - 21.0 | 480.0 - 750.0 |
| 12 | Mean ± S.D | 5.10 ± 1.61 | 0.55 ± 0.13 | 1.06 ± 0.36 | 21.33 ± 5.87 | 7.72 ± 0.56 | 1.18 ± 0.77 | 496.5 ± 863.1 | 1190.7 ± 452.4 |
| | Min - Max | 14.57 - 19.38 | 0.43 - 1.91 | 1.08 - 1.42 | 13 - 30 | 6.78 - 8.7 | 1.2 - 2.96 | 13.1 - 830.0 | 455.0 - 975.0 |
| 13 | Mean ± S.D | 6.21 ± 1.65 | 0.56 ± 0.23 | 1.34 ± 0.26 | 21.21 ± 5.74 | 7.76 ± 0.39 | 0.97 ± 0.56 | 507.9 ± 431.9 | 1253.6 ± 275.0 |
| | Min - Max | 8.43 - 15.06 | 0.51 - 1.80 | 0.92 - 1.70 | 13 - 30 | 7.45 - 8.64 | 1.56 - 4.63 | 20.0 - 533.0 | 526.0 - 988.0 |
| 14 | Mean ± S.D | 5.24 ± 1.44 | 0.83 ± 0.35 | 1.48 ± 0.31 | 21.21 ± 5.73 | 7.69 ± 0.35 | 1.14 ± 0.52 | 313.2 ± 244.0 | 1345.3 ± 541.8 |
| | Min - Max | 9.34 - 14.21 | 0.20 - 1.01 | 3.09 - 5.97 | 11--29 | 7.36 - 8.75 | 0.94 - 5.59 | 12.2 - 127.8 | 436.0 - 898.0 |
| 15 | Mean ± S.D | 12.36 ± 1.56 | 1.02 ± 0.46 | 1.52 ± 0.15 | 21.33 ± 5.88 | 7.91 ± 0.46 | 3.65 ± 1.39 | 127.6 ± 83.8 | 1159.4 ± 353.4 |
| | Min - Max | 15.25 - 18.60 | 0.43 - 2.00 | 1.46 - 2.40 | 13 - 30 | 7.3 - 7.85 | 1.18 - 3.89 | 46.7 - 376.2 | 612.0 - 783.0 |
| 16 | Mean ± S.D | 15.71 ± 2.07 | 1.07 ± 0.51 | 1.59 ± 0.22 | 21.21 ± 5.73 | 7.89 ± 0.36 | 3.32 ± 1.56 | 102.4 ± 96.3 | 795.2 ± 227.1 |
| | Min - Max | 17.50 - 20.87 | 0.41 - 1.67 | 1.50 - 2.00 | 12--30 | 7.58 - 8.01 | 0.31 - 2.13 | 20.8 - 68.0 | 680.0 - 563.0 |
| 17 | Means ± S.D | 19.59 ± 3.46 | 0.97 ± 0.62 | 1.33 ± 0.17 | 24.83 ± 3.25 | 7.55 ± 0.24 | 3.02 ± 1.38 | 139.6 ± 135.3 | 617.5 ± 143.4 |
| | Min - Max | 9.35 - 14.59 | 0.29 - 1.85 | 1.24 - 1.70 | 14 - 29 | 7.41 - 9.71 | 0.9 - 5.99 | 24.4 - 246.1 | 605.0 - 1801.0 |
| 18 | Means ±S.D | 18.20 ± 3.05 | 0.80 ± 0.24 | 1.67 ± 0.32 | 21.50 ± 6.06 | 7.99 ± 0.46 | 3.08 ± 1.21 | 154.4 ± 147.5 | 763.4 ± 216.5 |
| | Min - Max | 14.81 - 23.87 | 0.41 - 1.12 | 1.44 - 2.40 | 13 - 30 | 7.5 - 8.73 | 1.02 - 5.16 | 20.8 - 491.6 | 488.0 - 1096.0 |

Table 3.1  continue…
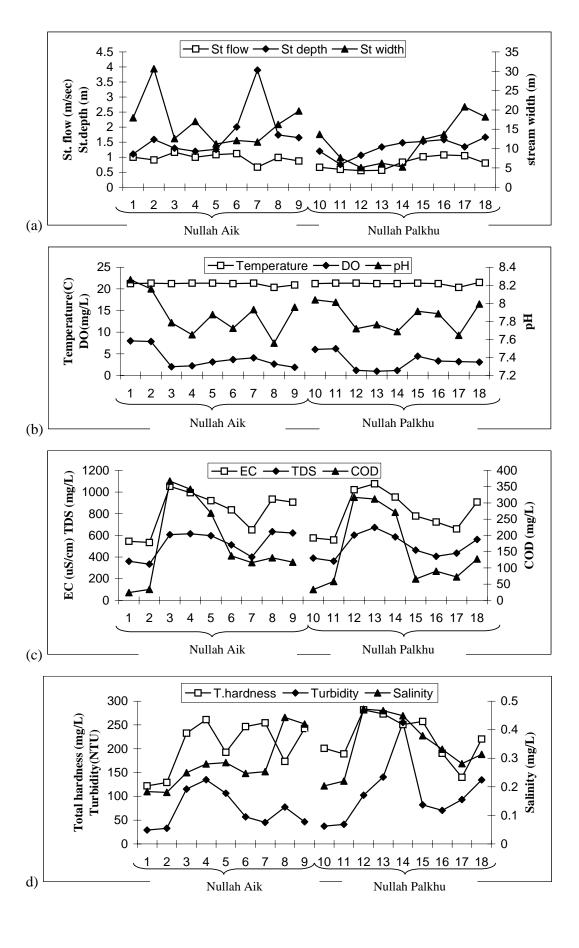
| St # | | TDS | Salinity | T. Hardness | Turbidity | NO$_3^-$ | PO$_4^{3-}$ | Cl | S$^{2-}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Means ± S.D | 353.5 ± 74.0 | 0.18 ± 0.06 | 194.5 ± 77.0 | 63.2 ± 125.6 | 1.07 ± 0.29 | 0.54 ± 0.48 | 23.38 ± 14.40 | 0.008 ± 0.006 |
| | Min - Max | 246.0 - 93.0 | 0.09 - 0.3 | 65.4 - 352.2 | 2.6 - 406.0 | 0.48 - 1.60 | 0.01 - 1.87 | 2.99 - 59.50 | 0.000 - .021 |
| 2 | Means ± S.D | 328.7 ± 74.5 | 0.18 ± 0.06 | 189.1 ± 73.9 | 31.6 ± 23.5 | 1.29 ± 0.47 | 1.36 ± 1.64 | 26.76 ± 16.90 | 0.015 ± 0.009 |
| | Min - Max | 226.0 - 469.0 | 0.08 - 0.29 | 73.3 - 291.5 | 4.5 - 74.5 | 0.56 - 2.03 | 0.04 - 6.78 | 5.13 - 84.50 | 0.002 - 0.031 |
| 3 | Mean ± S.D | 434.5 ± 177.3 | 0.25 ± 0.10 | 232.7 ± 106.0 | 75.8 ± 43.7 | 1.40 ± 1.14 | 1.78 ± 1.28 | 41.75 ± 31.97 | 0.069 ± 0.051 |
| | Min - Max | 235.0 - 855.0 | 0.1 - 0.4 | 115.3 - 450.4 | 26.2 - 167.0 | 0.24 - 4.05 | 0.38 - 3.59 | 10.40 - 98.84 | 0.007 - 0.159 |
| 4 | Mean ± S.D | 440.1 ± 116.7 | 0.28 ± 0.10 | 261.1 ± 150.1 | 68.1 ± 58.0 | 1.01 ± 0.77 | 2.07 ± 1.15 | 50.42 ± 40.32 | 0.058 ± 0.049 |
| | Min - Max | 320.0 - 695.0 | 0.147 - .47 | 61.0 - 578.1 | 12.4 -190.0 | 0.26 - 2.69 | 0.62 - 4.32 | 10.62 - 31.21 | 0.021 - 0.151 |
| 5 | Means  ± S.D | 498.0 ± 117.2 | 0.28 ± 0.10 | 192.8 ± 89.6 | 68.8 ± 85.3 | 1.09 ± 0.49 | 2.26 ± 1.84 | 52.33 ± 47.10 | 0.131 ± 0.113 |
| | Min - Max | 312.0 - 670.0 | 0.189 - 0.5 | 37.3 - 286.1 | 6.9 - 280.0 | 0.40 - 1.89 | 0.51 - 6.83 | 9.94 - 139.16 | 0.017 - 0.401 |
| 6 | Mean ± S.D | 426.8 ± 82.7 | 0.25 ± 0.09 | 209.1 ± 85.7 | 28.5 ± 27.4 | 1.31 ± 1.42 | 1.62 ± 1.58 | 57.79 ± 21.68 | 0.053 ± 0.039 |
| | Min  - Maxi | 321.0 - 610.0 | 0.13 - 0.4 | 64.2 - 329.0 | 6.3 - 96.8 | 0.20 - 4.13 | 0.59 - 6.25 | 29.92 - 00.39 | 0.007 - 0.125 |
| 7 | Mean ± S.D | 399.8 ± 65.8 | 0.25 ± 0.09 | 200.5 ± 85.3 | 31.3 ± 28.3 | 0.51 ± 0.19 | 1.35 ± 0.64 | 54.72 ± 32.41 | 0.022 ± 0.016 |
| | Min - Max | 329.0 - 550.0 | 0.11 - 0.4 | 72.4 - 294.0 | 6.0 - 99.5 | 0.23 - 0.87 | 0.51 - 2.75 | 14.25 - 32.74 | 0.005 - 0.067 |
| 8 | Means ± S.D | 438.6 ± 62.3 | 0.44 ± 0.21 | 173.4 ± 84.0 | 77.2 ± 53.0 | 1.23 ± 0.98 | 1.19 ± 0.69 | 73.57 ± 36.70 | 0.124 ± 0.183 |
| | Min - Max | 375.0 - 530.0 | 0.18 - 0.8 | 52.0 - 255.0 | 16.4 - 160.0 | 0.35 - 3.15 | 0.41 - 2.63 | 45.30 - 44.13 | 0.018 - .456 |
| 9 | Means  ± S.D | 716.3 ± 285.1 | 0.53 ± 0.34 | 150.3 ± 75.3 | 61.3 ± 20.5 | 1.85 ± 0.79 | 1.28 ± 0.45 | 125.81 ± 76.36 | 0.088 ± 0.075 |
| | Min - Max | 460.0 - 075.0 | 0.21 - 0.9 | 53.6 - 216.7 | 34.6 - 94.5 | 0.98 - 2.58 | 0.84 - 1.84 | 50.46 - 11.72 | 0.023 - 0.188 |
| 10 | Means ±S.D | 519.9 ± 123.6 | 0.20 ± 0.09 | 200.8 ± 74.3 | 79.1 ± 140.6 | 2.72± 1.63 | 1.15 ± 1.03 | 49.46 ± 26.12 | 0.024 ± 0.022 |
| | Min - Max | 279.0 - 691.0 | 0.07 - 0.4 | 77.9 - 325.1 | 2.5 - 455.0 | 1.07 - 7.10 | 0.02 - 4.56 | 14.91 - 115.30 | 0.002 - 0.064 |
| 11 | Means ±S.D | 482.6 ± 139.0 | 0.22 ± 0.09 | 189.1 ± 77.2 | 53.5 ± 75.9 | 4.44 ± 2.72 | 1.03 ± 0.83 | 41.50 ± 29.95 | 0.025 ± 0.028 |
| | Min - Max | 325.0 - 667.0 | 0.08 - 0.41 | 54.9 - 298.7 | 5.4 - 270.0 | 1.23 - 9.80 | 0.21 - 3.78 | 4.37 - 102.38 | 0.000 - 0.087 |
| 12 | Mean ± S.D | 702.5 ± 286.3 | 0.47 ± .30 | 323.5 ± 203.8 | 102.5 ± 130.9 | 0.82 ± 0.49 | 2.29 ± 1.77 | 275.6 ± 644.49 | 0.400 ± 0.309 |
| | Min - Max | 256.0 - 1180.0 | 0.06 - 0.98 | 89.1 - 729.7 | 21.8 - 646.0 | 0.32 -2.01 | 0.53 - 4.86 | 27.1 - 2463.1 | 0.043 - 1.103 |
| 13 | Mean ± S.D | 783.8 ± 138.9 | 0.47 ± 0.22 | 273.3 ± 88.3 | 140.6 ± 91.2 | 2.39 ± 2.86 | 5.23 ± 4.07 | 117.46 ± 69.53 | 0.741 ± 1.015 |
| | Min - Max | 453.0 - 935.0 | 0.19 - 0.96 | 132.5 - 391.1 | 14.5 - 342.0 | 0.21 - 7.78 | 0.65 - 12.56 | 32.60 - 264.46 | 0.024 - 3.412 |
| 14 | Mean ± S.D | 836.5 ± 295.3 | 0.45 ± 0.20 | 250.7 ± 80.8 | 255.1 ± 483.5 | 0.73 ± 0.35 | 5.03 ± 4.57 | 103.60 ± 52.90 | 0.494 ± 0.409 |
| | Min - Max | 456.0 - 564.0 | 0.14 - 0.7 | 119.7 - 381.9 | 34.5 - 1526.0 | 0.26 - 1.60 | 0.53 - 14.67 | 22.03 - 186.24 | 0.021 - 1.595 |
| 15 | Mean ± S.D | 689.5 ± 151.5 | 0.38 ± 0.19 | 257.3 ± 84.9 | 91.0 ± 108.8 | 0.70 ± 0.20 | 4.22 ± 3.71 | 90.60 ± 47.37 | 0.392 ± 0.431 |
| | Min - Max | 428.0 - 855.0 | 0.13 - 0.6 | 123.4 - 400.0 | 13.0 - 369.0 | 0.40 - 1.10 | 0.67 - 10.42 | 32.92 - 175.56 | 0.018 - 1.324 |
| 16 | Mean ± S.D | 447.7 ± 117.6 | 0.33 ± 0.15 | 190.3 ± 66.5 | 47.0 ± 44.9 | 0.81 ± 0.46 | 2.49 ± 2.44 | 182.0 ± 238.07 | 0.130 ± 0.104 |
| | Min - Max | 303.0 - 688.0 | 0.14 - 0.78 | 77.6 - 262.4 | 11.4 - 170.0 | 0.13 - 1.85 | 0.02 - 6.52 | 16.99 - 93.12 | 0.008 - 0.348 |
| 17 | Means ± S.D | 420.7 ± 97.6 | 0.30 ± 0.11 | 127.3 ± 1.3 | 61.1 ± 53.9 | 1.89 ± 1.29 | 2.14 ± 1.54 | 41.01 ± 23.25 | 0.036 ± 0.018 |
| | Min - Max | 325.0 - 510.0 | 0.19 - 0.4 | 57.8 - 211.0 | 14.6 - 145.0 | 0.72 - 4.12 | 0.63 - 3.62 | 18.70 - 66.60 | 0.021 - 0.066 |
| 18 | Means ±S.D | 485.3 ± 130.7 | 0.31 ± 0.13 | 220.3 ± 10.8 | 244.0 ± 525.9 | 1.25 ± 0.63 | 1.10 ± 0.94 | 65.55 ± 48.09 | 0.110 ± 0.112 |
| | Min - Max | 310.0 - 684.0 | 0.19 - 0.6 | 58.5 - 442.4 | 16.5 - 2002.0 | 0.12 - 1.98 | 0.04 - 2.85 | 17.00 - 142.14 | 0.001 - 0.332 |
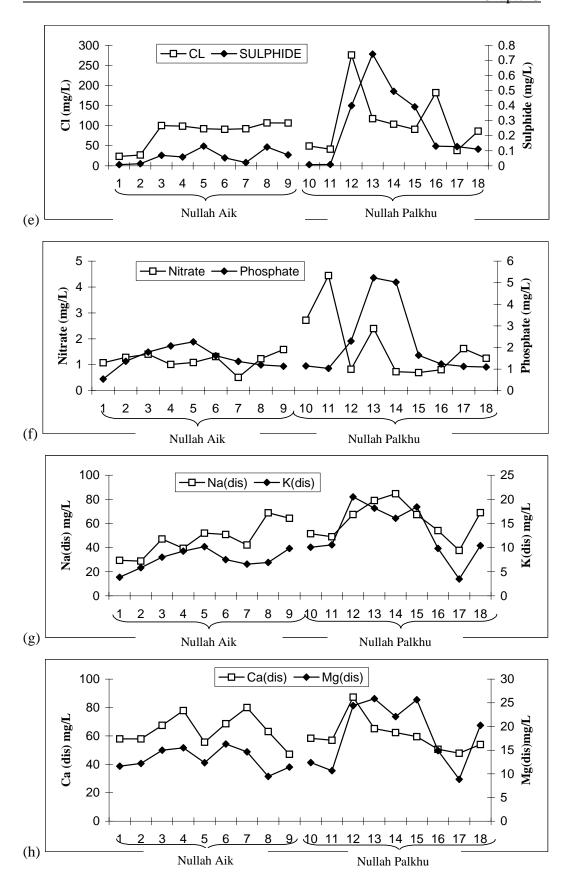
Table. 3.2          Means ± S.D. and Ranges (min– max) of dissolved metals in stream water at various sampling sites located on Nullah Aik and Nullah Palkhu
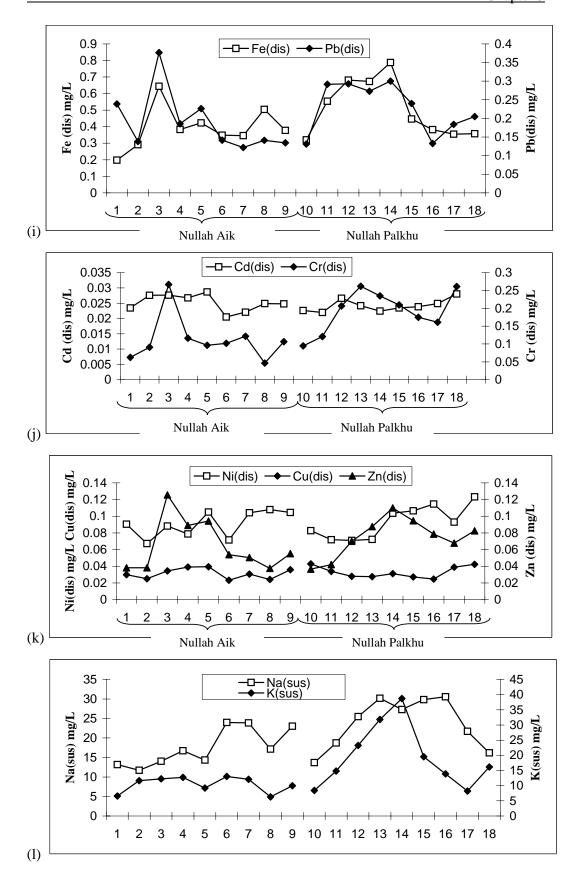
| St # | | Na (dis) | K (dis) | Ca (dis) | Mg (dis) | Fe (dis) | Pb (dis) | Cd (dis) | Cr(dis) | Ni (dis) | Cu (dis) | Zn (dis) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Means ± S.D | 29.41 ± 11.90 | 3.87 ± 3.07 | 57.92 ± 19.63 | 11.61 ± 9.59 | 0.198 ± 0.430 | 0.111 ± 0.070 | 0.023± 0.031 | 0.062 ± 0.083 | 0.090 ± 0.122 | 0.030 ± 0.062 | 0.038 ± 0.030 |
|   | Min - Max | 17.6 - 57.1 | 0.2 - 10.0 | 15.7 - 86.5 | 0.4 - 42.5 | 0.003 - .542 | 0.007 - .288 | 0.002 - .098 | 0.003 - .340 | 0.005 - .356 | 0.000 - .196 | 0.004 - .093 |
| 2 | Means ± S.D | 28.85 ± 10.12 | 5.84 ± 6.71 | 57.92 ± 18.80 | 12.18 ± 9.12 | 0.292 ± 0.406 | 0.129 ± 0.095 | 0.028 ± 0.029 | 0.091 ± 0.160 | 0.067 ± 0.069 | 0.025 ± 0.051 | 0.038 ± 0.029 |
|   | Min - Max | 19.3 - 58.9 | 0.6 - 23.1 | 19.5 - 86.9 | 0.1 - 29.9 | 0.018 - 1.330 | 0.029 - 0.421 | 0.003 - 0.098 | 0.002 - 0.504 | 0.008 - 0.256 | 0.000 - 0.173 | 0.002 - 0.085 |
| 3 | Mean ± S.D | 47.14 ± 30.57 | 8.02 ± 4.77 | 67.43 ± 28.19 | 14.98 ± 0.61 | 0.644 ± 0.769 | 0.660 ± 0.649 | 0.028 ± 0.032 | 0.267 ± 0.388 | 0.088 ± 0.063 | 0.034 ± 0.055 | 0.126 ± 0.183 |
|   | Min - Max | 14.5 - 130.4 | 0.4 - 5.7 | 30.9 - 130.6 | 1.6 - 31.2 | 0.002 - 2.526 | 0.031 - 1.922 | 0.003 - 0.108 | 0.006 - 1.146 | 0.004 - 0.224 | 0.000 - 0.187 | 0.015 - 0.689 |
| 4 | Mean ± S.D | 39.46 ± 16.32 | 9.25 ± 10.71 | 77.79 ± 42.36 | 15.49 ± 12.48 | 0.383 ± 0.513 | 0.445 ± 0.745 | 0.027 ± 0.031 | 0.116 ± 0.149 | 0.079 ± 0.078 | 0.039 ± 0.069 | 0.089 ± 0.088 |
|   | Min - Max | 12.0 - 69.0 | 1.0 - 37.9 | 16.7 - 170.3 | 1.7 - 37.2 | 0.020 - 1.693 | 0.039 - 2.605 | 0.001 - 0.109 | 0.006 - 0.489 | 0.002 - 0.239 | 0.001 - 0.203 | 0.018 - 0.415 |
| 5 | Means ± S.D | 52.01 ± 31.82 | 10.19 ± 15.41 | 55.60 ± 24.64 | 12.34 ± 8.19 | 0.423 ± 0.522 | 0.600 ± 0.818 | 0.029 ± 0.035 | 0.096 ± 0.139 | 0.105 ± 0.101 | 0.040 ± 0.069 | 0.094 ± 0.137 |
|   | Min - Max | 11.1 - 122.1 | 0.3 - 51.4 | 13.2 - 89.5 | 1.0 - 24.9 | 0.020 - 2.300 | 0.062 - 3.538 | 0.003 - 0.116 | 0.001 - 0.610 | 0.004 - 0.325 | 0.000 - 0.234 | 0.000 - 0.452 |
| 6 | Mean ± S.D | 50.92 ± 21.06 | 7.52 ± 2.51 | 56.53 ± 20.44 | 16.29 ± 10.92 | 0.349 ± 0.452 | 0.692 ± 0.904 | 0.021 ± 0.027 | 0.102 ± 0.153 | 0.072 ± 0.068 | 0.023 ± 0.041 | 0.054 ± 0.070 |
|   | Min - Maxi | 22.4 - 97.8 | 3.0 - 13.3 | 14.9 - 82.0 | 1.2 - 36.9 | 0.021 - 1.689 | 0.042 - 3.378 | 0.000 - 0.089 | 0.004 - 0.525 | 0.002 - 0.197 | 0.001 - 0.125 | 0.004 - 0.237 |
| 7 | Mean ± S.D | 42.20 ± 19.83 | 6.56 ± 2.17 | 54.95 ± 21.26 | 14.63 ± 10.26 | 0.346 ± 0.425 | 0.692 ± 0.849 | 0.022 ± 0.026 | 0.122 ± 0.178 | 0.104 ± 0.136 | 0.031 ± 0.055 | 0.050 ± 0.065 |
|   | Min - Max | 18.5 - 77.1 | 2.1 - 9.7 | 16.5 - 89.1 | 1.0 - 32.0 | 0.008 - 1.534 | 0.016 - 3.068 | 0.002 - 0.091 | 0.005 - 0.504 | 0.001 - 0.436 | 0.000 - 0.183 | 0.003 - 0.216 |
| 8 | Means ± S.D | 68.88 ± 56.10 | 6.92 ± 5.20 | 63.08 ± 10.11 | 9.45 ± 4.48 | 0.505 ± 0.647 | 0.747 ± 1.017 | 0.049 ± 0.058 | 0.046 ± 0.046 | 0.179 ± 0.189 | 0.141 ± 0.202 | 0.027 ± 0.023 |
|   | Min - Max | 19.8 - 173.5 | 0.2 - 12.6 | 45.1 - 76.5 | 3.2 - 13.6 | 0.045 - .405 | 0.044 - 2.162 | 0.001 - 0.130 | 0.014 - 0.108 | 0.001 - 0.424 | 0.011 - 0.532 | 0.000 - 0.056 |
| 9 | Means ± S.D | 66.60 ± 46.16 | 7.98 ± 8.43 | 61.46 ± 4.75 | 6.41 ± 3.41 | 0.862 ± 0.805 | 1.325 ± 1.239 | 0.067 ± 0.066 | 0.134 ± 0.145 | 0.055 ± 0.035 | 0.142 ± 0.150 | 0.019 ± 0.016 |
|   | Min - Max | 23.6 - 113.6 | 0.2 - 16.9 | 53.1 - 67.0 | 3.1 - 10.2 | 0.106 - 1.856 | 0.163 - 2.855 | 0.004 - 0.131 | 0.002 - 0.279 | 0.021 - 0.092 | 0.007 - 0.347 | 0.001 - 0.038 |
| 10 | Means ±S.D | 51.48 ± 25.28 | 10.05 ± 7.67 | 58.34 ± 21.85 | 12.37 ± 7.17 | 0.321 ± 0.532 | 0.210 ± 0.237 | 0.023 ± 0.030 | 0.195 ± 0.326 | 0.083 ± 0.092 | 0.043 ± 0.112 | 0.037 ± 0.029 |
|   | Min - Max | 16.5 - 112.0 | 0.2 - 26.5 | 20.6 - 96.4 | 1.2 - 20.9 | 0.020 - 1.784 | 0.021 - 0.989 | 0.001 - 0.099 | 0.011 - 1.013 | 0.003 - 0.281 | 0.000 - 0.475 | 0.001 - 0.096 |
| 11 | Means ±S.D | 49.03 ± 22.31 | 10.56 ± 7.63 | 56.92 ± 23.10 | 10.67 ± 6.88 | 0.553 ± 0.664 | 0.113 ± 0.083 | 0.022 ± 0.028 | 0.161 ± 0.250 | 0.072 ± 0.098 | 0.034 ± 0.078 | 0.042 ± 0.026 |
|   | Min - Max | 20.0 - 94.8 | 0.2 - 25.0 | 11.2 - 96.4 | 1.1 - 22.7 | 0.042 - 1.886 | 0.025 - .331 | 0.002 - 0.092 | 0.006 - 0.725 | 0.000 - 0.326 | 0.000 - 0.263 | 0.006 - 0.096 |
| 12 | Mean ± S.D | 67.44 ± 30.00 | 20.51 ± 12.52 | 87.25 ± 48.76 | 24.40 ± 20.44 | 0.682 ± 0.496 | 0.712 ± 0.774 | 0.027 ± 0.034 | 0.206 ± 0.328 | 0.071 ± 0.073 | 0.028 ± 0.055 | 0.070 ± 0.054 |
|   | Min - Max | 15.7 - 111.6 | 6.0 - 40.8 | 23.7 - 186.1 | 1.5 - 4.3 | 0.033 - 1.541 | 0.047 - 2.371 | 0.001 - 0.124 | 0.015 - 0.958 | 0.002 - 0.245 | 0.000 - 0.185 | 0.005 - 0.190 |
| 13 | Mean ± S.D | 79.09 ± 45.76 | 18.16 ± 8.20 | 65.07 ± 17.10 | 25.86 ± 13.38 | 0.673 ± 0.646 | 0.624 ± 0.959 | 0.024 ± 0.030 | 0.262 ± 0.302 | 0.072 ± 0.065 | 0.028 ± 0.050 | 0.088 ± 0.080 |
|   | Min - Max | 30.3 - 160.3 | 9.5 - 36.6 | 28.4 - 93.2 | 4.5 - 48.6 | 0.075 - 2.369 | 0.038 - 3.645 | 0.002 - 0.103 | 0.016 - 0.797 | 0.001 - 0.231 | 0.000 - 0.159 | 0.004 - 0.259 |
| 14 | Mean ± S.D | 84.63 ± 56.18 | 16.08 ± 7.10 | 62.39 ± 17.67 | 22.09 ± 11.96 | 0.787 ± 0.573 | 0.640 ± 0.790 | 0.022 ± 0.025 | 0.235 ± 0.258 | 0.104 ± 0.104 | 0.031 ± 0.057 | 0.110 ± 0.098 |
|   | Min - Max | 31.3 - 203.9 | 6.8 - 32.7 | 34.1 - 86.0 | 5.7 - 47.5 | 0.031 - 1.892 | 0.016 - 2.911 | 0.002 - 0.098 | 0.015 - 0.795 | 0.002 - 0.332 | 0.000 - 0.185 | 0.014 - 0.362 |
| 15 | Mean ± S.D | 67.32 ± 31.23 | 18.44 ± 14.47 | 59.50 ± 18.34 | 25.63 ± 13.85 | 0.446 ± 0.457 | 0.893 ± 0.914 | 0.023 ± 0.027 | 0.210 ± 0.255 | 0.106 ± 0.126 | 0.027 ± 0.056 | 0.095 ± 0.099 |
|   | Min - Max | 33.5 - 123.7 | 6.1 - 58.8 | 22.0 - 81.3 | 6.8 - 51.4 | 0.042 - 1.569 | 0.084 - 3.138 | 0.002 - 0.097 | 0.015 - 0.675 | 0.002 - 0.414 | 0.001 - 0.186 | 0.002 - 0.485 |
| 16 | Mean ± S.D | 54.10 ± 33.91 | 9.81 ± 6.16 | 50.63 ± 16.34 | 14.89 ± 8.20 | 0.382 ± 0.552 | 0.764 ± 1.104 | 0.024 ± 0.027 | 0.175 ± 0.202 | 0.115 ± 0.285 | 0.025 ± 0.050 | 0.079 ± 0.062 |
|   | Min - Max | 16.9 - 126.4 | 3.0 - 23.3 | 20.6 - 72.5 | 0.1 - 26.9 | 0.012 - 1.904 | 0.024 - 3.808 | 0.002 - 0.093 | 0.012 - 0.575 | 0.001 - 1.426 | 0.001 - 0.185 | 0.012 - 0.213 |
| 17 | Means ± S.D | 39.41 ± 15.73 | 2.58 ± 2.60 | 47.45 ± 5.88 | 8.86 ± 3.08 | 0.820 ± 0.775 | 1.640 ± 1.550 | 0.068 ± 0.067 | 0.028 ± 0.024 | 0.075 ± 0.093 | 0.109 ± 0.109 | 0.024 ± 0.009 |
|   | Min - Max | 24.8 - 58.1 | 0.2 - 5.4 | 42.6 - 58.4 | 3.8 - 12.6 | 0.086 - .693 | 0.172 - 3.386 | 0.004 - 0.131 | 0.006 - 0.053 | 0.000 - 0.231 | 0.007 - 0.215 | 0.014 - 0.036 |
| 18 | Means ±S.D | 68.95 ± 37.58 | 10.43 ± 6.13 | 54.00 ± 20.87 | 20.23 ± 21.79 | 0.357 ± 0.404 | 0.426 ± 0.618 | 0.028 ± 0.038 | 0.261 ± 0.321 | 0.123 ± 0.231 | 0.042 ± 0.101 | 0.082 ± 0.071 |
|   | Min - Max | 5.5 - 103.7 | 0.2 - 21.2 | 17.6 - s87.2 | 0.1 - 66.8 | 0.013 - 1.460 | 0.007 - 2.246 | 0.002 - 0.125 | 0.004 - 1.034 | 0.003 - 0.896 | 0.000 - 0.412 | 0.006 - 0.219 |

Table 3.3    Means ± S.D. and Ranges (min – max) of suspended metals in stream water at various sampling sites located on Nullah Aik and Nullah Palkhu

| St # | | Na (sus) | K (sus) | Ca (sus) | Mg (sus) | Fe (sus) | Pb (sus) | Cd (sus) | Cr (sus) | Ni (sus) | Cu (sus) | Zn (sus) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Means ± S.D | 13.192 ± 10.224 | 6.580 ± 6.920 | 13.694 ± 10.201 | 17.181 ± 8.242 | 4.563 ± 2.716 | 0.651 ± 0.516 | 0.049 ± 0.035 | 0.180 ± 0.132 | 0.300 ± 0.224 | 0.076 ± 0.084 | 0.544 ± 0.805 |
| | Min - Max | 1.755 - 31.097 | 0.124 - 22.457 | 0.987 - 39.064 | 0.531 - 26.97 | 2.011 - .718 | 0.000 - .700 | 0.008 - .129 | 0.022 - .375 | 0.070 - .774 | 0.003 - .344 | 0.000 - .415 |
| 2 | Means ± S.D | 11.708 ± 5.910 | 11.624 ± 8.379 | 36.284 ± 27.307 | 42.197 ± 30.688 | 10.600 ± 4.597 | 0.874 ± 0.360 | 0.056 ± 0.053 | 0.505 ± 0.465 | 0.348 ± 0.271 | 0.147 ± 0.210 | 0.802 ± 0.700 |
| | Min - Max | 2.361 - 22.457 | 0.235 - 28.467 | 1.854 - 76.383 | 3.163 - 91.659 | 3.548 - 7.623 | 0.212 - 1.580 | 0.004 - 0.196 | 0.041 - 1.814 | 0.023 - 0.885 | 0.001 - 0.695 | 0.072 - 1.989 |
| 3 | Mean ± S.D | 14.017 ± 9.820 | 12.255 ± 7.626 | 31.143 ± 25.800 | 47.965 ± 28.789 | 22.382 ± 10.445 | 1.023 ± 0.381 | 0.070 ± 0.050 | 1.398 ± 1.044 | 0.631 ± 0.554 | 0.170 ± 0.094 | 1.306 ± 1.129 |
| | Min - Max | 5.234 - 35.158 | 2.568 - 31.712 | 6.533 - 97.425 | 23.561 - 133.558 | 4.797 - 1.023 | 0.127 - 1.850 | 0.008 - 0.185 | 0.045 - 4.231 | 0.001 - 1.968 | 0.029 - 0.359 | 0.259 - 3.658 |
| 4 | Mean ± S.D | 16.717 ± 9.941 | 12.749 ± 9.674 | 28.187 ± 24.272 | 51.988 ± 30.511 | 30.940 ± 22.706 | 1.045 ± 0.435 | 0.102 ± 0.138 | 2.828 ± 2.782 | 0.806 ± 0.824 | 0.163 ± 0.111 | 2.427 ± 2.906 |
| | Min - Max | 5.048 - 37.39 | 1.798 - 31.745 | 3.996 - 88.961 | 16.457 - 97.774 | 0.759 - 1.054 | 0.526 - 1.963 | 0.016 - 0.710 | 0.073 - 8.124 | 0.100 - 3.168 | 0.008 - 0.412 | 0.165 - 8.529 |
| 5 | Means ± S.D | 14.336 ± 9.356 | 9.202 ±10.725 | 32.535 ± 26.644 | 27.542 ± 8.813 | 10.386 ± 5.770 | 0.987 ± 0.289 | 0.061 ± 0.051 | 3.132 ± 3.044 | 0.626 ± 0.510 | 0.156 ± 0.149 | 0.898 ± 0.592 |
| | Min - Max | 5.604 - 39.263 | 0.075 - 37.12 | 2.301 - 96.895 | 14.392 - 39.087 | 2.185 - 3.235 | 0.635 - 1.610 | 0.011 - 0.175 | 0.058 - 8.457 | 0.123 - 1.961 | 0.008 - 0.523 | 0.137 - 1.975 |
| 6 | Mean ± S.D | 23.966 ± 18.623 | 13.013 ± 9.973 | 29.785 ± 24.865 | 46.023 ± 26.316 | 10.208 ± 6.178 | 0.990 ± 0.550 | 0.073 ± 0.082 | 1.080 ± 0.781 | 0.581 ± 0.743 | 0.098 ± 0.133 | 0.724 ± 0.661 |
| | Min - Maxi | 5.986 - 59.09 | 0.695 - 27.018 | 2.001 - 78.244 | 16.604 - 99.531 | 1.230 - 8.478 | 0.265 - 1.895 | 0.002 - 0.403 | 0.024 - 2.559 | 0.032 - 3.261 | 0.023 - 0.630 | 0.036 - 1.989 |
| 7 | Mean ± S.D | 23.873 ± 20.619 | 12.091 ± 10.496 | 24.883 ± 20.654 | 46.092 ± 41.057 | 7.369 ± 5.097 | 1.082 ± 0.691 | 0.055 ± 0.044 | 0.598 ± 0.510 | 0.376 ± 0.294 | 0.092 ± 0.080 | 0.680 ± 0.924 |
| | Min - Max | 4.546 - 69.243 | 0.623 - 24.465 | 1.828 - 66.474 | 15.23 - 151.34 | 1.023 - 5.326 | 0.075 - 2.624 | 0.014 - 0.167 | 0.024 - 1.978 | 0.096 - 0.964 | 0.004 - 0.262 | 0.111 - 3.024 |
| 8 | Means ± S.D | 21.150 ± 12.441 | 10.569 ± 8.401 | 19.308 ±16.258 | 14.284 ± 1.235 | 7.528 ± 7.590 | 0.711 ± 0.643 | 0.083 ± 0.070 | 1.613 ± 1.991 | 0.634 ± 0.582 | 0.104 ± 0.110 | 0.220 ± 0.173 |
| | Min - Max | 5.333 - 35.69 | 1.186 - 24.212 | 1.024 - 44.235 | 12.137 - 16.564 | 1.231 - 8.425 | 0.012 - 1.527 | 0.013 - 0.197 | 0.099 - 5.263 | 0.088 - 1.634 | 0.015 - 0.267 | 0.099 - 0.635 |
| 9 | Means ± S.D | 23.001 ± 10.279 | 9.954 ± 7.400 | 17.182 ± 22.836 | 25.374 ± 13.975 | 5.267 ± 0.755 | 0.186 ± 0.154 | 0.067 ± 0.071 | 0.542 ± 0.267 | 0.116 ± 0.037 | 0.081 ± 0.040 | 0.109 ± 0.043 |
| | Min - Max | 9.959 - 36.457 | 1.207 - 19.457 | 1.23 - 49.863 | 12.71 - 45.718 | 4.127 - .351 | 0.029 - 0.433 | 0.001 - 0.141 | 0.089 - 0.859 | 0.074 - 0.174 | 0.040 - 0.126 | 0.056 - 0.152 |
| 10 | Means ±S.D | 13.681 ± 9.820 | 8.427 ± 5.941 | 27.940 ± 26.447 | 29.237 ±18.793 | 5.750 ± 2.909 | 0.805 ± 0.423 | 0.057 ± 0.064 | 0.308 ± 0.203 | 0.382 ± 0.264 | 0.090 ± 0.116 | 0.696 ± 0.554 |
| | Min - Max | 3.04 - 33.034 | 0.862 - 19.413 | 1.024 - 85.284 | 9.451 - 77.531 | 1.665 - 10.156 | 0.021 - 1.968 | 0.005 - 0.229 | 0.017 - 0.630 | 0.121 - 0.924 | 0.005 - 0.395 | 0.052 - 1.815 |
| 11 | Means ±S.D | 18.745 ± 15.488 | 14.812 ± 15.236 | 23.603 ±14.575 | 39.201 ± 14.853 | 12.299 ± 10.015 | 0.749 ± 0.435 | 0.050 ± 0.049 | 0.549 ± 0.471 | 0.505 ± 0.419 | 0.106 ± 0.126 | 0.683 ± 0.547 |
| | Min - Max | 4.912 - 48.4147 | 0.135 - 42.052 | 4.268 - 53.879 | 0.123 - 73.3676 | 3.485 - 37.124 | 0.089 - 1.536 | 0.007 - 0.197 | 0.071 - 1.856 | 0.121 - 1.745 | 0.006 - 0.456 | 0.109 - 1.634 |
| 12 | Mean ± S.D | 25.492 ± 15.333 | 23.265 ± 13.713 | 45.828 ± 30.462 | 75.650 ±70.124 | 16.877 ± 6.350 | 1.111 ± 0.356 | 0.062 ± 0.049 | 1.576 ± 2.373 | 0.660 ± 0.447 | 0.303 ± 0.486 | 1.217 ± 1.019 |
| | Min - Max | 6.125 - 51.4635 | 3.562 - 42.004 | 1.181 - 92.283 | 33.257 - 310.804 | 2.486 - 29.780 | 0.568 - 1.953 | 0.012 - 0.196 | 0.032 - 8.503 | 0.163 - 1.523 | 0.040 - 1.856 | 0.068 - 3.624 |
| 13 | Mean ± S.D | 30.181 ± 16.114 | 31.831 ± 13.012 | 50.839 ± 29.865 | 112.28 ± 132.07 | 27.312 ± 11.148 | 1.053 ± 0.514 | 0.065 ± 0.046 | 10.13 ± 10.68 | 1.029 ± 0.565 | 0.734 ± 0.887 | 3.620 ± 2.984 |
| | Min - Max | 8.96 - 59.4021 | 10.236 - 49.56 | 2.028 - 99.9731 | 50.291 - 468.716 | 10.172 - 45.124 | 0.055 - 1.908 | 0.014 - 0.175 | 0.082 - 25.17 | 0.368 - 2.206 | 0.065 - 3.201 | 0.596 - 9.562 |
| 14 | Mean ± S.D | 27.300 ±15.478 | 38.701 ± 32.960 | 51.879 ± 31.551 | 80.310 ± 60.146 | 20.942 ± 7.156 | 1.227 ± 0.681 | 0.106 ± 0.163 | 5.636 ± 6.264 | 0.912 ± 1.184 | 0.393 ± 0.284 | 3.180 ± 1.563 |
| | Min - Max | 0.04 - 57.3971 | 4.025 - 123 | 5.267 - 89.741 | 43.039 - 279.253 | 10.080 - 32.512 | 0.485 - 3.456 | 0.001 - 0.832 | 0.10 - 20.948 | 0.121 - 4.213 | 0.145 - 1.054 | 0.426 - 6.126 |
| 15 | Mean ± S.D | 29.816 ± 19.109 | 19.507 ± 15.681 | 52.793 ± 33.945 | 68.216 ± 41.518 | 19.653 ± 15.372 | 1.089 ± 0.580 | 0.083 ± 0.047 | 4.569 ± 7.628 | 0.905 ± 1.079 | 0.138 ± 0.085 | 1.974 ± 1.675 |
| | Min - Max | 1.96 - 68.784 | 2.013 - 48.121 | 2.014 - 94.71 | 44.912 - 189.16 | 3.458 - 53.596 | 0.289 - 2.361 | 0.009 - 0.161 | 0.119 - 26.47 | 0.025 - 4.235 | 0.023 - 0.295 | 0.129 - 5.012 |
| 16 | Mean ± S.D | 30.586 ± 25.374 | 13.866 ± 12.208 | 36.571 ± 26.956 | 62.306 ± 25.629 | 13.016 ± 6.831 | 0.907 ± 0.416 | 0.065 ± 0.042 | 0.794 ± 0.544 | 0.480 ± 0.356 | 0.125 ± 0.111 | 0.899 ± 0.897 |
| | Min - Max | 6.275 - 85.486 | 1.042 - 32.095 | 1.024 - 82.653 | 23.421 - 106.97 | 3.002 - 2.728 | 0.314 - 1.789 | 0.013 - 0.175 | 0.054 - 2.001 | 0.045 - 1.086 | 0.042 - 0.459 | 0.123 - 3.457 |
| 17 | Means ± S.D | 16.518 ± 8.821 | 3.551 ± 2.539 | 14.178 ± 15.548 | 13.393 ± 2.104 | 6.560 ± 4.197 | 0.252 ± 0.207 | 0.079 ± 0.084 | 0.375 ± 0.322 | 0.225 ± 0.189 | 0.026 ± 0.019 | 0.188 ± 0.078 |
| | Min - Max | 7.544 - 26.45 | 1.235 - 7.568 | 1.0784 - 39.564 | 11.421 - 16.124 | 2.421 - 11.450 | 0.069 - 0.544 | 0.001 - 0.163 | 0.062 - 0.845 | 0.051 - 0.416 | 0.008 - 0.052 | 0.089 - 0.313 |
| 18 | Means ±S.D | 16.170 ± 11.917 | 16.140 ± 14.929 | 29.973 ± 33.416 | 51.334 ± 34.691 | 9.891 ± 5.245 | 1.423 ± 1.004 | 0.073 ± 0.060 | 3.232 ± 5.593 | 0.720 ± 0.439 | 0.158 ± 0.097 | 1.615 ± 1.629 |
| | Min - Max | 6.02 - 38.458 | 1.023 - 56.528 | 1.031 - 101.836 | 12.419 - 135.46 | 2.551 - 18.683 | 0.129 - 4.012 | 0.011 - 0.225 | 0.025 - 18.23 | 0.052 - 1.374 | 0.010 - 0.301 | 0.106 - 4.562 |

(a)

(b)

(c)

d)

(e)



(f)



(g)



(h)

(i)



(j)



(k)



(l)

(m)



(n)



(o)



(p)

Fig. 3.1    Mean values of stream morphology (a), physiochemical (a - e), nutrients (g), metals in dissolved form (g-k) and metals in suspended particulate form (l- p) measured at various sites (names of sites are given in Fig 2.7) located on Nullah Aik and Nullah Palkhu

(a)


(b)


(c)


(d)


(e)


(f)

(g)

(h)

(i)



(j)
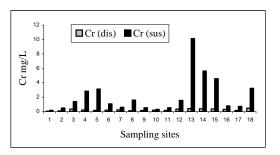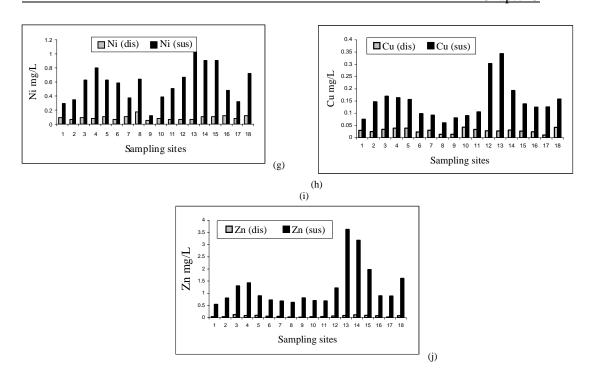
Fig. 3.2    Comparison of metals (a- j) in dissolved and suspended particulate form measured in water samples collected from Nullah Aik and Nullah Palkhu.

**Inter- relationships among Metals**

The metal correlations were observed in two forms i.e. dissolved and suspended particulate matter. Pearson correlation matrix of dissolved metals and suspended particulate matter are given in Table 3.4a & b. In case of dissolved form of metal, significant positive correlation of Fe (dis) was observed with Na (dis; r = 0.527), Pb (dis; r = 0.586) and Cu (dis; r = 0.481)   Pb (dis) exhibited positive correlations with K (dis; r = 0.432), Cr (dis; r = 0.516) and Zn (dis; r = 0.517). Cd (dis) showed negative correlation with Mg (dis; r =-0.479) and Zn (dis; r =-0.479) and positive with Cu (dis; r = 0.859). Cr (dis) exhibited strong positive correlation with K (dis; r = 0.704) and Zn (dis; r = 0.684) while negative with Cu (dis; r = 0.445). Cu (dis) was found to be negatively correlated with Mg (dis; r =0.445) and positively with Zn (dis; r = 0.864). Zn (dis) showed positive correlation with K (dis; r = 0.543) and Mg (dis; r = 0.667),   Metals such as Ca and Ni were not correlated with any other metal (Fig. 3.3a).

Fe (sus) exhibited strong positive correlation with K (sus; r =  0.598), Ca (sus; r = 0.514), Mg (sus; r =  0.641), Ni (sus; r = 0.595), Cu (sus; r = 0.668) and Zn (sus; r = 0.644). Cr (sus) was strongly correlated with Ca (sus; r = 0.624), Mg (sus; r = 0.759), Ni (sus; r = 0.757), Cu (sus; r = 0.843) and Zn (sus; r = 0.919). Ni (sus) exhibited positive correlation with K (sus; r = 0.510), Mg (sus; r = 0.694), Cu (sus; r = 0.712) and Zn (sus; r = 0.737). Zn (sus) showed strong correlation with K (sus; r = 0.797), Ca (sus; r = 711) and Mg (sus; r = 0.877). A significant correlation was observed between major metals (Na, K, Ca, and Mg). Similarly, among heavy metals (Fe, Cr, Ni, Cu and Zn) also exhibited positive correlation.

The results of correlation analysis were further confirmed by HACA (Fig. 3.3). Three clusters of dissolved metals were formed. Cluster 1 consisted of Na, K, Mg, Cr and Zn. Second cluster was made up of Ca and Ni, whereas, third cluster comprised of Fe, Pb, Cd and Cu. Metal in suspended particulate form showed two distinct clusters. First cluster consisted of major metals (Na, K, Ca and Mg), whereas, heavy metals such as Fe, Cr, Ni, Cu and Zn formed cluster 2. Pb and Cd in particulate matter were identified as outlier.

(a)



(b)
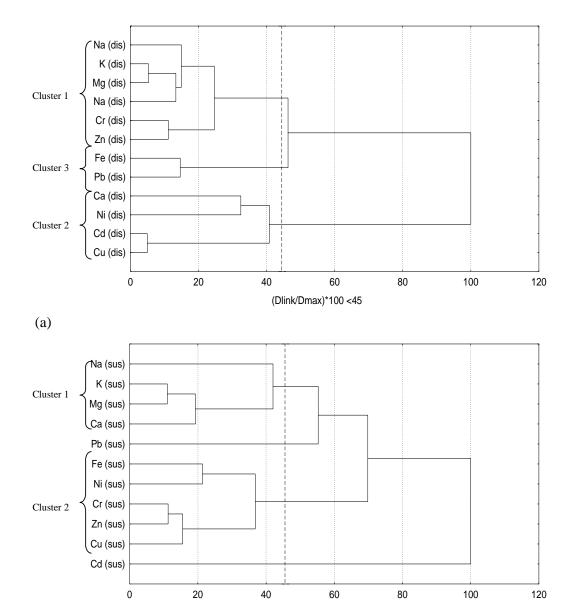
Fig. 3.3    Dendrogram showing association of metals (a) Dissolved form, (b) Suspended form in water samples collected from Nullah Aik and Nullah Palkhu

Table 3.4    Pearson's correlation matrix indicating the association within dissolved
             metals (a) and within suspended metals (b)

(a)

|     | Na | K | Ca | Mg | Fe | Pb | Cd | Cr | Ni | Cu |
|-----|------|--------|-------|--------|--------|--------|--------|--------|-------|--------|
| **K** | **0.733** | | | | | | | | | |
| **Ca** | 0.275 | 0.386 | | | | | | | | |
| **Mg** | **0.564** | **0.850** | 0.277 | | | | | | | |
| **Fe** | **0.527** | 0.287 | 0.142 | 0.054 | | | | | | |
| **Pb** | 0.313 | **0.423** | 0.188 | 0.320 | **0.586** | | | | | |
| **Cd** | 0.233 | -0.197 | 0.199 | **-0.479** | 0.426 | -0.024 | | | | |
| **Cr** | 0.592 | **0.704** | 0.079 | **0.701** | 0.212 | **0.516** | -0.237 | | | |
| **Ni** | 0.283 | -0.048 | 0.081 | 0.012 | -0.234 | -0.263 | 0.105 | -0.079 | | |
| **Cu** | 0.154 | -0.370 | 0.083 | **-0.588** | 0.481 | -0.117 | **0.859** | **-0.445** | 0.239 | |
| **Zn** | 0.346 | **0.543** | 0.137 | **0.669** | 0.067 | **0.517** | **-0.409** | **0.684** | 0.110 | **-0.563** |

(b)

|     | Na | K | Ca | Mg | Fe | Pb | Cd | Cr | Ni | Cu |
|-----|------|--------|--------|--------|--------|--------|--------|--------|-------|--------|
| **K** | 0.393 | | | | | | | | | |
| **Ca** | **0.452** | **0.618** | | | | | | | | |
| **Mg** | **0.611** | **0.658** | **0.749** | | | | | | | |
| **Fe** | 0.388 | **0.598** | **0.514** | **0.641** | | | | | | |
| **Pb** | 0.21 | -0.058 | 0.203 | -0.027 | -0.306 | | | | | |
| **Cd** | -0.118 | 0.352 | -0.230 | 0.147 | 0.092 | 0.185 | | | | |
| **Cr** | 0.382 | 0.356 | **0.624** | **0.759** | 0.416 | 0.172 | 0.387 | | | |
| **Ni** | 0.402 | **0.510** | 0.437 | **0.694** | **0.595** | 0.094 | -0.163 | **0.757** | | |
| **Cu** | 0.425 | **0.847** | **0.701** | **0.855** | **0.668** | -0.054 | 0.283 | **0.843** | **0.712** | |
| **Zn** | 0.412 | **0.797** | **0.711** | **0.877** | **0.644** | -0.08 | 0.405 | **0.919** | **0.737** | **0.900** |

Strong correlation < 0.400 at p = 0.05

## Classification of Sampling Sites

HACA performed on the water quality data set to evaluate spatial variation among sampling sites resulted in grouping of sampling sites into three groups (Fig. 3.4). The sites within each group have similar water quality parameters. These groups were designated based upon impairment of sites from different pollutants as relatively unimpaired region (RUR), moderately impaired region (MIR) and impaired region (IR). Sites 1, 2, 10 and 11 were classified in RUR group. Sites (1 and 2) were located in upstream of Nullah Aik, whereas, remaining two sites (10 and 11) in upstream of Nullah Palkhu. Sites grouped in this cluster were categorized with low level of COD, EC, TDS, $PO_4^{3-}$, $Cl^-$, Pb, Cr, Ni and Zn and high level of DO. These sites were located in upstream of the Sialkot city and were least affected from industrial effluents and municipal sewage. Sites such as 3, 4, 5, 8, 9, 12, 13, 14, and 18 were grouped in IR and located close to mid stream of Nullah Aik (3, 4, 5, 8 and 9) and Nullah Palkhu (12, 13, 14, and 18). This group was regarded as impaired region (IR) of streams, which exhibited low level of DO and high concentration of COD, EC, TDS, turbidity, Cl-, S2-, Na, Fe, Pb, Cr, Ni and Zn. These sites received effluents from multiple pollution sources.
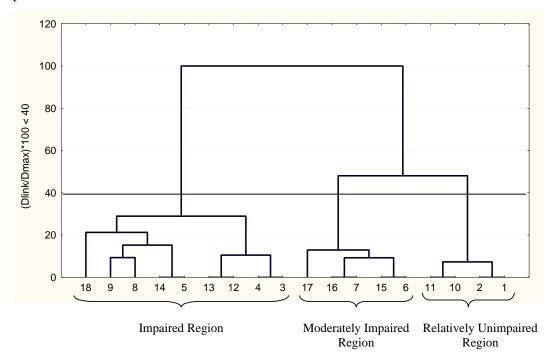


Fig. 3.4    Dendrogram showing different clusters of sampling sites located on Nullah Aik and Nullah Palkhu.

This group exhibited relatively higher concentrations of the DO and lower concentrations of COD, EC, TDS, turbidity, $PO_4^{3-}$, $Cl^-$, $S^{2-}$, Na, Fe, Pb, Cr, Ni and Zn compared to IR. Sites such as 6, 7, 15, 16 and 17 were included in MIR group, whereas, located far downstream of Nullah Aik (6, 7) and Nullah Palkhu (15, 16 and 17).

## Spatial Variations in Surface Water Quality of Nullah Aik and Nullah Palkhu

For spatial variations among different stream zones such as RUR, IR and LIR defined by HACA. Table 3.5 presents DFs and spatial classification accuracies of 90.9%, 90.4% and 87.6% for standard and forward stepwise and backward stepwise mode DFA. Backward stepwise mode DFA deleted least significant and discriminated 11 significant variables via; stream flow, stream depth, DO, COD, TDS, $NO_3^-$, $PO_4^{3-}$, Pb (dis), Cr (dis), Na (sus), and Ni (sus).
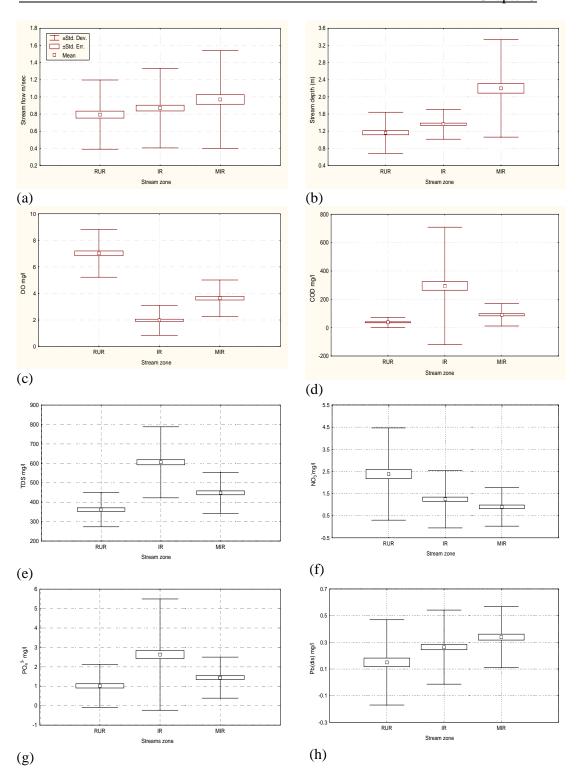
Box and whisker plots of significantly important parameters recognized by backward step mode of spatial DFA are presented in Fig. 3.5. Stream flow gradually improved from upstream towards downstream i.e. it was minimum in RUR and maximum in MIR (Fig. 3.5a). Similar to stream flow, stream depth was also maximum at MIR, whereas, minimum at RUR (Fig. 3.5b). Highest concentration of DO was found in RUR which declined in IR and gradually increased in MIR (Fig. 3.5c). In contrary to DO, maximum value of COD was measured in IR, whereas, minimum in RUR (Fig. 3.5d). TDS also exhibited similar spatial pattern (Fig. 3.5e).

In case of $NO_3^-$, highest concentration was measured in RUR followed by IR reaching to its lowest level in MIR (Fig. 3.5f). RUR exhibited lowest concentration of $PO_4^{3-}$ and highest concentration in IR (Fig. 3.5g). Lowest concentration of Pb (dis) was found in RUR, which gradually increased in IR and reached at highest concentration in MIR (Fig. 3.5h). Cr (dis) exhibited lowest concentration in RUR followed by highest concentration in IR and decreasing trend was found in MIR (Fig. 3.5i). Minimum concentration of Mg in suspended particulate matter was recorded in RUR that gradually increased towards IR and attain higher concentration in IR (Fig. 3.5j). Maximum concentration of Ni in suspended particulate matter was recorded in IR, lowest concentration in RUR (Fig. 3.5k).

Table 3.5    Classification functions for Discriminant Function Analysis of spatial variations in water quality data of Nullah Aik and Nullah Palkhu

| Variable | Standard Mode | | | Forward Step Mode | | | Backward Step Mode | | |
|---|---|---|---|---|---|---|---|---|---|
| | RUR coefficient | MIR coefficient | IR coefficient | RUR coefficient | MIR coefficient | IR coefficient | RUR coefficient | MIR coefficient | IR coefficient |
| Stream width | 0.668 | 0.543 | 0.477 | 0.636 | 0.517 | 0.453 | | | |
| Stream velocity | -9.701 | -3.714 | -4.430 | -4.241 | -2.095 | -0.411 | -4.221 | 1.744 | 0.747 |
| Stream depth | -2.590 | -0.459 | 1.230 | -7.403 | -1.408 | -2.153 | -0.630 | 1.144 | 2.741 |
| Temperature | 3.879 | 3.519 | 3.641 | 2.756 | 2.427 | 2.549 | | | |
| pH | 53.723 | 53.748 | 52.605 | 50.445 | 50.526 | 49.367 | 45.797 | 46.112 | 44.874 |
| DO | 8.776 | 4.697 | 5.780 | 6.550 | 2.481 | 3.567 | 6.343 | 2.946 | 3.815 |
| COD | 0.062 | 0.067 | 0.060 | 0.046 | 0.050 | 0.043 | 0.011 | 0.016 | 0.011 |
| E.C. | 0.053 | 0.054 | 0.053 | | | | | | |
| TDS | -0.039 | -0.025 | -0.032 | 0.034 | 0.048 | 0.040 | 0.027 | 0.039 | 0.033 |
| Salinity | 20.011 | 18.249 | 20.054 | | | | | | |
| Hardness | 0.093 | 0.102 | 0.105 | | | | | | |
| Turbidity | -0.004 | -0.001 | -0.002 | 0.113 | 0.122 | 0.125 | | | |
| $NO_3^-$ | 2.059 | 1.021 | 0.867 | 0.954 | -0.062 | -0.227 | 1.415 | 0.475 | 0.302 |
| $PO_4^{3-}$ | 1.012 | 0.233 | 0.330 | 2.872 | 2.028 | 2.110 | 0.286 | -0.298 | -0.293 |
| $Cl^-$ | 0.001 | 0.000 | 0.003 | 0.003 | 0.003 | 0.005 | | | |
| $S^{2-}$ | -8.210 | -6.403 | -7.557 | -6.989 | -5.187 | -6.229 | | | |
| Na (dis) | 0.271 | 0.271 | 0.267 | | | | | | |
| K (dis) | 0.136 | -0.016 | 0.032 | 0.344 | 0.187 | 0.239 | | | |
| Ca (dis) | 0.074 | 0.028 | 0.041 | 0.090 | 0.043 | 0.055 | | | |
| Mg (dis) | -0.416 | -0.478 | -0.422 | | | | | | |
| Fe (dis) | -8.479 | -9.413 | -9.843 | -1.632 | -1.627 | -2.134 | | | |
| Pb (dis) | -23.779 | -19.640 | -15.557 | -14.751 | -11.010 | -7.2467 | -5.798 | -2.759 | -1.196 |
| Cd (dis) | 0.565 | 31.683 | 47.448 | -19.602 | -15.650 | -16.786 | | | |
| Cr (dis) | 35.568 | 22.262 | 22.887 | 36.966 | 23.890 | 24.543 | 19.922 | 9.236 | 9.973 |
| Ni (dis) | -10.212 | -6.328 | -5.686 | -2.927 | 1.274 | 1.861 | | | |
| Cu (dis) | 57.323 | 56.200 | 44.735 | 50.880 | 48.580 | 38.993 | | | |
| Zn (dis) | -49.897 | -39.883 | -38.945 | -46.508 | -35.664 | -35.075 | | | |
| Na (sus) | 0.257 | 0.279 | 0.335 | 0.237 | 0.255 | 0.305 | | | |
| K (sus) | -0.252 | -0.262 | -0.280 | | | | | | |
| Ca (sus) | -0.017 | 0.024 | 0.001 | -0.063 | -0.023 | -0.043 | | | |
| Mg (sus) | 0.040 | 0.062 | 0.056 | 0.048 | 0.068 | 0.062 | 0.018 | 0.049 | 0.073 |
| Fe (sus) | 0.196 | 0.165 | 0.208 | 0.170 | 0.145 | 0.186 | | | |
| Pb (sus) | 3.173 | 5.652 | 4.864 | 2.951 | 5.426 | 4.647 | 5.925 | 8.513 | 7.890 |
| Cd (sus) | 58.826 | 55.296 | 56.767 | 99.785 | 130.72 | 147.75 | | | |
| Cr (sus) | -0.598 | -0.497 | -0.381 | -0.083 | -0.009 | 0.083 | | | |
| Ni (sus) | 8.022 | 7.631 | 7.695 | | | | | | |
| Cu (sus) | 8.460 | 7.397 | 6.936 | | | | | | |
| Zn (sus) | -2.575 | -2.604 | -3.104 | -3.005 | -3.166 | -3.745 | | | |
| Constant | -322.92 | -308.11 | -304.09 | -281.49 | -268.21 | -264.36 | -52.56 | -41.81 | -41.52 |
| Class Accuracy | 97.917 | 92.063 | 82.353 | 97.917 | 92.063 | 84.314 | 93.750 | 90.476 | 76.471 |
| Total Accuracy | 90.956 | | | 91.473 | | | 87.597 | | |

**RUR** (Relatively Unimpaired Region), **MIR** (Moderately polluted Region), and **IR** (Impaired Region)

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)



(j)



(k)
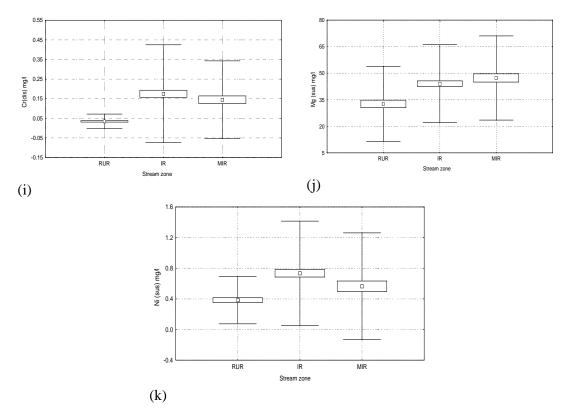
Fig. 3.5    Box and Whisker plots of some variables (a. stream flow, b. stream depth, c. DO, d. COD, e. TDS, f. $NO_3^-$, g. $PO_4^{3-}$, h. Pb dis, i. Cr dis, j. Mg sus, and k. Ni sus) separated by spatial Discriminant analysis associated with water quality data of Nullah Aik and Nullah Palkhu.

**Temporal Variations in Surface Water Quality of Nullah Aik and Nullah Palkhu**

After segregating raw data into four seasonal groups (post monsoon, winter, pre monsoon and monsoon), DFA was applied for the evaluation of temporal variations. DFs and classification matrix for temporal variations were obtained from standard, forward stepwise and backward stepwise of DFA for 38 water quality variables (Table 3.6). Temporal classification accuracies ranged from 97.9% to 94.7% for standard, forward stepwise and backward stepwise mode. Backward stepwise DFA discriminated 13 variables viz; stream flow, temperature, EC, salinity, total hardness, Na (dis), K (dis), Ca (dis), Mg (dis), Fe (dis), Cd (dis), Cu (dis) and Na (sus) as significant variables.

Box and whisker plots of variables discriminated using DFA are given in Fig. 3.6. Streams exhibited maximum flow in monsoon season that gradually decreased till pre monsoon season (Fig. 3.6a). Maximum temperature was found during monsoon season and lowest in winter (Fig. 3.6b). Highest level of EC was observed in winter season, which gradually reduced to its lowest level in monsoon season (Fig. 3.6c). Like EC, Total hardness, salinity and Na also showed similar trend i.e. maximum in pre monsoon and lowest in monsoon season (Fig. 3.6d, e, f). Highest concentration of total hardness of stream water was recorded in winter season which decreases during monsoon season. Concentration of K increases from post monsoon to pre monsoon, when it reaches to its maximum level (Fig. 3.6g). Lowest concentration of Ca was recorded in post monsoon, whereas, highest concentration in winter season followed by pre monsoon and monsoon season (Fig. 3.6h). Highest concentration of Mg was observed during pre monsoon, whereas lowest in monsoon season (Fig. 3.6i). Fe (dis), Cd (dis) and Cu (dis) also exhibited seasonal differences, minimum concentrations were recorded in post monsoon and maximum level during monsoon season (Fig. 3.6j k & l). Among suspended particulate matter, Na varied significantly between seasons. Minimum concentration of Na was recorded during monsoon, which increased till winter when its concentration becomes at highest level (Fig.3.6m).
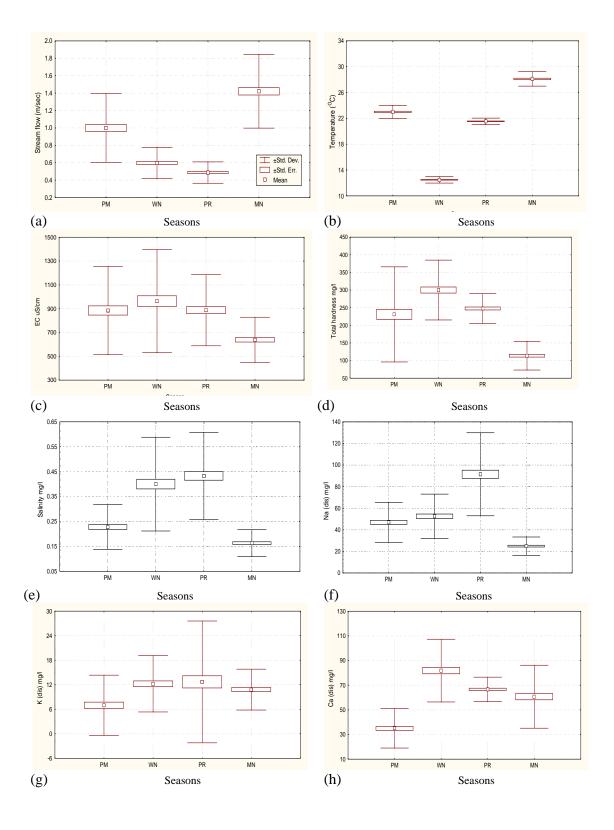
Table 3.6  Classification functions for discriminent function analysis of temporal variations in water quality data of Nullah Aik and Nullah Palkhu

| Seasons | Standard mode | | | | Forward stepwise mode | | | | Backward stepwise mode | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient |
| Stream width | 0.618 | 0.871 | 0.994 | 0.991 | 0.549 | 0.786 | 0.917 | 0.929 | | | | |
| Stream velocity | 4.774 | 4.294 | -0.181 | 15.161 | 5.989 | 5.135 | 1.073 | 16.425 | 4.249 | 4.841 | 0.748 | 11.103 |
| Stream depth | 0.998 | 1.724 | 1.490 | 1.323 | | | | | | | | |
| Temperture | 16.918 | 9.315 | 15.632 | 20.953 | 16.710 | 9.229 | 15.472 | 20.758 | 14.485 | 7.627 | 13.502 | 17.923 |
| pH | 54.212 | 54.893 | 54.234 | 56.852 | 53.377 | 53.997 | 53.316 | 56.055 | | | | |
| DO | 3.004 | 2.869 | 2.380 | 1.108 | 2.975 | 2.954 | 2.440 | 1.049 | | | | |
| COD | 0.038 | 0.057 | 0.053 | 0.033 | 0.034 | 0.051 | 0.048 | 0.029 | | | | |
| E.C. | 0.039 | 0.025 | 0.015 | 0.011 | 0.041 | 0.025 | 0.016 | 0.013 | 0.009 | 0.006 | 0.005 | 0.001 |
| TDS | -0.010 | -0.003 | 0.012 | 0.013 | -0.013 | -0.004 | 0.010 | 0.011 | | | | |
| Salinity | -28.044 | 10.365 | -3.579 | -33.907 | -28.592 | 11.317 | -3.512 | -34.321 | -25.558 | -2.250 | -10.923 | -23.813 |
| Hardness | 0.142 | 0.079 | 0.097 | 0.111 | 0.146 | 0.086 | 0.103 | 0.115 | 0.076 | 0.042 | 0.053 | 0.048 |
| Turbidity | -0.008 | -0.001 | -0.005 | -0.007 | | | | | | | | |
| $NO_3^-$ | -0.462 | 1.404 | 0.389 | 0.694 | -0.877 | 0.847 | -0.083 | 0.236 | | | | |
| $PO_4^{3-}$ | -0.307 | 0.506 | 0.021 | -0.085 | -0.465 | 0.243 | -0.212 | -0.186 | | | | |
| $Cl^-$ | -0.009 | -0.004 | -0.008 | -0.015 | -0.010 | -0.004 | -0.008 | -0.016 | | | | |
| $S^{2-}$ | 1.058 | -4.076 | -2.593 | 5.377 | 0.536 | -4.676 | -2.895 | 4.794 | | | | |
| Na (dis) | 0.289 | 0.261 | 0.370 | 0.231 | 0.268 | 0.231 | 0.342 | 0.209 | 0.069 | 0.030 | 0.131 | 0.036 |
| K (dis) | 0.392 | 0.361 | 0.423 | 0.816 | 0.472 | 0.426 | 0.506 | 0.885 | 0.175 | 0.099 | 0.099 | 0.538 |
| Ca (dis) | -0.086 | 0.126 | 0.054 | 0.041 | -0.101 | 0.104 | 0.037 | 0.027 | -0.020 | 0.127 | 0.067 | 0.079 |
| Mg (dis) | -1.134 | -0.697 | -0.824 | -1.450 | -1.174 | -0.774 | -0.891 | -1.485 | -0.465 | -0.161 | -0.257 | -0.689 |
| Fe (dis) | -2.950 | -2.505 | -5.021 | 7.316 | -2.175 | -1.635 | -4.222 | 8.025 | -9.822 | -6.556 | -9.461 | -4.221 |
| Pb (dis) | -24.591 | -18.057 | -18.735 | -29.486 | -24.601 | -19.219 | -19.034 | -30.010 | | | | |

Continued …

| Seasons | Standard mode | | | | Forward stepwise mode | | | | Backward stepwise mode | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient | Post Monsoon coefficient | Winter coefficient | Spring coefficient | Pre Monsoon coefficient |
| Cd (dis) | 279.089 | -19.136 | 137.604 | 154.050 | 343.614 | 56.249 | 208.393 | 215.908 | 390.341 | 126.883 | 275.472 | 345.717 |
| Cr (dis) | 9.990 | 19.691 | 17.951 | 6.262 | 6.190 | 14.650 | 13.137 | 3.488 | | | | |
| Ni (dis) | 12.487 | 14.734 | 19.261 | 28.491 | 6.711 | 6.807 | 12.116 | 24.087 | | | | |
| Cu (dis) | 41.602 | 78.222 | 57.838 | 81.998 | 43.754 | 78.157 | 57.414 | 85.786 | -57.949 | -4.415 | -34.313 | -29.202 |
| Zn (dis) | -21.750 | -35.185 | -27.679 | -19.046 | | | | | | | | |
| Na (sus) | 0.364 | 0.429 | 0.341 | 0.467 | 0.325 | 0.376 | 0.294 | 0.436 | | | | |
| K (sus) | 0.259 | -0.143 | 0.077 | 0.335 | 0.212 | -0.148 | 0.047 | 0.299 | 0.450 | 0.112 | 0.276 | 0.500 |
| Ca (sus) | -0.061 | -0.027 | -0.081 | -0.073 | -0.064 | -0.036 | -0.087 | -0.078 | | | | |
| Mg (sus) | 0.084 | 0.082 | 0.076 | 0.109 | 0.111 | 0.107 | 0.104 | 0.136 | | | | |
| Fe (sus) | 0.254 | 0.205 | 0.270 | 0.235 | 6.712 | 7.011 | 7.900 | 8.749 | | | | |
| Pb (sus) | 6.690 | 7.297 | 8.073 | 8.529 | | | | | | | | |
| Cd (sus) | 63.252 | 56.811 | 58.677 | 62.917 | | | | | | | | |
| Cr (sus) | -0.164 | -0.402 | -0.271 | -0.084 | | | | | | | | |
| Ni (sus) | 1.586 | 8.240 | 3.079 | 3.307 | 0.414 | 7.002 | 1.608 | 2.370 | -3.023 | 3.446 | -1.037 | -1.355 |
| Cu (sus) | 17.088 | 14.653 | 15.231 | 21.451 | 14.884 | 10.888 | 11.795 | 20.020 | | | | |
| Zn (sus) | -6.331 | -5.638 | -5.984 | -8.663 | -5.171 | -4.811 | -4.760 | -7.611 | | | | |
| Constant | -456.695 | -343.30 | -435.88 | -588.20 | -447.45 | -333.78 | -426.16 | -579.54 | -180.19 | -64.40 | -159.97 | -274.20 |
| Accuracy (%) | 96.774 | 100.000 | 98.039 | 100.000 | 96.774 | 100.000 | 98.039 | 100.000 | 96.774 | 100.000 | 95.098 | 100.000 |
| Overall Accuracy (%) | 98.708 | | | | 98.708 | | | | 97.933 | | | |

(a)  Seasons

(b)  Seasons

(c)  Seasons

(d)  Seasons

(e)  Seasons

(f)  Seasons

(g)  Seasons

(h)  Seasons

(i) Seasons  (j) Seasons

(k) Seasons  (l) Seasons

(m) Seasons
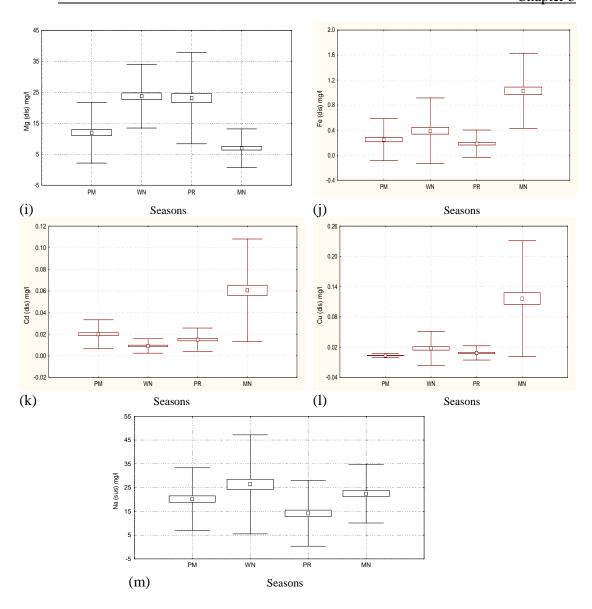
Fig. 3.6    Box and Whisker plots of some variables (a. stream flow, b. temperature, c. EC, d. Salinity, e. Total hardness, f. Na, g. K, h.Ca, i. Mg, j. Fe dis, k. Cd dis, l. Cu dis, m. Na sus) separated by temporal discriminant analysis associated with water quality data of Nullah Aik and Nullah Palkhu.

PM (post monsoon), WN (winter), PR (pre monsoon) and MN (Monsoon)

**Principal Component Analysis/ Factor Analysis (PCA/ FA)**

PCA/FA was applied separately on raw water quality data matrix for the three different regions, viz., RUR, IR and MIR identified by HACA to compare the distribution pattern between water samples to recognize factors that influence water quality of streams. The Data matrices used for PCA/FA were (38 × 96) for RUR, (38 × 186) for IR, and (38 × 108) for MIR. PCA/FA of the three regions produced 10 VFs for RUR and IR and nine for MIR with eigenvalues greater than 1 and explained 81.7%, 73.6% and 79.8% of the total variance of water quality variables.

Ten VFs were found significant for RUR (Table 3.7). VF1 explained 19.5% of total variance and was positively correlated with COD (r = 0.789), Ni (dis; r = 0.808) and Ni (sus; r = 0.820). This factor represented industrial and municipal source of pollution. VF2, explained 12.1% of the total variance, showed positive loadings on Zn (dis; r = 0.789) and Na (sus, r = 0.870) representing the impact of tanneries effluents and municipal sewage. VF3 was positively correlated with Cd (dis; r = 0.763) and Fe (sus; r= 841), which contributed 10.7% of the total variance. This factor is related to non-point source (surface runoff) and parent rock material. VF4 contributed 10.4% of the total variation which was negatively correlated to turbidity(r = -0.746), Pb (dis; r = -0.836), Cu (dis; r = - 0.918) and Cd (sus; r = - 0.808). This factor was related to municipal sewage and urban runoff. VF5 explained 6.4% of total variance, was negatively correlated to Cu (sus r = -0.760) which indicated the contribution from municipal waste and surface runoff. VF6 shared 6.1% of the total variance and negatively correlated to two variables stream width(r = -0.850) and stream depth(r = - 0.884), indicating the importance of stream morphology. VF7 contributed 5.3% of total variation with negatively correlated to EC (r = - 0 907 and TDS (r = -0.850). This factor can be related mainly with tanneries and from parent rock material (with lesser extent). VF8 contributed 4.2% of total variation and showed significant positive correlation to Cl⁻ (r = 0.839), K (dis; r = 0.780) and Cr (dis; r = 0.767) and was related to effluents coming from tanneries located in different locations in Sialkot. Presence of Fe in suspended particulate matter can be associated mainly with parent rock material. VF9 corresponded to 3.5% of the total explained variance and showed negative correlation with Mg (dis r = -0.815) contributed by municipal sewage and surface runoff. VF10, which accounted for 2.7% of total explained variance showed

negative correlation with $PO_4^{3-}$ (r = -0.806) indicating the origin of $PO_4^{3-}$ is mainly from municipal waste and also from tanneries effluent with lesser extent.

PCA/FA of IR separated eight significant VFs (Table 3.7). VF1 accounted for 24.01% of total explained variance and was positively correlated with $PO_4^{3-}$ (r = 0.772), $S^{2-}$ (r = 0.702) and K (dis r = 0.819). This factor can be related with municipal waste and tanneries effluent. VF2 explained about 8.5% of total variation, negatively correlated with Cr (dis; r = -0.841), Zn (dis; r = -0.793) and Cd (sus; r = -0.745) attributed by miscellaneous sources. VF3, shared about 8.1% of total variation and was positively correlated to Pb (dis; r = 0.841) and Zn (sus; r = 0.737) representing the contribution of surface run off from roads and urban area. VF4 contributed 7.0% of the total variations and showed positive correlation with Cr (dis r = 0.743), indicating their source related with effluents from tanneries. VF5 showed positive correlation with turbidity (r = 0.813), K (sus; r = 0.819) and Cr (sus; r = 0.764) explained 6.9% of total variations along this factor may be due to due to load of suspended solid waste from municipal sewage and tanneries effluents. VF6 was positively correlated to Na (sus; r = 0.852) explaining 5.7% of total variations. This factor identified tanneries effluents and municipal waste as major sources. VF7 contributed about 4.1% of total variance, with positive loadings on EC (r = 0.788) and TDS (r = 0.860) indicating from tanneries and parent rock material as major contributor. VF8 contributed about 3.8% of total variation showing negative correlation with Cl⁻ (r = -0.714) influenced from tanneries and municipal waste.

For MIR, FA/PCA segregated nine VFs as significant contributors (Table 3.7). VF1 correspond to 24.6% of the total explained variance and showed negative correlation with Pb (dis; r = -0.866) and Cu (dis; r = -0.882). This factor was found related to non-point sources like surface runoff. VF2 accounted for 14.1% of the total variance and showed negative correlation with Na (sus; r = -0.875), Mg (sus; r = -0.820) and Ca (sus; r = -0.827) representing the contribution of parent rock material, and tanneries effluents. VF3 was responsible for 9.1% of the total variance with positive correlation with turbidity(r = 0.917), and Cr (sus; r = 0.921) indicating the contribution of tanneries effluents. EC(r = 0.832) and TDS(r = 0.882) were positively correlated with VF4, which accounted for 7.2% of the total variance associated with untreated tanneries effluents and parent rock material. VF5 corresponds to 6.6% of the total explained variance and showed positive correlation with Mg (dis; r = 0.876).

This factor explained the load of Mg coming from municipal sewage and agricultural runoff. VF6 accounted for 3.9% and showed positive correlation Ca (dis; r = 0.776), Mg (sus; r = 0.815) and Cd (sus; r = 0.745) exhibiting the contribution of surface runoff and parent rock material. No variable was found strongly correlated with VF7, which is accounted for 3.9% of the total explained variance. VF8 corresponds to 3.5% of the total explained variance and showed negative with stream width (r = -0.721) and positive with stream depth (r = 0.778). This factor is influenced by stream morphology. VF9, which accounts for 3.0% of the total explained variance showing negative correlation with $NO_3^-$ (r = 0.756) indicating the origin of this factor from agricultural runoff.

Table 3.7    Loadings of environmental variables on the first ten VFs for RUR and IR, and nine VFs for MIR water quality data collected from of Nullah Aik and Palkhu

| Stream Region | Relatively Unimpaired Region | | | | | | | | | | Impaired Region | | | | | | | | Less Impaired Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF9 | VF10 | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF9 |
| **Stream width** | -0.064 | -0.072 | 0.049 | -0.051 | 0.013 | **-0.850** | 0.037 | -0.299 | -0.066 | 0.146 | -0.565 | -0.082 | -0.041 | -0.186 | -0.248 | -0.282 | 0.084 | 0.188 | -0.098 | -0.106 | -0.078 | -0.115 | -0.185 | -0.185 | -0.247 | **-0.721** | -0.164 |
| Stream velocity | -0.223 | 0.149 | -0.049 | -0.203 | 0.264 | -0.678 | -0.089 | -0.269 | 0.131 | -0.327 | -0.450 | -0.266 | -0.305 | -0.364 | -0.092 | 0.061 | -0.200 | 0.130 | -0.414 | 0.103 | 0.017 | -0.183 | -0.374 | 0.127 | -0.412 | 0.032 | -0.440 |
| Stream depth | -0.054 | 0.064 | -0.079 | -0.157 | 0.007 | **-0.884** | -0.012 | -0.050 | -0.082 | 0.027 | -0.062 | -0.139 | -0.223 | -0.093 | 0.081 | 0.043 | -0.109 | 0.236 | 0.018 | -0.096 | -0.156 | -0.173 | 0.041 | -0.113 | -0.109 | 0.778 | -0.195 |
| Temperture. | -0.333 | 0.216 | -0.002 | -0.402 | 0.698 | -0.246 | 0.021 | 0.061 | -0.069 | -0.247 | 0.015 | -0.367 | -0.218 | -0.568 | -0.122 | 0.012 | -0.098 | 0.151 | -0.298 | -0.075 | -0.230 | -0.282 | -0.316 | 0.102 | -0.336 | -0.032 | -0.120 |
| pH | -0.157 | -0.105 | 0.209 | 0.098 | 0.027 | 0.081 | 0.098 | -0.197 | 0.589 | 0.024 | -0.249 | 0.201 | 0.273 | -0.152 | 0.045 | 0.146 | -0.294 | -0.114 | -0.564 | 0.155 | 0.028 | -0.192 | 0.051 | 0.180 | 0.128 | 0.312 | -0.116 |
| DO | 0.155 | -0.070 | 0.119 | 0.571 | -0.310 | -0.267 | 0.072 | -0.465 | 0.218 | 0.176 | -0.540 | 0.190 | 0.085 | -0.137 | -0.260 | -0.028 | -0.132 | 0.106 | -0.086 | 0.343 | -0.042 | -0.441 | -0.094 | 0.188 | 0.197 | 0.127 | -0.408 |
| COD | **0.789** | 0.172 | 0.011 | 0.158 | -0.190 | 0.219 | 0.218 | 0.091 | -0.135 | -0.051 | 0.166 | 0.091 | 0.205 | 0.463 | 0.233 | -0.032 | 0.173 | 0.233 | 0.450 | -0.002 | -0.147 | 0.200 | 0.585 | -0.130 | 0.120 | 0.074 | 0.036 |
| E.C. | -0.076 | -0.088 | 0.065 | 0.063 | 0.061 | -0.079 | **-0.907** | 0.117 | -0.065 | -0.091 | 0.151 | 0.091 | 0.118 | 0.306 | 0.030 | -0.027 | **0.788** | -0.236 | 0.230 | -0.030 | 0.205 | **0.832** | -0.019 | 0.064 | 0.123 | -0.066 | -0.011 |
| TDS | -0.103 | 0.036 | -0.021 | 0.112 | 0.126 | 0.083 | **-0.850** | 0.259 | 0.148 | 0.021 | 0.205 | 0.096 | 0.103 | 0.209 | 0.035 | 0.012 | **0.860** | -0.162 | 0.141 | 0.044 | -0.025 | **0.882** | 0.050 | 0.096 | -0.079 | -0.055 | 0.231 |
| Salinity | 0.254 | -0.131 | 0.057 | 0.422 | -0.158 | 0.228 | -0.142 | 0.665 | 0.037 | 0.184 | 0.595 | 0.040 | 0.226 | 0.404 | 0.219 | 0.073 | 0.225 | -0.262 | 0.266 | 0.136 | 0.254 | 0.314 | 0.036 | 0.179 | 0.150 | -0.101 | 0.661 |
| Hardness | 0.329 | -0.104 | 0.029 | 0.426 | -0.265 | 0.316 | -0.024 | 0.507 | -0.072 | -0.099 | 0.127 | 0.536 | 0.301 | 0.238 | 0.098 | 0.359 | 0.236 | -0.064 | 0.379 | 0.189 | 0.040 | 0.209 | 0.232 | 0.004 | 0.625 | 0.065 | 0.249 |
| Turbidity | -0.030 | 0.113 | 0.203 | **-0.746** | 0.217 | -0.212 | -0.079 | -0.035 | -0.281 | -0.091 | -0.149 | 0.007 | 0.069 | 0.247 | **0.813** | -0.181 | -0.136 | -0.086 | -0.110 | -0.024 | **0.917** | 0.019 | 0.085 | -0.068 | -0.044 | -0.150 | 0.087 |
| **$NO_3^-$** | 0.032 | 0.625 | -0.043 | -0.122 | -0.229 | 0.491 | -0.112 | -0.099 | -0.290 | -0.124 | -0.155 | -0.077 | -0.130 | -0.011 | 0.036 | 0.078 | 0.047 | -0.090 | -0.207 | 0.005 | 0.009 | 0.109 | -0.101 | -0.022 | -0.772 | -0.050 | **-0.756** |
| $PO_4^{3-}$ | 0.177 | 0.064 | -0.065 | 0.018 | -0.038 | -0.030 | 0.066 | 0.113 | 0.033 | **0.806** | **0.772** | 0.135 | 0.102 | 0.002 | 0.140 | 0.170 | 0.287 | 0.132 | 0.118 | 0.012 | -0.153 | 0.514 | 0.164 | 0.102 | 0.089 | 0.029 | 0.452 |
| $Cl^-$ | 0.028 | 0.156 | 0.249 | -0.083 | -0.141 | 0.056 | -0.088 | **0.839** | -0.130 | -0.024 | 0.122 | 0.017 | 0.104 | -0.085 | -0.016 | 0.050 | 0.288 | **-0.714** | 0.192 | -0.041 | -0.008 | 0.146 | 0.193 | 0.106 | -0.167 | 0.020 | 0.154 |
| $S^{2-}$ | -0.126 | -0.009 | 0.491 | 0.231 | -0.431 | -0.156 | -0.091 | 0.034 | -0.061 | 0.426 | **0.704** | 0.330 | 0.057 | -0.113 | 0.054 | -0.058 | -0.129 | -0.137 | 0.013 | 0.073 | 0.404 | 0.025 | -0.098 | 0.658 | 0.108 | -0.050 | 0.493 |
| Na (dis) | 0.016 | -0.124 | -0.036 | 0.057 | -0.670 | 0.116 | 0.266 | 0.113 | 0.093 | -0.164 | 0.325 | -0.087 | 0.195 | 0.616 | 0.143 | -0.004 | 0.224 | 0.313 | 0.126 | 0.195 | 0.200 | 0.597 | 0.153 | -0.225 | 0.028 | 0.092 | 0.289 |
| K (dis) | 0.207 | -0.013 | 0.009 | 0.220 | 0.180 | 0.298 | -0.163 | **0.780** | 0.051 | 0.256 | **0.819** | 0.135 | 0.183 | 0.119 | -0.070 | -0.201 | 0.193 | 0.037 | 0.271 | 0.098 | -0.068 | 0.256 | 0.227 | 0.316 | 0.158 | -0.071 | 0.606 |
| Ca (dis) | 0.298 | 0.648 | -0.146 | -0.357 | 0.145 | 0.061 | -0.008 | 0.062 | -0.243 | 0.276 | 0.424 | 0.537 | -0.245 | -0.064 | 0.099 | 0.042 | 0.115 | -0.441 | -0.232 | 0.163 | 0.118 | 0.223 | -0.004 | **0.776** | 0.021 | 0.085 | 0.348 |
| Mg (dis) | 0.555 | -0.469 | 0.212 | -0.139 | -0.338 | 0.073 | -0.267 | 0.095 | -0.031 | 0.151 | 0.035 | 0.111 | -0.328 | 0.647 | 0.068 | 0.077 | 0.054 | -0.032 | -0.043 | 0.041 | 0.092 | 0.122 | **0.825** | -0.108 | 0.083 | 0.194 | 0.123 |
| Fe (dis) | 0.162 | 0.072 | -0.137 | 0.344 | -0.455 | 0.077 | 0.383 | 0.450 | 0.117 | 0.270 | 0.440 | **0.719** | 0.077 | 0.210 | -0.033 | 0.087 | 0.140 | -0.085 | -0.130 | 0.520 | 0.044 | 0.214 | 0.466 | 0.283 | 0.283 | -0.015 | 0.374 |
| Pb (dis) | -0.071 | 0.197 | 0.304 | **-0.836** | 0.057 | -0.014 | 0.137 | 0.053 | 0.143 | -0.109 | -0.140 | -0.012 | -0.535 | 0.134 | -0.101 | 0.610 | 0.117 | 0.023 | **-0.866** | 0.167 | -0.060 | -0.097 | -0.075 | -0.219 | -0.307 | 0.000 | -0.003 |
| Cd (dis) | 0.026 | -0.214 | **0.763** | -0.054 | -0.152 | 0.157 | 0.064 | 0.284 | 0.201 | -0.077 | 0.025 | 0.252 | 0.127 | 0.453 | -0.151 | 0.592 | 0.309 | 0.271 | -0.191 | 0.662 | -0.248 | 0.100 | -0.029 | -0.126 | 0.135 | -0.138 | 0.429 |
| Cr (dis) | -0.091 | -0.286 | 0.274 | 0.006 | 0.058 | 0.055 | -0.121 | **0.767** | -0.162 | -0.139 | 0.076 | 0.012 | 0.157 | **0.743** | 0.209 | -0.030 | 0.198 | 0.075 | 0.305 | -0.258 | 0.434 | 0.207 | -0.119 | -0.161 | 0.026 | 0.143 | 0.626 |
| Ni (dis) | **0.808** | -0.204 | -0.077 | 0.218 | 0.110 | 0.066 | 0.049 | 0.017 | 0.118 | 0.260 | 0.128 | 0.489 | 0.171 | 0.269 | 0.136 | -0.601 | 0.008 | -0.020 | 0.212 | -0.210 | 0.364 | -0.127 | 0.350 | 0.194 | -0.081 | 0.003 | 0.102 |

Continued…

| Stream Region Variables | Relatively Unimpaired Region | | | | | | | | | | Impaired Region | | | | | | | | Less Impaired Region | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF9 | VF10 | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF1 | VF2 | VF3 | VF4 | VF5 | VF6 | VF7 | VF8 | VF9 |
| **Cu (dis)** | -0.046 | -0.079 | -0.147 | **-0.918** | 0.001 | -0.057 | 0.107 | -0.056 | 0.022 | -0.049 | -0.114 | -0.167 | **-0.793** | 0.030 | -0.133 | 0.055 | -0.129 | 0.012 | **-0.882** | -0.149 | 0.004 | -0.149 | 0.056 | -0.206 | -0.228 | -0.074 | -0.021 |
| **Zn (dis)** | 0.214 | **0.798** | 0.129 | 0.128 | 0.127 | -0.247 | 0.088 | -0.022 | 0.034 | 0.106 | 0.011 | **0.737** | 0.119 | 0.071 | -0.097 | 0.023 | 0.059 | 0.126 | 0.086 | 0.480 | -0.062 | -0.058 | 0.573 | 0.336 | 0.018 | -0.188 | 0.005 |
| **Na (sus)** | -0.144 | **0.873** | -0.009 | -0.057 | -0.197 | 0.057 | -0.023 | -0.179 | -0.051 | -0.084 | 0.090 | 0.227 | 0.175 | -0.003 | 0.045 | **0.852** | -0.104 | -0.109 | 0.077 | **0.875** | 0.006 | 0.100 | 0.005 | 0.052 | -0.068 | 0.106 | -0.087 |
| **K (sus)** | 0.001 | 0.688 | 0.554 | 0.190 | 0.141 | 0.050 | -0.022 | 0.067 | -0.018 | -0.038 | 0.231 | 0.119 | 0.292 | 0.100 | **0.819** | 0.144 | -0.075 | -0.034 | 0.270 | **0.820** | 0.050 | -0.060 | 0.122 | 0.274 | -0.020 | 0.017 | -0.093 |
| **Ca (sus)** | 0.125 | 0.422 | 0.032 | 0.227 | -0.236 | -0.254 | 0.311 | -0.046 | -0.209 | 0.485 | 0.209 | 0.576 | 0.108 | -0.192 | 0.143 | 0.424 | -0.102 | -0.214 | -0.014 | **0.827** | 0.236 | -0.040 | -0.009 | 0.308 | 0.039 | 0.010 | -0.002 |
| **Mg (sus)** | -0.059 | 0.035 | 0.214 | 0.154 | 0.184 | 0.033 | 0.210 | 0.014 | **-0.815** | 0.029 | 0.152 | 0.124 | -0.398 | -0.040 | 0.006 | -0.046 | -0.036 | 0.060 | 0.154 | 0.173 | -0.066 | -0.087 | 0.048 | **0.815** | -0.161 | 0.202 | -0.021 |
| **Fe (sus)** | -0.116 | 0.180 | **0.841** | 0.096 | 0.101 | -0.048 | -0.083 | 0.152 | -0.135 | -0.078 | 0.068 | 0.226 | 0.136 | -0.402 | 0.308 | -0.012 | 0.422 | 0.214 | 0.027 | 0.299 | 0.065 | 0.057 | 0.042 | **0.745** | 0.103 | -0.153 | -0.188 |
| **Pb (sus)** | 0.585 | -0.054 | -0.146 | 0.013 | -0.173 | 0.086 | -0.060 | -0.229 | 0.028 | 0.593 | 0.036 | 0.680 | -0.068 | -0.007 | 0.158 | 0.008 | 0.013 | -0.002 | -0.116 | 0.116 | 0.160 | -0.078 | 0.699 | 0.439 | 0.266 | 0.131 | -0.089 |
| **Cd (sus)** | 0.099 | -0.176 | -0.232 | **-0.858** | 0.036 | -0.043 | 0.077 | -0.177 | 0.047 | 0.093 | -0.140 | 0.085 | **-0.745** | -0.106 | 0.010 | -0.118 | -0.068 | 0.006 | **-0.729** | -0.129 | 0.077 | 0.013 | -0.117 | 0.230 | 0.108 | -0.058 | -0.256 |
| **Cr (sus)** | 0.598 | 0.135 | 0.062 | -0.016 | 0.254 | -0.105 | 0.431 | 0.246 | -0.241 | 0.026 | 0.243 | -0.068 | -0.047 | 0.034 | **0.764** | -0.153 | 0.237 | 0.066 | 0.047 | 0.033 | **0.921** | 0.093 | -0.045 | 0.183 | -0.003 | 0.049 | 0.026 |
| **Ni (sus)** | **0.820** | 0.210 | -0.121 | -0.108 | 0.031 | 0.017 | 0.024 | 0.105 | -0.014 | 0.072 | -0.069 | 0.350 | -0.048 | 0.469 | 0.630 | 0.075 | -0.011 | -0.107 | -0.101 | 0.270 | 0.622 | 0.173 | 0.168 | 0.049 | 0.174 | 0.026 | -0.030 |
| **Cu (sus)** | -0.176 | 0.353 | 0.071 | 0.175 | **-0.760** | -0.035 | -0.054 | -0.039 | 0.053 | 0.319 | 0.389 | -0.177 | 0.062 | 0.037 | 0.527 | 0.184 | 0.363 | 0.321 | 0.009 | 0.455 | -0.219 | 0.096 | 0.153 | 0.239 | 0.324 | -0.061 | 0.144 |
| **Zn (sus)** | 0.000 | 0.567 | -0.208 | 0.360 | 0.203 | -0.128 | 0.258 | 0.240 | 0.101 | 0.276 | 0.274 | 0.302 | 0.127 | -0.467 | 0.531 | 0.161 | 0.337 | 0.251 | 0.085 | 0.579 | -0.058 | -0.152 | 0.245 | 0.605 | 0.111 | -0.083 | 0.189 |
| **Eigenvalues** | 7.616 | 4.726 | 4.175 | 4.089 | 2.496 | 2.405 | 2.096 | 1.674 | 1.368 | 1.085 | 9.367 | 3.353 | 3.165 | 2.866 | 2.695 | 2.218 | 1.627 | 1.482 | 9.698 | 5.507 | 3.567 | 2.809 | 2.575 | 2.050 | 1.525 | 1.370 | 1.192 |
| **Variance** | 19.529 | 12.119 | 10.705 | 10.486 | 6.401 | 6.166 | 5.375 | 4.293 | 3.507 | 2.781 | 24.018 | 8.598 | 8.115 | 7.348 | 6.911 | 5.688 | 4.172 | 3.800 | 24.866 | 14.121 | 9.146 | 7.203 | 6.602 | 5.256 | 3.909 | 3.512 | 3.056 |
| **Eigenvalues** | 7.616 | 12.343 | 16.517 | 20.607 | 23.103 | 25.508 | 27.604 | 29.278 | 30.646 | 31.731 | 9.367 | 12.720 | 15.885 | 18.751 | 21.446 | 23.664 | 25.291 | 26.773 | 9.698 | 15.205 | 18.772 | 21.581 | 24.156 | 26.206 | 27.731 | 29.100 | 30.292 |
| **% of variance** | 19.529 | 31.647 | 42.352 | 52.838 | 59.238 | 65.404 | 70.779 | 75.073 | 78.580 | 81.361 | 24.018 | 32.616 | 40.731 | 48.079 | 54.990 | 60.678 | 64.849 | 68.649 | 24.866 | 38.987 | 48.133 | 55.336 | 61.938 | 67.195 | 71.104 | 74.616 | 77.672 |
| **Sources** | SR, IW | TN, MS | MS, IW | Misc | MN, AG | TN | EC | TN | MS | EC, TN | TN | SR, MS | Misc | TN | Misc | SR | EC | SR | SR | SR, IW | TN | TN | AG, MS | SR | SR, | AG, EC | AG, EC |

SR(Surface runoff), IW (Industrial waste), TN (Tanneries effluent), MS (Municipal waste) EC(Earth crust), AG (Agricultural runoff) and Misc (Miscellaneous)

## Discussion

### Spatial Variations in Surface Water Quality

The spatial and temporal variations in streams water quality are highly influenced by natural and anthropogenic activities (Sullivan & Drever, 2001; De Carlo *et al*, 2005). Anthropogenic activities in the catchment area of any stream highly degrade the stream water quality. This process of degradation becomes severe in streams traversing from densely populated and industrial areas (Paul & Meyer, 2001). Nullah Aik and Nullah Palkhu are facing severe degradation from anthropogenic activities in the catchment area. The water quality was relatively better in upstream of Nullah Aik and Nullah Palkhu lies within 15 kilometres above the Sialkot city. The upstream segment was least impacted from point sources receive pollutants from agricultural runoff. Water quality of mid stream and downstream segments was degraded as streams passes through Sialkot city, where they receive pollutants from multiple point sources. Water quality at mid stream sites was highly degraded, which improve as it flows towards far downstream due to sedimentation and dilution factors.

Less variations in water quality parameters were observed in RUR, whereas, more variations exhibited by MIR and IR. In RUR, variations in water quality parameters were resulted from seasonal variations in stream discharge and parent rock material in IR. Effluents load from point sources decreased in MIR that reduced the variations within water quality parameters. Most of the point sources are concentrated in IR that drains effluents in streams. The sites in IR were located in downstream of Sialkot city, which receive effluents and raw sewage throughout the year.

Spatial variations in water quality were due to stream flow, depth, DO, COD, TDS, $NO_3^-$, $PO_4^{3-}$, Pb (dis), Cr (dis), Mg (sus) and Ni (sus). Stream flow showed an increasing trend from RUR to MIR. Qadir *et al.* (2008) reported that stream water flow and discharge plays a vital role in determination of pollutants load. Stream depth varies from site to site. Maximum stream depth was observed at site 7(Wain) located in MIR due to construction of small headworks, which control and divert the stream water for irrigation purposes.

The results showed that high concentration of DO in RUR (6.3mg/L), low concentration at IR(1.97mg/L), which gradually increased in LIR(3.63mg/L). The concentration of DO in IR and LIR were below the standard limits of fresh water organisms like fishes (5.0 - 9.0mg/L) described by CEC (1978). The concentration of

DO of present study were below the DO(7.4mg/L) as reported by Sharma & Ghosh (1987) and above the concentration (0.51mg/L) in Hudiara drain (Khan *et al*., 2003). Low concentration of DO in IR is associated with the direct discharge of untreated industrial effluents and municipal sewage from Sialkot and Sambrial towns situated along the Nullah Aik and Nullah Palkhu. Municipal sewage and industrial effluents contain organic substances, which are biodegradable, require a large amount of oxygen for oxidation process by micro-organisms and causes depletion of DO. In aquatic systems, excessive organic and inorganic input (from industrial and urban waste) may reduce the availability of DO. Recommended concentration of DO is 4.0 mg/L for fishes, however, most species are distressed when it falls between 2.0 - 4.0 mg/L. Low level of DO (less than 2.0 mg/L) can cause fish mortality (McNeil & Closs, 2007).

Municipal sewage and industrial effluents decrease DO level and increase COD level in stream water (Singh *et al*., 2005a). Lowest level of COD was recorded in RUR (38.02 mg/L) and highest in IR (90.01 mg/L). High level of COD may be due to the discharge of high volume of untreated industrial and municipal effluents into streams. Khan *et al*. (2003) and Singh *et al*. (2005a) observed similar trend in COD values in Hudiara drain (Pakistan) and Gomti River (India). The level of COD was higher in IR, where its level exceeded the permissible level (50mg/L) of effluent discharge in fresh water (UNEP, 2004). The COD level in present study was higher to those reported by Singh *et al*. (2005a) and lower to those of Khan *et al*. (2003).

Similarly, high concentration of TDS was recorded in IR (585.1 mg/L), which was below the permissible level (500mg/L) described by USEPA (1998), whereas, concentration of TDS in RUR(418.9mg/L) and LIR ( 486.8 mg/L) were below the permissible level. The level of TDS was high as compared to the concentration of TDS recorded by PCRWR (2004) and Singh *et al*., (2005a), however, below TDS values recorded by Khan *et al*. (2003). High values of TDS can be resulted due to effluent containing higher concentration of soluble salts related with natural and anthropogenic sources. Stream system of Nullah Aik and Nullah Palkhu receives effluents from tanneries, which contain higher concentration of sodium chloride, which affects the freshwater aquatic life, when its concentration becomes high.

Highest concentration of $NO_3^-$ (2.19mg/L) was measured in RUR, which can be associated with agricultural activities in the catchment area. Elevated concentration

of $NO_3^-$ comes from agricultural fields after surface runoff. Yang *et al.* (2004) and Morrison *et al.* (2001) also reported similar reason of high level of $NO_3^-$ in stream water. The level of $NO_3^-$ was reported in the present study was higher to those in River Chenab (PCRWR, 2004) and Gomti River, India (Singh *et al.*, 2005a). Concentration of $NO_3^-$ in stream water of Nullah Aik and Nullah Palkhu was also higher than concentration (0.05 - 0.2mg/L) reported by Chapman (1996) for natural stream water.

In contrast, highest level of $PO_4^{3-}$ was observed in IR (2.78mg/L) and lowest in RUR (1.02mg/L). Municipal sewage was identified as the possible source of $PO_4^{3-}$. The elevated concentration of $PO_4^{3-}$ is due to human impacts on streams and may be associated with direct discharge of raw sewage (Singh *et al.*, 2005a). Higher level of $PO_4^{3-}$ may also occur as a consequence of the use of detergents (Perona *et al.*, 1999; Sundaray *et al.*, 2006). Debels *et al.* (2005) reported higher level of $PO_4^{3-}$ in downstream sites as compared to upstream sites of Chillan River and municipal sewage being blamed as its source. The level of $PO_4^{3-}$ concentration (2.19mg/L) in surface water was higher than the concentration (0.002 - 0.025mg/L) measured in pristine streams (Chapman *et al.,* 1996). Higher concentration of $PO_4^{3-}$ was also reported in River Chenab (0.3mg/L; PCRWR, 2004), River Ganga (0.21mg/L; Sharma & Ghosh, 1987) and River Gomti (0.1 - 10mg/L). Higher concentrations of $PO_4^{3-}$ and $NO_3^-$ may cause eutrophication in streams and lakes (Morrison *et al.*, 2001).

Pb in dissolved form was recorded maximum in MIR (0.812mg/L) and lowest at RUR (0.141mg/L). Pb concentration increases from upstream towards downstream sites. Atmospheric deposition in urban areas and surface runoff can be main sources of Pb in stream water. During heavy rainfall, Pb on soil surface becomes dissolved, which makes its way into the stream (Hurst *et al.*, 1996; Sutherland *et al.*, 2003). Manufacturing processes (battery plants), paints and pigments, incineration of municipal solid wastes and hazardous wastes are the other sources of Pb (FDA, 1993). Pb comes from automobile exhaust and deposited on soil form atmosphere especially near major highways (FDA, 1993; Tomazelli *et al.*, 2003). Concentration of Pb in water of Nullah Aik and Nullah Palkhu exceeded the permissible limits (0.01mg/L) for freshwater (Chapman, 1996). In present study, high concentration of Pb was compared to the studies of Khan *et al.* (2003), Singh *et al.* (2004) and Tariq *et al.*

(1996), whereas, its concentrations were lower to those reported by Javed (1999) and Khan *et al*. (2003).

High concentration of Cr measured in present study is mainly related with effluents from tanneries and electroplating industries discharged into streams, which may accumulated in sediment with higher concentrations (Nriagu & Nieboere, 1988). Maximum concentration of Cr was recorded from those sites, which were close to the Sialkot city. The results showed high level of Cr (dis) were recorded in IR (0.196mg/L) and MIR (0.145mg/L), which can be attributed to heavy load of contaminants from tanneries. Cr concentration in IR and MIR was above the permissible limit (0.05mg/L) described by CEC (1978) and USEPA (1993). Large volume of effluents is produced during tanning process, which contain high concentration of chromium and soluble salts such as $NaCl$ (Moten & Sami, 2000). Indiscriminate use of Cr salts in tanneries is one of the main source of its increase level in drains and streams of Sialkot. Cr is mainly found in industrial waste from the chrome tanning process, about 70% of the total amount is taken up by the hides and about 30% remains unabsorbed, which goes into the effluent and sludge. Continuous discharges of Cr, even in low concentrations, have been reported to have toxic effects on aquatic life and can disrupt the food chain in aquatic ecosystems (Bosnic *et al*., 2000). High pH allows Cr to convert into complex substances and become part of suspended particulate matter, which settles down as effluents travels the distance from the source. Significantly higher concentration of Cr (0.196mg/L) was observed in IR region. Higher concentration of Cr in sediment is toxic for aquatic organisms in general and particularly for bottom dweller organisms. Cr concentration in studied streams was higher than the permissible level (0.022mg/L) for freshwater described by Wright & Welbourn, (2002). Similarly, concentration of present study was above to those reported by Tariq *et al*. (1996), Javed (1999), Khan *et al*. (2003) and Abbas *et al*. (2004).

The distribution of Mg in suspended particulate matter depicted an increasing trend from upstream to downstream. The origin of Mg is from both natural and anthropogenic activities. Relatively high concentrations of Mg in moderately impaired sites may indicate the effect of human activities in the catchment area. Tzankov *et al*. (2007) reported that higher concentration of Mg in stream water is strongly influenced by weathering of parent materials.

Ni is relatively less toxic to organism in comparison to other metals and toxicity of Ni can be masked by other metals in aquatic environment (Kszos *et al.,* 1992; Wright & Welbourn, 2002). Ni is mainly used for alloy formation and metal plating. Industrial effluent and municipal waste are the main sources that increases level of Ni in stream water (Ntengwe & Maseka, 2006). Highest level of Ni in suspended particulate matter was found in IR that can be as result of discharge of municipal sewage and industrial effluents that contain waste from electroplating units, where surgical instruments are manufactured. In alkaline pH, dissolved Ni metal reacts with suspended particulate and organic matter that settles down in streambed. The concentration of Ni decreases with an increase of distance from the source (Wright & Welbourn, 2002). Sometimes, Ni deposited at remote sites from its sources indicating that its source may be resulted from atmospheric deposition (Sarkar, 2002). Concentrations of Ni recorded from all sampling zones were recorded within safe limits (0.065mg/L) as described for freshwater by Chapman (1996). During present study, concentration of Ni was recorded higher than the concentrations of Ni reported by Tariq *et al*. (1996), Abbas *et al*. (2004) and Singh *et al*. (2005a). In contrary to results of present study, Javed (1999) studied higher concentrations of Ni (0.8mg/L) in River Ravi.

**Temporal Variations in Surface Water Quality**

The results highlight temporal variability of various pollutants. Pollutants from non-point sources exhibited maximum concentration during monsoon season due to surface runoff and pollutants from point sources showed highest concentration when the stream flow reduced to its minimum level. In present study, stream flow, temperature, EC, salinity, total hardness, Na (dis), K (dis), Ca (dis), Mg (dis), Fe (dis), Cd (dis), Cu (dis) and Na( sus) showed marked seasonal variations. Distinct seasonal variations in concentration of pollutants may be due to fluctuation in the stream discharge level. During monsoon season, due to high discharge level the effect of effluent becomes diluted, whereas, in winter and early summer, studied streams experiences extreme low discharge level. The amount of effluents remains same throughout the year in the catchment area and pollutants concentration is generally higher during low stream flow. Similar results were obtained by Tsai *et al.* (2003). Maximum stream discharge level was observed in monsoon season and minimum discharge level was observed during winter and pre monsoon seasons (Fig. 3.7).

During monsoon season, the catchment area receives major share of rainfalls. High discharge level of streams during heavy rains causes local flooding (Plate. 3.1a & b). This may result in dilution of toxic chemicals like heavy metals coming from point sources, whereas, the concentration of metals coming from non-point sources (atmospheric deposition, urban and agricultural runoff) increases in stream water during rainy season. During dry season, heavy metals deposits on the soil surface through atmospheric deposition and flushed away by surface runoff into streams during rainy season. There is a strong relationship between stream flow and rainfall in their catchment area. Low stream flow prevails in pre monsoon season (early summer) that recovered rapidly in monsoon season (mid June - mid September). During winter season, occasional rainfall may increases stream flow. About 80% of rainfalls occur during monsoon season when streams flow at their full swing. During early monsoon rains, fast flowing water erodes the contaminated sediments deposited in stream bed and transported to River Chenab. Floodwater also carries a huge volume of non degradable solid (plastic bottles, polythene bags and packing material) ultimately discharged into River Chenab (Plate. 3.2a & b).

The results indicated marked seasonal variations in EC, which was recorded high in winter (915.8µS/cm) and pre monsoon (974.5µS/cm) and lowest (622.5µS/cm) in monsoon season. High values of EC can be due to high discharge of soluble salts along with tanneries effluents. Stream system of Nullah Aik and Nullah Palkhu receive effluents from tanneries, which contain higher concentration of sodium chloride, which in turn affects freshwater aquatic life. EC value of present study was relatively higher to those reported in different regional studies, such as, Khan *et al*. (2003), Singh *et al*. (2005a) and PCRWR (2004).

Salinity and hardness affects the availability of metals to organism (Wright & Welbourn, 2002). At high level of salinity trace metals present in dissolved form react with suspended particulate matter and become insoluble, which settle down in sediments. In natural stream, presence of soluble salts indicates that salts originate from parent rock material in catchment area (Crosa *et al*., 2006). The results showed that maximum salinity was recorded in pre monsoon (0.43mg/L) and minimum in monsoon season (0.16mg/L). High stream discharge level reduce the level of salinity in stream water thus, the temporal variations in salinity can be associated with high quantity of soluble salts used in tanneries, which are discharged into streams.

Plate 3.1a    An overview of site 2 showing the reduced stream flow during pre monsoon season, 2005.



Plate 3.1b An overview of site 2 showing the highest stream discharge (35,000Cs) during monsoon season, 2005.

Plate 3.2a. The stream water turn into black after erosion of deposited sediments at stream bed during early monsoon season



Plate 3.2b. The stream water return to its normal colour during monsoon season after heavy rainfall in the catchment area

Plate 3.3a    Solid waste (plastic bottles, rubber and packing material) at site 7 on Nullah Aik



Plate 3.3b    Entangled polythene bags on branches of stream bank plants during high stream flow.

Ca and Mg are major determining factors for total hardness in freshwater, however, other factors also contribute an increase in total hardness. Stream water of Nullah Aik and Nullah Palkhu can be categorized as moderately hard water, whereas, stream water of post monsoon and pre monsoon season as hard water. According to Wright & Welbourn (2002) based on hardness, four classes of fresh water can be recognized viz. soft water (0-75 mg/L), moderately hard (75-150 mg/L), hard (150-300 mg/L)

and very hard (above 300 mg/L). The results depicted that maximum total hardness was recorded in winter when the stream flow become low and effluents coming from tanneries containing higher concentration of calcium salts discharge into streams. Lowest values of total hardness were recorded in monsoon season, when streams flow at full swing and dilute Ca level in water. Main natural sources of Ca are gypsum, pyroxenes, calcite, dolomite, and clay minerals (Nanda, 2005). Generally, Ca content of natural waters is less than 100 mg/L. Parent rock material contains higher level of Ca in catchment area, which gets dissolved after weathering (Marofi & Maryanaji, 2007; Girija et al., 2007). In pristine water, concentration of Ca ranged between 0.06 to 210 mg/L (Chapman, 1996). PCRWR (2004) reported mean concentration of Ca (30mg/L) in water samples collected from River Chenab. Highest level of Mg (23.74mg/L) was recorded during winter, whereas, lowest concentration (6.959mg/L) was recorded during monsoon season. In natural running water, concentration of Mg ranged from 0.05 to 80mg/L (Chapman, 1996). The concentration of Mg recorded during present study was within the range of natural waters (0.05 to 80mg/L). The concentration of Mg was recorded in present study was higher to those of River Chenab (12.0 mg/L; PCRWR 2004).

Concentration of K increases from post monsoon (6.903mg/L) to pre monsoon (12.714mg/L), however, stream samples showed a slight decline during monsoon season (10.715mg/L), probably due to dilution factor. The concentration of K was higher to those reported by Chapman (1996) for natural streams. Possible source of K are from human activities (municipal sewage and agricultural sources) and natural input (Girija et al., 2007). Na has natural sources, but it is also be enriched by anthropogenic processes. The concentration of Na was higher than K and is related with TDS. Na and K are essential elements play vital role in various physiological processes in organisms. Minimum concentration of Na (24.89mg/L) was recorded during monsoon and maximum (91.49mg/L) during pre monsoon. Higher concentration can be attributed by the use of NaCl in tanning activities and other human uses. NaCl is used for hide and skin preservation process. Tanning industry in Sialkot produces considerable quantities of salts, which may result in higher concentration of Na in drinking water. Increased salt content in groundwater, especially in areas of high industrial density has been reported to cause environmental hazard (Bosnic et al., 2000). Concentration of Na measured in present study was

lower to those reported by Chapman (1996). Similarly, the concentration of Na was below the permissible level (200mg/L) and (150mg/L) as described by CEC (1978) and USEPA (1993) for drinking purposes.

Maximum concentration of dissolved Fe (1.018mg/L) was recorded during monsoon season, which is higher than the permissible value (0.3mg/L) for fresh water (Wright & Welbourn, 2002). Similarly, Taboada-Castro *et al*. (2002) also reported higher dissolved Fe content when stream flow was highest. This also support that inflow of Fe in dissolved form can be as a result of its remobilisation from earth crust in streams. Boman *et al.* (2002) stated that possible reason of higher concentration of Fe in stream water can be linked to its geological origin. Alther (1981) also described that presence of Fe in stream water is related to parent rock material. Fe may be originated with either organic or inorganic wastewater generated from municipal and industrial activities in the study area; however, major share of Fe comes from natural sources. Fe concentration measured in present study was higher to those reported in River Indus (Tariq *et al.,* 1996) and lower to those of Gomti and Ravi rivers (Singh *et al.,* 2004; Javed (1999).

Mean concentration of Cd was very low at most of the sites but maximum concentration was recorded during monsoon. The possible sources of Cd are atmospheric deposition, surface runoff, electroplating industries, solid waste disposal and the use of fertilizers (He & Singh, 1994; Jonsson *et al.*, 2002). Highest mean concentration of Cd (0.06mg/L) was recorded in monsoon season, which  was above the standards for drinking water as described by CEC (1978), USEPA (1993) and WHO (1993) Similarly, Cd concentration was also above the permissible level of freshwaters water (0.002mg/L) and for aquatic life (0.017mg/L) as described by Wright & Welbourn (2002).

Like Cd, highest mean concentration of dissolved Cu (0.116mg/L) was also recorded during monsoon season, which was higher than the threshold values for surface water (0.043mg/L) and aquatic life (0.056mg/L). The major pathway of Cu in stream water is from non-point sources (urban and agricultural runoff and atmospheric deposition). Stream water is a main vector for Cu transportation to soil, ground water and biota. Cu is less toxic in hard water whereas it has significant toxic effect in soft water (Nriagu, 1980). Fish and crustaceans are susceptible to copper toxicity as compared to birds and mammals (Wright & Welbourn, 2002). During present study,

higher level of dissolved Fe, Cu and Cd were recorded in monsoon when water discharge level becomes high. Kimball *et al*. (1995) studied increased concentration of colloidal iron, copper, and lead loads during high flow, likely resulting from re-suspension of iron colloids settled onto bed sediments.

## Conclusions

Water quality of Nullah Aik and Nullah Palkhu is highly impaired due to unwise and wide spread human activities in the catchment area. The anthropogenic activities have converted pristine condition of studied stream into fragile and unacceptable state. On the basis of spatial classification, three groups of sites were identified. First group (RUR) showed comparatively good water quality, second group of sites (IR) indicated highest level of stream degradation and third group (MIR) showed water quality was comparatively less degraded. Spatial and seasonal variations were observed in water quality parameters. Multivariate techniques discriminated the most influencing factors and identified their possible sources. DFA identified 11 water quality parameters viz., stream flow, stream depth, DO, COD, TDS, $NO_3^-$, $PO_4^{3-}$, Pb (dis), Cr (dis), Mg (sus), and Ni (sus), which showed significant variations along spatial gradient. Seasonal variations were recorded due to stream flow, temperature, EC, salinity, total hardness, Na (dis), K (dis), Ca (dis), Mg (dis), Fe (dis), Cd (dis), Cu (dis) and Na (sus). Main factors that bring changes in chemical composition of water are either related to anthropogenic and/or natural factors. Point sources such as industrial, tanneries, municipal sewage and non-point sources (atmospheric deposition, urban and agricultural runoff) were identified the most important factors. However, concentration of some contaminants such as Ca, Mg and Fe were due to the contribution of parent rock material and related to geo-chemical processes. The results of present study also showed parameters such as COD, TDS, $NO_3^-$ Fe, Pb, Cd, Cr and Cu exceeded the permissible level for fresh water, aquatic life, irrigation and domestics use. Present study highlighted the spatio-temporal variations in water quality of Nullah Aik and Nullah Palkhu caused due to anthropogenic activities. The statistical techniques used in present study pointed out the factors responsible for degradation of water quality. These results will help the environmental managers to develop restoration activities with minimum operational cost and expenditures.

**Chapter 4**

# Bioaccumulation of Heavy Metals in Selected Fishes of Nullah Aik and Nullah Palkhu

## Introduction

Higher level of pollutants had severe impact directly/ indirectly on the health of an aquatic ecosystem. Aquatic organisms respond to changes in their environment, which in turn reflect the prevailing health status of aquatic ecosystem (Kotze *et al.,* 1999). Heavy metals are important group of toxic pollutants (Amin, 1996), which are chemicals of great concern (Qadir *et al.,* 2008). Heavy metal pollution has gained serious attention from public and scientific community in relation with their toxicity to aquatic organisms and ultimate affect on well being of humans (Yayıntas *et al.,* 2007). Continuous release of heavy metals from point and non-point sources is putting the aquatic ecosystem under stress (Dural *et al.,* 2006). Heavy metals e.g. iron, copper, chromium and zinc are essential for regulation of metabolic activities but become toxic at higher concentrations, whereas, lead and cadmium have no documented role in living organism (Canli & Atli, 2003). Essential and non-essential metal at higher concentration can disrupt metabolic and physiological activities of aquatic organisms (Batley, 1983). Essential metals also become toxic if their concentration exceeds the permissible level (Wright & Welbourn, 2002).

Fishes are most important organisms in the aquatic food chain, which are sensitive to heavy metals contamination (Pickering & Pottinger, 1995). Most of the freshwater fishes are confined to specific microhabitat within inter connected river/ stream system. If such system becomes contaminated by heavy metals, fish species either shift to less polluted segment of river/stream system or die off which ultimately disturb the food chains (Rashed, 2001). High level of heavy metals has apparent lethal and chronic effects on fishes (Kotze *et al.,* 1999). Thus, fish not only indicates the pollution status of aquatic ecosystem but have significant impact on the food web (Qiao-qiao *et al.,* 2007). It is one of the main source of protein-enriched food all over the world (Mansour & Sidky, 2002). Consumption of contaminated fish with heavy metals can result hazardous effects on human health (Mai *et al.,* 2006).

Various pathways of metal accumulation in fish include such as ingestion of food, suspended particulate matter, metal ion exchange through gills and skin (Liang *et al.,* 1999; Wright & Welbourn, 2002). Nussey *et al.* (2000) also identified five routes through which heavy metals enter into fish viz; food, suspended particle, gills, intake of water and integuments. From these pathways, metals get absorbed into blood and transported to various organs for either storage or excretion. Level of trace metals in different organs of fish is used as an index of metal pollution in an ecosystem, which is considered as an important tool to highlight the role of elevated level of metals in aquatic organisms (Tarrio *et al.,* 1991; Mendil *et al.,* 2005). Concentration of heavy metals in different tissues/organs of fishes is directly influenced by contamination in aquatic environment, uptake, regulation and elimination inside the fish body (Bervoets *et al.,* 2001). Liver stores either heavy metals or excretes through bile. Other routes of heavy metal regulation are either kidneys or gills (Heath, 1991; Nussey *et al.,* 2000). Accumulation of metals in various organs and tissues depends upon the way of exposure such as through diet or their elevated level in surrounding environment (Pourang, 1995, Nussey *et al.,* 2000; Alam *et al.,* 2002). Morphological and behavioural abnormalities such as alteration in sensory reception, reduced responses to normal olfactory function (feeding, mating, selection, or homing), reduction in swimming performance, gills purge, ventilation, coughs, learning impairment, loss of equilibrium that lapsed into paralysis, loss of reproductive efficiency and irregular metamorphosis appeared as symptom of toxic exposure of trace metals (Atchison *et al.,* 1987). Concentration of metals becomes toxic to the fish when its level exceeds the permissible level (Langston, 1990). This threshold limit not only varies from metal to metal but also from one species to another (Langston, 1990; Kalay & Canli, 2000). Toxic effects of metals become more pronounced when various metabolic activities inside organism body fail to detoxify (Nussey *et al.,* 2000).

Heavy metals exhibit different accumulation pattern in organs (Jezierska & Witeska, 2007). Gills, liver and kidneys accumulate heavy metals in higher concentration in comparison to muscles, which exhibit lowest levels of metals accumulation (Jezierska & Witeska, 2007). Among different organs, liver accumulates higher concentrations of metals comparatively and has been used widely to investigate the process of bioaccumulation. Kidneys also play a vital role in excretion of trace metal ions (Langston *et al.,* 1998). Exchange of gases and

absorption of heavy metals takes place from external aquatic to internal body environment through gills (Wepener *et al.,* 2001).

The concentration of heavy metals in fish is highly influenced by spatial and seasonal variations, which are directly related to metal contents in different organs (Farkas *et al.,* 2002). The concentration of heavy metals in different fish species have been studied on spatial and seasonal basis (Nussey *et al.,* 2000; Avenant-Oldewage & Marx, 2000). Besser *et al.* (2007) documented spatial and temporal variations in heavy metal concentration in stream biota from mining areas, Viburnum, United States. Tawari-Fufeyin & Ekaye (2007) highlighted metal pollution in different segments of river Ikpoba River, Nigeria. Kojadinovic *et al.* (2007) studied comparative concentration of heavy metal among different fishes. Bajc *et al.* (2005) investigated distribution of heavy metals with in fish body and differences in heavy metals accumulation among different fish species. Agarwal *et al.* (2007) studied heavy metal accumulation pattern in wild fishes of Gomti River. However, no systematic study has been conducted on spatial and temporal variations of heavy metal contents in different fish species in Nullah Aik and Nullah Palkhu. Due to lack of information about the bioaccumulation pattern of heavy metals in different fish species, there is a dire need to investigate the deleterious effect of heavy metal on local fish fauna. The focus of this chapter is to provide baseline information about heavy metal contents (Fe, Pb, Cd, Cr, Ni, Cu and Zn) in eight species to regulate the aquatic ecosystem for restoration and management.

The main objectives are:

- to highlight spatial, seasonal and specific variations of heavy metals in different organs of selected eight fishes.
- to evaluate trends of heavy metal accumulation in fish species and compare with international guidelines for human consumption.

## Materials and Methods

The details of fish sampling and metal analysis in fish organs are given in Chapter 2. The bioaccumulation of heavy metals was studied in eight fish species collected from upstream and downstream of Nullah Aik and Nullah Palkhu. All samples of fish were collected on seasonal basis (post and pre monsoon) from 18 sites located at Nullah Aik and Nullah Palkhu commencing from September, 2004 and culminate at April, 2006. Out of 18 sampling sites, fish was recorded from only 14 sites and no fish was found from sites (3, 4, 12, and 14). Site 13 was also deleted because only one specimen of *Channa punctata* was captured from this site. Sites with no fish catch were located in close proximities of Sialkot city and were named as mid-stream sites (Fig 4.1). The sites from where fishes were grouped into four zones.

1.      Upstream of Nullah Aik (1and 2 site)
2.      Upstream of Nullah Palkhu (10 and 11site)
3.      Downstream of Nullah Aik (5, 6, 7, 8 and 9 site)
4.      Downstream of Nullah Palkhu (15, 16, 17 and 18 site)

## Statistical analysis

The basic statistics such as mean, standard deviation (SD) and range of heavy metal concentration in fishes of Nullah Aik and Nullah Palkhu was calculated using Microsoft Excel. Two-way analysis of variance ANOVA was used to indicate the statistically significant differences in mean metal concentration between organs, sampling zones, and species (Pourang, 1995; Besser *et al.,* 2007).
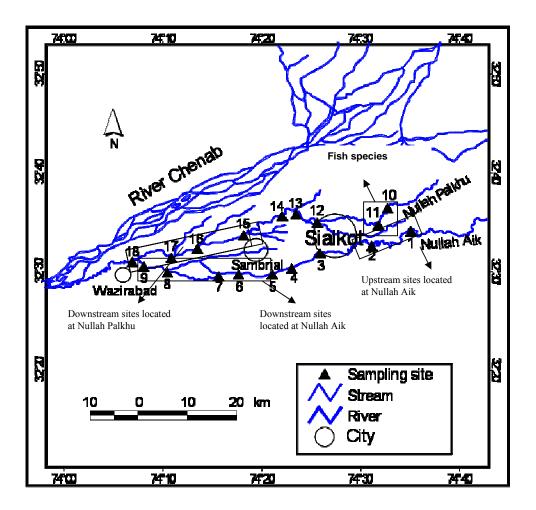
Fig. 4.1     Map of study area showing sampling sites located on Nullah Aik and
             Nullah Palkhu; tributaries of River Chenab, Pakistan.

## Results

A total of eight fish species (*Channa punctata, Cirrhinus reba, Labeo rohita, Heteropneustes fossilis, Mystus cavasius, Oreochromis niloticus, Puntius sophore* and *Wallago attu*) were selected to highlight spatio-temporal variations in heavy metal concentrations among different organs (gills, liver, kidneys and muscles). Mean concentrations and standard deviations of metals (Fe, Pb, Cd, Cr, Ni, Cu and Zn) in gills, kidneys, liver and muscles of fish from four sampling zones during post monsoon and pre monsoon seasons are given in Table 4.1 a & b. Metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn were statistically significant in four organs (gills, liver, kidneys and muscles) between fish species. Similarly, significant spatial differences were found in accumulation of Cr and Pb. Mean concentration of Fe, Cd, Cr and Cu were also significantly different between two seasons (Table. 4.2).

**Bioaccumulation of Heavy Metals**

**Iron**

Significant seasonal and inter-specific variations in bioaccumulation of Fe were found, whereas, non-significant spatial differences were found between upstream and downstream (Table. 4.2). Highest accumulation of Fe was recorded during pre monsoon and minimum in post monsoon. Maximum Fe accumulation was recorded in *Wallago attu,* followed by *Mystus cavasius* and minimum concentration in *Labeo rohita* (Fig. 4.2). Maximum mean concentration of Fe was analyzed in liver and minimum mean concentration in muscles. The general trend of mean Fe concentration in different organs of eight species was: liver > gills > kidneys > muscles (Fig. 4.3a). Maximum mean Fe concentration ($566.720 \pm 30.376\mu g/g$) was recorded in liver of *Wallago attu* captured at downstream of Nullah Aik and minimum ($109.866 \pm 86.149$) in *Oreochromis niloticus* from same stream segment. In gills, maximum mean Fe concentration ($320.456 \pm 57.362\mu g/g$) was recorded of *Puntius sophore* sampled from upstream of Nullah Aik and minimum ($53.065 \pm 46.058\mu g/g$) in *Labeo rohita* from downstream of Nullah Aik. Kidneys showed higher mean concentration ($249.007 \pm 27.494\mu g/g$) in *Heteropneustes fossilis* captured from downstream of Nullah Palkhu, whereas, lowest mean concentration was measured in *Labeo rohita* from downstream of Nullah Aik. Maximum mean Fe concentration

(92.152 ± 22.399µg/g) in muscles was observed in *Channa punctata* from downstream of Nullah Aik and minimum (24.421 ± 14.699µg/g) in *Labeo rohita* sampled from downstream of Nullah Palkhu (Fig 4.2a).

During pre monsoon, maximum mean Fe (372.715 ± 55.331µg/g) was accumulated in liver of *Wallago attu* captured from upstream of Nullah Aik followed by *Channa punctata* (509.206 ± 41.827µg/g) collected from downstream of Nullah Aik and minimum was recorded in liver (277.268 ± 36.824µg/g) of *Labeo rohita* collected from upstream of Nullah Aik. Mean concentration of Fe in gills (370.215 ± 21.361µg/g) and kidneys (372.715 ± 55.331µg/g) maximum in *Wallago attu* was observed in case of liver but minimum mean Fe contents were observed in gills (130.808 ± 28.888µg/g) and kidneys (88.999 ± 68.293µg/g) of *Cirrhinus reba* collected from upstream of Nullah Aik. Maximum mean Fe concentration (118.947 ± 45.122µg/g) in muscles were observed in *Mystus cavasius* while minimum concentration (49.464 ± 52.862µg/g) was observed in *Labeo rohita* sampled from upstream of Nullah Aik.

Table 4.1a     Means ± standard deviation of heavy metals (µg/g) in different organs of eight fish species sampled from four sampling zones located at Nullah Aik and Nullah Palkhu during post monsoon seasons (September, 2004 – April, 2006)

| Species | Zone | Organ | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| *Channa* | 1 | gills | 206.050 ± 33.703 | 12.356 ± 3.148 | 4.665 ± 0.588 | 6.328 ± 5.221 | 4.678 ± 0.933 | 2.149 ± 1.256 | 38.287 ± 16.926 |
| *punctata* | | Kidneys | 186.280 ± 18.322 | 8.715 ± 2.114 | 4.085 ± 0.759 | 3.464 ± 2.144 | 5.027 ± 1.941 | 1.703 ± 0.776 | 33.358 ± 16.143 |
| | | Liver | 338.196 ± 46.844 | 11.575 ± 2.170 | 3.509 ± 0.898 | 6.649 ± 4.765 | 4.874 ± 1.950 | 5.955 ± 2.323 | 41.484 ± 17.411 |
| | | Muscles | 66.875 ± 35.733 | 1.719 ± 2.402 | 1.612 ± 1.131 | 2.160 ± 2.171 | 1.489 ± 0.588 | 0.406 ± 0.041 | 11.361 ± 3.322 |
| | 2 | gills | 122.868 ± 48.426 | 11.878 ± 1.322 | 3.504 ± 0.607 | 4.312 ± 1.807 | 5.212 ± 0.358 | 3.162 ± 1.710 | 16.133 ± 10.750 |
| | | kidneys | 122.934 ± 28.201 | 5.323 ± 1.618 | 4.373 ± 0.254 | 3.762 ± 2.337 | 5.075 ± 2.648 | 2.457 ± 1.351 | 29.189 ± 12.389 |
| | | Liver | 214.874 ± 28.495 | 9.314 ± 2.205 | 4.453 ± 1.084 | 5.243 ± 1.218 | 3.937 ± 1.403 | 4.041 ± 1.563 | 43.844 ± 11.305 |
| | | Muscles | 51.367 ± 35.966 | 1.527 ± 2.019 | 1.112 ± .101 | 1.205 ± 0.957 | 1.253 ± 0.140 | 0.715 ± 0.146 | 14.659 ± 5.006 |
| | 3 | gills | 202.740 ± 33.782 | 15.146 ± 2.719 | 4.093 ± 1.381 | 10.840 ± 3.467 | 6.926 ± 1.197 | 2.650 ± 1.122 | 47.633 ± 11.247 |
| | | Kidneys | 161.920 ± 28.674 | 7.153 ± 2.056 | 3.465 ± 0.564 | 5.794 ± 1.732 | 5.566 ± 4.014 | 2.254 ± 0.258 | 24.288 ± 8.864 |
| | | Liver | 377.373 ± 22.485 | 16.927 ± 2.162 | 4.366 ± 0.857 | 9.079 ± 1.295 | 5.365 ± 2.184 | 5.901 ± 1.382 | 39.541 ± 11.870 |
| | | Muscles | 92.152 ± 22.400 | 2.299 ± 2.285 | 1.509 ± 0.187 | 2.015 ± 0.559 | 1.596 ± 0.811 | 1.382 ± 0.149 | 19.554 ± 4.456 |
| | 4 | gills | 189.785 ± 23.786 | 17.224 ± 3.061 | 3.833 ± 0.797 | 9.737 ± 4.006 | 6.852 ± 3.225 | 3.283 ± 1.991 | 36.414 ± 15.003 |
| | | Kidneys | 133.590 ± 16.021 | 9.273 ± 2.106 | 3.699 ± 1.620 | 8.979 ± 5.194 | 6.411 ± 2.101 | 2.828 ± 1.882 | 27.851 ± 13.924 |
| | | Liver | 227.225 ± 27.113 | 13.909 ± 2.233 | 4.697 ± 1.235 | 9.695 ± 2.896 | 6.539 ± 2.180 | 5.762 ± 2.014 | 31.883 ± 17.046 |
| | | Muscles | 64.062 ± 29.217 | 1.855 ± 2.387 | 1.480 ± 0.727 | 2.054 ± 1.200 | 1.473 ± 1.156 | 1.531 ± 0.389 | 15.796 ± 4.295 |
| | 1 | gills | 108.128 ± 27.430 | 8.027 ± 4.478 | 5.101 ± 1.732 | 4.574 ± 3.296 | 4.452 ± 2.286 | 3.976 ± 1.910 | 22.331 ± 15.354 |
| *Cirrhinus reba* | | Kidneys | 122.044 ± 22.642 | 6.181 ± 1.887 | 5.400 ± 1.558 | 4.310 ± 2.499 | 4.492 ± 2.455 | 6.080 ± 1.705 | 19.393 ± 13.738 |
| | | Liver | 171.076 ± 25.331 | 7.944 ± 2.491 | 5.650 ± 1.102 | 5.685 ± 2.262 | 3.507 ± 1.857 | 6.103 ± 2.225 | 34.080 ± 21.298 |
| | | Muscles | 68.444 ± 18.001 | 1.043 ± 1.045 | 2.093 ± 1.036 | 1.786 ± 0.869 | 0.909 ± 0.703 | 0.520 ± 0.496 | 13.691 ± 4.545 |
| | 2 | gills | 146.467 ± 33.178 | 10.748 ± 1.632 | 5.286 ± 1.150 | 5.062 ± 2.606 | 9.815 ± 1.135 | 7.292 ± 4.043 | 23.707 ± 13.900 |
| | | kidneys | 136.867 ± 13.685 | 6.404 ± 1.694 | 5.412 ± 0.705 | 7.826 ± 8.146 | 9.323 ± 7.819 | 6.608 ± 1.301 | 16.166 ± 12.734 |
| | | Liver | 146.818 ± 29.816 | 9.350 ± 2.140 | 5.548 ± 0.742 | 7.268 ± 1.486 | 8.651 ± 2.169 | 8.344 ± 1.940 | 35.080 ± 18.442 |
| | | Muscles | 81.570 ± 17.452 | 1.633 ± 1.034 | 1.807 ± 0.180 | 3.187 ± 2.465 | 2.090 ± 1.213 | 1.018 ± 0.746 | 14.780 ± 2.565 |
| | 4 | gills | 128.859 ± 39.284 | 11.687 ± 2.127 | 3.751 ± 0.286 | 5.427 ± 1.201 | 9.458 ± 0.329 | 7.154 ± 2.025 | 27.718 ± 10.597 |
| | | Kidneys | 169.117 ± 36.438 | 7.871 ± 1.371 | 5.439 ± 0.207 | 9.135 ± 1.012 | 8.819 ± 1.292 | 8.813 ± 2.037 | 18.600 ± 10.537 |
| | | Liver | 185.918 ± 46.714 | 10.960 ± 2.116 | 5.549 ± 0.615 | 2.918 ± 0.636 | 6.226 ± 2.233 | 9.197 ± 1.015 | 39.489 ± 10.278 |
| | | Muscles | 53.809 ± 33.046 | 3.105 ± 2.024 | 1.683 ± 0.127 | 1.830 ± 0.970 | 2.033 ± 0.186 | 1.527 ± 0.023 | 5.994 ± 3.173 |
| *Labeo rohita* | 1 | gills | 119.126 ± 50.819 | 14.602 ± 3.124 | 4.395 ± 1.741 | 6.234 ± 3.973 | 7.269 ± 1.436 | 5.199 ± 2.064 | 36.181 ± 17.391 |
| | | Kidneys | 83.924 ± 29.564 | 10.273 ± 4.386 | 3.223 ± 4.139 | 4.601 ± 6.967 | 6.946 ± 1.621 | 4.725 ± 1.951 | 32.352 ± 18.527 |
| | | Liver | 197.203 ± 36.117 | 7.473 ± 2.283 | 4.151 ± 1.500 | 6.215 ± 3.247 | 6.283 ± 3.938 | 9.874 ± 3.166 | 44.558 ± 17.974 |
| | | Muscles | 27.354 ± 16.327 | 3.738 ± 2.844 | 1.535 ± 1.821 | 3.659 ± 1.174 | 1.803 ± 1.594 | 1.306 ± 0.665 | 15.493 ± 6.196 |
| | 3 | gills | 53.065 ± 46.058 | 15.062 ± 3.742 | 4.925 ± 0.186 | 22.036 ± 10.83 | 10.957 ± 1.376 | 8.169 ± 1.171 | 34.684 ± 11.001 |
| | | Kidneys | 75.845 ± 48.655 | 6.042 ± 1.228 | 2.199 ± 0.249 | 11.311 ± 1.728 | 9.994 ± 3.456 | 5.763 ± 1.276 | 41.455 ± 11.674 |
| | | Liver | 274.083 ± 8.454 | 8.611 ± 2.115 | 3.998 ± 0.610 | 16.757 ± 1.267 | 8.040 ± 0.376 | 12.183 ± 1.171 | 47.986 ± 10.893 |
| | | Muscles | 44.649 ± 23.768 | 4.353 ± 2.013 | 0.890 ± 0.069 | 3.639 ± 0.413 | 2.005 ± 0.117 | 1.854 ± 0.076 | 18.346 ± 7.395 |
| | 4 | gills | 59.809 ± 41.547 | 11.615 ± 2.864 | 4.180 ± 1.648 | 15.517 ± 6.122 | 9.713 ± 1.249 | 7.746 ± 2.011 | 22.947 ± 10.321 |
| | | Kidneys | 93.523 ± 48.389 | 9.328 ± 1.034 | 4.896 ± 0.721 | 17.458 ± 1.935 | 8.622 ± 1.065 | 8.052 ± 3.030 | 32.903 ± 11.742 |
| | | Liver | 286.305 ± 13.202 | 9.117 ± 2.122 | 4.214 ± 0.647 | 17.822 ± 3.663 | 7.828 ± 3.090 | 12.081 ± 0.008 | 42.302 ± 10.881 |
| | | Muscles | 24.421 ± 14.699 | 3.338 ± 2.005 | 0.771 ± 0.027 | 3.604 ± 0.530 | 2.242 ± 1.214 | 1.479 ± 0.010 | 15.709 ± 4.328 |
| *Heteropneustes* | 3 | gills | 124.842 ± 19.591 | 12.877 ± 2.293 | 2.837 ± 0.281 | 22.073 ± 6.368 | 7.934 ± 0.781 | 3.074 ± 1.351 | 29.089 ± 10.298 |
| *fossilis* | | Kidneys | 198.622 ± 21.328 | 7.647 ± 1.248 | 2.059 ± 0.261 | 8.619 ± 1.276 | 6.809 ± 2.516 | 3.696 ± 1.020 | 14.559 ± 10.419 |
| | | Liver | 223.295 ± 34.770 | 12.389 ± 2.077 | 3.146 ± 0.408 | 21.112 ± 1.747 | 5.577 ± 1.557 | 4.490 ± 1.006 | 35.085 ± 10.312 |
| | | Muscles | 38.492 ± 19.965 | 2.026 ± 2.343 | 0.308 ± 0.051 | 2.798 ± 0.214 | 1.573 ± 0.244 | 0.593 ± 0.016 | 6.343 ± 3.509 |
| | 4 | gills | 198.690 ± 29.492 | 12.552 ± 1.884 | 2.960 ± 0.286 | 24.511 ± 7.386 | 6.687 ± 2.172 | 5.061 ± 5.082 | 27.785 ± 13.768 |
| | | Kidneys | 249.007 ± 27.494 | 10.038 ± 2.754 | 2.125 ± 0.331 | 7.013 ± 2.297 | 5.365 ± 3.032 | 3.114 ± 1.481 | 25.161 ± 14.917 |
| | | Liver | 358.440 ± 29.707 | 11.911 ± 2.268 | 1.968 ± 1.422 | 19.824 ± 3.971 | 5.699 ± 4.381 | 5.077 ± 1.471 | 36.916 ± 14.786 |
| | | Muscles | 75.534 ± 23.856 | 1.523 ± 2.063 | 0.640 ± 0.585 | 2.363 ± 3.646 | 1.515 ± 0.411 | 0.998 ± 0.206 | 11.218 ± 5.136 |
| *Mystus* | 1 | gills | 236.519 ± 88.126 | 7.350 ± 2.705 | 5.303 ± 1.571 | 6.610 ± 1.901 | 4.658 ± 2.212 | 3.119 ± 2.005 | 28.744 ± 19.832 |
| *cavasius* | | Kidneys | 225.973 ± 36.501 | 10.368 ± 2.631 | 5.540 ± 1.157 | 5.740 ± 4.356 | 4.494 ± 2.163 | 4.009 ± 2.887 | 32.194 ± 17.738 |

| | | | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| | | Liver | 382.010 ± 27.173 | 8.918 ± 2.699 | 5.914 ± 1.702 | 4.698 ± 2.568 | 5.200 ± 1.477 | 3.835 ± 2.196 | 40.988 ± 11.884 |
| | | Muscles | 45.153 ± 67.773 | 1.926 ± 2.635 | 1.515 ± 0.716 | 1.260 ± 1.353 | 1.238 ± 3.760 | 1.130 ± 0.132 | 10.526 ± 3.568 |

Table 4.1a          Continue …

| Species | Zone | Organ | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| *Mystus* | 2 | gills | 263.521 ± 49.888 | 6.561 ± 2.304 | 6.017 ± 0.538 | 7.618 ± 3.853 | 6.063 ± 1.689 | 2.451 ± 1.303 | 27.861 ± 12.609 |
| *cavasius* | | kidneys | 187.851 ± 27.430 | 6.024 ± 2.058 | 6.783 ± 1.124 | 5.703 ± 3.053 | 5.779 ± 1.412 | 3.381 ± 2.383 | 35.574 ± 13.648 |
| | | Liver | 362.318 ± 33.212 | 6.104 ± 2.079 | 5.434 ± 0.419 | 7.076 ± 2.382 | 4.818 ± 1.376 | 5.629 ± 2.126 | 43.159 ± 16.276 |
| | | Muscles | 27.353 ± 22.548 | 1.593 ± 2.024 | 1.396 ± 0.127 | 1.093 ± 0.390 | 1.231 ± 0.403 | 1.064 ± 0.162 | 8.096 ± 3.560 |
| *Mystus* | 4 | gills | 218.660 ± 37.683 | 7.095 ± 1.749 | 3.983 ± 0.403 | 17.650 ± 4.005 | 6.633 ± 1.886 | 4.050 ± 1.388 | 45.851 ± 23.787 |
| *cavasius* | | Kidneys | 208.084 ± 38.045 | 7.165 ± 2.197 | 3.382 ± 0.491 | 16.000 ± 4.017 | 6.438 ± 2.594 | 3.274 ± 1.026 | 48.793 ± 16.550 |
| | | Liver | 459.230 ± 42.528 | 10.874 ± 2.085 | 13.137 ± 0.449 | 13.590 ± 6.099 | 5.179 ± 2.397 | 6.255 ± 0.241 | 60.389 ± 14.743 |
| | | Muscles | 42.094 ± 39.607 | 2.223 ± 2.406 | 2.799 ± 0.383 | 5.553 ± 1.669 | 1.532 ± 1.074 | 1.436 ± .177 | 9.397 ± 3.735 |
| *Oreochromis* | 1 | gills | 184.787 ± 16.139 | 9.558 ± 1.847 | 4.651 ± 1.556 | 3.449 ± 2.934 | 5.281 ± 1.299 | 3.299 ± 3.423 | 23.310 ± 13.227 |
| *niloticus* | | Kidneys | 126.989 ± 33.997 | 6.860 ± 2.675 | 5.019 ± 0.928 | 2.813 ± 2.046 | 4.407 ± 1.954 | 3.294 ± 0.946 | 20.302 ± 13.046 |
| | | Liver | 449.212 ± 65.412 | 10.594 ± 2.108 | 4.520 ± 0.572 | 2.681 ± 2.640 | 3.715 ± 1.426 | 5.104 ± 5.523 | 36.876 ± 15.053 |
| | | Muscles | 55.559 ± 10.338 | 3.122 ± 2.640 | 1.567 ± 0.739 | 1.415 ± 1.344 | 1.556 ± 1.527 | 0.556 ± 0.103 | 8.579 ± 1.683 |
| | 2 | gills | 180.774 ± 40.651 | 8.872 ± 3.164 | 4.703 ± 0.371 | 14.257 ± 4.324 | 7.226 ± 1.448 | 3.783 ± 4.360 | 16.819 ± 23.734 |
| | | Kidneys | 138.617 ± 46.894 | 6.794 ± 2.338 | 5.162 ± 1.791 | 2.531 ± 1.749 | 6.219 ± 2.959 | 3.940 ± 1.579 | 16.582 ± 18.491 |
| | | Liver | 482.107 ± 65.588 | 9.849 ± 2.306 | 4.819 ± 1.622 | 3.551 ± 2.306 | 4.056 ± 1.563 | 6.232 ± 4.758 | 61.206 ± 13.630 |
| | | Muscles | 59.413 ± 20.617 | 3.480 ± 3.072 | 1.100 ± 0.382 | 1.770 ± 0.986 | 1.453 ± 2.009 | 1.436 ± 3.031 | 11.414 ± 15.287 |
| | 3 | gills | 179.856 ± 3.010 | 13.911 ± 1.148 | 6.851 ± 0.509 | 5.038 ± 3.769 | 7.842 ± 1.567 | 3.242 ± 1.005 | 15.658 ± 10.327 |
| | | Kidneys | 177.621 ± 50.812 | 7.431 ± 2.076 | 5.333 ± 0.403 | 3.664 ± 1.419 | 6.664 ± 3.101 | 4.391 ± 1.006 | 10.116 ± 10.142 |
| | | Liver | 109.866 ± 86.149 | 14.905 ± 2.039 | 5.460 ± 0.207 | 5.048 ± 1.774 | 5.201 ± 1.659 | 4.349 ± 0.002 | 53.060 ± 10.371 |
| | | Muscles | 56.631 ± 81.261 | 2.384 ± 2.018 | 2.569 ± 0.095 | 1.619 ± 0.122 | 2.194 ± 1.033 | 1.256 ± 0.007 | 9.461 ± 1.390 |
| | 4 | gills | 140.852 ± 65.907 | 13.220 ± 1.747 | 6.173 ± 1.039 | 8.931 ± 2.419 | 7.942 ± 2.302 | 4.591 ± 1.429 | 22.272 ± 9.464 |
| | | Kidneys | 195.709 ± 20.090 | 8.497 ± 2.655 | 5.273 ± 0.819 | 4.170 ± 1.776 | 7.476 ± 2.455 | 5.019 ± 2.223 | 17.369 ± 11.505 |
| | | Liver | 206.221 ± 33.932 | 14.260 ± 2.192 | 5.774 ± 1.015 | 3.965 ± 0.946 | 6.267 ± 2.123 | 5.789 ± 1.511 | 33.361 ± 11.621 |
| | | Muscles | 52.528 ± 135.142 | 3.664 ± 2.028 | 1.506 ± 0.146 | 1.843 ± 0.714 | 1.977 ± 1.128 | 1.425 ± 0.138 | 10.177 ± 6.733 |
| *Puntius* | 1 | gills | 320.457 ± 57.362 | 18.997 ± 1.858 | 3.021 ± 0.347 | 6.093 ± 5.197 | 6.863 ± 1.785 | 7.456 ± 7.718 | 20.366 ± 16.796 |
| *sophore* | | Kidneys | 180.438 ± 22.085 | 12.342 ± 2.273 | 2.735 ± 1.444 | 8.508 ± 3.170 | 5.445 ± 1.929 | 5.669 ± 1.372 | 23.175 ± 14.895 |
| | | Liver | 249.323 ± 26.569 | 14.843 ± 2.282 | 3.168 ± 1.495 | 5.293 ± 1.459 | 5.320 ± 1.712 | 10.262 ± 1.613 | 59.953 ± 15.263 |
| | | Muscles | 41.077 ± 21.086 | 2.743 ± 4.441 | 3.605 ± 6.435 | 2.985 ± 1.628 | 1.401 ± 0.490 | 1.048 ± 0.300 | 9.450 ± 4.640 |
| | 2 | gills | 108.67 ± 104.900 | 8.577 ± 2.089 | 2.501 ± 1.580 | 5.490 ± 3.729 | 7.790 ± 3.854 | 8.586 ± 6.591 | 13.251 ± 3.427 |
| | | kidneys | 154.728 ± 20.628 | 12.766 ± 2.057 | 2.868 ± 0.299 | 5.548 ± 1.510 | 7.292 ± 1.371 | 6.679 ± 3.845 | 24.294 ± 16.480 |
| | | Liver | 248.742 ± 24.955 | 15.096 ± 3.120 | 4.586 ± 0.933 | 6.897 ± 6.622 | 6.452 ± 2.984 | 11.860 ± 3.704 | 57.986 ± 15.745 |
| | | Muscles | 36.462 ± 31.202 | 4.560 ± 2.526 | 1.473 ± 0.135 | 1.709 ± 1.697 | 1.398 ± 0.529 | 1.319 ± 0.081 | 15.627 ± 7.297 |
| | 3 | gills | 256.409 ± 62.014 | 15.255 ± 1.574 | 2.600 ± 0.338 | 11.329 ± 2.406 | 9.261 ± 1.551 | 5.987 ± 1.290 | 12.690 ± 4.664 |
| | | Kidneys | 153.853 ± 23.185 | 8.386 ± 2.086 | 5.066 ± 0.456 | 14.203 ± 3.034 | 7.536 ± 1.993 | 6.755 ± 2.034 | 23.320 ± 10.679 |
| | | Liver | 346.898 ± 40.023 | 14.540 ± 2.057 | 2.053 ± 0.302 | 11.100 ± 1.809 | 7.679 ± 0.993 | 14.828 ± 2.162 | 67.123 ± 11.036 |
| | | Muscles | 53.939 ± 27.209 | 4.044 ± 2.030 | 1.828 ± 0.160 | 2.646 ± 0.098 | 1.581 ± 0.237 | 1.419 ± 0.012 | 15.534 ± 5.925 |
| | 4 | gills | 162.934 ± 63.975 | 13.960 ± 1.619 | 4.410 ± 0.415 | 4.025 ± 1.569 | 10.185 ± 3.059 | 6.529 ± 1.875 | 18.780 ± 6.129 |
| | | Kidneys | 187.763 ± 25.530 | 6.577 ± 2.117 | 5.365 ± 0.622 | 3.281 ± 0.832 | 9.187 ± 2.079 | 6.450 ± 2.148 | 17.280 ± 11.368 |
| | | Liver | 373.609 ± 21.251 | 12.872 ± 2.063 | 4.053 ± 0.336 | 4.361 ± 1.844 | 7.707 ± 3.929 | 14.150 ± 2.012 | 46.559 ± 11.371 |
| | | Muscles | 42.254 ± 25.323 | 3.808 ± 2.360 | 1.206 ± 0.143 | 1.855 ± 0.525 | 1.773 ± 0.452 | 1.087 ± 0.111 | 11.642 ± 7.738 |
| *Wallago attu* | 1 | gills | 124.360 ± 50.393 | 15.655 ± 2.410 | 4.395 ± 1.818 | 12.700 ± 3.706 | 4.719 ± 1.265 | 0.824 ± 0.123 | 28.143 ± 10.201 |
| | | Kidneys | 130.604 ± 41.751 | 11.050 ± 3.130 | 4.492 ± 5.989 | 7.596 ± 3.204 | 4.551 ± 2.459 | 0.813 ± 0.105 | 29.596 ± 15.513 |
| | | Liver | 336.637 ± 32.230 | 16.003 ± 3.479 | 3.635 ± 2.339 | 13.689 ± 6.568 | 4.446 ± 1.411 | 0.944 ± 0.073 | 65.427 ± 29.669 |
| | | Muscles | 43.297 ± 36.069 | 2.470 ± 3.032 | 2.221 ± 1.970 | 1.977 ± 1.321 | 1.689 ± 0.645 | 0.471 ± 0.071 | 16.969 ± 4.494 |
| | 3 | gills | 168.200 ± 42.077 | 20.596 ± 1.503 | 4.932 ± 0.551 | 31.274 ± 6.289 | 7.746 ± 3.278 | 2.284 ± 0.363 | 30.537 ± 15.960 |
| | | Kidneys | 159.214 ± 28.242 | 15.789 ± 2.079 | 4.020 ± 0.416 | 23.180 ± 8.740 | 5.008 ± 1.996 | 1.582 ± 0.335 | 27.141 ± 13.736 |
| | | Liver | 566.721 ± 30.377 | 18.734 ± 2.096 | 4.561 ± 0.506 | 25.716 ± 8.942 | 5.944 ± 1.164 | 2.675 ± 0.185 | 52.847 ± 12.447 |
| | | Muscles | 56.090 ± 14.050 | 4.053 ± 2.064 | 1.841 ± 0.339 | 5.173 ± 1.589 | 1.179 ± 0.305 | 0.959 ± 0.149 | 11.934 ± 4.180 |
| | 4 | gills | 133.598 ± 42.628 | 12.411 ± 1.490 | 2.622 ± 1.794 | 14.022 ± 3.074 | 6.715 ± 2.270 | 1.234 ± 0.718 | 30.638 ± 17.848 |
| | | Kidneys | 136.948 ± 29.224 | 10.386 ± 2.158 | 5.409 ± 0.837 | 5.389 ± 3.360 | 6.679 ± 6.715 | 1.531 ± 0.115 | 42.512 ± 16.694 |

| | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|
| Liver | 335.899 ± 26.193 | 16.917 ± 2.149 | 5.589 ± 0.790 | 20.988 ± 4.065 | 6.210 ± 1.467 | 1.147 ± 0.265 | 51.774 ± 28.766 |
| Muscles | 59.494 ± 24.968 | 3.784 ± 2.708 | 1.377 ± 0.975 | 2.850 ± 1.611 | 1.783 ± 0.986 | 0.405 ± 0.084 | 8.364 ± 4.711 |

Table 4.1b      Means ± standard and deviation of heavy metals (µg/g) in different organs of eight fish species sampled from four sampling zones located at Nullah Aik and Nullah Palkhu during pre monsoon seasons from (September, 2004 – April, 2006)
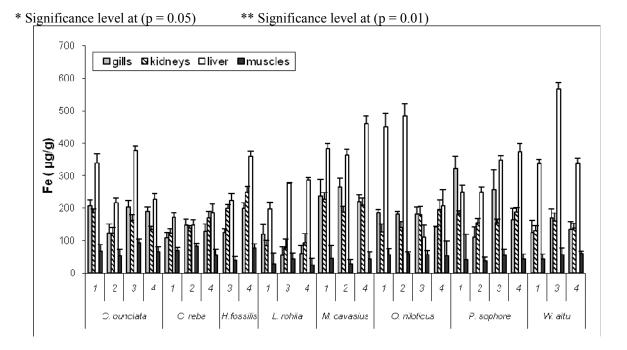
| Species | Zone | Organ | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| *Channa* | 1 | gills | 281.790 ± 40.691 | 16.497 ± 3.003 | 4.947 ± 3.270 | 7.745 ± 3.182 | 9.701 ± 1.385 | 3.281 ± 1.215 | 44.985 ± 10.71 |
| *punctata* | | Kidneys | 245.793 ± 46.883 | 12.215 ± 4.627 | 4.831 ± 0.930 | 5.825 ± 1.863 | 10.538 ± 2.200 | 6.139 ± 1.855 | 52.010 ± 20.445 |
| | | Liver | 475.106 ± 23.631 | 13.294 ± 6.013 | 5.833 ± 0.600 | 5.720 ± 2.097 | 15.826 ± 2.270 | 12.32 ± 1.153 | 69.264 ± 8.59 |
| | | Muscles | 60.282 ± 64.962 | 3.987 ± 2.044 | 1.857 ± 0.865 | 2.735 ± 3.497 | 9.643 ± 1.350 | 1.772 ± 0.527 | 49.905 ± 4.865 |
| | 2 | gills | 314.140 ± 71.233 | 22.142 ± 9.120 | 6.161 ± 3.463 | 11.380 ± 5.841 | 12.050 ± 3.445 | 4.923 ± 1.990 | 65.475 ± 12.375 |
| | | Kidneys | 224.066 ± 46.213 | 18.521 ± 4.871 | 4.881 ± 2.375 | 15.660 ± 5.474 | 11.001 ± 4.739 | 3.936 ± 2.138 | 51.944 ± 7.608 |
| | | Liver | 418.548 ± 225.20 | 15.640 ± 8.178 | 6.284 ± 0.628 | 9.620 ± 3.368 | 12.814 ± 1.250 | 6.688 ± 1.649 | 76.312 ± 11.96 |
| | | Muscles | 87.867 ± 35.289 | 4.236 ± 3.141 | 1.778 ± 0.653 | 3.881 ± 3.996 | 8.376 ± 3.163 | 1.566 ± 0.410 | 34.469 ± 8.35 |
| | 3 | gills | 303.290 ± 45.448 | 29.912 ± 6.192 | 5.808 ± 1.990 | 19.730 ± 2.014 | 13.056 ± 2.810 | 5.119 ± 1.634 | 54.378 ± 14.93 |
| | | Kidneys | 272.311 ± 46.212 | 14.400 ± 3.064 | 5.364 ± 0.308 | 16.584 ± 5.583 | 4.726 ± 1.960 | 4.418 ± 1.176 | 60.425 ± 10.25 |
| | | Liver | 509.207 ± 41.828 | 15.773 ± 8.029 | 8.180 ± 0.580 | 18.868 ± 5.355 | 12.897 ± 4.013 | 5.470 ± 1.555 | 88.107 ± 9.405 |
| | | Muscles | 68.056 ± 33.298 | 6.961 ± 7.770 | 2.581 ± 2.263 | 13.693 ± 4.156 | 11.696 ± 2.703 | 1.646 ± 1.095 | 36.670 ± 11.79 |
| | 4 | gills | 294.668 ± 26.103 | 18.713 ± 7.784 | 6.456 ± 1.630 | 16.862 ± 3.468 | 14.379 ± 2.426 | 4.466 ± 1.665 | 73.905 ± 12.433 |
| | | Kidneys | 218.348 ± 36.450 | 16.958 ± 1.564 | 6.060 ± 1.013 | 14.372 ± 3.805 | 5.282 ± 1.430 | 5.480 ± 2.238 | 60.378 ± 23.3 |
| | | Liver | 475.426 ± 24.361 | 13.805 ± 5.927 | 7.409 ± 1.395 | 14.537 ± 4.127 | 8.182 ± 2.220 | 5.649 ± 2.743 | 92.258 ± 11.672 |
| | | Muscles | 69.630 ± 44.018 | 5.674 ± 7.681 | 2.151 ± 1.715 | 8.834 ± 1.125 | 14.424 ± 2.338 | 1.603 ± 1.105 | 42.598 ± 10.78 |
| | 1 | gills | 130.808 ± 28.889 | 18.334 ± 7.735 | 6.072 ± 3.183 | 16.791 ± 3.670 | 6.377 ± 2.720 | 4.104 ± 2.077 | 39.176 ± 18.2725 |
| *Cirrhinus reba* | | Kidneys | 89.000 ± 68.293 | 15.001 ± 3.441 | 5.476 ± 0.705 | 8.051 ± 4.084 | 8.854 ± 1.998 | 5.756 ± .905 | 74.621 ± 7.61 |
| | | Liver | 295.784 ± 36.440 | 15.074 ± 5.317 | 6.017 ± 1.815 | 12.690 ± 2.624 | 16.236 ± 5.613 | 6.755 ± 1.296 | 90.237 ± 24.685 |
| | | Muscles | 55.525 ± 66.148 | 3.380 ± 6.136 | 1.627 ± 1.463 | 2.998 ± 5.573 | 14.536 ± 3.088 | 1.700 ± 1.069 | 33.010 ± 8.3975 |
| | 2 | gills | 160.085 ± 28.601 | 17.15 ± 12.901 | 6.019 ± 1.695 | 19.939 ± 2.705 | 12.838 ± 3.705 | 3.248 ± 1.752 | 52.841 ± 20.165 |
| | | Kidneys | 83.431 ± 53.593 | 16.370 ± 7.574 | 5.883 ± 0.470 | 10.099 ± 5.472 | 5.761 ± 1.660 | 5.585 ± 1.239 | 72.970 ± 7.93 |
| | | Liver | 373.078 ± 22.328 | 14.009 ± 2.408 | 6.079 ± 1.485 | 14.987 ± 1.521 | 9.219 ± 1.315 | 4.794 ± 1.040 | 93.421 ± 22.28 |
| | | Muscles | 51.884 ± 57.702 | 4.764 ± 7.266 | 1.386 ± 2.120 | 5.305 ± 2.223 | 10.700 ± 2.305 | 1.360 ± 0.567 | 32.221 ± 9.765 |
| | 4 | gills | 170.229 ± 33.803 | 18.302 ± 7.234 | 6.600 ± 3.400 | 19.331 ± 5.936 | 12.013 ± 3.885 | 4.433 ± 2.862 | 48.407 ± 9.705 |
| | | Kidneys | 130.205 ± 67.513 | 18.620 ± 4.725 | 4.418 ± 1.465 | 14.898 ± 3.749 | 11.292 ± 1.580 | 5.386 ± 2.169 | 75.986 ± 8.755 |
| | | Liver | 327.655 ± 50.854 | 15.030 ± 2.117 | 7.325 ± 1.050 | 16.683 ± 4.068 | 13.854 ± 2.585 | 6.300 ± 2.309 | 95.624 ± 36.375 |
| | | Muscles | 51.470 ± 32.970 | 3.732 ± 4.984 | 1.118 ± 1.385 | 1.647 ± 2.700 | 13.744 ± 2.960 | 1.849 ± 0.923 | 33.383 ± 7.4 |
| *Labeo rohita* | 1 | gills | 160.191 ± 38.913 | 18.688 ± 5.901 | 5.831 ± 1.373 | 10.163 ± 1.402 | 13.987 ± 2.988 | 5.401 ± 1.632 | 42.747 ± 10.7625 |
| | | Kidneys | 109.827 ± 44.778 | 14.099 ± 5.124 | 5.196 ± 1.565 | 6.320 ± 4.651 | 9.516 ± 2.015 | 3.625 ± 1.547 | 37.501 ± 11.925 |
| | | Liver | 277.269 ± 36.824 | 13.003 ± 3.504 | 7.713 ± 0.848 | 8.074 ± 0.650 | 8.790 ± 2.473 | 8.151 ± 0.428 | 40.954 ± 16.03 |
| | | Muscles | 49.464 ± 52.863 | 3.554 ± 8.741 | 1.602 ± 0.868 | 4.570 ± 2.405 | 12.642 ± 4.650 | 1.339 ± 0.526 | 13.250 ± 9.5575 |
| *Heteropneustes* | 3 | gills | 212.803 ± 48.759 | 30.062 ± 10.645 | 2.860 ± 2.475 | 32.770 ± 4.856 | 9.940 ± 6.065 | 2.809 ± 1.742 | 50.536 ± 7.375 |
| *fossilis* | | Kidneys | 145.463 ± 45.274 | 18.824 ± 1.806 | 2.406 ± 0.470 | 12.635 ± 0.212 | 14.389 ± 3.005 | 2.688 ± 1.221 | 59.463 ± 10.93 |
| | | Liver | 323.298 ± 27.109 | 18.221 ± 5.456 | 1.734 ± 2.195 | 30.668 ± 1.881 | 8.780 ± 2.630 | 5.258 ± 1.929 | 68.809 ± 13.545 |
| | | Muscles | 92.911 ± 53.532 | 9.534 ± 5.131 | 0.309 ± 3.355 | 4.247 ± 1.292 | 15.345 ± 3.205 | 1.588 ± 0.572 | 11.130 ± 14.51 |
| | 4 | gills | 211.882 ± 68.012 | 24.449 ± 6.264 | 2.983 ± 3.780 | 28.566 ± 6.854 | 9.563 ± 9.280 | 3.190 ± 2.804 | 49.359 ± 7.66 |
| | | Kidneys | 140.968 ± 31.953 | 21.799 ± 1.442 | 2.299 ± 1.285 | 12.472 ± 0.216 | 10.978 ± 1.575 | 4.390 ± 1.085 | 66.354 ± 6.04 |
| | | Liver | 361.893 ± 27.949 | 19.486 ± 4.784 | 1.712 ± 1.325 | 27.455 ± 6.318 | 7.507 ± 1.905 | 7.085 ± 0.396 | 69.166 ± 18.455 |
| | | Muscles | 85.995 ± 79.732 | 4.227 ± 9.037 | 0.908 ± 1.320 | 5.548 ± 0.185 | 14.609 ± 1.040 | 2.118 ± 0.485 | 47.745 ± 13.995 |

Table 4.1b Continue…

| Species | Zone | Organ | Fe | Pb | Cd | Cr | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|---|---|
| *Heteropneustes* | 3 | gills | 212.803 ± 48.759 | 30.062 ± 10.645 | 2.860 ± 2.475 | 32.770 ± 4.856 | 9.940 ± 6.065 | 2.809 ± 1.742 | 50.536 ± 7.375 |
| *fossilis* | | Kidneys | 145.463 ± 45.274 | 18.824 ± 1.806 | 2.406 ± 0.470 | 12.635 ± 0.212 | 14.389 ± 3.005 | 2.688 ± 1.221 | 59.463 ± 10.93 |
| | | Liver | 323.298 ± 27.109 | 18.221 ± 5.456 | 1.734 ± 2.195 | 30.668 ± 1.881 | 8.780 ± 2.630 | 5.258 ± 1.929 | 68.809 ± 13.545 |
| | | Muscles | 92.911 ± 53.532 | 9.534 ± 5.131 | 0.309 ± 3.355 | 4.247 ± 1.292 | 15.345 ± 3.205 | 1.588 ± 0.572 | 11.130 ± 14.51 |
| | 4 | gills | 211.882 ± 68.012 | 24.449 ± 6.264 | 2.983 ± 3.780 | 28.566 ± 6.854 | 9.563 ± 9.280 | 3.190 ± 2.804 | 49.359 ± 7.66 |
| | | Kidneys | 140.968 ± 31.953 | 21.799 ± 1.442 | 2.299 ± 1.285 | 12.472 ± 0.216 | 10.978 ± 1.575 | 4.390 ± 1.085 | 66.354 ± 6.04 |
| | | Liver | 361.893 ± 27.949 | 19.486 ± 4.784 | 1.712 ± 1.325 | 27.455 ± 6.318 | 7.507 ± 1.905 | 7.085 ± 0.396 | 69.166 ± 18.455 |
| | | Muscles | 85.995 ± 79.732 | 4.227 ± 9.037 | 0.908 ± 1.320 | 5.548 ± 0.185 | 14.609 ± 1.040 | 2.118 ± 0.485 | 47.745 ± 13.995 |
| *Mystus cavasius* | 1 | gills | 267.835 ± 44.795 | 18.607 ± 7.428 | 5.742 ± 1.485 | 6.398 ± 1.175 | 16.143 ± 4.475 | 3.091 ± 1.779 | 44.747 ± 21.79 |
| | | Kidneys | 240.935 ± 19.672 | 16.734 ± 5.446 | 5.512 ± 0.755 | 5.727 ± 0.923 | 14.463 ± 1.030 | 3.898 ± 1.180 | 70.134 ± 5.345 |
| | | Liver | 477.837 ± 24.441 | 15.488 ± 3.087 | 8.970 ± 0.290 | 8.661 ± 0.671 | 5.409 ± 1.500 | 4.814 ± 0.257 | 89.642 ± 9.295 |
| | | Muscles | 118.947 ± 45.122 | 3.863 ± 4.056 | 1.990 ± 0.405 | 4.453 ± 2.129 | 12.154 ± 2.865 | 1.608 ± 0.743 | 45.241 ± 9.89 |
| | 2 | gills | 251.884 ± 37.702 | 15.764 ± 11.242 | 1.386 ± 0.840 | 5.305 ± 6.939 | 8.635 ± 1.310 | 1.360 ± 1.149 | 32.221 ± 11.91 |
| | | Kidneys | 217.033 ± 12.523 | 15.294 ± 6.916 | 6.487 ± 2.525 | 3.904 ± 1.017 | 8.065 ± 3.890 | 2.277 ± 3.281 | 76.613 ± 13.295 |
| | | Liver | 428.359 ± 36.427 | 14.548 ± 3.977 | 12.815 ± 0.785 | 9.445 ± 1.265 | 14.547 ± 2.080 | 5.342 ± 0.500 | 97.594 ± 21.56 |
| | | Muscles | 104.976 ± 32.939 | 3.755 ± 1.260 | 1.800 ± 0.200 | 4.784 ± 0.914 | 3.412 ± 3.720 | 1.933 ± 0.216 | 44.381 ± 7.42 |
| *Oreochromis* | 1 | gills | 226.342 ± 48.928 | 16.077 ± 9.958 | 3.690 ± 1.340 | 14.901 ± 2.207 | 6.306 ± 2.120 | 6.860 ± 2.037 | 27.070 ± 9.262 |
| *niloticus* | | Kidneys | 209.939 ± 38.339 | 15.005 ± 5.646 | 4.093 ± 1.478 | 12.010 ± 3.493 | 9.641 ± 3.450 | 4.328 ± 1.549 | 34.741 ± 15.542 |
| | | Liver | 332.253 ± 64.025 | 15.562 ± 8.682 | 6.384 ± 0.545 | 13.636 ± 2.571 | 5.673 ± 2.730 | 10.35 ± 0.162 | 59.133 ± 18.775 |
| | | Muscles | 72.081 ± 28.223 | 3.365 ± 2.426 | 1.683 ± 0.305 | 4.240 ± 2.113 | 2.966 ± 1.153 | 1.549 ± 0.463 | 17.446 ± 6.972 |
| *Puntius sophore* | 1 | gills | 292.593 ± 31.830 | 20.315 ± 5.491 | 4.949 ± 1.805 | 3.265 ± 2.934 | 13.437 ± 3.145 | 4.912 ± 2.086 | 32.379 ± 13.54 |
| | | Kidneys | 150.867 ± 45.479 | 18.759 ± 5.058 | 5.052 ± 0.585 | 3.469 ± 1.555 | 11.469 ± 1.703 | 3.910 ± 1.315 | 29.876 ± 22.757 |
| | | Liver | 439.446 ± 19.464 | 16.265 ± 2.560 | 6.519 ± 1.743 | 2.758 ± 0.866 | 8.504 ± 1.293 | 4.628 ± 0.689 | 55.501 ± 15.455 |
| | | Muscles | 54.348 ± 40.198 | 3.773 ± 3.559 | 1.305 ± 0.833 | 2.745 ± 1.321 | 2.822 ± 0.933 | 1.769 ± 0.162 | 23.409 ± 8.255 |
| | 2 | gills | 324.055 ± 27.725 | 19.604 ± 9.851 | 4.034 ± 2.723 | 5.444 ± 2.108 | 11.114 ± 2.923 | 5.704 ± 3.427 | 31.396 ± 24.83 |
| | | Kidneys | 148.676 ± 47.499 | 17.033 ± 2.755 | 4.960 ± 0.570 | 4.307 ± 0.383 | 5.055 ± 1.255 | 3.735 ± 1.076 | 29.521 ± 8.982 |
| | | Liver | 489.738 ± 37.769 | 16.377 ± 3.889 | 5.607 ± 1.420 | 6.391 ± 1.517 | 7.452 ± 3.083 | 8.281 ± 0.857 | 68.563 ± 10.912 |
| | | Muscles | 57.500 ± 31.798 | 4.922 ± 6.832 | 1.655 ± 1.138 | 3.657 ± 0.414 | 1.855 ± 0.278 | 1.340 ± 0.368 | 16.660 ± 14.487 |
| *Wallago attu* | 1 | gills | 370.215 ± 21.361 | 15.71 ± 10.080 | 4.788 ± 2.065 | 14.195 ± 4.653 | 13.696 ± 1.610 | 1.725 ± 1.170 | 36.415 ± 6.095 |
| | | Kidneys | 372.715 ± 55.331 | 13.006 ± 4.732 | 5.771 ± 0.080 | 6.107 ± 0.707 | 4.885 ± 1.385 | 4.211 ± 1.243 | 30.334 ± 6.975 |
| | | Liver | 613.383 ± 20.318 | 14.442 ± 5.887 | 6.665 ± 1.255 | 11.866 ± 7.452 | 9.720 ± 15.310 | 6.175 ± 2.401 | 60.675 ± 26.785 |
| | | Muscles | 48.277 ± 20.079 | 2.824 ± 1.072 | 2.057 ± 0.445 | 1.804 ± 0.104 | 1.215 ± 0.175 | 1.494 ± 0.329 | 12.003 ± 12.585 |

Table 4.2      Significance of metal accumulation in organs, sampling zones, season and species in eight fishes captured from Nullah Aik and Nullah Palkhu through ANOVA.
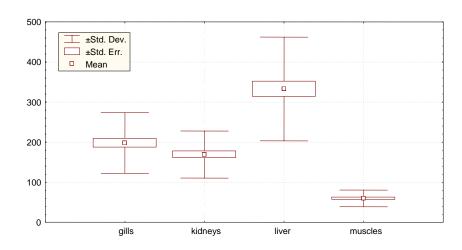
| Metals | Organs | | | Sampling Zone | | | Season | | | Species | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F value* | *Prob.* | *Sign. level* | *F value* | *Prob.* | *Sign. level* | *F value* | *Prob.* | *Sign. level* | *F value* | *Prob.* | *Sign. level* |
| Fe | 100.8 | 0.000 | ** | 0.23 | 0.877 | NS | 9.13 | 0.003 | ** | 2.16 | 0.040 | * |
| Pb | 74.00 | 0.000 | ** | 0.78 | 0.047 | * | 34.06 | 0.560 | NS | 1.39 | 0.091 | * |
| Cd | 51.33 | 0.000 | ** | 1.31 | 0.273 | NS | 5.45 | 0.021 | * | 4.28 | 0.000 | ** |
| Cr | 19.35 | 0.000 | ** | 11.57 | 0.000 | ** | 6.26 | 0.013 | * | 6.50 | 0.000 | ** |
| Ni | 42.51 | 0.000 | ** | 2.75 | 0.210 | NS | 62.60 | 0.931 | NS | 2.08 | 0.059 | * |
| Cu | 49.69 | 0.000 | ** | 0.30 | 0.825 | NS | 0.01 | 0.000 | ** | 5.75 | 0.000 | ** |
| Zn | 39.43 | 0.000 | ** | 0.19 | 0.905 | NS | 68.54 | 0.664 | NS | 2.71 | 0.011 | * |

* Significance level at (p = 0.05)          ** Significance level at (p = 0.01)



(a)



(b)

Fig. 4.2    Spatial variations in bioaccumulation of Fe (µg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during (a) post monsoon and (b) pre monsoon at upstream and downstream of Nullah Aik and Nullah Palkhu.
*Upstream of Nullah Aik (1), Upstream of Nullah Palkhu (2), Downstream of Nullah Aik (3) and Downstream of Nullah Palkhu (4).*

(a)



(b)



(c)

Fig. 4.3    Box and whisker plots of showing comparative accumulation of Fe (μg/g) concentration in (a) organs, (b) seasons, and (c) fish species collected from Nullah Aik and Nullah Palkhu.

**Lead**

Mean Pb concentration was significantly different between up and downstream zones. Maximum concentration of Pb was recorded in downstream of Nullah Aik and minimum at upstream of Nullah Aik (Fig.4.5b). Similarly, significant variations in mean concentration of Pb were found between species. Highest Pb accumulation was recorded in *Heteropneustes fossilis* and *Wallago attu,* and lowest in *Mystus cavasius* (Fig.4.5c).

Like Fe, highly significant variations in mean Pb concentration were observed between various organs from pre and post monsoon seasons (Table 4.1a & b; Fig 4.5a). The results showed that the highest mean concentration of Pb was found in gills followed by liver, kidneys and muscles during post monsoon. Maximum mean concentration of Pb ($20.595 \pm 1.506$μg/g) was measured in the gills and liver ($18.734 \pm 2.095$μg/g) of *Wallago attu* recorded from downstream of the Nullah Aik, whereas, minimum in gills ($6.561 \pm 2.307$μg/g) and liver ($6.104 \pm 2.079$μg/g) was recorded in *Mystus cavasius* captured from upstream of Nullah Palkhu. In kidneys, maximum mean concentration of Pb ($15.788 \pm 2.078$μg/g) was measured in *Wallago attu* captured from downstream of Nullah Aik and minimum ($1.033 \pm 2.034$μg/g) in *Cirrhinus reba* from upstream of Nullah Palkhu. Maximum mean concentration of Pb ($4.56 \pm 2.525$μg/g) was found in muscles of *Puntius sophore* at upstream of Nullah Palkhu, whereas, minimum mean concentration ($1.043 \pm 1.044$μg/g) in *Cirrhinus reba* sampled from upstream of Nullah Aik (Fig 4.4a).

In pre monsoon, highest concentration of Pb was recorded in gills ($30.062 \pm 10.645$μg/g) and muscles ($9.534 \pm 5.131$μg/g) of *Heteropneustes fossilis* sampled from downstream of Nullah Aik and lowest concentration was recorded in gills ($15.717 \pm 10.08$μg/g) of *Wallago attu* and in muscles ($4.227 \pm 9.037$μg/g) of *Channa punctata* captured from upstream of Nullah Aik. In liver ($19.486 \pm 4.784$μg/g) and kidneys ($21.799 \pm 11.442$μg/g) highest Pb concentration was recorded in *Heteropneustes fossilis* and lowest concentration in liver ($12.215 \pm 4.627$μg/g) and kidneys ($13.006 \pm 4.732$μg/g) of *Wallago attu* from upstream of Nullah Aik (Fig. 4.4b).

(a)



(b)
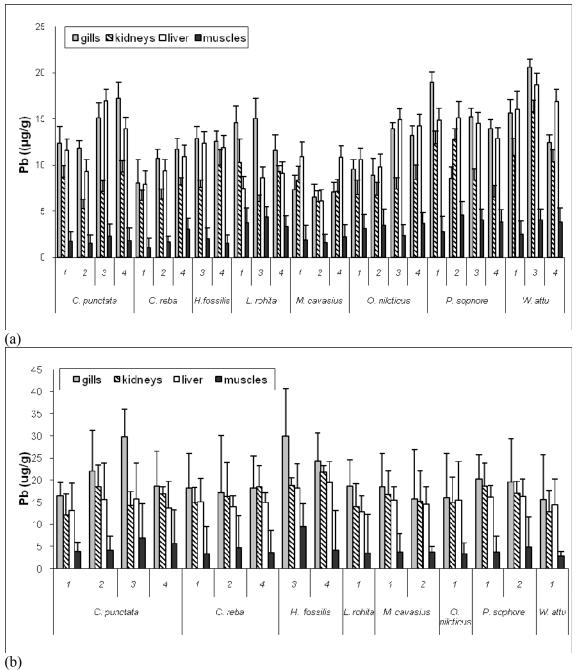
Fig. 4.4    Spatial  variations  in  bioaccumulation  of  Pb  (µg/g)   in  four  organs
(gills, kidneys, liver and muscles) of eight fish species during (a) post
monsoon and (b) pre monsoon at upstream and downstream of Nullah
Aik and Nullah Palkhu.

(a)



(b)
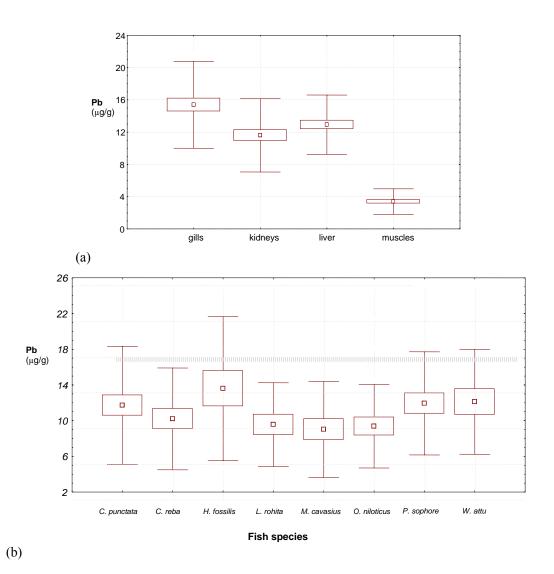
Fig.4.5    Box and whisker plots of showing comparative accumulation of Pb (µg/g) concentration in (a) organs (b) fish species collected from Nullah Aik and Nullah Palkhu.

**Cadmium**

Upstream and downstream sampling zones were non-significantly different in Cd concentration; however, significant differences were recorded between two seasons. Similarly, inter-specific variations in mean concentration of Cd were found (Table 4.2). Minimum accumulation of Cd was recorded during post monsoon and maximum in pre monsoon (Fig. 4.7b). *Mystus cavasius* showed highest level of Cd accumulation followed by *Cirrhinus reba* and minimum concentration in *Heteropneustes fossilis* (Fig. 4.7c).

Mean concentration of Cd in various organs was diminutive. Pattern of Cd accumulation was in this order: liver> gills > kidneys > muscles (Fig. 4.7a). Mean concentration of Cd ($13.136 \pm 0.448\mu g/g$) was highest in the liver of *Mystus cavasius* at downstream of Palkhu and lowest ($1.968 \pm 1.421\mu g/g$) in liver of *Heteropneustes fossilis* from downstream of Nullah Aik (Table 4.1a & b). Maximum mean concentration of Cd ($6.851 \pm 0.509\mu g/g$) in gills was recorded in *Oreochromis niloticus* at downstream of Nullah Palkhu and minimum ($2.837 \pm 0.281\mu g/g$) was exhibited by *Heteropneustes fossilis* captured from downstream of Nullah Aik. Maximum mean Cd concentration ($6.782 \pm 1.123\mu g/g$) in kidneys of *Mystus cavasius* at upstream of Nullah Palkhu and minimum ($2.058 \pm 0.261\mu g/g$) was recorded in *Heteropneustes fossilis* at downstream of Nullah Aik (Fig 4.6a). Maximum mean Cd concentration ($2.798 \pm 0.383\mu g/g$) was recorded in muscles of *Mystus cavasius* from downstream of Nullah Palkhu and minimum ($0.64 \pm 0.285\mu g/g$) in *Heteropneustes fossilis* from downstream Nullah Aik.

*Channa punctata* accumulated highest mean concentration of Cd during pre monsoon in gills ($6.456 \pm 1.63\mu g/g$), kidneys ($6.060 \pm 1.0125\mu g/g$) and muscles ($2.505 \pm 1.715\mu g/g$) captured from downstream of Nullah Palkhu. *Mystus cavasius* also showed high mean concentration of Cd in liver ($12.815 \pm 0.785\mu g/g$) from upstream of Nullah Palkhu, whereas, minimum in liver ($1.734 \pm 2.195\mu g/g$), gills ($2.86 \pm 2.475\mu g/g$), kidneys ($2.406 \pm 0.47\mu g/g$) and muscles ($1.309 \pm 0.835\mu g/g$) of *Heteropneustes fossilis* captured from downstream of streams (Fig. 4.6b).

(a)



(b)
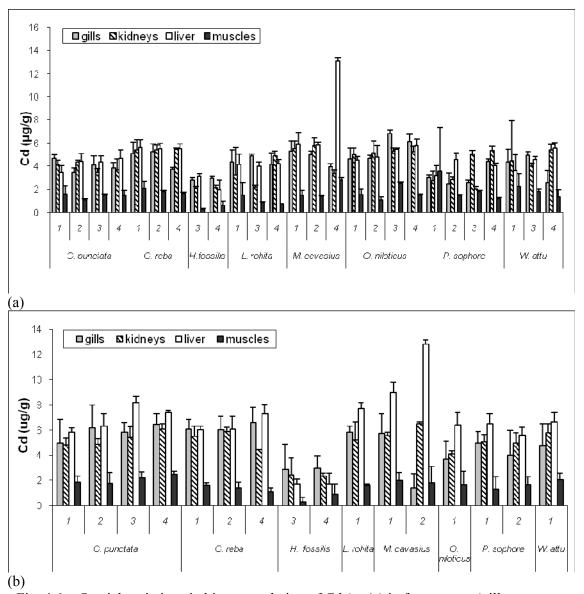
Fig. 4.6    Spatial variations in bioaccumulation of Cd (µg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during (a) post monsoon and (b) pre monsoon at upstream and downstream of Nullah Aik and Nullah Palkhu.

(a)



(b)



**Fish species**

(c)

Fig. 4.7    Box and whisker plots of showing comparative accumulation of Cd (µg/g) concentration in (a) organs, (b) seasons, and (c) fish species collected from Nullah Aik and Nullah Palkhu
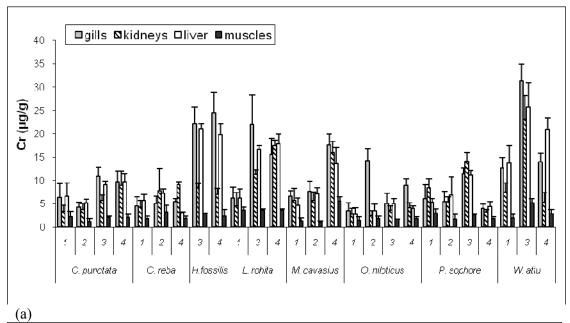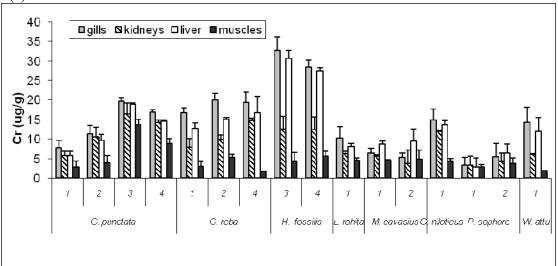
**Chromium**

Mean Cr concentration varied significantly between seasons and species (Table 4.2). Highest accumulation of Cr was observed in downstream of Nullah Aik followed by Nullah Palkhu and lowest at upstream of Nullah Aik (Fig. 4.9b). Highest mean concentration of Cr was recorded in *Heteropneustes fossilis* followed by *Wallago attu* and minimum in *Puntius sophore* (Fig. 4.9d).

Gills, liver, kidneys and muscles showed marked variations of Cr accumulation of Cr during post monsoon (Table 4.2). Overall Cr accumulation was in order: gills ≥ liver> kidneys > muscles (Fig. 4.9a). In post monsoon, maximum mean Cr concentration was recorded in gills (31.274 ± 6.289μg/g), followed by liver (25.7155 ± 8.942μg/g) and kidneys (23.18 ± 8.739μg/g) in *Wallago attu* sampled from downstream of Nullah Aik. Minimum mean Cr concentration was recorded in gills (3.448 ± 2.933), liver (4.52 ± 0.572μg/g) and kidneys (1.415 ± 1.3435μg/g) of *Oreochromis niloticus* inhabiting at upstream of Nullah Aik (Table 4.2a & b). Maximum mean Cr concentration (5.173 ± 1.589μg/g) was measured in muscles of *Wallago attu* from downstream of Nullah Aik and minimum (1.415 ± 1.343μg/g) in *Mystus cavasius* captured from upstream of Nullah Aik (Fig. 4.8b).
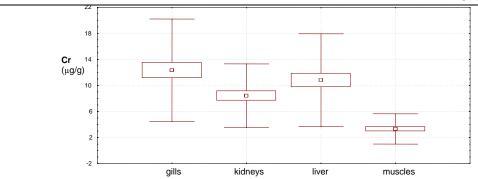
In pre monsoon, maximum mean concentration of Cr was recorded in gills (32.77 ± 4.855μg/g) and liver (30.668 ± 1.881μg/g) of *Heteropneustes fossilis* from downstream of studied streams, whereas, highest mean concentration of Cr in kidneys (16.584 ± 5.583μg/g) and muscles (13.693 ± 4.156μg/g) was recorded in *Channa punctata* dwelling in downstream of Nullah Aik (Fig. 4.8b). *Puntius sophore* captured from upstream showed lowest accumulation of Cr metals in gills (3.2645 ± 2.933μg/g) followed by liver (6.390 ± 1.5165μg/g), kidneys (3.468 ± 1.554μg/g) and muscles (3.656 ± 0.414μg/g).

(a)



(b)

Fig. 4.8  Spatial variations in bioaccumulation of Cr (µg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during (a) post monsoon and (b) pre monsoon at upstream and downstream of Nullah Aik and Nullah Palkhu.

(a)

(b)



(c)



**Fish species**
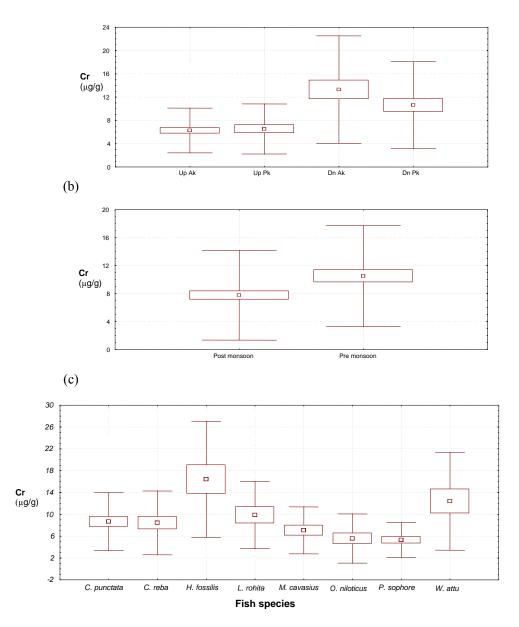
Fig. 4.9 Box and whisker plots of showing comparative accumulation of Cr (µg/g) concentration in organs, (b) sampling zone, (c) seasons and (d) fish species collected (d)from Nullah Aik and Nullah Palkhu

## Bioaccumulation of Nickel

The accumulation of Ni among different sampling zones and seasons were found non-significant differences, whereas, significant in inter-specific comparison (Table 4.2). In case of inter-specific Ni accumulation, *Cirrhinus reba* and *Labeo rohita* showed highest accumulation of Ni and minimum in *Wallago attu* (Fig. 4.11).

The pattern of Ni accumulation in different organs was: gills > kidneys > liver > Muscles during post monsoon (Fig.11a). Maximum mean concentration of Ni in

gills (10.957±1.376μg/g), kidneys (9.994 ± 3.455μg/g) liver (8.04 ± 0.376μg/g) and muscles (2.205 ± 0.117μg/g) was recorded in *Labeo rohita* inhabiting downstream of Nullah Aik, whereas, minimum in gills (4.452 ± 2.285μg/g), kidneys (4.492 ± 2.45μg/g) liver (3.506 ± 1.857μg/g) and muscles (0.909 ± 0.703μg/g) of *Cirrhinus reba* captured from upstream of Nullah Aik (Fig. 4.10a).

In pre monsoon, highest mean Ni concentration (14.379 ± 2.426μg/g) was recorded in gills of *Channa punctata* collected from downstream of Nullah Palkhu and kidneys (14.489 ± 3.005μg/g) of *Heteropneustes fossilis* and liver (16.236 ± 5.612μg/g) of *Cirrhinus reba* captured from downstream of Nullah Aik. Maximum mean Ni concentration (6.335 ± 3.162μg/g) was reported in muscles of *Labeo rohita* collected from upstream of Nullah Aik. Minimum mean Ni concentration in gills (6.571 ± 1.61μg/g), kidneys (2.459 ± 1.385μg/g) liver (5.139 ± 1.31μg/g) and muscles (1.215 ± 0.84μg/g) was accumulated in *Wallago attu* captured from downstream of Nullah Palkhu (Fig. 4.10b).
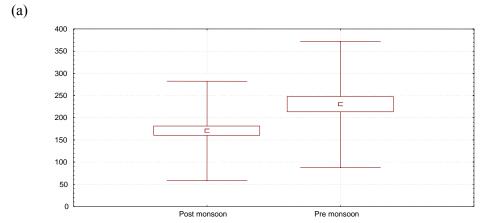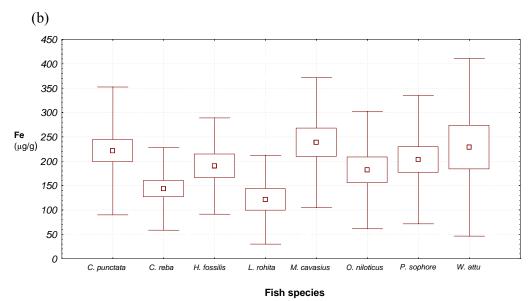
(a)



(b)
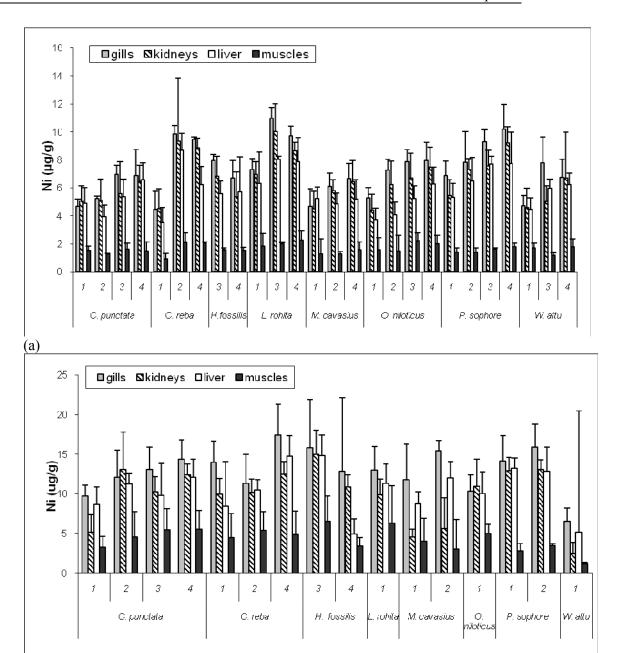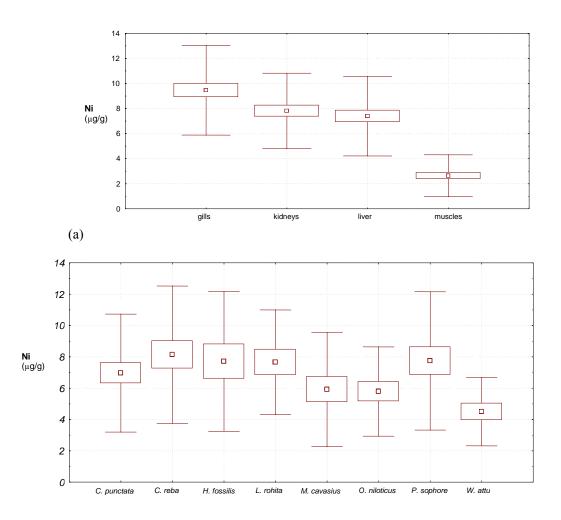
Fig. 4.10  Spatial variations in bioaccumulation of Ni (μg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during post monsoon (a) and pre monsoon (a) at upstream and downstream of Nullah Aik and Nullah Palkhu

(a)



(b)

Fig.4.11   Box and whisker plots of showing comparative accumulation of Ni
(µg/g) concentration in organs, (b) sampling zone and (c) fish
species collected from Nullah Aik and Nullah Palkhu.

**Copper**

Non-significant variations in Cu concentration were recorded between sampling zones as well as between two seasons (Table 4.2). However, significant inter-specific variations for Cu concentration were observed. Maximum mean Cu concentration was found in *Puntius sophore, Labeo rohita* and minimum in *Wallago attu* (Fig. 4.13b).

The accumulation trend of Cu in different organs during post monsoon was highest in liver followed by gills, kidneys and muscles (Fig. 4.13a). Maximum mean Cu concentration (14.827 ± 2.161µg/g) was observed in liver of *Puntius sophore* captured from downstream of Nullah Aik (Fig. 4.12a). *Labeo rohita* collected from downstream of Aik showed maximum mean Cu concentration (8.169 ± 1.171µg/g) in gills, whereas, maximum mean concentration (8.052 ± 3.030µg/g) in kidneys of *Labeo rohita* captured from downstream of Nullah Palkhu (Table 4.1a & b). Maximum mean Cu concentration (1.854 ± 0.076µg/g) in muscles was recorded in *Labeo rohita* collected from downstream of Nullah Aik. Minimum mean Cu concentration was accumulated in liver (0.943 ± 0.073µg/g), gills (0.823 ± 0.123µg/g), kidneys (0.813 ± 0.105µg/g) and muscles (0.471± 0.071µg/g) of *Wallago attu* inhabiting in upstream of Nullah Aik.

During pre monsoon, maximum Cu concentration was accumulated in liver (6.139 ± 1.855µg/g) and kidneys (5.795 ± 2.237µg/g) of *Channa punctata*, whereas, maximum Cu concentration in gills (10.357 ± 0.162µg/g) was observed in *Oreochromis niloticus* captured from upstream of Nullah Aik. *Mystus cavasius* collected from upstream of Nullah Aik, showed minimum accumulation in liver (4.814 ± 0.256µg/g), kidneys (2.277 ± 3.280µg/g), and gills (1.36 ± 1.048µg/g). Maximum mean Cu concentration in muscles (2.118 ± 0.485 µg/g) of *Heteropneustes fossilis* was recorded from downstream of Nullah Aik, whereas, minimum Cu concentration in muscles (1.339 ± 0.526µg/g) of *Labeo rohita* captured from upstream of Nullah Palkhu (Fig. 4.12b).

(a)



(b)
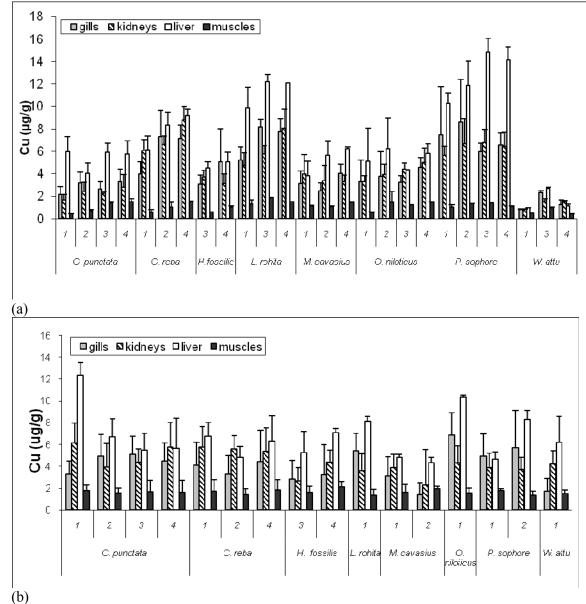
Fig. 4.12 Spatial variations in bioaccumulation of Cu (µg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during (a) post monsoon and (b) pre monsoon at upstream and downstream of Nullah Aik and Nullah Palkhu

(a)

(b)
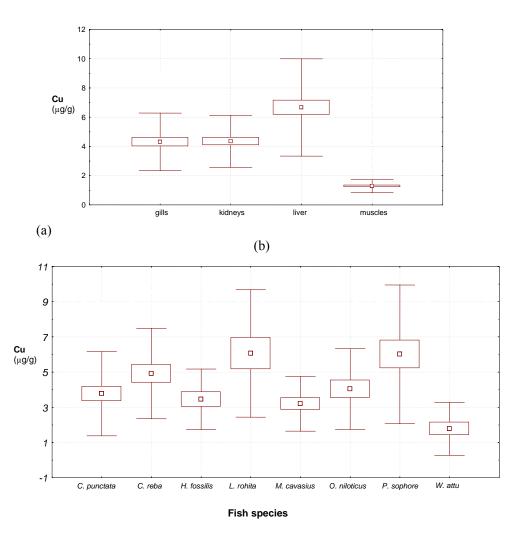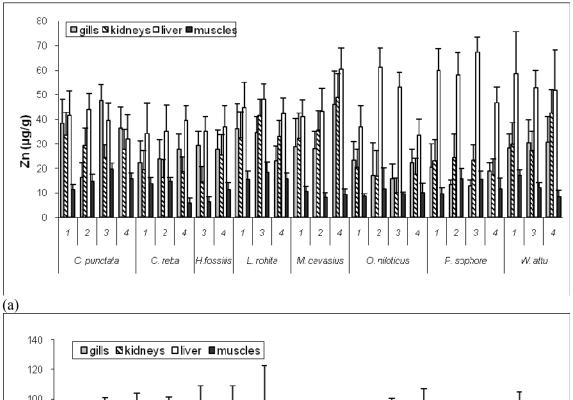
Fig. 4.13  Box and whisker plots of showing comparative accumulation of Cu (µg/g) concentration in (a) organs, and (b) fish species collected from Nullah Aik and Nullah Palkhu

**Zinc**

Non-significant variations in accumulation of Zn were found between sampling zones, whereas, significant seasonal and inter-specific variations were recorded (Table. 4.3). *Mystus cavasius, Channa punctata, Cirrhinus reba* showed higher accumulation of Zn and lowest in *Wallago attu* (Fig. 4.15c).

The accumulation of Zn in different organs during post monsoon was in the order: liver > gills ≥ kidneys > muscles (Fig. 4.15a). Maximum mean Zn concentration was recorded in liver (65.427 ± 29.669µg/g) of *Wallago attu* dwelling in upstream of Nullah Aik. Maximum mean Zn concentration in gills (45.851 ± 23.787µg/g) and kidneys (48.793 ± 16.55µg/g) was recorded in *Mystus cavasius* captured from downstream of Nullah Aik. Maximum mean concentration of Zn (19.554 ± 4.456µg/g) was recorded in muscles of *Channa punctata* collected from downstream of Nullah Aik. Minimum mean Zn concentration in liver (31.883 ± 17.046µg/g) of *Channa punctata* sampled from downstream of Nullah Palkhu, whereas, minimum in kidneys (10.116 ± 10.142µg/g) of *Oreochromis niloticus* captured from downstream of Nullah Aik. Minimum mean Zn concentration was recorded in gills (13.251 ± 3.427µg/g) of *Puntius sophore* from downstream of Nullah Aik (Fig. 4.14a). In muscles, minimum mean Zn concentration (6.343 ± 3.503µg/g) was found in *Heteropneustes fossilis* captured from downstream of Nullah Palkhu (Table 4.1a & b).

In pre monsoon, Zn accumulation pattern in different organs was: liver > kidneys > gills > muscles. During pre monsoon, highest mean Zn concentration was recorded in liver (97.594 ± 21.56µg/g), kidneys (76.613 ± 13.295µg/g) and muscles (45.241 ± 9.890µg/g) of *Mystus cavasius* and highest mean concentration in gills (73.905 ± 12.434µg/g) of *Channa punctata* collected from upstream of Nullah Palkhu. Minimum mean concentration of Zn was observed in liver (55.501 ± 15.455µg/g) and kidneys (29.520 ± 8.982µg/g) of *Puntius sophore*, whereas, minimum mean concentration of Zn was observed in gills (27.07±9.262µg/g) of *Oreochromis niloticus* dwelling in upstream of Nullah Aik (Fig. 4.14b). *Heteropneustes fossilis* showed minimum accumulation in muscles (21.13 ± 14.51µg/g) captured from downstream of Nullah Aik.

(a)



(b)
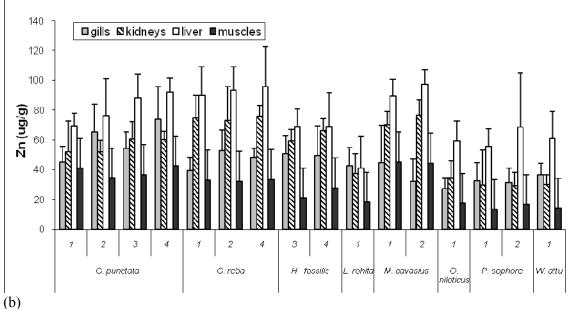
Fig. 4.14  Spatial variations in bioaccumulation of Zn (µg/g) in four organs (gills, kidneys, liver and muscles) of eight fish species during (a) post monsoon  and (b) pre monsoon  at upstream and downstream of Nullah Aik and Nullah Palkhu
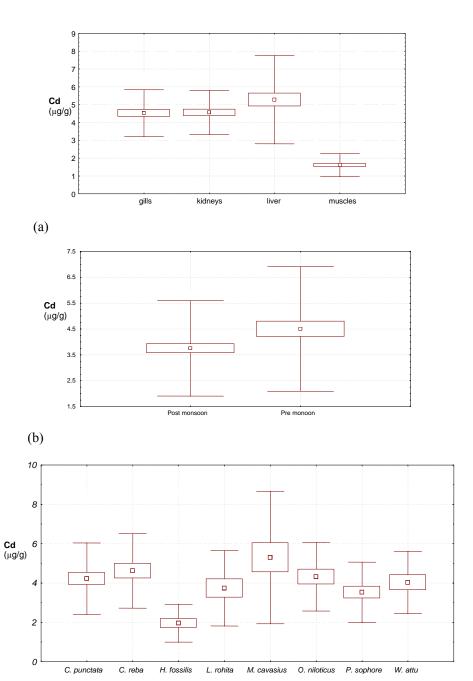
(a)



(b)

(c)
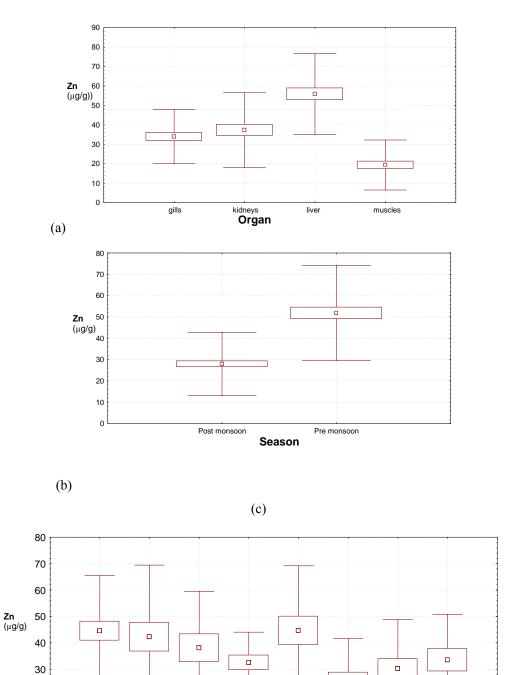


Fig. 4.15 Box and whisker plots of showing comparative accumulation of Zn (µg/g) concentration in (a) organs, (b) seasons and (c) fish species collected from Nullah Aik and Nullah Palkhu.

## Discussion

### Spatio-temporal Variations in Accumulation of Metals in Fish Organs

Fish intake heavy metals through different organs (gills and digestive tract), which are transported by blood to various body parts where, metal ion bind with different proteins (Kotze *et al.,* 1999). Presence of metal-binding proteins is an indication of toxic metal pollution in an aquatic environment (Hennig, 2008). Fish regulate metal ions through excretion via kidneys and gills. Most of the organisms regulate and maintain level of essential heavy metals due to metabolic requirements (Sorensen, 1991). The intensity of heavy metals uptake is influenced by physiochemical and ecological factors. Physiochemical factors such as temperature, pH, TDS and salinity influenced the availability of heavy metals. Higher concentrations of anions, chloride, fluoride and phosphate have been reported to play a vital role in regulating concentration of metals in an aquatic ecosystem (Avenant-Oldewage & Marx, 2000). Ecological requirements of every species differ from each other in terms of habitat preferences, genetic variations, feeding and breeding behaviour. Accumulation of metals in organs of different fish species can be related to differences in physiological functions such as uptake, absorption, storage, regulation and excretion abilities of fish species (Kotze *et al.,* 1999).

The results highlighted that overall accumulation pattern of trace metals in different organs (liver, gills, kidneys and muscles) was: Fe > Zn > Cr > Pb > Ni > Cu > Cd. Similar accumulation pattern was found in the study of Javaid & Mehmood (2000) and Zyadah & Chouikhi (1999) in fishes collected from Ravi River and Aegean Sea (Turkey), respectively. Furthermore, Yilmaz *et al.* (2007) also described the sequence of metal accumulation Zn > Fe > Cu > Pb > Co > Cd. According to Terra *et al.* (2008) metal such as Fe and Zn, which are essential for the metabolic activities accumulated in higher concentration. Cr, Ni and Cu which are required in trace amounts for metabolic activities showed high bioaccumulation in fish species. Higher Fe concentration was recorded in all organs as compared to Pb, Cd, Cr, Ni, Cu and Zn. Avenant-Oldewage & Marx (2000) indicated that higher concentration of Fe in water and sediments increases the rate of accumulation in fish organs. *Wallago attu* accumulated relatively higher Fe concentration in liver. Fe is basic component of haemoglobin protein, which is stored in liver and reuse in genesis of red blood cells.

High concentration of Fe was accumulated in liver followed by gills indicating the metabolic and physiological role in fish body (Avenant-Oldewage & Marx, 2000).

Kojadinovic *et al.* (2007) described the general principle for accumulation of metal i.e., higher in liver, followed by gills, kidneys and muscles. Liver and gills generally accumulated higher concentration of metals compared to other organs. Similarly, the results of present studies indicated relatively high concentration of heavy metals in liver and gills of all fishes captured from Nullah Aik and Nullah Palkhu. All organs showed significant variations in accumulations of metals. Cd and Zn showed similar accumulation pattern in fish organs as earlier recorded for Fe in eight fish species.

Cadmium is highly toxic to organisms and its biological function is unknown (Wright & Welbourn, 2002). Trace amount of Cd can cause anomalies such as reduction in development and growth rates and skeletal ossification even death of organism (Hollis *et al.*, 1999). During present study lowest concentration of Cd was detected in all organs as compare to the other metals.

Pb accumulated with higher concentration in gills, followed by liver, kidneys and muscles. El-Shaikh *et al.* (2005); Canli & Kalay (1998); Akhtar *et al.* (2005); Kargin (1998) also reported high concentration of Pb in gills in comparison to other organs. According to Pastor *et al.* (1998) highest percentage of Pb accumulated in gills, whereas, other tissues such as liver, kidneys and muscles accumulate least quantity. Metals can easily enter into fish through gills from external medium (El-Shaikh *et al.,* 2005).

The results showed highest Cu concentration in liver which was followed by gills, kidneys and muscles. Similar pattern of Cu accumulation was recorded by Kargin (1998), Canli & Kalay (1998) and Avenant-Oldewage & Marx (2000). However, Akhtar *et al.* (2005) reported maximum concentration of Cu in gills followed by liver and muscles. McCarter & Roch (1983) also reported highest accumulation of Cu in liver. Excessive amount of Cu in liver also excreted through bile duct and kidneys (Heath, 1987). Gills can accumulate Cu due to large surface area from aquatic medium and facilitates the absorption into blood (Stagg & Shuttleworth, 1982). Cu in dissolved form may bind with respiratory surface directly exposed to external medium water. The results demonstrated lowest concentration of Cu in muscles. Avenant-Oldewage & Marx (2000) also indicated that muscles accumulate less amount of Cu even if fish is exposed to high levels of metal

contamination. In contrast, Campenhout *et al.* (2004); Chatterjee *et al.* (2006) reported higher concentration of Cu in liver, followed by gills and muscles.

The concentration of Pb, Cr, Ni and Cu displayed accumulation in this order: gills > liver > kidneys > muscles. Similar accumulation patterns among various organs. Canli & Kalay (1998), Avenant-Oldewage & Marx (2000), Nussey *et al.* (2000) and Akhtar *et al.* (2005) reported similar trend of metal concentration in different organs. Cr absorbed readily by gills and liver as compared to kidneys and muscles. Liver detoxifies and excretes Cr through digestive system to regulate the concentration inside the body (Avenant-Oldewage & Marx, 2000). Fish dwelling in contaminated water with higher load of Cr and Ni accumulate higher concentration in gills and liver due to slow excretion rate (Avenant-Oldewage & Marx, 2000; Heath, 1991). Generally, fishes accumulate Cr to the largest extent in organs such as gills, liver, kidneys and spleen, whereas, relatively lower concentration in muscles. Higher concentration of Cr was observed at the lowest trophic levels (Eisler, 1986). Similar accumulation pattern of Ni in different organs of fish species was reported by several authors (Canli & Atli, 2003; Deheyn *et al.,* 2005; Bajc *et al.,* 2005; Kojadinovic *et al.,* 2007; Akhtar *et al.,* 2005).

The results demonstrated higher concentration of Zn in liver followed by gills, kidneys and muscles. Javaid & Mehmood (2000) described analogous pattern of Zn accumulation in organs of fishes captured from River Ravi. Tekin-Özan & Kır (2006) also reported high concentration of Zn in liver, gills and muscles of fishes. The study of Deb & Santra (1997); Farkas *et al.* (2000); Andreji *et al.* (2006); Farombi *et al.* (2007) also confirmed the findings of present study.

Metal accumulation in fish species provided evidences of constant exposure to metals contaminated aquatic environment (Kotze *et al.,* 1999). Mean concentration of Cr and Pb was significantly different between upstream and downstream stream zones. Distinct variations in metal accumulation were observed for metals originated from point sources. Nullah Aik and Nullah Palkhu receive Cr from tanneries effluents. The results showed non-significant spatial differences in mean concentration of metals such as Fe, Cd, Ni, Cu and Zn, which were mainly related with non-point sources of contamination. Van Eeden (2003) also reported non-significant variations in accumulation of Pb, Ni and Cu between reference and least polluted sites.

The concentration of Fe, Cd, Cr and Cu were significantly different between two seasons. During present study, maximum concentration of metals (Fe, Cd, Cr, and

Cu) was recorded in pre monsoon, while lowest concentration in post monsoon. Non-significant seasonal variations were recorded for Pb, Ni and Zn accumulation in fishes of Nullah Aik and Nullah Palkhu. Differences in metal concentrations in fishes can be attributed by the presence of metal contaminant in surrounding medium. Tekin-Özan & Kir (2007) described that bioavailability of metals may influenced by physiological activities of fish during different seasons. Nussey *et al*. (2000) indicated highest metal accumulation in fish species during spring and summer as compared to autumn and winter season. Farkas *et al*. (2000) also described that seasonal variations in metal accumulation is related to feeding behaviour of fish species (Pourang, 1995). Kargin (1998) reported an increasing trend of metal accumulation in fishes from autumn to summer in Seyhan River in Turkey. Seasonal variations in metal accumulation may be influenced by stream conditions, pollutants load, water chemistry and other environmental factors which affect the availability of metal (Johnson, 1988; Heiny & Tate, 1997).

Simkiss & Mason (1983) indicated that seasonal differences in temperature, rainfall, salinity and stream flow can influence metal accumulation and their bioavailability. Roesijadi & Robinson (1994) reported that metal concentration in surface water varies due to sources of metal, rainfall pattern, surface runoff, feeding behaviour and metabolic activities. High stream flow in post monsoon season may dilute the concentration of contaminants. In winter, stream discharge is relatively low and streams experience elevated level of heavy metals. Highest accumulation of heavy metals was measured during pre monsoon season. Nussey *et al*. (2000) also recorded high metal accumulation in pre monsoon, which can also be related with high temperature favourable for optimum metabolic activities of surrounding medium during pre monsoon season. High metabolic rate reduces oxygen concentration in blood and increase accumulation of pollutants (Grobler *et al.,* 1989; Nussey *et al*., 2000). Most of the fish species breed during pre monsoon season and high metabolic rate with elevated level of heavy metals in surrounding medium may influence the embryonic development of fish individuals (Campbell, 1995).

Mean Fe concentration did not show significant variations between in upstream and downstream zones. Similar results were reported by Avenant-Oldewage & Marx, (2000). Fe in stream water originates from natural as well as anthropogenic sources. Fe concentration varied significantly between seasons. Kargin (1998); Ozmen *et al*. (2006); Avenant-Oldewage & Marx (2000) also reported that Fe

accumulation varied significantly between seasons in freshwater fishes of South Africa. Avenant-Oldewage & Marx, (2000) also stated that high concentration of Fe in stream water can be related with high level of organic matter and humic acids.

Mean Pb concentration showed spatial significant variations in fish organs from four sampling zones located on Nullah Aik and Nullah Palkhu. Minimum mean Pb concentration in muscles was reported in upstream of Nullah Aik (8.774 μg/g) and maximum at downstream of Nullah Aik (9.182μg/g) followed by Nullah Palkhu (10.768 μg/g). Downstream of Nullah Aik (12.320μg/g) and Nullah Palkhu receive pollutants from urban runoff and municipal sewage that increases the concentration of Pb in stream water. Van Aardt & Erdmann (2004) reported significant spatial variations in accumulation of Pb in fishes captured from polluted freshwater bodies. Tryfonas *et al*. (2006) also reported the same results of Pb accumulation in fishes dwelling in Yamuna River. Similarly, Canli & Kalay (1998); Javed (2005), Javid *et al.* (2007), Karadede-Akin & Ünlü (2007) and Terra *et al.* (2008) reported high spatial variations of Pb accumulation in less and high polluted streams and rivers. During present study, non-significant seasonal variations in Pb accumulation were recorded. In contrary, Mwashote (2003) and Mansour & Sidky (2002) reported significant seasonal variations in Pb accumulation in fishes from African waters.

Mean Cd concentration did not show pronounced variations between upstream and downstream zones. Campbell (1995), Nnaji *et al.* (2007) reported similar results. Goldstein & DeWeese (1999) also found non-significant spatial variations of Cd concentration in Red River. In contrary, Besser *et al.* (2007) reported significant spatial variations of Cd concentration in fishes from streams polluted from cadmium mines in United States. Cd accumulation was significantly different between post and pre monsoon downstream seasons. Tekin-Özan & Kir (2007) reported seasonal variations in Cd accumulation between fishes of Beyşehir Lake, Turkey. High concentration of Cd in fish is an indicator of the environmental contamination of surrounding medium (Kojadinovic *et al.,* 2007).

Fish species captured from downstream of Nullah Aik showed high level of Cr accumulation and low concentration in upstream sampling zone. Significant spatial and seasonal variations were recorded in Cr concentration between fish species. In contrary, Avenant-Oldewage & Marx (2000) and Ozmen *et al*. (2006) reported non-significant spatial and seasonal variation in Cr accumulation. High concentrations of

Cr in fishes indicate the contribution of tanneries effluents which bring large amount of Cr salts that may concentrate in the aquatic organism like fishes.

Ni concentration in different fishes did not showed significant variations between seasons. These results further confirmed the findings of Lefkovitz *et al.* (2000) and Nussey *et al.* (2000), whereas, Canli & Kalay (1998) reported significant spatial differences in Ni accumulation in the Seyhan River, Turkey. Nussey *et al.* (2000) also reported significant seasonal variations in Ni accumulation.

Cu and Zn concentration in four sampling zones did not showed significant differences in accumulation. Similar results were reported by Campbell (1995), Goldstein & DeWeese (1999) and Youssef & Tayel (2004). However, Canli & Kalay (1998), Kotze *et al.* (1999) and Beldi *et al.* (2006) found significant spatial differences in Cu accumulation in fishes. In present study, Cu concentration in fishes was significantly different between seasons. Avenant-Oldewage & Marx (2000) and Tekin-Özan & Kir (2007) reported significant seasonal variations in Cu accumulation.

Harrison & KlaverkKamp (1990); Schmitt *et al.* (2002; 2007) reported significant spatial variation in Zn accumulation in freshwater fishes. In present study, non-significant seasonal variations were recorded. Similar results were also reported by Velcheva (2006) and Tekin-Özan & Kir (2007).

## Inter-specific Variations in Accumulation of Metals

Inter-specific variations in heavy metals accumulation are influenced by life history patterns such as trophic position in food chain, geographic variations, exposure to contaminated environment and internal metabolic activities (Allen-Gill & Martynov, 1995). Fishes occupy various positions (herbivore to top carnivore) in the food chain (Mansour & Sidky, 2002) and species at lower trophic level accumulate low metal concentration in comparison to those occupy higher trophic level (Peakall & Burger, 2003; Burger *et al.,* 2001). Amundsen *et al*. (1997) and Canli & Kalay (1998) reported inter-specific variation of heavy metal accumulation. Concentration of metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn were significantly different between fish species. Highest concentration of Fe accumulated in *Wallago attu,* whereas, lowest in *Labeo rohita.* Similarly, Highest Fe concentration in carnivorous fish species and lowest in herbivore fish species.

*Heteropneustes fossilis* showed highest concentration of Pb and *Mystus cavasius* accumulated lowest Pb concentration. Fishes are highly sensitive to Pb toxicity. Higher concentrations of Pb in bottom feeder fish species was recorded to those of predatory fishes (Campbell, 1995).

Among metals lowest level of Cd accumulation was recorded in all fish species; though, it varies significantly from one fish species to another. Similarly Youssef & Tayel (2004) found low concentration of Cd in *Tilapias*. In present study, highest concentration of Cd was recorded in *Mystus cavasius,* which is carnivore species and lowest concentration of Cd in *Heteropneustes fossilis,* which is an omnivore species. Similar results were also reported by Amundsen *et al*. (1997) and Andreji *et al*. (2006). Burger *et al*. (2001) and Terra *et al*. (2008) found higher level of metals in carnivorous species instead of herbivores and omnivores. Furthermore, bottom dweller fish species showed higher concentration of Cd.

Among fish species, *Wallago attu* and *Heteropneustes fossilis* showed highest accumulation of Cr while lowest accumulation was recorded in *Oreochromis niloticus. Heteropneustes fossilis* is a bottom dweller omnivore fish found in downstream sites and *Wallago attu* feed on small fish species and macro-invertebrates. Terra *et al*. (2008) and Tekin-Özan & Kir (2006) recorded high level of Cr accumulation in carnivore species followed by omnivore species. *Heteropneustes fossilis* and *Oreochromis niloticus* are omnivorous species but have different habitat preferences. *Heteropneustes fossilis* is bottom dweller showed preferences for downstream sites contaminated with industrial effluents and urban sewage and *Oreochromis niloticus* is column dweller and prefer upstream sites (less polluted). Fish dwelling in contaminated water with higher load of Cr metal from tanneries, exhibit higher level of Cr in gills and liver due to slow excretion rate by liver under stress conditions. Chromium in aquatic medium found in two forms trivalent ($Cr^{+3}$) or hexavalent ($Cr^{+6}$). $Cr^{+3}$ are less toxic as compared to $Cr^{+6}$ and can disturb the permeability of membrane by oxidation process (USEPA, 1980).

Highest concentration of Ni was recorded in *Cirrhinus reba* and lowest in *Wallago attu.* Present study indicated that lowest Ni concentration in carnivorous species as compared to herbivore, which can accumulate higher concentration. Campbell (1995) also reported similar results.

High Cu accumulation was recorded in invertevore (*Puntius sophore*) and herbivore species (*Labeo rohita*), whereas, lowest accumulation in carnivore species (*Wallago attu*). Silva & Shimizu (2004) reported omnivorous fishes can accumulate higher Cu concentration as compared to carnivorous fishes. In contrary to our results, Terra *et al.* (2008) reported that carnivore fishes accumulate highest level of Cu to those of herbivore species. Phillips (1990) reported that carnivore fish species can accumulate Cu at higher concentration than herbivorous and omnivorous and indicated that this pattern could be changed due to pollution exposure, feeding habits and fish breeding behaviour. Higher accumulation of Cu in omnivore species could be due to pollution stress and feeding patterns (Phillips, 1990).

During present study, highest concentration of Zn was recorded in *Mystus cavasius* and lowest in *Oreochromis niloticus.* According to Kotze *et al.* (1999) inter-specific differences in Zn bioaccumulation could be due to differences in feeding habits and behaviour of fish species. *Oreochromis niloticus* is an omnivore species feeds on aquatic vegetation and detritus matter (Greger & Deacon, 1988), whereas, *Mystus cavasius* feed on small fishes and small invertebrate. High concentration of Zn was recorded in *Channa punctata* (Mirza & Bhatti, 1993).

**Toxic Effects of Metals on Fishes**

Elevated level of metals in different organs of fish is mainly absorbed from aquatic resources polluted by industrial effluent and municipal sewage (Asuquo *et al.*, 2004). Heavy metals discharged from multiple sources into aquatic ecosystems are responsible for fish physiological and histological abnormalities (Sehgal & Saxena, 1986). Toxic level of metals may affect growth, impaired movements, behavioural, physiological and reproductive abnormalities in fishes (Atchison *et al.*, 1987). During present study, skin and fin rot diseases were observed *Channa punctata* and *Mystus cavasius* (Plate 4.1). Species like *Wallago attu* captured from less polluted sites and polluted sites also showed prominent variations in skin colour (Plate 4.2). These negative effects of heavy metals affect fish health, leading to abnormalities and even death.

Iron is rarely toxic (Atchison *et al.*, 1987; USEPA, 1999) and hardly toxic to aquatic ecosystem. Bury *et al.* (2003) reported that excessive amount of iron can be harmful for fish health including gills clogging and respiratory perturbations. Mean Fe

concentration in fishes of Nullah Aik and Nullah Palkhu ranged from 121.0 to 238.3 (μg/g).

The toxic effect of Pb decreases with an increase in water hardness. High level of water hardness reduces bioavailability of heavy metal (Wright & Welbourn, 2002). Factors such as age, sex and food and interference of Pb with other chemical present in mixture of effluents affect the adsorption process of Pb in fishes (Eisler, 1988; USEPA, 1999). In present study, mean dissolved Pb concentration varied from 188.2 to 206.9 (μg/L). USEPA (1999) reported chronic (11.86μg/L) and acute effect (316.64μg/L) of Pb concentration for fish species at water hardness 290 (mg/L). According to Davies *et al.* (1976) and Atchison *et al.* (1987) Pb concentration of 32 (μg/L) and LC 50 for fish species for 96 hours at 1320 (μg/L) of water hardness can cause abnormalities such as hepatic lesions, splenic hemosiderosis and malfunctioning of kidneys in freshwater fishes (Pattee *et al.,* 2006). The LC50 (lethal concentration to kill 50% population of an organism) of Pb concentration for fishes was 542 (mg/L) at 290 (mg/L) water hardness (USEPA, 1985). Pb concentration in water of Nullah Aik and Nullah Palkhu was above Pb concentration that can cause chronic effects, however, below the concentration that can cause acute effects on fish fauna. Sub-lethal effects of Pb on fish fauna appears if its concentration ranged from 2300 to 145.72 (mg/L) in aquatic ecosystem (USEPA, 1999). Mean Pb concentration, which ranged from 9.012 to 13.597 of (μg/g) in muscles of fishes captured from Nullah Aik and Nullah Palkhu was above the permissible level (4.0μg/g) in fish muscles for human consumption (USEPA, 1999).

Cd toxicity of fish varied from species to species, developmental stages, interference of toxicants and water hardness (USEPA, 1999). Mean Cd concentration in water of Nullah Aik and Nullah Palkhu ranged between 22.3 and 33.6 (μg/L) at water hardness 122 to 288 (mg/L). These values were higher to Cd level, which can produce chronic and acute effects in freshwater fishes (USEPA, 1999). Sastry & Shukla (1994) described the LC50 of Cd, which is 11,200,000 (μg/L) for *Channa punctata* at 165 (mg/L) level of water hardness for 96 hours exposure. Hontela *et al.* (1996) noted abnormal production of fish thyroxin and cortisol hormones at exposure of fish juveniles to Cd concentrations of 400-2,400 μg/L for 2 hours to 1 week at 110 mg/l water hardness. USEPA (1999) described Cd concentration (300- 400,000μg/L) at which sub-lethal effects appears which altered normal functioning of enzymes and physiological activities of catfishes, whereas, lethal effects appear at Cd concentration

ranged from 338.3- 405,000μg/L. During present study, average concentration of Cd in the fish body ranged from 1.953 to 5.295 (μg/g). Cd concentration should not exceed the standards (5.0μg/g) for human consumption developed by Food and Drug Administration (1993). Higher concentration of Cd was recorded in *Mystus cavasius* captured from downstream sites of Nullah Palkhu.

According to (USEPA, 1980) $Cr^{+6}$ concentration of 16 to 21(μg/L) in freshwater can reduce growth in teleosts (bony fishes). $Cr^{+6}$ have higher biological activity and are more toxic to those of $Cr^{+3}$. The Canadian water quality guideline (Canadian Council for Ministers for Environment CCME, 1999) for $Cr^{+3}$ for freshwater fishes is 8.9 (μg/L), whereas, it's least observed effect concentration (LOEC) is 0.089 (μg/L). CCME (1999) reported chronic Cr toxicity appear in freshwater fishes at concentration ranged from 6– 110000 (μg/L) while acute toxicity appear at concentration ranged from 3300- 77500 (μg/L). In present study, the level of Cr in surface water of Nullah Aik and Nullah Palkhu ranged between 76.7 – 196.8 (μg/L), which is below the concentration in freshwater that cause acute toxicity level described by the Benoit (1975) and CCME (1999). In present study, mean concentration of Cr in fish body ranged from 2.30 to 16.42(μg/g). Eisler (1986) described the maximum permissible concentration of Cr (4.0 μg/g) in fishes for human consumption. Higher concentration of Cr causes abnormal development of fish embryos, over production of mucous and blood serum, malfunction of liver and chromosomal aberration.

Nickel is considered less toxic in comparison to Cd and Pb (Clark, 2001). The symptoms of nickel toxicity in fishes are fast mouth, opercula (Beraldo *et al.,* 1995) and convulsive movements and loss of equilibrium before death (Khangarot & Ray, 1990; Eisler, 1998). Higher concentration of Ni reduces respiration rate and causes death due to blood hypoxia (Ellgaard *et al*., 1995; Eisler, 1998). Ni concentration in water of Nullah Aik and Nullah Palkhu ranged from 78.8 to 100.8 (μg/L), which were below the concentration that causes the chronic (160μg/L) and acute toxic effect levels (1400μg/L) described by USEPA (1999). Higher Ni concentration affects growth, reproduction, teratogenicity, and carcinogenicity (Eisler, 1998). Ni concentration was also less than LOEC (134μg/L) and LC50 (8100μg/L) as given by Nebeker *et al*. (1985). In present study, mean Ni concentration in fish muscles ranged from 4.50 to 8.14(μg/g), which was below the permissible level of Ni (10.0μg/g) in fishes for human consumption (Eisler, 1998).

*Channna punctata*

**(a)**



*Mystus cavasius*

**(b)**

Plate 4.1    Diseased specimens *Channa punctata* (a) and *Mystus cavasius* (b) captured from Nullah Aik near Bhopalwala and Nullah Palkhu near Jathikay.



*Wallago attu*

**(a)**



*Wallago attu*

**(b)**

Plate 4.2    Skin colour variations in two individuals of *Wallago attu* captured from (a) upstream(less polluted site) and (b) downstream (polluted site) on Nullah Aik.

The level of Cu in fish tissues reflects indirectly its bio-availability in aquatic environment. The toxic effect of Cu in fishes is highly influenced by water hardness,

organic matter and developmental stage of freshwater fishes (USEPA, 1999). During present study, concentration of dissolved Cu in stream water ranged between 27.5 (μg/L) and 46.1 (μg/L). These concentrations were below to the concentration that can produce acute and chronic effects (USEPA, 1999). Pickering *et al*. (1977) studied LOEC of Cu (37μg/L) and LC 50 (460mg/l). Buhl & Hamilton (1996) reported mortality in sward fish at 363-10,000 (μg/L) of Cu concentration. According to ANZFA (The Australian, New Zealand Food Authority), the concentration of Cu should not exceed 10.0(μg/g) in fish body (Van den Broek *et al.,* 2002). The concentration of Cu muscles of fishes from Nullah Aik and Nullah Palkhu ranged from 1.779 to 6.059 (μg/g) and was within the permissible (10.0μg/g) concentration for human consumption (USEPA, 1999).

Zinc becomes toxic for fish in water with low pH, alkalinity, dissolved oxygen and high temperature (Eisler, 1993). Majority of fish species accumulate excessive amount of Zn than required for metabolic activities (USEPA, 1999). Shukla & Pandey (1986) reported reduced growth of *Channa punctata* at Zn concentration of 12000 (μg/L) in surface water. According to Bengeri & Patil (1986) fishes showed reduced production of liver glycogen at Zn concentration of 65,000 (μg/L). According to USEPA (1999) chronic and acute toxic effects appear at Zn concentration of 10 (μg/L) and 100 (μg/L). The chronic effects of Zn such as changes in enzymes production and alteration in blood and tissues appear when its concentration ranged between 500 and 130,000 (μg/L) on catfishes (USEPA, 1999). USEPA (1987) reported LC 50 of Zinc for freshwater fishes ranged from 840 to 22,600 (μg/L). According to ANZFA (The Australian, New Zealand Food Authority) Zn concentration in fish tissues should not exceed 150(μg/g) within tissues of fishes (Van den Broek *et al.,* 2002). Van den Broek *et al.* (2002) and Eisler (1993) reported that the concentration of Zn in whole fish body should be below 80(μg/g). In present study, mean concentration of Zn in fish body was ranged from 25.25 to 44.6(μg/g) which is within safe limits for human consumption described by Van den Broek *et al.* (2002) and Eisler (1993).

**Comparison with Guidelines for Human Consumption**

Metal contents in muscles are most important because people, consume the muscular parts of fish as food after removing liver, gills and kidneys (Mai *et al.*, 2006). Lowest accumulation of Fe, Pb, Cd, Cr, Ni, Cu and Zn was found in muscles. Fish species such as *Channa punctata, Wallago attu, Cirrhinus reba, Labeo rohita* and *Heteropneustes fossilis* are commonly used as food in the catchment area of Nullah Aik and Nullah Palkhu either captured by game fishermen or professional fishermen. *Channa punctata* is the most abundant species and is preferably used for food (plate 4.3). The concentration of Fe in the muscles of all eight species during post monsoon and pre monsoon was below the standards of (FAO/WHO, 1987; USEPA, 1999) ranged from 20 to 150 (µg/g). However, Pb (4.0µg/g) and Cr (8.0µg/g) during each season exceeded the permissible level described by USEPA (1999) and FAO (1983). The Pb concentration in *Labeo rohita* (4.353 ± 2.013µg/g) captured from downstream of Nullah Aik was higher to standard limits (4.0 µg/g), whereas, *Puntius sophore* collected from upstream of Nullah Palkhu (4.044 ± 2.03µg/g) and downstream of Nullah Aik (4.560 ± 2.526µg/g) and *Wallago attu* (4.05 ± 2.06µg/g) sampled from downstream of Nullah Aik also exceeded Pb concentration. During pre monsoon season, Pb concentration in muscles of *Channa punctata* captured from upstream (4.24 ± 3.14µg/g) and downstream (6.96 ± 7.77µg/g) of Nullah Aik and Nullah Palkhu (5.67 ± 7.68µg/g) were above the standard limits of 4.0 (µg/g). Similarly, Pb concentration in muscles of *Labeo rohita* captured from downstream of studied streams (9.534 ± 5.131µg/g, 4.227 ± 9.037µg/g) and *Puntius sophore* (4.922 ± 6.832µg/g) dwelling in upstream of Nullah Palkhu exceeded the standard limits (USEPA, 1999; FAO, 1983).

*Cirrhinus reba* (2.09 ± 1.04µg/g) captured from upstream of Nullah Aik and *Oreochromis niloticus* (2.57 ± 0.10µg/g) from downstream of Nullah Aik during post monsoon, whereas, *Channa punctata* from downstream of Nullah Aik (2.581 ± 2.26µg/g) and Nullah Palkhu (2.15 ± 1.71µg/g) during pre monsoon were higher than the permissible limits (2.0µg/g) described by (FAO/WHO 1987). Cr concentration in muscles of studied fish species did not exceed the standard limits (8.0µg/g) during post monsoon, however, exceeded in *Channa punctata* captured from downstream of Nullah Aik (13.693 ± 4.156µg/g) and Nullah Palkhu (8.834 ± 1.125µg/g) during pre monsoon. The present study highlighted the intensity of heavy metal contamination in fishes, which need to be addressed urgently.

*Wallago attu*

*Channa punctata*

Plate 4.3    A fish sale point at Sambrial town located in lower catchment area of Nullah Aik and Nullah Palkhu.

In Sialkot City, heavy metal contamination in Nullah Aik and Nullah Palkhu is the consequence from industrial effluents, municipal sewage and surface runoff. Effluents discharge into streams contains heavy metals (Qadir *et al.*, 2008), which may provide conducive condition for bio-concentration of metals in fish fauna of Nullah Aik and Nullah Palkhu. The finding of present study indicated that fishes dwelling in Nullah Aik and Nullah Palkhu are under risk of metal accumulation, which is an ultimate threat to local human population. If existing situation remain unchanged, it will not only affect the fish life but also well being of humans.

**Conclusions**

In conclusion, the results of this chapter indicated that all seven metals studies showed significant variations in accumulation in different organs such as gills, liver, kidneys and muscles. Significant spatial differences in Cr concentration were observed, whereas, non-significant spatial variation were recorded in Fe, Pb, Cd, Ni and Cu. All studied heavy metals showed significant variation between different fish species. Among heavy metals, Fe showed high concentration and Cd lowest concentration in fish species. Metals like Pb, Cr, Ni and Cu showed highest accumulation in gills followed by liver, kidneys and lowest in muscles, whereas, Fe, Cd and Zn showed metal accumulation pattern in order: liver > gills > kidneys > muscles. General pattern of metal accumulation was: Fe > Zn > Cr > Pb > Ni > Cu > Cd. Higher concentration of metals like Fe, Cd, Cr and Cu was recorded in pre monsoon season. Most of the metals in stream water were above the LOEC and below the level of acute toxic effects of heavy metals on fish species. Pb concentration in muscles of *Labeo rohita, Puntius sophore* and *Wallago attu*, Cd concentration in muscles of *Cirrhinus reba, Oreochromis niloticus* and *Channa punctata* and Cr concentration in muscles of *Channa punctata* were recorded above the standard limits for human consumption as described by FAO/WHO (1987) and USEPA (1999). These findings suggested that consumption of fishes captured from downstream of Nullah Aik and Nullah Palkhu may cause human health hazard. Therefore, it is recommended that fishes captured from downstream sites of each stream should be avoided as human diet.

**Chapter 5**

# Patterns of Fish Assemblage and Distribution under the Influence of Environmental Variables in Nullah Aik and Nullah Palkhu

## Introduction

The diversity and spatio-temporal distribution pattern of fish assemblage in stream is highly influenced by variations in lotic ecosystem (Taylor *et al*., 1997). These variations are resulted due to environmental factors inside the stream (Taylor *et al.,* 2006). In intermittent streams environmental variations are of great importance for fish assemblage as compared to continuously flowing streams (Pires *et al*., 1999). Environmental factors such as physiochemical parameters of water quality and habitat structure determine the structure and composition of fish assemblages inside the stream (Casatti *et al*., 2006; Aparicio *et al.,* 2000) and result in change of the ecological equilibrium (Karr, 1996; Lyons, 1996). Habitat structure and physiochemical quality of stream water have been widely used to explore the relationships with fish assemblage (Casatti *et al.,* 2006). Fishes aggregate into co-existing groups to form complex assemblages of species in pristine aquatic ecosystem (Pusey & Kennard 1996; Lamouroux *et al*., 2002). Association of different species in food web is a delicate and complex phenomenon that has evolved over long time period. Physical habitat and stream water quality are very sensitive to human pollution (Casatti *et al*., 2006). Aquatic communities, especially fishes reflect the intensity of degradation in streams and rivers (Wichert & Rapport, 1998; Das & Chakrabarty, 2007).

Streams which experience irregular flow are more vulnerable to anthropogenic activities (Paul & Meyer, 2001) such as industrial effluents, municipal sewage, and surface runoff from urban and agricultural land, which alter the water quality and habitat structure of stream (Singh *et al.,* 2005a; Qadir *et al*., 2008). Change in water quality due to untreated industrial effluent and municipal sewage not only increases the load of pollutants to streams but also put the fish fauna under stress (Matthews, 1998; Davis *et al.,* 2003).

Fish assemblages have been used as an indicator of environmental degradation (Scott & Hall, 1997; Arunachalam, 2000). Fish diversity in streams and rivers is considered as a diagnostic tool to highlight the impact of environmental changes (Das & Chakrabarty, 2007). Loss of fish species diversity determines the severity of habitat degradation of an aquatic ecosystem (Ganasan & Hughes, 1998).

Structure and composition of fish assemblage in temperate streams exhibit prominent temporal variations influenced by habitat characteristics and physico-chemical characteristics of water (Magalhães *et al*., 2002). The temporal distribution patterns of fish assemblages and water quality are directly or indirectly influenced by the flow regime (Grossman *et al.,* 1990; Godoy *et al.,* 2002). Most of the streams and rivers in oriental region exhibit high flow during monsoon season and experience low flow or drought during pre monsoon season, which determine the structure of fish communities in the streams (Pires *et al.,* 1999). Fish assemblages of such streams are adapted to low and high stream flow to attain ecological equilibrium in fish population. However, anthropogenic factors such as pollution load from industries and municipalities, water abstraction and introduction of alien species highly destabilize the equilibrium of fish assemblage (Pires *et al.,* 1999). Unwise human activities in the catchment area deteriorate the water quality and disrupt the integrity of fish assemblage, which results, vanishing of native fish species or appearance of exotic species in an ecosystem (Karr *et al*., 1986; Lyons, 1996). Any change in species composition due to addition or deletion of species may disrupt the functional and functional integrity of an aquatic ecosystem (Lyons, 1996).

Influence of environmental factors on structure and function of fish assemblages in streams have been studied in several countries (Gafny *et al.,* 2000; Bhat, 2004; Magalhães *et al*., 2002; Li & Gelwick, 2005; Snodgrass *et al*., 1996; Davis *et al.,* 2003; Belliard *et al.,* 1999). Diversity indices and multivariate statistical techniques are commonly used to highlight the role of various important environmental factors that contributes in explanation of fish diversity and distribution along longitudinal gradients (Koel & Peterka, 2003). Multivariate techniques like Canonical correspondence analysis (CCA) provide information regarding current status of fish distribution in relation to environmental variables (Inoue & Nakano, 2001). Multivariate techniques have been useful in decision making for the restoration and management of degraded streams and to assess the impact of disturbance and identification of factors contribute in deterioration of stream ecosystem for effective

stream management (Paul & Meyer, 2001). Major restoration efforts for intermittent streams are underway in many parts of the world (Paul & Meyer, 2001) and restoration efforts have been successfully implemented. Unfortunately, in developing countries like Pakistan, no restoration efforts for streams and rivers have been made so far. Information related to role of environmental factors in shaping the distribution pattern of fishes in streams and rivers is completely lacking. It is important to collect baseline data about biotic (fish assemblage) and abiotic (environmental) components of stream/river ecosystem before taking any step towards restoration and management of a streams or rivers. The objectives of this chapter are:

- to assess the fish species diversity in Nullah Aik and Nullah Palkhu,
- to explain the pattern of spatio-temporal variations in diversity and composition of fish assemblage and
- to explore the role of environmental variables in fish distribution.

## Materials and Methods

### Site Selection and Fish Sampling

Detailed information about the fish sampling and environmental variables is given in Chapter 2.

### Statistical Analysis

Shannon index (H´) and Simpson index (D) were used to calculate fish species diversity (Magurran, 1991; Lima-Junior, *et al.*, 2006) at different sampling sites during post and pre monsoon seasons.

CCA which is a direct gradient analysis was used to highlight the relationship between fish distribution and environmental variables (Malik & Hussain, 2008). CCA is an eigenvalue based technique applied on species abundance data in relation to environmental variables (stream morphology, physiochemical and metal concentration) during post monsoon and pre monsoon seasons at 18 sampling sites located on Nullah Aik and Nullah Palkhu. Strongly correlated variables were excluded from this analysis using Pearson correlation and 13 variables were used (Pires *et al*., 1999). Environmental variables viz; stream morplogy (stream flow, depth and width), physiochemical variables (DO, COD, turbidity and $NO_3^-$) and six metals (Na, Ca, Fe, Pb, Cr and Cu) were subjected to CCA. The sites representing zero fish catch were also excluded from CCA. Two separate CCAs were performed on fish abundance data with environmental data sets recorded during post and pre monsoon season (Ter Braak & Verdonschot, 1995). First CCA was applied to find out the relationship between (a) sites and environmental variables (14 sites x 13environmental variables) and (b) between species and environmental variables (24 species x 13environmental variables) for post monsoon. CCA for pre monsoon season was performed on site matrix including 12 sites and x 13 environmental variables and species matrix which included 17 x 13 environmental variables (Eggleton, 2004; Malik & Hussain, 2008). Monte Carlo permutation test with 1000 permutations was performed to check wheather environmental parameters were significancantly correlated with fish species composition of plots (Pires *et al.,* 1999). For Fish diversity analysis and CCA, Multivariate Statistical Package (MVSP) was used (Kovach, 1999).

**Index for Change in Scenarios of Fish Communities**

The coefficient of community loss depict the range of scenarios to measure the intensity of change caused by pollution which is a useful tool to take decision for the management of species diversity in an aquatic ecosystem was calculated using following formula as:

$$l = (a-c)/b$$

where (1) is coefficient of community loss, (a) total number of texa from polluted section, (b) total number of texa from unpolluted section and (c) number of texa common in polluted and unpolluted zones.

## Results

### Fish Diversity

A total of 1506 individuals of 24 species (Plate 5.1) belonging to 12 families and 19 genera were recorded in two seasons (post monsoon and pre monsoon) from Nullah Aik and Nullah Palkhu (Table 5.1a & b). About 50.6% of individuals belonged to ten species of family Cyprinidae followed by 19.6% of individual of *Channa punctata* belonging to family Channidae. The remaining 29.8% of relative abundance was contributed by family Bagridae (8.7%), Heteropneustidae (6.7%), Ambassidae (2.7%), Notopteridae (2.3%), Siluridae (2.3%), Osphronemidae (1.7%), Mastacembelidae (0.1%) and Sisoridae (0.1%). The highest species richness and abundance was recorded in post monsoon after rainy season, whereas, lowest in pre monsoon before rainy season.
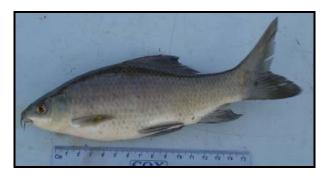
During post monsoon season, 1141 fish specimens were captured from 14 sites. Maximum species richness (14), Simpson (2.29) and Shannon diversity index (0.86 and 0.88) were recorded from upstream sites (1 and 2) of Nullah Aik, which decrease to zero at sites located in close vicinity of Sialkot city. Fish species reappear in far downstream of studied stream (Fig 5.1). *Channa punctata* and *Puntius sophore* species were the dominant species recorded from 12 sites (Table 5.1b).

A total of 375 fish specimens were captured from 12 sites during pre monsoon. None of fish was recorded from six sites (3, 4, 5, 12, 13 and 14). Maximum species richness (13) was recorded at site 2 located at upstream of Nullah Aik with Simpson (2.19) and Shannon (0.85) diversity indices and *Channa punctata* and *Puntius ticto* were recorded in high abundance from nine and eight sampling sites, respectively (Fig 5.1).

### Changes in Species Composition along longitudinal Gradient and Distributional Patterns of Feeding Guilds

Four trophic groups of fishes were identified viz; invertivores, herbivores, omnivores and carnivores from studied streams. Relative abundance for different feeding groups recorded from Nullah Aik was in order: invertivores 36.2% > herbivore 24.6 > Carnivore 24.0% > omnivore 15.1%, whereas, Nullah Palkhu

exhibited different trend: Carnivore 44.9 % > invertivores 36.5% > omnivore 11.3% > herbivore7.3%.



*Labeo calbasu* **(Hamilton, 1822)**



*Labeo rohita* **(Hamilton, 1822)**



*Labeo  dero*  **(Hamilton, 1822)**



*Cyprinus carpio carpio*   **(Linnaeus, 1758)**



*Puntius  sophore*  **(Hamilton, 1822)**



*Puntius  ticto*  **(Hamilton, 1822)**



*Salmostoma bacaila*  **(Hamilton, 1822)**



*Garra  gotyla*  **(Gray, 1830)**

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*Cirrhinus reba* (**Hamilton, 1822**)



*Cirrhinus cirrhosus* (**Bloch, 1795**)



*Osteobrama cotio cotio* (**Hamilton, 1822**)



*Mystus cavasius* (**Hamilton, 1822**)



*Rita rita* (**Hamilton, 1822**)



*Sperata seenghala* (**Sykes, 1839**)



*Parambassis ranga* (**Hamilton, 1822**)



*Polyacanthus fasciatus* (**Bloch & Schneider, 1801**)
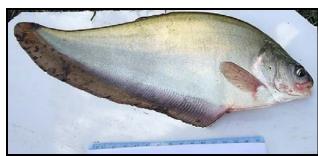
*Heteropneustes fossilis* **(Bloch, 1794)**



*Gagata cenia* (**Hamilton, 1822)**



*Oreochromis niloticus niloticus* **(Linnaeus, 1758)**



*Notopterus notopterus* **(Pallas, 1769)**



*Channa punctata* **(Bloch, 1793)**



*Wallago attu* **(Bloch & Schneider, 1801)**



*Mastacembelus armatus* **(Hamilton, 1822)**



*Xenentodon cancila* **(Hamilton, 1822)**

Plate 5.1    Fish species captured from Nullah Aik and Nullah Palkhu during study period.

Table 5.1    Richness (S), evenness (E), Simpson's diversity (H) and Shannon diversity (D) indices of (a) site and (b) fish species from Nullah Aik and Nullah Palkhu

(a)

| Season | Post monsoon | | | Pre monsoon | | |
|---|---|---|---|---|---|---|
| Site # | S | H | D` | S | H | D` |
| 1 | 14 | 2.20 | 0.86 | 8 | 1.4 | 0.67 |
| 2 | 14 | 2.29 | 0.88 | 13 | 2.19 | 0.85 |
| 5 | 4 | 1.22 | 0.65 | 0 | 0 | 0 |
| 6 | 4 | 1.15 | 0.62 | 1 | 0 | 0 |
| 7 | 7 | 1.47 | 0.68 | 3 | 0.99 | 0.60 |
| 8 | 3 | 1.01 | 0.61 | 1 | 0 | 0 |
| 9 | 4 | 1.09 | 0.58 | 1 | 0 | 0 |
| 10 | 7 | 1.76 | 0.80 | 4 | 1.23 | 0.66 |
| 11 | 5 | 1.39 | 0.73 | 2 | 0.68 | 0.48 |
| 13 | 1 | 0 | 0 | 0 | 0 | 0 |
| 15 | 13 | 2.16 | 0.84 | 3 | 1.02 | 0.62 |
| 16 | 10 | 1.99 | 0.83 | 3 | 0.9 | 0.56 |
| 17 | 12 | 2.10 | 0.84 | 1 | 0 | 0 |
| 18 | 5 | 1.39 | 0.72 | 2 | 0.34 | 0.19 |

(b)

| Family | Species | Post monsoon | | | Pre monsoon | | |
|---|---|---|---|---|---|---|---|
| | | S | H | D` | S | H | D` |
| Ambassidae | *Parambassis ranga* | 2 | 0.29 | 0.15 | 1 | 0 | 0 |
| Bagridae | *Mystus cavasius* | 7 | 1.73 | 0.80 | 2 | 0.66 | 0.47 |
| | *Rita rita* | 4 | 1.32 | 0.72 | 1 | 0 | 0 |
| | *Sperata seenghala* | 2 | 0.56 | 0.37 | 0 | 0 | 0 |
| Belonidae | *Xenentodon cancila* | 2 | 0.63 | 0.44 | 1 | 0 | 0 |
| Channidae | *Channa punctata* | 12 | 2.17 | 0.86 | 8 | 1.69 | 0.78 |
| Cichlidae | *Oreochromis niloticus* | 5 | 1.30 | 0.68 | 2 | 0.38 | 0.22 |
| Cyprinidae | *Cirrhinus cirrhosus* | 5 | 1.49 | 0.75 | 1 | 0 | 0 |
| | *Cyprinus carpio* | 5 | 1.46 | 0.74 | 0 | 0 | 0 |
| | *Cirrhinus reba* | 1 | 0 | 0 | 3 | 0.80 | 0.49 |
| | *Garra gotyla* | 1 | 0 | 0 | 0 | 0 | 0 |
| | *Labeo calbasu* | 1 | 0 | 0 | 1 | 0 | 0 |
| | *Labeo dero* | 1 | 0 | 0 | 1 | 0 | 0 |
| | *Labeo rohita* | 4 | 1.18 | 0.64 | 2 | 0.58 | 0.39 |
| | *Osteobrama cotio* | 2 | 0.69 | 0.49 | 1 | 0 | 0 |
| | *Puntius ticto* | 11 | 2.22 | 0.87 | 9 | 2.06 | 0.85 |
| | *Puntius sophore* | 12 | 2.29 | 0.88 | 4 | 1.11 | 0.59 |
| | *Salmostoma bacaila* | 2 | *0.59* | *0.40* | *2* | *0.41* | *0.24* |
| Heteropneustidae | *Heteropneustes fossilis* | 7 | 1.69 | 0.79 | 2 | 0.69 | 0.49 |
| Mastacembelidae | *Mastacembelus armatus* | 1 | 0 | 0 | 0 | 0 | 0 |
| Notopteridae | *Notopterus notopterus* | 4 | 1.27 | 0.69 | 0 | 0 | 0 |
| Osphronemidae | *Polyacanthus fasciatus* | 4 | 1.30 | 0.71 | 0 | 0 | 0 |
| Siluridae | *Wallago attu* | 7 | 1.77 | 0.81 | 1 | 0 | 0 |
| Sisoridae | *Gagata cenia* | 1 | 0 | 0 | 0 | 0 | 0 |
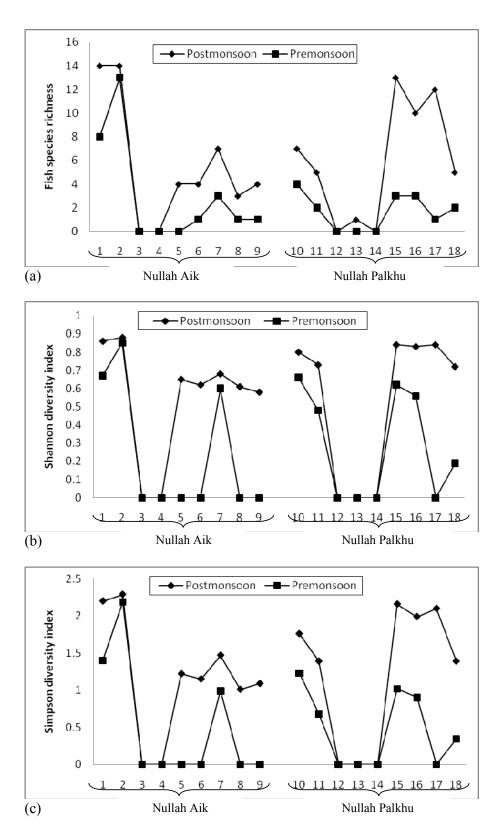
Fig. 5.1   (a)Species richness, (b)Shannon diversity Indices and (c) Simson diversity indices recorded from various sites recorded from Nullah Aik and Nullah Palkhu.

Invertivore fishes were generally small sized fishes, which mainly feed on insect larvae and other small invertebrates. Among invertivores, two species (*Puntius ticto* and *Puntius sophore*) were found as ubiquitous species in upstream and downstream sites, whereas, *Osteobrama cotio* and *Parambassis ranga* showed restricted distribution in upstream of Nullah Aik. *Polyacanthus fasciatus,* which an invertevore species was distributed from upstream and downstream of Nullah Palkhu, whereas, no specimen of this species was captured from Nulluh Aik. Five herbivore fish species were sampled throughout the study area. Two species (*Labeo calbasu* and *Labeo dero*) were restricted in upstream of Nullah Aik, whereas, other three species (*Labeo rohita, Cirrhinus reba* and *Cirrhinus cirrhosus)* were ubiquitous in upstream and downstream of studied streams. Omnivore trophic group of fishes was consisting of four species. Out of these, two species (*Oreochromis niloticus* and *Salmostoma bacaila*) were common in up and downstream sites but preferably present in upstream sites. One omnivore species (*Garra gotyla*) was restricted to downstream of Nullah Aik, whereas, *Heteropneustes fossilis* was common in downstream of Nullah Aik and Nullah Palkhu. The largest trophic guild was carnivore species consisting of nine fish species. Among these carnivore species, *Channa punctata, Mystus cavasius, Rita rita* and *Wallago attu* were distributed in upstream and downstream of Nullah Aik and Nullah Palkhu. *Sperata seenghala* and *Xenentodon cancila* were distributed at upstream sites, whereas, *Mastacembelus armatus, Gagata cenia* and *Notopterus notopterus* were restricted in downstream of Nullah Palkhu.

The relative abundance of feeding groups of fish in up and downstream of streams in post monsoon and pre monsoon is given in Table 5.2. Among these feeding groups, maximum relative abundance of herbivores (54.2%) captured from upstream of Nullah Aik, whereas, no herbivorous species was recorded from downstream of Nullah Aik and Palkhu during pre monsoon season. Highest relative abundance of invertivores (57.4%) was recorded from downstream of Nullah Palkhu during pre monsoon. Insectivorous individuals were more abundant in upstream during post monsoon, whereas, abundant in downstream sites during pre monsoon. Highest relative abundance of omnivores (21.6%) was observed in downstream of Nullah Aik during pre monsoon season. Carnivore species were more abundant in downstream zone throughout the sampling period.

Table 5.2    Relative abundance of different feeding group in up and downstream of Nullah Aik and Nullah Palkhu

| Season | Sampling zone | Feeding group (%) | | | |
|--------|---------------|-----------|-----------|-------------|----------|
| | | Carnivore | Herbivore | Invertivore | Omnivore |
| **Post** | Upstream of Nullah Aik | 20.2 | 16.8 | 49 | 14 |
| **monsoon** | Downstream of Nullah Palkhu | 46.7 | 6.1 | 28 | 19.2 |
| | Upstream of Nullah Palkhu | 34.3 | 8.7 | 54.7 | 2.1 |
| | Downstream of Nullah Palkhu | 49.4 | 7.4 | 28.4 | 14.8 |
| **Pre** | Upstream of Nullah Aik | 8.5 | 54.2 | 25.5 | 11.9 |
| **monsoon** | Downstream of Nullah Palkhu | 56 | 0 | 21.6 | 21.6 |
| | Upstream of Nullah Palkhu | 52.5 | 10 | 37.5 | 0 |
| | Downstream of Nullah Palkhu | 27.7 | 0 | 57.4 | 14.8 |

## Environmetal Relationship of Fish Assemblage during post monsoon and pre monsoon

CCA was applied on 13 significant environmental variables to highlight their relationship with distribution of fish during post monsoon and pre monsoon in studied streams. The ordination plot of site and species scores produced from CCA shows the distribution of sites and species in ordination (Fig. 5.2 & 5.3).

## Post Monsoon

First three axes of sites ordination for post monsoon accounted 51.38% of total variance with more than 0.1 eigenvalues (Table 5.3a). First axis explained about 29.94 % of the total variations with 0.4 eigenvalue and was correlated to COD (r = 0.59), Pb (r = 0.51) and DO (r = -0.90). Axis 2 descibed 11.6 of the total variations with 0.15 eigenvalue was correlated to stream width (r = 0.67), Cr (r = 0.59) and stream depth (r = 0.75) (Table 5.3b).

CCA ordinations for sampling sites during post monsoon season are given in Fig 5.2a-d). The sampling sites located at upstream of Nullah Aik and Nullah Palkhu were closely associated and grouped together on the left side of CCA axis 1. Furthermore, sampling sites located in downstream of Nullah Aik and Nullah Palkhu on the right side of CCA axis 1. The sites located in downstream of Nullah were found to be closely associated with each other and showing the similar composition of fish assemblages, whereas, sites located at downstream of Nullah Aik were dispersed on right side of CCA axis 1. These dispersed sites indicate variations in composition of

fish assemblages of sites. Therefore, sites located close to each other on CA plot exhibit the same fish species composition than sites located far from each other and showed differences in fish species composition. Stream flow, stream width, DO and $NO_3^-$ and Pb were associated with upstream site of Nullah Aik and Nullah Palkhu, whereas, stream depth, COD, Na, Ca, Fe, Cr and Cu were related with downstream of Nullah Aik and Nullah Palkhu.

CCA species ordination (species x environment) identified three different groups of fish species for post monsoon (Fig. 5.2c-d). Group 1 comprised of species, which were dwelling in upstream site consisting of seven species (*Parambassis ranga, Labeo rohita, Labeo dero, Labeo calbasu, Osteobrama cotio, Xenentodon cancila* and *Sperata seenghala*) highly correlated to DO, $NO_3^-$ and stream flow. Out of seven, five species (*Parambassis ranga, Labeo rohita, Labeo dero, Labeo calbasu* and *Osteobrama cotio*) belonged to family Cyprinidae, whereas, remaining two *Xenentodon cancila* and *Sperata seenghala* represented the families Belonidae and Bagridae, respectively. Ten species (*Cirrhinus reba, Cirrhinus cirrhosus, Puntius sophore, Puntius ticto, Salmostoma bacaila, Mystus cavasius, Rita rita, Channa punctata, Oreochromis niloticus* and *Wallago attu*) were widely distributed in upstream and downstream and clustered in group 2. Among these species, species such as *Cirrhinus reba, Cirrhinus cirrhosus, Puntius sophore, Puntius ticto* and *Salmostoma bacaila* were represented by family Cyprinidae, whereas, *Mystus cavasius* and *Rita rita* belonged to family Bagridae. Remaining four species viz; *Channa punctata, Oreochromis niloticus* and *Wallago attu* belonged to families viz; Channidae, Cichlidae and Siluridae, respectively. These species were ubiquitous in upstream and downstream and located at the centre of the biplot (Fig. 5.2c & d). Group 3 was represented by *Cyprinus carpio, Garra gotyla, Heteropneustes fossilis Mastacembelus armatus, Notopterus notopterus* and *Gagata cenia* belonged to Cyprinidae, Mastacembelidae, Notopteridae and Sisoridae, which were distributed in downstream sites and absent at upstream sites. Species belonging to third group were tolerant to COD, turbidity, Na, Ca, Fe, Pb, Cr and Cu.

## Pre Monsoon

CCA plot ordination of pre monsoon season explained about 37.48% of the total variation for first three axes with more than 0.1 eigenvalues (Table 5.3a). First

axis explained 37.5% variation with 0.68 eigenvalue and positively correlated with stream flow (r = 0.56), turbidity (r = 0.65), nitrates (r = 0.66), Na(r = 0.82) and Cr (r = 0.86), whereas, negative with DO (r = -0. 90). Second axis contributed about 20.30% of the total variations with 0.36 and negatively correlated with stream flow (r = -0.75) and nitrate (r = -0.55). COD (r = -0.65) exhibited positive correlation with third axis that explained about 12.9 % variation with 0.23 eigenvalue (Table 5.3b).

CCA plot ordination for pre monsoon, sites located at upstream of Nullah Aik were grouped together on the left side of CCA axis1 and were strongly influenced with Stream flow, DO and $NO_3^-$. Sites located in upstream of Nullah Palkhu, downstream of Nullah Aik and Palkhu were plotted on right side of CCA axis 1. The upstream sites of Nullah Aik were correlated with stream width, DO and $NO_3^-$ . Stream depth, flow, COD, turbidity, Na, Ca, Fe, Cr and Cu were related to downstream sites of studied streams.

CCA species ordination provides pattern of fish assemblage (Fig. 5.3c & d). Three clusters of fish species were recognized. First group was represented by nine species of upstream sites (*Cirrhinus cirrhosus, Cirrhinus reba, Labeo dero, Labeo calbasu, Labeo rohita, Osteobrama cotio, Parambassis ranga, Xenentodon cancila,* and *Wallago attu)*. Out of nine species, six species (*Cirrhinus cirrhosus, Cirrhinus reba, Labeo dero, Labeo calbasu, Labeo rohita* and *Osteobrama cotio)* were represented by family Cyprinidae, whereas, remaining four species (*Parambassis ranga, Xenentodon cancila* and *Wallago attu*) belong to families Ambassidae, Belonidae and Siluridae, respectively. These species were positively correlated with stream width, DO and $NO_3$. Second group comprized of three species (*Channa punctata, Puntius sophore* and *Puntius ticto)*, which was evenly distributed in up and downstream sites. Third group was represented by one fish species (*Heteropneustes fossilis*), which belongs to family Heteropneustidae restricted in downstream sites. Fish species showed correlation with COD, turbidity, Na, Ca, Fe, Pb, Cr and Cu. CCA plot for pre monsoon season differ from pre monsoon. The upstream fish species did not show significant variation, whereas, significant reduction in fish species richness in second group (ubiquitous species) and in third group (downstream cluster) during pre monsoon. However, species in first group showed an increase in number of species and restricted to upstream area during pre monsoon season.

Table 5.3a    Results of the canonical correspondence analysis on species abundance and environmental variables on 14 sites located on Nullah Aik and Nullah Palkhu

| Season | Post monsoon | | | Pre monsoon | | |
|---|---|---|---|---|---|---|
| Axis | Axis 1 | Axis 2 | Axis 3 | Axis 1 | Axis 2 | Axis 3 |
| Eigen values | 0.40 | 0.15 | 0.13 | 0.68 | 0.36 | 0.23 |
| Variance in species data | 29.94 | 11.60 | 9.83 | 37.48 | 20.30 | 12.9 |
| % of variance explained | 29.94 | 41.54 | 51.38 | 37.48 | 57.79 | 70.69 |
| Cumulative % explained | 45.88 | 63.65 | 78.73 | 44.96 | 69.32 | 84.80 |
| Correlation, Spp-Envt* | 0.97 | 0.93 | 0.91 | 0.97 | 0.98 | 0.88 |

* Species vs Environment

Table 5.3b    Canonical coefficients (A) and Inter-set correlations (B) of environmental variables canonical correspondence analysis on species abundance and environmental variables matrices in Nullah Aik and Nullah Palkhu.

| Season | Post monsoon | | | | | | Pre monsoon | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Axis | Axis 1 | | Axis 2 | | Axis 3 | | Axis 1 | | Axis 2 | | Axis 3 | |
| | A | B | A | B | A | B | A | B | A | B | A | B |
| S. Width | -0.52 | -0.17 | 0.77 | **0.67*** | 0.33 | 0.43 | -0.21 | -0.22 | 0.15 | -0.9 | 0.76 | 0.16 |
| S. Flow | 0.12 | -0.08 | -0.46 | -0.08 | 0.3 | 0.43 | 0.65 | **0.56*** | -0.97 | 0.14 | -0.82 | 0.35 |
| S. Depth | 0.46 | 0.32 | 0.49 | **0.75*** | -0.24 | -0.13 | 0.53 | 0.4 | -1.45 | **-0.50*** | -1.63 | -0.06 |
| DO | -0.84 | **-0.90*** | 0.12 | 0 | -0.63 | -0.19 | -0.73 | **-0.90*** | 0.22 | -0.01 | 0.55 | -0.23 |
| COD | -0.03 | **0.59*** | -0.03 | -0.1 | 0.21 | 0.16 | -0.5 | 0.37 | 0.37 | -0.1 | 1.04 | **0.65*** |
| Turbidity | 0.14 | 0.42 | 0.13 | 0.02 | -0.78 | -0.16 | -0.05 | **0.65*** | 0.54 | -0.32 | 1.25 | 0.41 |
| NO$_3$ | 0.2 | -0.3 | -0.03 | 0.05 | 0.65 | 0.47 | 0.15 | **-0.66*** | -1.33 | **-0.55*** | -0.9 | 0.14 |
| Na | -0.24 | 0.42 | 3.71 | 0.12 | 0.53 | -0.24 | 2.29 | **0.82*** | -1.05 | -0.08 | 8.66 | -0.38 |
| Ca | -0.89 | 0.04 | -1.17 | -0.39 | 0.54 | 0.25 | -0.88 | 0.08 | 0.82 | -0.18 | -3.69 | -0.09 |
| Fe | 0.80 | 0.48 | -0.81 | -0.19 | 1.79 | -0.18 | -1.34 | 0.40 | 1.04 | -0.36 | -6.98 | -0.45 |
| Pb | -0.47 | **0.51*** | -1.53 | 0.27 | 1.16 | -0.18 | 0.80 | 0.40 | -1.43 | -0.19 | 2.95 | -0.35 |
| Cr | -0.20 | -0.07 | 0.32 | **0.57*** | -0.08 | -0.41 | -3.00 | **0.86*** | 4.15 | 0.05 | -16.18 | -0.04 |
| Ni | 0.72 | 0.02 | 3.39 | 0.08 | 3.13 | 0.00 | 0.07 | -0.12 | -0.67 | 0.05 | -0.96 | -0.17 |
| Cu | -1.39 | 0.28 | 0.55 | -0.21 | -2.54 | -0.24 | 1.05 | 0.49 | -1.25 | 0.35 | 3.12 | 0.27 |

**(a)**

Upstream of Nullah Aik
Downstream of Nullah Aik
Upstream of Nullah Palkhu
Downstream of Nullah Aik

Axis 2

COD
S.Flow
DO   NO3   Turbidity
S.Width
S. Depth

Axis 1

**(b)**

Pb
Cr
Na
Axis 2
Fe
Cu
Ca

Axis 1

**Group 1 (upstream)**
*Parambassis ranga, Sperata seenghala, Xenentodon cancila Labeo dero,Labeo calbasu* and *Osteobrama cotio.*

**Group 2(Ubiquitous species)**
*Mystus cavasius, Rita rita, Channa punctata, Oreochromis niloticus, Cirrhinus reba, Cirrhinus cirrhosus, Polyacanthus fasciatus, Labeo rohita, Puntius sophore, Puntius ticto, Salmostoma bacaila*, and *Wallago attu*

**(c)**

Upstream group          Ubiquitous group
Sal bas
Lab cal
Lab der          Lab roh   Pun sop   Mac pan   COD
Spe see          Ore nil   Pun ti   Pol fas   Gag cen
                 Cir cir   Rit rit Not not   Turbidity
DO   NO3   S.Flow   Wal attu Cha pun
Ost cot   Mys cav   Het fos
Par ran   Cyp reb          Cyp car
Xen can                    Gar got
S.Width                    Downstream group
S. Depth

Axis 2
Axis 1

**Group 3 (downstream)**
Cyprinus carpio *Garra gotyla Heteropneustes fossilis Macrognathus pancalus Notopterus notopterus* and *Gagata cenia*

**(d)**

Pb          Ubiquitous group
Upstream group
Sal bas          Rit rit          Cr
Par ran   Xen can   Pun sop   Mac pan   Na
Ost cot   Mys cav   Cyp reb   Pun tic   Not not Cyp car
Spe see          Cir cir   Pol fas Cha pun
Lab der   Ore nil   Wal att   Het fos
Lab cal Lab roh          Gag cen   Gar got   Fe
Ca                      Cu
                        Downstream group
Axis 2
Axis 1

Fig. 5.2    Canonical correspondence analysis (CCA) plots showing site scores (a &b) and species (c &d) and effect of environmental factors on fish assemblages during postmonsoon.
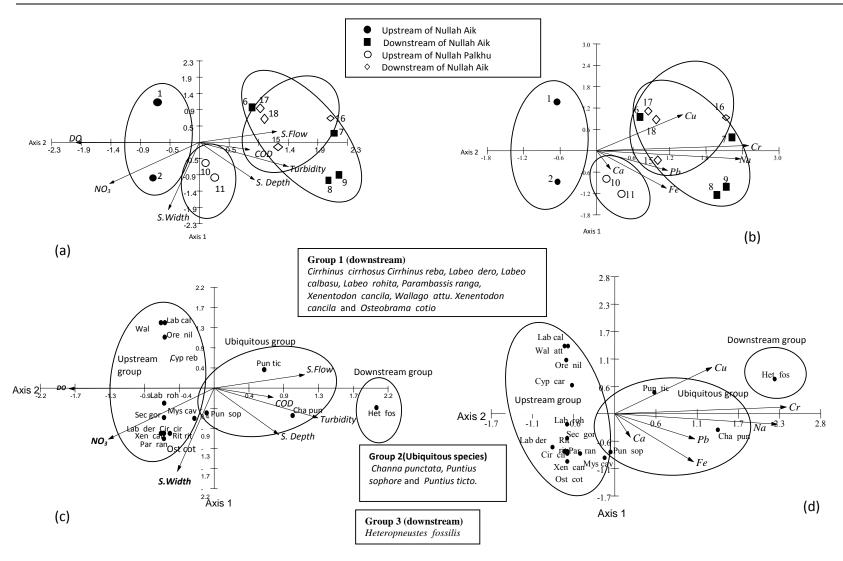
Fig. 5.3 Canonical correspondence analysis (CCA) plots showing site scores (a & b) and species (c & d) and effect of environmental factors on fish assemblages during premonsoon.

## Discussion

Fish responds to changes in its environment whether it is human induced or natural (Han, *et al.*, 2007). Local environmental factors play a vital role in structuring stream fish assemblage (Pires *et al.*, 1999) and have significant impacts on stream habitat as well as fish assemblage (Wang *et al.*, 2001). Poor water quality is one of the important factor, which has been reported to alter abiotic and biotic component of intermittent streams (Moses & Morris, 1998; Davis *et al.*, 2003). During present study, studied streams are facing natural and anthropogenic disturbances. Natural disturbances are mainly based on climatic and geomorpholoical factors such as stream flow, depth, width and rainfall pattern in the catchment area, whereas, discharge of industrial effluents and municipal sewage are resulted due to human activities, which remain throughout the year. During present study, major changes in fish assemblage were resulted due to discharge of industrial effluents and municipal sewage from Sialkot city. The severe impacts of anthropogenic activities were observed at sites (3, 4, 12 and 14) located in close vicinity of Sialkot, where no fish specimen was captured. The continuous human stress on streams can completely vanish the whole fish fauna, whereas, pristine streams exhibited little variations in fish assemblage over long time period (Paller, 2002).

Deterioration of water quality due to anthropogenic activities has contributed in replacement and disappearance of sensitive fish species in streams and rivers (Karr *et al.*, 1986). Degradation of water quality increases stress on fish assemblage that reduces species diversity and abundance (Pollino *et al.*, 2004). Boët *et al.* (1994) and Belliard *et al.* (1999) highlighted that water quality degradation in downstream of urban stream, profoundly affects distribution and movements of fishes. Extinction or missing of any species provokes the ecological disturbance at the community as well as ecosystem level. During present study, fish assemblage in upstream of Nullah Aik was stable in post monsoon and pre monsoon season, whereas, fish assemblage in upstream of Nullah Palkhu became affected due to reduced stream discharge, which reduce the diversity of fishes during pre monsoon. The fish assemblage in downstream of Nullah Aik and Nullah Palkhu are severely affected from increased level of pollutant in stream water. Highest fish diversity was recorded at upstream sites of Nullah Aik during post monsoon. In upstream of studied streams, 18 fish species (*Parambassis ranga, Sperata seenghala, Xenentodon cancila Labeo dero, Labeo*

*calbasu, Osteobrama cotio, Mystus cavasius, Rita rita, Channa punctata, Oreochromis niloticus, Cirrhinus reba, Cirrhinus cirrhosus, Polyacanthus fasciatus, Labeo rohita, Puntius sophore, Puntius ticto, Salmostoma bacaila* and *Wallago attu)* were captured and species diversity reduced to 13 fish species (*Cirrhinus cirrhosus, Cirrhinus reba, Labeo dero, Labeo calbasu, Labeo rohita, Parambassis ranga, Xenentodon cancila, Wallago attu, Channa punctata, Puntius sophore, Puntius ticto* and *Osteobrama cotio*) in pre monsoon. Highest variations in species richness were recorded in downstream of Nullah Palkhu. Eighteen fish species were recorded in downstream during post monsoon, whereas, only four fish species (*Channa punctata, Puntius sophore*, *Puntius ticto and Heteropneustes fossilis*) were recorded from down stream. Similarly, Paul & Meyer (2001) and Gafny *et al.* (2000) reported significant reduction in fish diversity during dry season at close downstream sites of cities. Decline in fish diversity and abundance become more pronounced in downstream of towns and cities, however, the relative abundance of tolerant taxa increases in moderately disturbed downstream (Paul & Meyer, 2001). In response to pollution, sensitive species disappear sharply, whereas, individuals of tolerant fish species can continue to exist in polluted water up to some extent (Gafny *et al.,* 2000).

Gradual increase in species richness and distribution of fish assemblage, from upstream to downstream have been reported in pristine streams and rivers (Bhat, 2004; Lima-Junior *et al.*, 2006), however, in intermittent streams, species richness decreases with increase in anthropogenic activities (Paul & Meyer, 2001). The results showed that Nullah Aik has maximum diversity in upstream region that reaches to its ebb (zero fish catch) at sampling sites (3 and 4) near to Sialkot city, which receive high pollutant load from industries and municipalities. In downstream of Nullah Aik, some species like *Channa punctata, Puntius sophore* and *Wallago attu* reappear to establish fish assemblage. According to Bhat (2004) natural streams become modified due to municipal and industrial effluents as well as construction of barriers for divergences of water. Presence of small head works at site 7 (Wain) is also an important reason for reduction of the fish diversity in downstream. Stream water of Nullah Aik is diverted into sub-channels and hundreds of pumps have been established on stream banks to suck stream water for the irrigation purpose. Reduced of stream flow at downstream of Wain cannot support fish assemblage to re-establish, resulting less diversity in downstream of Nullah Aik.

Streams and rivers exhibit changes in distribution of different feeding groups along longitudinal gradient (Bhat, 2004). High relative abundance of herbivore species in upstream was due to presence of algae and other aquatic plants on which these species feed. During pre monsoon season, herbivore fishes shift towards upstream segment due to high load of pollutants in downstream segment. According to Ganasan & Hughes (1998) herbivore species are sensitive as compared to invertivore, which disappears as pollution stress increases. Bhat (2004) also reported that insectivorous species are ubiquitous in upstream and downstream of rivers. The results indicated seasonal variations in distribution of invertivore species. Hoyt *et al*. (2001) reported that concentration of specific feeding guild at upstream or downstream depends upon the feeding and reproductive activities. Omnivores and carnivores were found in up and downstream but preferably present at downstream sites. Bhat (2004) calculated higher relative abundance of carnivore in downstream sites. Generally, carnivore species are sensitive and respond to any change in water quality. However, carnivore species are also tolerant like *Channa punctata* can survive in unfavaourable conditions by developing some adaptation to survive in turbid condition and low level of oxygen (Anctil & Ali, 1976; Narayanan & Khan, 1995).

Streams exhibited great variations in stream morphological conditions influenced by stream size and discharge (Vlach *et al*., 2005). Stream morphological parameters include water flow; depth and width greatly influence the fish community assemblages and are critical in the maintenance of fish populations (Pires *et al.,* 1999; Paul & Meyer, 2001). Bhat (2004) showed stream depth is correlated with species richness in the stream of Western Ghat, India. Stream depth and width contributes in structuring the fish assemblage (Lima-Junior *et al*., 2006). Stream depth also affects on fish assemblage in streams facing regular wet and dry seasons (Mesquita *et al*., 2006) and provides good spawning, feeding habitats and protection from predation (Angermeier & Winston, 1998; Jackson *et al.,* 2001a). During present study, maximum depth was observed during post monsoon while minimum in pre monsoon season due to variations in stream discharge. Stream sites exhibited depth ranged from 0.5 - 2.0m except, site 7.

Stream flow highly influences chemical properties of water and community patterns of fishes (Reash & Pigg, 1990). The structure of fish assemblage is characterized by the fluctuation in stream flow that is influenced by rainfall in the catchment area (Fraser, 1997; Castillo-Rivera *et al*., 2002). Stream flow has

significant effects on species composition, diversity and reproduction (Xenopoulos *et al.*, 2005). Regular stream flow provides better conditions for stability of fish communities. Low stream flow causes reduction in diversity of fishes in intermittent streams (Thompson & Larsen, 1994). Lowest stream flow rate was recorded in upstream of Palkhu during summer season. Reduction in stream flow affects the species richness and diversity in upstream of Nullah Palkhu and during dry period, fishes restrict to ditches and small pools. These ditches and small pools act as refuge sites, when water flow re-establishes in streams, fishes proliferate to maintain their population (Pires *et al.*, 1999). Only four species (*Channa punctata, Puntius sophore, Puntius ticto* and *Heteropneustes fossilis*) were collected from downstream sites, which can survive at low level of DO. *Cirrhinus cirrhosus Cirrhinus reba, Labeo rohita, Wallago attu* and *Osteobrama cotio)* were common species in upstream and downstream during post monsoon but restrict themselves in upstream due to input of pollutants in pre monsoon. Streams flow with highest discharge level due to heavy rains in the catchment area during monsoon season. High stream water flow dilutes concentration of pollutants and allows the fish to move from river to upstream for spawning and breeding. Movement of fishes from river to streams during monsoon facilitates the re-colonization of fish species in upstream region. After monsoon season catchment area experiences dry spell resulting reduced stream. High discharge of pollutants from industries and urban sewage acts as barrier, which restrict the sensitive fishes in upstream sites.

DO is one of the important variable in explaining the distribution of species (Fraser, 1997; Castillo-Rivera *et al.,* 2002). Depletion of DO makes the habitat unsuitable for fish life (Slavík & Bartŏs, 2001). In streams and rivers, DO define the pattern of fish assemblage on temporal and spatial scale (Mathews & Berg, 1997). DO play a vital role in all developmental stages of fish from embryo to adult (Thompson & Larsen, 1994). Low concentration of DO (below 4mg/L) causes reduced growth rate increases the risk of disease and even death (Thompson & Larsen, 1994).  Level of DO (2.0 mg/L) in aquatic ecosystem is the minimum level to prevent the mortality of fish Abegaz *et al.* (2005). Intolerant species cannot withstand extreme fluctuations of DO. In disturbed stream; sensitive fishes disappear first or shift to least disturbed conditions. Disappearance of sensitive species creates the space for tolerant and exotic species to proliferate in such streams. The results showed that sites with less amount of DO were least diverse. Depletion of DO is mainly caused by organic pollution

resulting in the process of decomposition of organic component inducing instability of DO concentration (Slavík & Bartŏs, 2001) and cause high COD value. High COD values determine chemical and organic pollution that severely affect the fish assemblage (Gafny *et al.,* 2000).

High turbidity level was recorded at downstream sites due to discharge of untreated effluents and sewage from Sialkot city. Preference of fish species varies with the change in the level of turbidity in stream water (Ludsin *et al.,* 2001). Turbidity affects water color and reduces light penetration, which ultimately change the composition of fish assemblage (Akin *et al.,* 2005). According to Costa *et al.* (2007) turbidity can influenced on the distribution of fishes in stream. Stream flow directly affects stream turbidity level, which may restrict fish distribution and movement of fish during breeding season. In present study, generally cyprinids species preferred to live in clear water, whereas, *Channa punctata* and *Heteropneustes fossilis* were found at turbid site with high relative abundance. *Channa punctata* and *Heteropneustes fossilis* which are tolerant species can survive in turbid condition (Ganasan & Hughes, 1998).
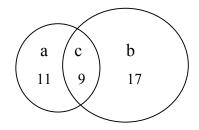
The concentration of nitrates in post monsoon and pre monsoon seasons was found correlated with upstream sites of Nullah Aik and Nullah Palkhu. The high concentration of nitrates in upstream sites was mainly contributed by fertilizers used in agricultural land (Rashleigh, 2004). Nitrates make their way into stream through surface runoff after heavy irrigation and rainfalls. Higher concentration of nitrates instream increases the production of native fishes because nitrates increase the production of aquatic plant especially algae (Wolgast & Stout, 1977). However, excessive amount of nitrates in stream may cause eutrophication (Addiscott, 1996).

High concentration of metals recorded in downstream sites have negative impacts on the fish assemblage, whereas, upstream sites with low level of metals represented higher number of fish species. In post monsoon season, stream discharge level becomes high, which dilute the concentration of heavy metals in stream water coming from industrial effluents and municipal sewage. During this season, fishes showed wide distribution in downstream sites especially in Nullah Palkhu, whereas, in pre monsoon season, only four species were recorded in downstream of Nullah Palkhu. Similar results were obtained by Tawari-Fufeyin & Ekaye (2007) in polluted Ikpoba River, Nigeria. This reduction in species richness in downstream sites could be attributed by human activities in the catchment area. Qadir *et al.* (2008) reported that

downstream sites of Nullah Aik and Nullah Palkhu are heavily polluted by industrial effluents coming from Sialkot city. These effluents contain many toxic metals such as Pb, Cd and Cr. The sensitive species showed strong negative correlation with heavy metals, if concentration of metals becomes high, fishes migrate to less polluted segment of river or stream (Tawari-Fufeyin & Ekaye, 2007). Svecevièius (1999) showed experimentally that sensitive fishes always avoid living in contaminated waters with heavy metals. However, some tolerant species have developed the tendency to tolerate the heavy metals up to certain limits. The reduction of fishes in downstream is due to increasing industrial and urban pressure (Gafny *et al.,* 2000). Pinto *et al.* (2006) reported significant reduction in species richness and abundance of fishes in downstream of Paraíba do Sul River in Brazil. Pfeiffer *et al.* (1986) studied the loss of fish species and reduction in diversity of Fish species in downstream of Volta Redonda city. Heavy metal pollution in stream has significant negative impacts on structure of fish assemblage in downstream of Nullah Aik and Nullah Palkhu. Similar results were obtained by Snodgrass & Meffe (1998) and Magalhães *et al.* (2002).
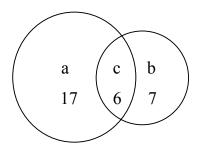
Seventeen fish species were recorded from upstream sites, whereas, 11 species were captured from downstream sites of Nullah Aik. Among these species, nine were common to upstream and downstream sites (Fig. 5.4). Seven species were recorded from upstream sites while seventeen species from downstream of Nullah Palkhu, whereas, six species were found in upstream as well as downstream of Nullah Palkhu. High coefficient of community loss was calculated for Nullah Palkhu as compared to Nullah Aik (0.012).

Courtemanch & Davies (1987) and Wright & Welboum (2000) described three possible scenarios (acceptable, criteria needed and unacceptable) of stream conditions on the basis of fish communities in downstream segment (Table 5.4). According to criteria developed by Courtemanch & Davies (1987) Nullah Aik is facing partial loss and replacement of fish species at downstream sites. Partial loss of fish species was observed in Nullah Palkhu and downstream showed maximum fish species. The existing situation of fish diversity and water quality in studied streams are highly degraded. Complete loss of fish species has been observed at mid stream sites, which is an alarming situation and unacceptable from ecological point of view. Therefore, this situation needs urgent measure to stop indiscriminate anthropogenic activities in the catchment area to improve water quality and restore the fish communities.

Coefficient of community loss = 0.012

**Nullah Aik**



Coefficient of community loss = 1.57

**Nullah  Palkhu**

Fig. 5.4     Change in Scenarios of Fish Communities in up and
             downstream of Nullah Aik and Nullah Palkhu

| | | |
|---|---|---|
| No change | **a b c** | Acceptable |
| No loss of texa increased richness in downstream | **a c** **b** | Acceptable |
| Partial loss of texa Partial replacement in downstream | **a** **c** **b** | Certain managerial steps needed |
| Partial loss of texa Partial replacement in downstream | **a** **c** **b** | Certain managerial steps needed |
| Partial loss of texa No replacement | **a** **b c** | Certain managerial steps needed |
| Total loss of texa increased richness in downstream | **a** **b** | Unacceptable but improbable |
| Total loss of texa Partial replacement in downstream | **a** **b** | Unacceptable |
| Total loss of texa replacement in downstream | **a** | Unacceptable |

Fig. 5.5 Possible scenarios for the community loss in the downstream of pollution source (Courtemanch & Davis, 1987; Wright & Welbourn, 2002).

**Conclusions**

Present study highlighted that stream habitat structure, water quality parameters and metals highly influence the fish assemblage in Nullah Aik and Nullah Palkhu. CCA results indicated strong relationship between species and environmental factors such as stream flow, depth, width, DO, COD, turbidity, $NO_3^-$, Na, Ca, Fe, Pb, Cr and Cu. Highest fish species richness was recorded in upstream of Nullah Aik and minimum at downstream of Nullah Aik. The fish assemblage was studied in upstream of Nullah Aik was least disturbed where fish assemblage did not showed significant seasonal variations in term of fish diversity. Seasonal variations in fish assemblage were observed at downstream of Nullah Aik and Nullah Palkhu due to anthropogenic activities that change the stream habitat characteristics, degrade water quality and become unfavourable for fishes. Industrial effluents and municipal sewage from Sialkot acts as a barrier between upstream and downstream fish communities and restrict the movement of fishes between stream segments. Present study highlighted the impact of natural as well as human activities on the fish assemblage of Nullah Aik and Nullah Palkhu. The coefficient of fish community loss indicated that there must be management criteria to restore the fish species loss in Nullah Aik and Nullah Palkhu.

**Chapter 6**


# Assessment of Stream Health of Nullah Aik and Nullah Palkhu Using an Index of Biotic Integrity (IBI)


## Introduction

The biological response and sensitivity of different organisms/communities to physical or chemical changes such as flow, depth and width of stream, light, turbidity, temperature, organic and inorganic carbon, oxygen, nutrients, and metals can be used as an indicator for the assessment of habitat quality (Karr, 1991). Physical and chemical factors may be related directly or indirectly to biological changes (such as absence of native species, intrusion of non-native species, decline in diversity and productivity, loss of integrity in fish assemblage etc) and may contribute in complexity and integrity of streams and rivers (Mullins, 1999). Biological indicators have significant importance in evaluation of environmental degradation due to anthropogenic activities and have long history of their use (Simon & Lyons, 1995). The use of biological indicators in freshwater ecosystem is becoming popular (Davis & Simon, 1995; Joy & Death, 2004), which may reflect the intensity of anthropogenic stress and have been used as a tool in risk assessment and evaluation of human induced changes in fresh water ecosystem (Toham & Teugels, 1999).

Assemblages of freshwater organisms such as fish, macro-invertebrates, and periphytons which respond to both environmental contaminants and habitat changes are commonly used to assess the biotic condition of streams, rivers lakes and wetlands because the integrity of these assemblages provides a direct measure of ecological conditions of water resources (Ode *et al*., 2005). Fish have been regarded as an effective biological indicator of environmental quality and anthropogenic stress in aquatic ecosystems (Fausch *et al*., 1990; Simon, 1991) not only because of its iconic value, but also because of sensitivity to subtle environmental changes (Karr, 1981) and represents a wide range of tolerance at community level (Plafkin *et al*., 1989). Fish is sensitive to changes in water chemistry due to different anthropogenic activities from their catchment.

Fish responses to environmental disturbances, including hydro-morphological factors, are different in time and space in comparison to simpler organisms, as they tend to be integrated over larger intervals. Fish has been identified as suitable for biological assessment due to its easy identification and economic value (Simon & Lyons, 1995; Smith *et al*., 1999; Siligato & Böhmer, 2001). Fish assemblages have widely been used as ecological indicator to assess and evaluate the level of degradation and health of rivers and streams at various spatial scales (Zampella *et al.,* 2006). Plafkin *et al*., (1989) observed that there are many advantages of using fish assemblage as biological indicator. Most of the streams exhibit variations in different sections especially from upstream to downstream and these variations become more remarkable in streams facing problem of habitat degradation particularly from direct discharge from untreated industrial and municipal effluents (Qadir *et al*., 2008). Upstream section are generally less degraded with relatively less changed physical, chemical and biological conditions of habitats (Drake, 2004). Sites located in upstream section have generally been considered as reference sites and can be compared with rest of sites for assessment of stream health (Joy & Death, 2004). Fish assemblages may differ on longitudinal gradient in streams according to various biological aspects such as species diversity, stress tolerance, habitat preferences, feeding behaviour and origin of species (Ganasan & Hughes, 1998). These variations depict the level and severity of degradation in stream health.

Both multimetric and multivariate methods have been used to characterize biotic conditions and to establish thresh holds of ecological impairment (Ode *et al*., 2005. The Index of Biotic Integrity (IBI) is most commonly used to assess stream health based on biological criteria (Mebane *et al.,* 2003). It is an index for quantitative assessment of the biological quality/health of streams (Ganasan & Hughes, 1998) and was formulated to integrate information from various aspects of an ecosystem into numerical value that indicate the quality rating for streams, rivers, lakes etc., (Ganasan & Hughes, 1998). This criterion has been widely used to assess and categorize the condition of streams and rivers by studying the structure and composition of fish assemblage (Simon & Lyons, 1995; Bozzetti & Schulz, 2004). The concept of IBI was developed by Karr (1981) to assess the ecological status of streams and rivers in mid western United States and has been widely applied in other parts of the world with modification either addition or deletion of

biological metrics depending upon biological conditions of an ecosystem (Ganasan & Hughes 1998). Ganasan & Hughes (1998) applied IBI to determine level of degradation in two rivers such as Khan and Kshipra in India. Fausch *et al*. (1990) and Simon & Lyons (1995) applied IBI criteria to study lakes, streams and rivers with some modification based on regional, geographic and climatic variation in fish distribution and assemblage structure. Toham & Teugels (1999) used IBI to quantify the impact of deforestation on freshwater biodiversity in Central African tropical streams. Kleynhans (1999) developed IBI in the form of fish assemblage integrity index (FAII) using metrics to explain the habitat quality of fish assemblages in different segments of Crocodile River, South Africa. Araújo (1998) used IBI to recognize the impacts of the effluents (heavy metals, pesticides and organic solvents) on fish assemblage from industrial areas into *Paraiba do Sul* River. Bozzetti & Schulz (2004) highlighted the ecological conditions of sub-tropical streams using IBI in southern parts of Brazil. Fish-based IBI was used to assess upstream brooks in Flanders, Belgium (Breine *et al*., 2004). River conditions at varying spatial scales were also evaluated in New Zealand (Joy & Death, 2004). In central India, Ganasan & Hughes (1998) studied the impact of municipal and industrial effluents on fish assemblage based on IBI criterion.

The IBI criteria used in this study is a combination of twelve metrics of fish assemblage which are grouped into four categories viz., species diversity and composition; trophic composition; and fish abundance and health (Ganasan & Hughes, 1998). IBI metrics were derived from previous studies conducted by various workers in different regions of the world on streams and rivers. IBI was developed for two tributaries of river Chenab: Nullah Aik and Nullah Palkhu which are under facing environmental degradation due to indiscriminant industrial, urban and agricultural pollution. Theses streams are located in densely populated and industrialized region of Punjab, Pakistan. Major activities in upstream region are characterized by agriculture sector, whereas industrial and municipal activities highly impacted the middle and downstream regions.

The results of this chapter will highlight the need for better future protection of fish fauna in small streams from anthropogenic activities. This initiative will be helpful to measure fish assemblage characteristics that are responsive to human-induced environmental changes.

Main objective of this chapter was

- to develop IBI for the tributaries of river Chenab to assess the impact of anthropogenic activities on fish assemblage structure.
- to quantify the quality stream health of Nullah Aik and Nullah Palkhu, tributaries of river Chenab in term of index of biotic integrity.
- to investigate the spatial variations of IBI in relation to biological conditions of streams and water quality parameters.

## Materials and Methods

The details of fish and water sampling and chemical analysis of water quality parameters is given in Chapter 2.

**Selection of Reference sites**

Reference condition of aquatic water resources such as streams and rivers generally reflects natural conditions with minimal disturbance from anthropogenic activities. Reference condition explains the undisturbed biological, chemical and physical components of a site or group of sites. In ecological assessment of fresh water resources, identification of reference sites is vital to highlight, the impact of human activities on aquatic ecosystem (Zhu & Chang, 2008). Identification of reference sites helps in understanding the relationship between human disturbance and biological condition of a stream (Fritz, 2004) and to scale bio-metrics appropriately. Reference sites are the segments of water bodies that reflect the natural conditions and least effected by anthropogenic activities (Reynoldson *et al*., 1997). The choice of an appropriate reference condition is a major problem in all ecological assessments (Hughes, 1994). The knowledge of metric responses to human impact is also appropriate for the selection of reference conditions. The reconstitution of a reference fish assemblage based on historical data (Hughes, 1994) is impossible in the studied streams. Identification and selection of reference sites in present study was difficult as the catchment area of Nullah Aik and Nullah Palkhu is densely populated and its fish fauna is poorly known because ecological and historical information about fish assemblage is extremely scarce / absent. Quantitative physical, chemical, as well biological standards are needed against which streams/rivers in the region can be compared to determine the influence of cumulative stressor (Radwell & Kwak, 2005). Selection of reference sites was done based on evaluation of the biological, physical habitat, and water quality data of streams as suggested by USEPA (2005) and Springe *et al*. (2006). Similarly, Zhu & Chang (2008) also used cumulative data set based on physical, chemical, and biological conditions of upper Yangtze River to define reference sites for calculation of biotic integrity. There is no information exists with regard of reference site identification in Pakistan and there is

need to develop reference condition for streams and rivers. In present study, sites with minimal disturbance or least impairment or best conditions in term of fish communities in Nullah Aik and Nullah Palkhu were treated as reference sites. Reference sites possess a maximum IBI score (Bozzetti & Schulz, 2004) which further compared with other sites located on the same stream or sites located on other streams of the same eco-regions (areas of homogenous ecological systems or areas that have the potential for similar biological communities) to quantify the anthropogenic stress on biological integrity of the ecosystem. Selection of least-disturbed/impairment sites was further confirmed by the application of biological (fish) data to cluster analysis (CA).

## Statistical Analysis

### Assessment of Habitat Degradation and Identification of Reference Sites in relation to underlying Ecological Gradients

Sub-tropical and temperate streams are highly variables in fish diversity in different seasons throughout the year (Ganasan & Hughes, 1998). It was highlighted that fish composition of a single season gives inadequate information to evaluate the scores for IBI metrics. Usually seasonal changes in fish assemblages are not considered (Bozzetti & Schulz, 2004) since Karr *et al.* (1986) in his first IBI study (Karr, 1981) did not find temporal dependence. Bozzetti & Schulz (2004) highlighted that seasonal differences in species composition do not reflect to influence general IBI results. Other studies of temporal variability in IBI score have also indicated minimal effects from among years and within season fluctuations in species composition (Hughes *et al.*, 1998; McCormick *et al.*, 2001). Pyron *et al.* (2008) addressed within temporal variation of IBI scores for two sampling event and indicated non-significant differences in IBI scores. Therefore, all fish individuals captured during different seasons from same site were used for analysis (Ganasan & Hughes, 1998). Similarly, Kushlan (1976) also supported pooling up seasonal species composition collected from the same site for further analysis and suggested that information related to species composition collected during one season may leads to inaccurate assessment of stream health. Each individual site should be sampled for fish species abundance many times in year, especially when the life history of different fish species is less known (LoweMcConnell, 1987). Long & Walker (2005)

also combined the data collected from a site to avoid seasonal and inter-annual variations for further analysis.

Cluster Analysis (CA) was used to group the sites on the basis of similarities within fish assemblage and for identification of reference sites. Ward's method was selected as a linkage method and Euclidean matrix as dissimilarity distance measure. Same method has been used to characterize fish abundance data by McCune & Grace (2002) and Bozzetti & Schulz (2004) into classification of sites into groups with different level of habitat degradation. Non-metric multidimensional scaling (NMDS) ordination was calculated to highlight the intensity of stress on fish abundance along longitudinal gradient and to confirm the sampling sites identified as reference sites using CA. Pair-wise similarity metrics was used in ordination analysis to calculate the stress level between the similarity matrix and ordination axes (Clarke & Warwick, 1994; Casatti *et al*. 2006). CA and NMDS ordination analysis was calculated using Statistica 5.5 (Soft stat Inc., 1999) and PC ORD ver. 4.0 (McCune & Mefford, 1999), respectively.

**Calculation of Index of Biological Integrity (IBI)**

Biological or biotic integrity which encompasses compositional, structural and functional levels of organization refers to the ability of biological system's to function and maintain itself and ultimately to evolve in the face of changes in environmental conditions (Angermeier & Karr, 1994). Biotic integrity has played an important role in an assessment of environmental health and in bio-monitoring of environmental degradation. IBI is multimetric approach which uses combination of metrics (Mebane *et al*., 2003) for biotic health of streams suffering with anthropogenic activities was developed to assess the health condition of studied streams. Biotic indices based on fish have become common tool in ecological monitoring (Scardi *et al*., 2008). While (multi-metric) biotic indices are commonly used, even the most successful ones have been criticized due to lack of their diagnostic capacity, unpredictable interactions between different metrics, the inherent circularity of selecting indicators that are supposed to respond to anthropogenic stressors (Scardi *et al*., 2008). In the present study, different metrics were calculated using biological information of fish species either with the help of literature and personal experiences. Twelve different metrics relevant to assessment of anthropogenic impacts on fish communities were calculated based on structure, composition, assemblage pattern of

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fish species. IBI scoring criteria was modified as proposed by Ganasan & Hughes (1998). IBI metrics were categorized into five main groups viz; fish species diversity, habitat preference, stress tolerance, trophic composition and origin of fish species (Table 6.1).

The information about various different ecological aspects of a fish species was extracted from existing local literature (Mirza, 2003; Talwar & Jhingran, 1991; Jayaram, 1999; http://www.fishbase.org). Fish species were classified into different ecological groups on the basis of taxonomic diversity, habitat preference, stress tolerance (intolerant, moderately intolerant, and tolerant species), feeding habits and origin of species (native and exotic species). The column and bottom feeder were included into the category of habitat preference. Fish feeding habits were determined by the following into four trophic groups (herbivore, omnivore, invertivore and carnivore) on the basis of their feeding preferences (Matthews 1998). Another important parameter related to the biological health streams i.e. infestation of exotic species was incorporated on the basis of origin of species. Higher number of the exotic individual and species indicates the ecological degradation of stream ecosystem (Moyle *et al.,* 2003).

Species diversity metrics were calculated to express fish species diversity which included number of native fish species, total number of fish families and total number of individuals of species. Metrics for number of native species were calculated as introduced by Steedman (1988) by replacing the total number of fish species used by Karr (1981). Metrics of number of native species and families have been used as an indicator of anthropogenic stress on fish assemblage by different authors. Sometimes due to long term exposure to anthropogenic activities in the catchment may cause complete elimination of a fish family or families from particular stream/river (Oberdorff & Hughes, 1992; Ganasan & Hughes, 1998). Therefore, metrix of number of native families was included as described by Ganasan & Hughes (1998). Matrix of total number of individuals of a species in the site which indicates species richness level and ecological conditions of an aquatic ecosystem was included without any modification as described by Ganasan & Hughes (1998) and Karr (1981).

Habitat preferences metrics which relate habitat quality of the sampling sites in term of water quality were calculated using number of benthic and water column species. Originally, this matrix was used to incorporate number of darter species and sunfish

species for mid-western streams of United States by Karr (1981). Later on Oberdorff & Hughes (1992) replaced these two metrics with number of benthic dweller species and number of column dweller species. Higher number of benthic and column dweller in habitats is associated with good habitat quality and showed a decreasing trend with an increase in turbidity, pollutants, reduced oxygen content and organic matter (Hughes *et al.*, 1998).

Fishes were grouped on the basis of stress tolerance into tolerant, moderately tolerant and intolerant by following the Oberdorff *et al.* (2002). Intolerant species were present at stream segment exhibiting good water quality and absent at degraded segment of stream. Tolerant species were present at most of the sites ranging least disturbed to moderately degraded sites dominant at moderately degraded sites. Moderately tolerant species were those species which found at least degraded sites as well as moderately degraded sites but have preferences for least degraded sites. Two metrics related to stress tolerance were calculated to assess the sensitivity of different species viz., number of intolerant species and proportion of individual as tolerant species. Number of intolerant species which provides information regarding the sensitivity of fish species to environmental degradation (Shinn, 2000) was calculated as described by Karr (1981).

Trophic composition related with trophic status of fish species were calculated based on literature and information extracted from www.fishbase.com. Four metrics such as proportion of individuals as herbivorous species, proportion of individuals as omnivorous species, proportion of individuals as insectivorous species, and proportion of individuals as carnivorous species were included as measure of trophic composition of a fish assemblage based on their feeding patterns. Three metrics such as proportion of individuals as omnivorous, invertivorous and carnivorous species were calculated as described by Karr (1981). Presence of introduced species in a natural ecosystem is always problematic for the equilibrium of ecosystem (Howard, 2002) and metric for proportion of individuals as non-native species was calculated based on presence of introduced species to assess the infestation intensity of non-native species in study area.

**Application of IBI**

Two methods such as stepped and continuous scoring metrics were used to assess biological health of stream at 18 monitoring sites. The scores values of different metrics calculated for the evaluation of stream conditions for studied streams are given in Table 6.2. Continuous IBI was calculated as proposed by Minns *et al*. (1994) and Ganasan & Hughes (1998) and ranged from 0 to 10 for metric score and 0 to 100 for IBI score. For all metric scores upper thresholds value of 10 was used for least disturbed habitat (reference condition) and lower values of 0 was used for highly degraded habitat. Each metric score was calculated by a ratio of observed metric site score (O) and score at reference site (R) and multiplied with 10 (Bozzetti & Schulz, 2004). For the tolerant, carnivore and non-native metrics, scoring criteria was reciprocal to all other metrics and calculated using equation (1).

$$\text{Individual IBIscore (tolerant, carnivore and non-native species)} = \left\lfloor 10 - \left( \frac{O}{R} \times 10 \right) \right\rfloor \text{ --- (1)}$$

In case of these metric scores, highest score (10) was assigned to lowest proportions and lowest score (0) to highest proportions of tolerant, carnivore and non-native species. Overall continuous metric score for each site was computed by a ratio of total of metric scores and total number of metrics and was multiplied by 100. Stepped IBI scoring criteria was also used along with continuous IBI and derived from Ganasan & Hughes (1998). All metric scores were treated the same as discussed in continuous IBI. Each metric score was scored as 1(impaired), 3(moderately impaired) and 5(less impaired) representing conditions of habitat that deviates from threshold level of reference sites (Fausch *et al*., 1990; Kleynhans, 1999). For the carnivores, tolerant and non-natives metrics scoring criteria was also reciprocal to all other metrics. Stepped IBI score for sites were ranged between 0-50 and calculated by the following equation (2):

$$\text{Stepped Individual IBIScore} = \left\lfloor \left( \frac{\text{stepped IBIscore of individual metrics}}{\text{Total number of metrics}} \right) \times 50 \right\rfloor \text{ --- 2)}$$

Table 6.1    Families, species, relative abundance, feeding behavior, tolerance level, habitat preferences and origin of ichtho-fauna recorded from Nullah Aik and Nullah Palkhu: tributaries of River Chenab.

| S# | Family | Name | Abbreviations | Relative abundance (%)[*] | Habitat preferences | Tolerance level | Feeding Behaviour | Origin |
|----|--------|------|---------------|------------------------|---------------------|-----------------|-------------------|--------|
| 1 | Ambassidae | *Parambassis ranga* (Hamilton, 1822) | *Para ran* | 2.39 | BD[a] | IT | Invertivore[c] | N[a] |
| 2 | Bagridae | *Mystus cavasius* (Hamilton, 1822) | *Mys cav* | 7.04 | CD[a] | MT | Carnivore | N[a] |
| | | *Sperata seenghala* (Sykes, 1839) | *Spe see* | 0.53 | BD[a] | IT | Carnivore | N[a] |
| | | *Rita rita* (Hamilton, 1822) | *Rit rit* | 1.13 | BD[a] | MT | Carnivore | N[a, b] |
| 3 | Belonidae | *Xenentodon cancila* (Hamilton, 1822) | *Xen can* | 0.13 | SF[a] | MT | Carnivore | N[a] |
| 4 | Channidae | *Channa punctata* (Bloch, 1793) | *Cha pun* | 19.59 | BD[a] | T[e] | Carnivore | N[a] |
| 5 | Cichlidae | *Oreochromis niloticus* (Linnaeus, 1758) | *Ore nil* | 6.11 | CD[a] | MT[f] | Omnivore[c, d] | E[a, b] |
| 6 | Cyprinidae | *Labeo rohita* (Hamilton, 1822) | *Lab roh* | 6.31 | CD[a] | IT[f] | Herbivore[c, d] | N[a] |
| | | *Labeo calbasu* (Hamilton, 1822) | *Lab cal* | 0.27 | CD[a] | IT[f] | Herbivore[c, d] | N[a] |
| | | *Labeo dero* (Hamilton, 1822) | *Lab der* | 0.20 | CD[a] | IT[f] | Herbivore[c, d] | N[a] |
| | | *Cirrhinus reba* Hamilton, 1822 | *Cir reba* | 8.83 | CD[a] | MT[f] | Herbivore[d] | N[a] |
| | | *Cyprinus carpio* (Linnaeus, 1758) | *Cyp car* | 0.2 | CD[a] | MT[f] | Herbivore[d] | E[a, b] |
| | | Cirrhinus cirrhosus (Bloch, 1795) | Cir cir | 1.26 | CD[a] | MT[f] | Herbivore[c, d] | N[a] |
| | | *Puntius sophore* (Hamilton, 1822) | *Pun sop* | 16.93 | CD[a] | T[f] | Invertivore[c] | N[a] |
| | | *Puntius ticto* (Hamilton, 1822) | *Pun tic* | 11.49 | CD[a] | T[f] | Invertivore[c] | N[a] |
| | | *Osteobrama cotio* (Hamilton, 1822) | *Ost cot* | 3.78 | CD[a] | IT[f] | Herbivore[c, d] | N[a] |
| | | *Salmostoma bacaila* (Hamilton, 1822) | *Sal bac* | 0.93 | SF[a] | MT[f] | Herbivore[c, d] | N[a] |
| | | *Garra gotyla (Gray, 1830)* | *Gar got* | 0.4 | BD[a] | MT[e] | Omnivorous[c, d] | N[a] |
| 7 | Heteropneustidae | *Heteropneustes fossilis* (Bloch, 1794) | *Het fos* | 6.04 | BD[a] | T[e] | Omnivorous[c, d] | N[a] |
| 8 | Mastacembelidae | *Mastacembelus armatus (Hamilton, 1822)* | *Mas arm* | 0.13 | CD[a] | MT[e] | Carnivore[d] | N[a] |
| 9 | Notopteridae | *Notopterus notopterus* (Pallas, 1769) | *Not not* | 2.26 | CD[a] | MT[e] | Carnivore[d] | N[a] |
| 10 | Osphronemidae | *Polyacanthus fasciatus* (Bloch & Schneider, 1801) | *Pol fas* | 1.73 | CD[a] | MT[f] | Invertivore[c] | N[a] |
| 11 | Siluridae | *Wallago attu* (Bloch & Schneider, 1801) | *Wat att* | 2.26 | CD[a] | MT[e] | Carnivore[d] | N[a] |
| 12 | Sisoridae | *Gagata cenia (Hamilton, 1822)* | *Gaga cen* | 0.07 | BD[a] | IT[f] | Carnivore[d] | N[a] |

BD : Bottom dweller, CD: column dweller, SF: surface Feeder, IT: Intolerant, MT: Moderately tolerant, T: Tolerant, N: Native and E: exotic (non native).

* Relative abundance was calculated by this formula

$$\text{Relative abundance} = \left| \left( \text{Individual of a species from study area} \middle/ \text{Total number of individuals of all species} \right) \right|$$

a (Information extracted from Mirza & Bhatti, 2003 and Talwar & Jhingran and 1991)
b (origin of species extracted from www.fishbase.com and Mirza & Bhatti, 1993)
c (Based on information provided by Mirza & Bhatti, 1993)
d (Information retrieved from www.fishbase.com)
e (Ganasan & Hughes, 1998)
f (status assigned during study based on presence in wide range of habitats within streams)

**Classification of Integrity Classes**

Many authors classified the integrity classes into three to five groups. For example Karr *et al*. (1986) grouped the IBI classes into five groups. These classes were categorized as very poor, poor, fair, good and excellent. Plafkin *et al*. (1989) classified the IBI qualitative classes in to four groups viz; non-impaired, slightly impaired, moderately impaired and severely impaired. In present study, on the basis of overall IBI score, all sites were categorized into three qualitative classes such as less impaired, moderately impaired and impaired as described by Ganasan & Hughes (1998). Overall IBI scores were ranged between 0 to 80 and 0 to 45 for continuous and stepped IBI classes, respectively. IBI scores for continuous integrity classes were characterized as impaired (<43), moderately impaired (43-63) and less impaired (>63). Overall IBI scores for three stepped integrity classes were ranged as impaired (<25), moderately impaired (25-35) and less impaired (>35). In present study, for the qualitative assessment of sites in terms of IBI were categorized into three classes. Large number of classes may cause some problem during restoration process for managers to decide for the management actions therefore, three classes were developed by following (Ganason & Hughes, 1998; Lyon *et al*., 2000).

Table 6.2    Scheme for rating of index of biological integrity (IBI) metrics for Nullah Aik and Palkhu tributaries of River Chenab.

| S# | Major Categories of Metrics | Scoring Criteria | Continuous IBI metrics Score* | | Stepped IBI metrics Scores | | |
|----|------|------|------|------|------|------|------|
| | | Individual metrics | 10 (Best) | 0 (worst) | 5 (Best) | 3 (Fair) | 1 (worst) |
| 1 | **Species diversity** | Number of native Species | 15 | 0 | >12 | 12 - 6 | <6 |
| 2 | | Number of Native Families | 8 | 0 | >7 | 4 - 7 | <4 |
| 3 | | Total number of Individuals | 306 | 0 | >250 | 100 - 250 | <100 |
| 4 | **Habitat preference** | Number of Bottom Feeder species | 4 | 0 | >4 | 2-4 | <2 |
| 5 | | Number of Column Feeder species | 11 | 0 | >9 | 4 - 9 | <4 |
| 6 | **Stress tolerance** | Number of intolerant species | 4 | 0 | >4 | 2- 4 | <4 |
| 7 | | Proportion of individuals as tolerant species | 0 | 100 | <40 | 40 - 70 | >70 |
| 8 | **Trophic composition (%)[a]** | Proportion of individuals as herbivore species | 0 | 45 | >40 | 20 - 40 | <20 |
| 9 | | Proportion of individuals as omnivore species | 0 | 28 | >15 | 15 - 25 | <25 |
| 10 | | Proportion of individuals as insectivore species | 0 | 71 | >40 | 20 - 40 | <20 |
| 11 | | Proportion of individuals as carnivore species | 100 | 20 | <50 | 25 -50 | >25 |
| 12 | **Origin of species (%)[a]** | Proportion of individuals as exotic species | 0 | 18 | <5 | 5 - 10 | >10 |
| 13 | **Health of fish** | Proportion of individual of with abnormalities | NA | NA | NA | NA | NA |
| | **IBI score** | | 80 | 0 | >43 | 43 - 23 | <23 |
| | **Integrity Class** | | Less impaired | Highly impaired | Less impaired | Impaired | Highly impaired |

\*    The range of continuous IBI method is ranged between 0 – 10. The values for each metrics were obtained from (n x 10). n=

NA    Not applied in present research paper.

a: information extracted from Karr (1986); Minns *et al.* (1994), Ganasan & Hughes (1998).

# Results

## Classification of Sites and Species in Clusters Based on Habitat Degradation and Identification of Reference Sites

CA identified three groups/clusters of sites based on spatial difference of fish abundance data (Fig. 6.1). Cluster 1 was consisted of two sites (1 and 2) situated in the upstream of Nullah Aik. This cluster was distinguished from other clusters on the basis of species diversity. Out of the total of 24 species recorded, a total of 58% and 63% species were recorded at site 1 and 2 and constituted 39.7% of the total fish abundance recorded from all studied sites. Among water quality parameters, highest average concentration of dissolved oxygen contents, lowest COD, and dissolved metal content were recorded from these sites. No point sources of pollution such effluents from municipal and industrial waste was identified in their catchment and was relatively least disturbed with good water quality. Only agricultural activities were found in the surrounding area. These sites were comparatively more diverse, exhibited complex fish assemblage and reflected low level of habitat degradation. Highest stream width of 28m was recorded to those of other clusters. These sites were also considered as reference sites because of least disturbance from anthropogenic activities. Cluster 2 comprised of seven sites which includes 3, 4, 5, 8, 10, 12 and 14 with highly degraded habitat condition with minimum species diversity. These sites receive contamination from industrial and urban effluents and surface runoff from urban area. Water quality of these sites was found highly contaminated (Qadir *et al*., 2008). Lowest dissolved oxygen, highest values of COD, salts and heavy metals were recorded in this cluster. These parameters make the habitat unsuitable for fish species. Species richness ranged between zero catch to five species. Most of the sites in this cluster represented the zero fish catch from four sites situated in close proximity of the Sialkot city. These sites receive huge volume of industrial effluents (especially from tanneries) and domestic sewages from different point sources. Cluster 3 consisted of nine sites (6, 7, 9, 10, 11, 15, 16, 17 and 18) located in far downstream sites of Sialkot city and showed moderate degradation of habitat conditions. After traversing distance, water quality gradually become improved due to sedimentation of suspended solid and improvement of dissolved oxygen contents in stream water. The major human activity in the catchment area of these sites was agricultural practices along with small contribution

of domestic sewage coming from small villages. These showed an improved ichtheo-fauna ranging from the four to 12 species. More number of fish species was recorded from sites located on Nullah Palkhu due to improvement of water quality through dilution process, whereas, site located on Nullah Aik did not show improvement in species diversity due to presence of small head works.
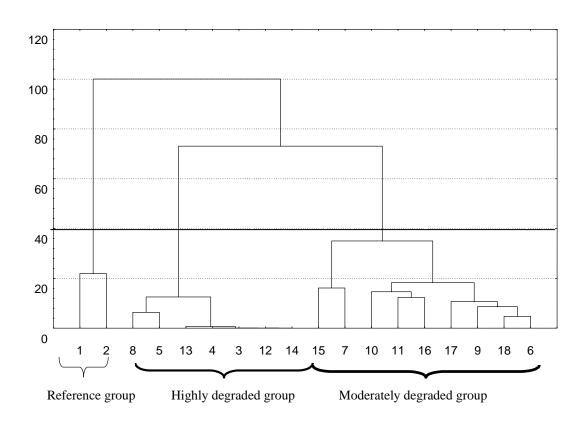


Fig. 6.1    Dendrogram showing major three clusters viz: Cluster 1 (reference sites) Cluster 2 and (impaired sites) and Cluster 3 (moderately impaired sites).

**Identification of Ecological Gradients**

Fig. 6.2 represented site ordination of NMDS which explained 73% of the total variance in relation to fish abundance at sites. First axis explained 39.8% of total variation whereas second and third axes accounted 13.5% and 10.8% respectively. Two ecological gradients were defined along first and second axes. First axis provided information related to water quality degradation and habitat impairment. Degradation of water quality and habitat impairment increases from lower to upper side along the axis of the ordination plot. Highly impaired sites which were located in close vicinity of Sialkot city were found on the lower side and least impaired sites on the upper side of the ordination plot. Second axis provided information about species richness along longitudinal gradient of streams (Fig. 6.3). NMDS grouped all sites into four groups. First group consisted of less impaired sites (1 and 2) with high species diversity. Second group was comprised with 6-14 fish species from sites (7, 9, 10, 11, 15, 16 and 17) and regarded as moderately impaired group. Third group consisted of site (5, 6, 8, 9, 13 and 18) which represent less than six species and considered as impaired group. Fourth group was made up of sites (3, 4, 12, and 14) with zero catch of fish specimen and ranked as highly impaired group.

Species ordination plot explained distribution and association of species dwelling in different habitat. Three groups of fish species (intolerant, moderately tolerant and tolerant) based on their ability to tolerate stress can be recognized (Fig. 6.4). First group consisted of intolerant species (*Labeo rohita, L. calbasu, L. dero, Osteobrama cotio, Sperata seenghala* and *Parambassis* ranga) which inhabiting at upstream sites. Second group consisted of species (*Mystus* cavasius*, Wallago attu, Cyprinus reba, Xenentodon* cancila*, Cirrhinus cirrhosus, Puntius sophore, P. ticto* and *Oreochromis* niloticus) which were distributed in both upstream and downstream sites. *Channa punctata, Heteropneustes fossilis, Notopterus notopterus* and *Polyacanthus fasciatus* were resistant/tolerant species residing downstream sites and formed third group. Along first axes tolerant species group was clearly segregated on right side whereas other two groups showed overlap. Second axis explained the spatial gradient of species distribution. Two groups of species which had the preferences in upstream and downstream can be recognized along this axis. Intolerant species group was identified on the upper side of the ordination diagram whereas other two groups showed overlap.
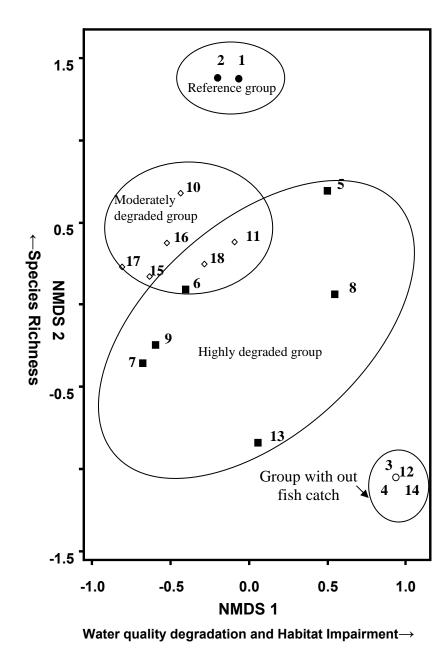
Fig. 6.2    Two-dimensional plot of sampling sites located on Nullah Aik and Palkhu showing the four groups based on ordination resulting from the NMDS based on a similarity Bray-Curtis coefficient.
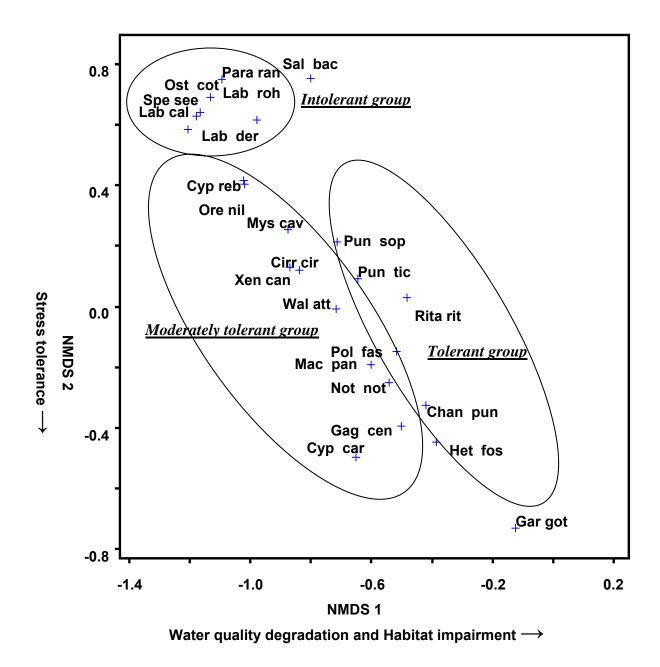
Fig. 6.3     Two-dimensional plot showing distribution of different fish species in Nullah Aik and Palkhu showing the four groups based on ordination resulting from the NMDS based on a similarity Bray-Curtis coefficient.

**Fish Abundance and Assemblage in relation to its Spatial Distribution**

A total of 24 fish species belonging to 12 families were identified from 18 sampling sites belonging to different habitats and trophic levels (Table 6.3). Five sites (3, 4, 12, 13 and 14) were recognized as the most polluted sites which do not support the fish fauna. Two sites (1 and 2) in upstream of Nullah Aik were identified as the least polluted sites which were treated as reference sites. The results showed that at most of the sampling sites, native fish species dominated studied streams. Reference sites (1 and 2) shared about 39.7% of the total relative fish abundance from all sites, whereas 61.3% individuals were collected from other 16 sites. Out of 24 fish species, 22 species were native which contributed 93.4% of the total fish species. Maximum number of fifteen native species was recorded from site 2. Similarly, maximum number of native families was also recorded from site 2. Two non-native species such as *Oreochromis niloticus* and *Cyprinus carpio* were also recorded from different sites which contributed 6.3% of total fish species. Among these, *O. niloticus* was found in upstream and downstream sites of Nullah Palkhu whereas *Cyprinus carpio* was recorded only from site 17 located at downstream of Nullah Palkhu. Among all fish species *Channa punctata* was the most abundant species with relative abundance of 19.6% of the total fish diversity.

Sixteen column dweller, five bottom dweller, and two surface feeder species were recorded which contributed 68.8%, 30.1%, and 1.1% of total fish abundance data of both streams (Table 6.3). Majority of column feeder and bottom feeder species were confined to upstream of Nullah Aik and downstream of Nullah Palkhu. Four resistant species such as *Channa punctata, Puntius sophore, P. ticto* and *Heteropneustes fossilis* were recorded which showed wide distribution from less impaired to impaired sites. These species shared about 54.1% of the total fish abundance data. Fish species which were restricted to upstream were categorized as intolerant/sensitive species. These species included *Labeo rohita, L. calbasu, L. dero, Osteobrama cotio, Gagata cenia*, *Sperata seenghala* and *Parambassis ranga* and contributed about 13.5% of the total fish abundance data. Intolerant species were confined to less impaired part of streams and absent in moderately and highly impaired section of the streams. Moderately tolerant species such as *Mystus cavasius, Wallago attu, Cyprinus reba, C. carpio, Rita rita*, *Polyacanthus fasciatus, Salmostoma bacaila, Garra gotyla, Xenentodon cancila, Mastacembelus armatus,*

*Cirrhinus cirrhosus,* and *Oreochromis niloticus* contributed 32.4 % of the total abundance data and dominated at less impaired sites but least abundant at moderately impaired sites. Herbivore species such as *L. rohita, L. calbasu, Cyprinus reba, C. carpio, Cirrhinus cirrhosus, Osteobrama cotio,* and *Salmostoma bacaila* showed narrow/restricted distribution and were recorded at reference sites. Among three omnivorous species, *Oreochromis niloticus,* and *Garra gotyla* were found in upstream sites, whereas, *Heteropneustes fossilis* was restricted at moderately impaired sites in downstream. Among four invertivore species; two species such as *Puntius sophore* and *P. ticto* showed even distribution at less impaired and moderately impaired sites. Nine carnivorous species were identified in downstream sites. These were *Mystus cavasius, Sperata seenghala, Rita rita, Xenentodon cancila, Mastacembelus armatus, Notopterus notopterus, Wallago attu, Gagata cenia* and *Channa punctata.*

**Metric Scores and IBI Scores**

The results of 12 individual metrics for continuous and stepped IBI criteria are given in Tables 6.2 and 6.3. Species diversity metrics (such as number of native species, number of native families and total number of species individuals) were used as a measure of species diversity. In term of metric scores, different trends were observed. Metric score for number of native species, number of native families and abundance of species were highest in "less impaired" integrity class, and lowest in "impaired" integrity class. In case of these three metrics of species diversity, minimum overlap was observed for three integrity classes (Fig. 6.4a, b & c). Higher metric scores related to habitat preferences (number of column and bottom dweller species) were recorded in "less impaired" integrity class which gradually decreased in "moderately impaired" and "impaired" integrity classes (Fig. 6.4d & e). This stresses that as the level of pollution increases, the number of column and bottom dweller species decreases.

Intolerant species (sensitive native species) colonized at reference sites and were rare at impaired sites. Metric score for stress intolerant species in "less impaired" integrity class was highest as compared to "moderately impaired" and "impaired" integrity classes (Fig. 6.4f). Absence of intolerant species from impaired sites reflected biological degradation of habitat and poor water quality of the sites. Proportion of tolerant species

was highest in "moderately impaired" integrity class and lowest in "less impaired" integrity class (Fig. 6.4g). High proportion of tolerant species in "moderately impaired" and "impaired" integrity classes and absence of intolerant species highlighted significant change in structure of fish assemblage in studied streams.

IBI score for proportion of herbivorous species was highest in "less impaired" integrity class which indicated that most of herbivorous species had preferences for the less impaired sites. Herbivorous species contributed about 45% of the total fish abundance in "less impaired" integrity class (Fig. 6.4h). Highest metric score for proportion of omnivores and insectivore species was observed in "moderately impaired" and "less impaired" integrity classes whereas lowest metric score in "impaired" integrity class (Fig. 6.4i & j). No clear distribution pattern of omnivore and insectivore in different integrity classes was observed. Higher proportion of carnivore was recorded in "moderately impaired" integrity class in comparison to other classes. About 50% of total abundance was shared by carnivore in "moderately impaired" integrity class (Fig. 6.4k). "Moderately impaired" integrity class had lowest metric score for proportion of carnivore and highest in "less impaired" integrity class. Box and whisker plots indicated that carnivore species were abundant in downstream sites rather than upstream sites. Maximum proportion of non-native species was recorded from less impaired site included in "less impaired" integrity class. Higher proportion of non-native species was assigned lowest metric score because presence of alien species represents biological disturbed habitat (Fig. 6.4l). Minimum metric score was measured in "less impaired" integrity class due to infestation by introduced species.

Three qualitative integrity classes viz; less impaired, moderately impaired, impaired were obtained from sum of stepped and continuous IBI scores. The range of scores for each class is given in Table. 6.3. Out of 18 sites, two sites (reference sites 1 and 2) depicted maximum score and were placed into less impaired integrity class. Five sites (8, 10, 15, 16 and 17) fall into moderately impaired integrity class whereas eleven sites (3, 4, 5, 6, 7, 9, 11, 12, 13, 14 and 18) were categorized in impaired integrity class (Table. 6.3).
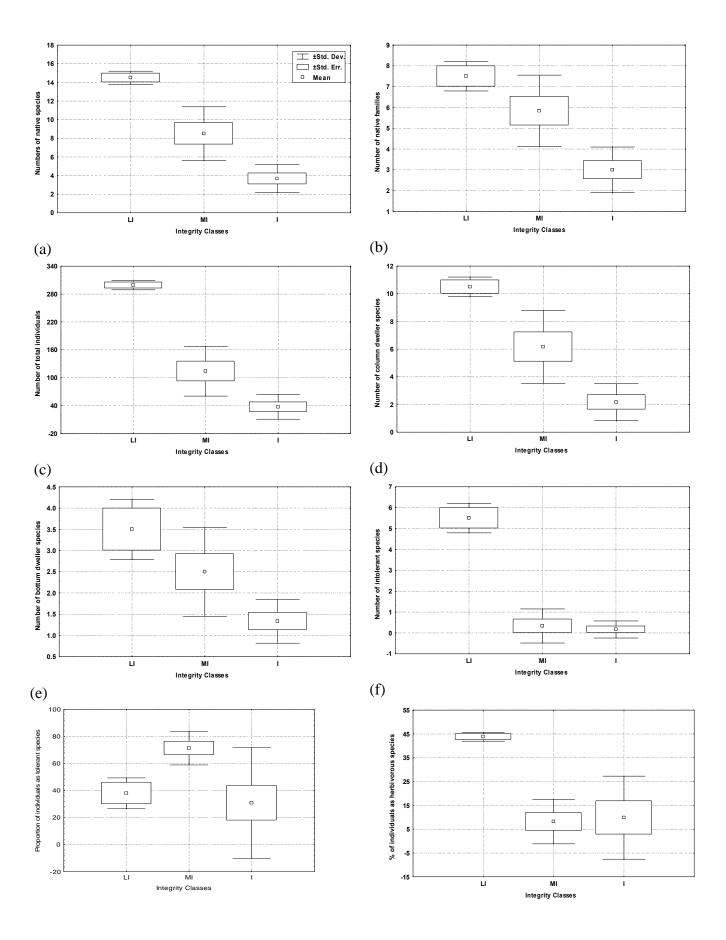
Table 6.3    Stepped and continuous IBI metrics values and qualitative classification evaluated on the basis of fish fauna recorded during Study period from Nullah Aik and Palkhu: tributaries of river Chenab

| eam | Nullah Aik | | | | | | | | | Nullah Palkhu | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | Upstream | | Down stream | | | | | | | Upstream | | Down stream | | | | | | |
| Metrics/ Sampling sites | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** |
| Number of native Species | 14 | 15 | 0 | 0 | 4 | 4 | 7 | 4 | 3 | 6 | 5 | 0 | 1 | 0 | 11 | 10 | 10 | 5 |
| A | **9.3** | **10** | **0** | **0** | **3.3** | **2.7** | **4.7** | **2.7** | **2** | **4** | **3.3** | **0** | **0.7** | **0** | **7.3** | **6.7** | **8** | **3.3** |
| B | *5* | *5* | *0* | *0* | *1* | *1* | *3* | *1* | *1* | *3* | *1* | *0* | *1* | *0* | *3* | *3* | *5* | *1* |
| Number of Native Families | 7 | 8 | 0 | 0 | 4 | 3 | 5 | 3 | 3 | 4 | 4 | 0 | 1 | 0 | 7 | 8 | 7 | 4 |
| A | **8.8** | **10** | **0** | **0** | **5** | **3.8** | **6.3** | **3.8** | **3.8** | **5** | **5** | **0** | **1.3** | **0** | **8.8** | **10** | **8.8** | **5** |
| B | *5* | *5* | *0* | *0* | *3* | *1* | *3* | *1* | *1* | *3* | *3* | *0* | *1* | *0* | *5* | *5* | *5* | *3* |
| Number of Bottom Feeder species | 3 | 4 | 0 | 0 | 1 | 1 | 3 | 2 | 2 | 1 | 1 | 0 | 1 | 0 | 4 | 2 | 3 | 2 |
| A | **7.5** | **10** | **0** | **0** | **2.5** | **2.5** | **7.5** | **5** | **5** | **2.5** | **2.5** | **0** | **2.5** | **0** | **10** | **5** | **7.5** | **5** |
| B | *3* | *5* | *0* | *0* | *1* | *1* | *3* | *1* | *1* | *1* | *1* | *0* | *1* | *0* | *5* | *1* | *3* | *1* |
| Number of Column Feeder species | 11 | 10 | 0 | 0 | 2 | 3 | 3 | 2 | 2 | 6 | 4 | 0 | 0 | 0 | 9 | 8 | 8 | 3 |
| A | **10** | **9.1** | **0** | **0** | **1.8** | **2.7** | **2.7** | **1.8** | **1.8** | **5.5** | **3.6** | **0** | **0** | **0** | **8.2** | **7.3** | **7.3** | **2.7** |
| B | *5* | *5* | *0* | *0* | *1* | *1* | *1* | *1* | *1* | *3* | *3* | *0* | *1* | *0* | *5* | *5* | *5* | *1* |
| Number of intolerant species | 6 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| A | **10** | **8.3** | **0** | **0** | **0** | **0** | **0** | **1.7** | **0** | **0** | **0** | **0** | **0** | **0** | **3.3** | **0** | **0** | **0** |
| B | *5* | *5* | *0* | *0* | *1* | *1* | *1* | *1* | *1* | *1* | *1* | *0* | *1* | *0* | *1* | *1* | *1* | *1* |
| % Individual of tolerant species | 46 | 30 | 0 | 0 | 68 | 94 | 86 | 57 | 100 | 56 | 89 | 0 | 100 | 0 | 74 | 71 | 58 | 83 |
| A | **5.4** | **7** | **0** | **0** | **3.2** | **0.6** | **1.4** | **4.3** | **0** | **4.4** | **1.2** | **0** | **0** | **0** | **2.6** | **2.9** | **4.2** | **1.7** |
| B | *5* | *5* | *0* | *0* | *3* | *1* | *1* | *3* | *1* | *3* | *1* | *0* | *1* | *0* | *1* | *1* | *1* | *1* |
| % Individual of Carnivore | 12.3 | 15.4 | 0 | 0 | 16.1 | 55.3 | 55.1 | 9.5 | 64.4 | 45.9 | 29.1 | 0 | 100 | 0 | 48.8 | 46 | 44 | 50 |
| A | **8.7** | **8.5** | **0** | **0** | **8.4** | **4.5** | **4.5** | **9.1** | **3.6** | **5.4** | **7.1** | **0** | **0** | **0** | **5.1** | **5.4** | **5.6** | **5** |
| B | *5* | *5* | *0* | *0* | *5* | *1* | *1* | *5* | *1* | *3* | *3* | *0* | *0* | *0* | *3* | *3* | *3* | *1* |
| % Individual of Herbivore species | 42.5 | 45.1 | 0 | 0 | 16.1 | 0 | 3.7 | 42.9 | 0 | 16.3 | 0 | 0 | 0 | 0 | 4.4 | 1.5 | 23.1 | 0 |

| Stream | Nullah Aik | | | | | | | | | Nullah Palkhu | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | Upstream | | Down stream | | | | | | | Upstream | | Down stream | | | | | | |
| **A** | **9.4** | **10** | **0** | **0** | **3.6** | **0** | **0.8** | **9.5** | **0** | **3.6** | **0** | **0** | **0** | **0** | **1** | **0.3** | **5.1** | **0** |
| *B* | *5* | *5* | *0* | *0* | *1* | *1* | *1* | *5* | *0* | *1* | *0* | *0* | *0* | *0* | *1* | *1* | *3* | *0* |
| % Individual of Invertivorous species | 27 | 33 | 0 | 0 | 68 | 45 | 13 | 33 | 11 | 35 | 71 | 0 | 0 | 0 | 28 | 40 | 18 | 48 |
| **A** | **3.8** | **4.6** | **0** | **0** | **9.6** | **6.3** | **1.8** | **4.6** | **1.5** | **4.9** | **10** | **0** | **0** | **0** | **3.9** | **5.6** | **2.5** | **6.8** |
| *B* | *3* | *3* | *0* | *0* | *5* | *3* | *1* | *3* | *1* | *3* | *5* | *0* | *0* | *0* | *3* | *3* | *1* | *3* |
| % Individual of Omnivorous species | 18 | 6 | 0 | 0 | 0 | 0 | 28 | 14 | 24 | 3 | 0 | 0 | 0 | 0 | 19 | 12 | 15 | 2 |
| **A** | **4.1** | **8.5** | **0** | **0** | **0** | **0** | **0** | **5.4** | **1.6** | **9.6** | **0** | **0** | **0** | **0** | **3.6** | **5.8** | **5** | **10** |
| *B* | *3* | *5* | *0* | *0* | *1* | *0* | *1* | *5* | *3* | *5* | *1* | *0* | *1* | *0* | *5* | *5* | *3* | *5* |
| Total number of Individuals | 292 | 306 | 0 | 0 | 31 | 47 | 107 | 21 | 45 | 98 | 79 | 0 | 1 | 0 | 205 | 137 | 91 | 46 |
| **A** | **9.5** | **10** | **0** | **0** | **1.0** | **1.5** | **3.5** | **0.7** | **1.5** | **3.2** | **2.6** | **0** | **0.0** | **0** | **6.7** | **4.5** | **2.8** | **1.5** |
| *B* | *5* | *5* | *0* | *0* | *1* | *1* | *3* | *1* | *1* | *1* | *1* | *0* | *1* | *0* | *3* | *3* | *1* | *1* |
| % Individual of non native species | 8 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 3 | 0 | 0 | 0 | 0 | 7 | 0 | 3 | 0 |
| **A** | **5** | **0** | **0** | **0** | **10** | **10** | **10** | **10** | **5.6** | **8.1** | **10** | **0** | **0** | **0** | **5.6** | **10** | **8.1** | **10** |
| *B* | *3* | *1* | *0* | *0* | *5* | *5* | *5* | *5* | *3* | *5* | *5* | *0* | *5* | *0* | *3* | *5* | *5* | *5* |
| Cont. IBI score a | 76 | 80 | 0 | 0 | 40 | 29 | 36 | 49 | 22 | 47 | 38 | 0 | 4 | 0 | 55 | 53 | 54 | 43 |
| Stepped IBI Score b | 43 | 45 | 0 | 0 | 23 | 14 | 20 | 27 | 13 | 27 | 21 | 0 | 11 | 0 | 32 | 30 | 30 | 19 |
| Integrity Class a | **LI** | **LI** | **HI** | **HI** | **HI** | **HI** | **HI** | **I** | **HI** | **I** | **HI** | **HI** | **HI** | **HI** | **I** | **I** | **I** | **HI** |
| Integrity Class b | *LI* | *LI* | *HI* | *HI* | *HI* | *HI* | *HI* | *I* | *HI* | *I* | *HI* | *HI* | *HI* | *HI* | *I* | *I* | *I* | *HI* |

**a** (**Bold**)  individual continuous metrics and *b* (*Italics*) individual stepped metrics, Continuous integrity classes    Highly impaired = below 46, impaired = 46 -66 and less impaired = above 66, Stepped integrity classes       Highly impaired = below 25, impaired = 25 - 35 and less impaired = above 35

(a)

(b)

(c)

(d)

(e)

(f)

(g)



(h)



(i)



(j)



(k)

(l)

LI = Less Impaired, I = Impaired and HI = Highly impaired
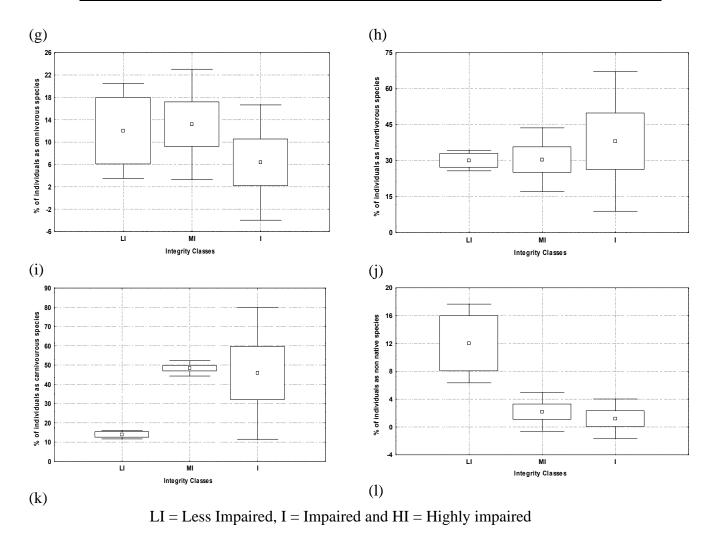
Fig. 6.4  Box and whisker plot showing scores individual IBI metrics in three integrity (a; number of native species, b; number of native families, c; total number of individuals, d; number of column dweller species, e; number of bottom dweller species, f; number of intolerant species, g; proportion of individuals as tolerant species, h; proportion of individuals as herbivore species, i; proportion of individuals as omnivore species, j; proportion of individuals as invertivore species k; proportion of individuals as carnivore species and l; proportion individuals as of exotic species).

**Assessment of IBI along Longitudinal Gradient of Nullah Aik and Nullah Palkhu and Relationship with Water Quality Parameters**

IBI scores for stepped and continuous integrity classes were highest in upstream sites of Nullah Aik (1 and 2) which decreased to its ebb in the city (site 3 and 4) and recovered in downstream sites such as 5, 6 and 7 (Fig. 6.5a and b). Maximum IBI scores for Nullah Palkhu were observed in downstream sites 15, 16 and 17 respectively. Sites 10 and 11 of upstream were moderately rich with fish fauna. Minimum values were observed at sites 12, 13 and 14 near the city. Upstream sites of Nullah Aik were the least disturbed sites while sites close to the city towards downstream were highly polluted with untreated discharge of industrial effluents as well as domestic sewage discharged from city. The IBI score at downstream sites of Nullah Palkhu increases as these sites receive different unpolluted water channels and gradually decrease till Wazirabad city. On the contrary to Nullah Aik, IBI score for downstream of Nullah Palkhu was greater (55). IBI scores varied along longitudinal gradient in both streams representing the intensity of anthropogenic stress. The results of stepped and continuous IBI scores showed longitudinal gradient for Nullah Aik and Palkhu (Fig: 6.5a & b). In case of Nullah Aik maximum IBI scores (80 and 76) was recorded at upstream sites (1 and 2) which decreased in mid-stream sites and regain in downstream sites.

Fig 6.6 showed that water quality parameters viz., COD, TDS, turbidity, and metals had negative correlation with IBI scores. IBI score was significantly positively correlated with DO and pH. Significantly negative correlation was found between DO, COD and TDS. Metals such as Cr, Zn and Fe were negatively associated with IBI scores. COD, TDS and turbidity showed positive correlation with temperature. The results of physiochemical parameters (such as temperature, pH, DO, COD, EC, TDS, turbidity, $NO_3^-$, $PO_4^{3-}$ and toxic metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn) of water quality of different sampling sites are illustrated in Table 6.4 and indicated that variability in water chemistry was related as a function of stream sites (i.e. unimpaired, moderately impaired, and severely impaired) and depended on the chemical properties.

The IBI score at downstream sites of Nullah Palkhu increases as these sites receive different unpolluted water channels and gradually decrease till Wazirabad. On the contrary to Nullah Aik, IBI score for downstream of Nullah Palkhu was greater (55). IBI scores varied along longitudinal gradient in both streams representing the intensity of anthropogenic stress. The results of both stepped and continuous IBI scores showed along longitudinal gradient for Nullah Aik and Palkhu (Fig. 6.5a & b). In case of Nullah Aik maximum IBI scores (80 and 76) was recorded at upstream sites (1 and 2) which decreased in mid-stream sites and regain in downstream sites. Fig 6.6 and Table 6.4 showed that water quality parameters viz., COD, TDS, turbidity, and metals had negative correlation with IBI scores. IBI score was significantly positively correlated with DO and pH. Significantly negative correlation was found between DO, COD and TDS. Metals such as Cr, Zn and Fe were negatively associated with IBI scores. COD, TDS and turbidity showed positive correlation with temperature. Table 6.4 illustrated the basic physiochemical parameters of water quality of different sampling sites. These parameters include temperature, pH, DO, COD, EC, TDS, turbidity, $NO_3^-$, $PO_4^{3-}$ and toxic metals such as Fe, Pb, Cd, Cr, Ni, Cu and Zn. The variability in water chemistry was related as a function of stream sites (i.e. unimpaired, moderately impaired, and severely impaired) and depended on the chemical properties. The mean temperature during the sampling ranged from 23.13-25.54°C. Small variations in temperature were observed between sites. TDS and pH ranged from 7.55-8.26 and 328.66 to 883.78 (mg/L). Large variations were found in COD and DO that varied between 26.74-494.75 (mg/L) and 0.99-7.99 (mg/L), respectively.

All water samples were highly turbid in nature and turbidity values ranged from 2849 to 255.12 (NTU). Nitrates which are end product of the aerobic decomposition of organic nitrogenous matter and are most highly oxidized form of nitrogen compounds ranged from 0.51 to 2.72 (mg/L) whereas $PO_4^{3-}$ contents ranged from 0.54 to 5.66 (mg/L). Significant positive correlation was found between different metals viz., Pb, Cu, Cr, Ni, Zn and Fe indicated their common origin or possible sources of origin. Average concentration of Fe was highest followed by Pb, Cr, Zn, Ni, Cu and Cd. Cr showed positive association with Fe, Pb, and Cd. Similarly, Cu was positively correlation with Fe, Pb, and Cd whereas Zn showed negative association with Cu, and Cd respectively.

Concentration of metals such as Fe, Cr, and Zn was highest in highly impaired group followed by impaired, moderately impaired and lowest in less impaired group. Similar trend was observed for COD, TDS, salinity and turbidity values. The results also showed DO and pH was highest in the least impaired and moderately impaired streams sites and lowest in highly impaired streams sites. Variation in $NO_3^-$, and $PO_4^{3-}$ contents were observed between different impaired groups.

## Discussion

### Variations in Individual IBI Metrics on Spatial Scale and Effect of Environmental Factors on Fish Assemblages

Impacts of anthropogenic activities related with industrialization, population expansion, urbanization and agriculture on biological integrity of water resources are very complex and long-lasting (Hu *et al*., 2007). Human influences, such as changes in water chemistry or physical habitat modifications, could alter fish assemblage and diversity by disrupting their structures and functions. Variations in fish assemblage could be detected through changes in components of the community, functional groups, species diversity, and relative abundance. The results showed high fish abundance at reference sites (less disturbed) in comparison to disturbed sites. These results are analogous to the results obtained by Karr *et al*. (1986), Oberdorff & Hughes (1992) and Toham & Teugels (1999). The results also illustrated that species diversity decreases as human impact increases (Belpaire *et al*., 2000, Breine *et al*., 2004). Sites located on both streams were affected by various anthropogenic stresses such as industrial effluents, municipal sewage, atmospheric deposition and surface runoff from agricultural and urban areas. These factors negatively influence the structure and composition of fish assemblage in streams passing through cities and can adversely affect their habitat which becomes vulnerable for fish survival. These results were in accordance with Wichert & Rapport (1998) and Casatti *et al*. (2006). Large number of bottom and column dweller species was recorded from least impaired sites and decreasing trend was observed towards downstream sites. Column dwellers are generally sensitive to bottom depletion of oxygen and sedimentation which affect their feeding; Oberdorff & Hughes (1992). Organic and inorganic chemicals form industrial and domestic sewage (Kestemont *et al*., 2000) are indiscriminately discharged into streams which consume dissolved oxygen during oxidation process resulting depletion of oxygen (Eklöv *et al*., 1998). Heavy metals and Persistent Organic Pollutants (POPs) in fresh water have been mainly associated with industrial and agricultural sources. Accumulation of these pollutants in body tissues of fish species may result in reduction of growth and cause disturbance in physiological processes (Lwanga *et al*., 2003). Upstream sites were less disturbed from anthropogenic activities with large number of intolerant species which declined toward downstream. Six intolerant species

were recorded from reference sites which were absent from degraded sites. Kennard *et al*. (2005) and Habit *et al*. (2006) also recorded intolerant species from reference sites which gradually disappear from the sites with high level of contamination. *Channa punctata, Puntius sophore, P. ticto* and *Heteropneustes fossilis* can tolerate pollution stress and were encountered at moderately degraded sites. Presence of intolerant fish species generally indicates species richness of sites which are relatively less disturbed by anthropogenic activities (Karr *et al*., 1986) in contrast; higher proportion of tolerant individuals reflects habitat degradation. Tolerant species are the last to disappear in disturbance conditions and reappear first in comparison to other species as habitat become favourable (Ganasan & Hughes, 1998). The percentage of carnivorous individuals was higher at moderately degraded sites in downstream, whereas, minimum proportion was recorded at least disturbed sites. High percentage of herbivore species was recorded at reference sites. Similar, results were also reported by Bhat (2003). However, Ganasan & Hughes (1998) found higher proportion of carnivorous species at reference sites. Stream width has been identified as important factor which influence richness of herbivore species i.e., more is the stream width, more will be the proportion of herbivorous species. The results showed that stream width was higher at reference sites with low flow rate. This in turn allows suspended sediment to settle down resulting clear water which favours the establishment of phytoplankton that forms the food of herbivorous fishes (Borges *et al*., 2003). Most herbivores species were found upstream and carnivores in the downstream sites. These results highlighted that more detailed studies on the feeding habits and preferences of these species are required for better understanding of spatial pattern in feeding guilds. The relative abundance of insectivorous species found during current study was almost the same at reference and degraded sites. These results are in consistent with the findings of Bhat (2004). Among three omnivore species, *O. niloticus* was found in the upstream, whereas, other two species such as *Garra gotyla* and *Heteropneustes* fossilis in downstream sites. Oberdorff & Hughes (1992) showed that omnivorous species had wide range of food selection and can switch from one food to another. Relatively higher proportion of omnivorous species at reference site may be due to more availability of food as compared to other sites. Toham & Teugels (1999) described that omnivorous species are comparatively more successful at reference sites

and degraded sites than any other trophic groups. Among non-native species, *O. niloticus* showed wide distribution in upstream as well as downstream of Nullah Palkhu. It is considered as a potential threat to diversity of native fish fauna of the studied streams. It was introduced to fresh water resources of Pakistan from River Nile basin in 1985 and now has been reported in most of rivers, streams, lakes and small ponds (Mirza, 2003). In contrary to other non-native species, *Cyprinus carpio* was captured only from site 17, located downstream of Nullah Palkhu. This species is intensively cultured in ponds and fish farms to fulfill the local demand of white meat. During monsoon season, due to heavy rains, fish farms overflow and this species makes its way to streams. According to Kasulo (2000) non-native species have the ability to spread from one ecosystems to another through inter connected water ways and have significant impact on local fish species by predation, resource competition and interference with reproduction. These species have been reported as a carrier of parasites of different diseases from one ecosystem to another and used as an ecological indicator of biological health of stream and river (Kennard *et. al.*, 2005). High richness of non-native species can be correlated with level of biological degradation. Intrusion of non-native species into freshwater ecosystem disrupt the food webs making the aquatic ecosystem more vulnerable for native fauna (Scrimgeour & Chambers, 2000; Wolter *et al.,* 2000; Habit *et al.*, 2006) and their presence is considered problematic for management and conservation of water recourses (Kennard *et al.*, 2005).

The results highlighted that fish diversity increases as streams moves downstream. Vila-Gispert *et al.*, (2002) stated that fish diversity increases from upstream to downstream of undisturbed streams. Pollutants from point and non-point source and other factors such as introduction of alien species, exploitation of water for irrigation purpose and habitat alteration by construction of dams and canals has been recognized as significant threats for loss of species diversity and changes in spatial trends of species distribution (Moyle & Leidy, 1992; Lyons *et al.*, 2000; Kouamélan *et al.*, 2003; Mebane *et al.,* 2003). Fish diversity of Nullah Aik and Palkhu was highly fragmented. Discharge of untreated pollutants form industries and urban waste, pumping of stream water for irrigation purposes and presence of small head works at site (7) are main causes of fragmentation of fish species. These threats may increases the risk of local extinction of

fish species (Cetra & Petrere, 2006). Surface water contamination could result in reduction in fish diversity and abundance as few tolerant species can only thrive in highly polluted water due to their adaptability and resistivity to water pollution (Pinto *et al.,* 2006). The results highlighted that upstream of Aik and Palkhu streams were mainly affected by agricultural activities, whereas, downstream were heavily polluted from discharge of untreated industrial effluents and domestic wastes. Walton *et al*. (2007) concluded that streams which passed through human settlements generally receive pollutants from point sources (industrial and municipal sewage) and non-point sources including agricultural and urban runoff and atmospheric deposition. These pollution sources inexorably affect directly or indirectly the biota of the streams in particular fish fauna. Heavy load of industrial and municipal effluents have been recognized to have negative effect on habitat conditions which become unfavorable for native/sensitive species and favourable for the opportunist/tolerant species (Pinto *et al.,* 2006). Tolerant species generally have the ability to survive in organically enriched environment from industrial and municipal discharges (Ganasan & Hughes, 1998). The results indicated that upstream sites were dominated by intolerant species as relatively undisturbed habitat conditions prevail. The fish diversity decreases as study streams passes through Sialkot metropolitan which lies in midstream region generates large volume of untreated industrial and municipal wastes. Impairment of sites also increases as study streams moves from upstream towards downstream. Impairment of study sites can be related to high level of COD, TDS, turbidity, and toxic metals viz., Cr, Zn, Cu, Pb, Ni, Fe, and Cd (Qadir *et al.,* 2008). The results found negative correlation of DO with turbidity, COD, TDS and toxic metals. Less impaired groups showed better water quality in terms of high level of DO, and low level of COD, turbidity, TDS and toxic metals.

**Validation of Reference Sites and Variations in Overall IBI Scores**

Defining a reference conditions against which other streams can be compared present a unique challenge because most of aquatic ecosystems have been alter to some extent by changes in physical, chemical or biological structure (e.g., impoundment, pollution, or introduction of exotic species). The selection of reference sites was based on findings of Sudaryanti *et al*., (2001), Hart *et al*. (2001) and Tejerina-Garro *et al*. (2005).

Sudaryanti *et al*. (2001) assessed the impact of different human activities on riverine ecology of river Brantas, Indonesia and indicated that it is difficult to find any un-disturbed reference site in developing countries due to anthropogenic activities in catchment area of aquatic resources. They suggested sites with relatively better ecological characteristics can provide baseline for ecological change in streams and can be considered as a reference sites because the choice of an appropriate reference condition is a major problem in most of ecological assessments. The decision about the health of stream is an essential part of biological assessment (Hughes, 1994). In order to study the health status of any stream, it should be compared with those stream segments having best ecological conditions such stream segments are called reference sites. These sites represent the minimal disturbed conditions indicating the good physio-chemical and biological quality of the stream (Qadir *et al*., 2008). In evaluation of ecological health of streams, it is essential to study various characteristics of the reference site. No completely undisturbed reference sites were identified in the catchment of Nullah Aik and Palkhu because of intensive utilization of land resources for urbanization, industrial activities and agriculture processes (Qadir *et al*., 2008). Site 1 and 2 were represented as reference sites were located at 247m and 245m height from the sea level with no apparent source of pollution in catchment. However, some agricultural activities were found. Thus runoff from agricultural field and atmospheric deposition of pollutants can be considered the only source of contamination. These sites showed relatively similar physical, chemical and biological characteristics. Maximum average width (25.46 and 30.64m) was recorded from these sites with an average depth (1.10m and 1.00m). Stream bank vegetation of the two site was very similar dominated by tall grasses mainly *Saccharum* spp. stream bed sediment were pre-dominantly sandy with least organic substances. From these sites fresh water bivalves were also recorded which were absent from other sites and water was clear with no unpleasant odor which is produced during decomposition process. Thus represent habitat condition suitable for flourishing of Fish fauna. The results also indicated that highest fish abundance was recorded from these sites and depicted relatively better ecological conditions with least impact from anthropogenic processes. The selection of reference sites was also validated by multivariate techniques such as CA and NMDS. Among three clusters of sites from streams (viz., less degraded, moderately degraded and

degraded sites) classified using CA, less degraded sites were considered as reference sites. Similarly, NMDS also separated sites into four groups based on the intensity of habitat impairment due to anthropogenic stress. Thus least impacted sites selected as reference sites were treated as threshold level of biological conditions of habitat in terms of quantitative values of IBI scores.

IBI provides multiple indicator evaluation system to describe biological conditions of streams (Groom *et al.,* 2005) and has been used to assess the level of impairment at different spatial scales. The results of metric scores and IBI scores indicated that degradation of the studied streams can strongly be related to anthropogenic activities. The results revealed upstream sites with better water quality had higher IBI scores than sites located midstream and downstream regions. Low IBI scores were related with environmental degradation and decline in site quality with downstream distance primarily to increasing impacts from direct discharge of industrial and municipal effluents in the streams. There is no treatment plant for industrial and sewage treatment in Sialkot city (Qadir *et al*., 2008). The effluents are directly discharged either into nearby streams, drains, ponds or/and agricultural land. Gammon (1998) also attributed agriculture and urban discharge as major factor responsible for habitat degradation in downstream to those of upstream region. Anthropogenic disturbance has been recognized as a major contributor for changing chemical composition of stream water (Qadir *et al*., 2008) which ultimately disturbs the ecological integrity of an ecosystem. The results showed significantly negative correlation of IBI scores with COD, TDS, turbidity, Fe, Cr, Zn and positive correlation with DO and pH (Fig. 6.6 and Table 6.4). The results stress the role of industrial and municipal effluents in deterioration of water quality which negatively influence ecological health of streams.

(a)



(b)
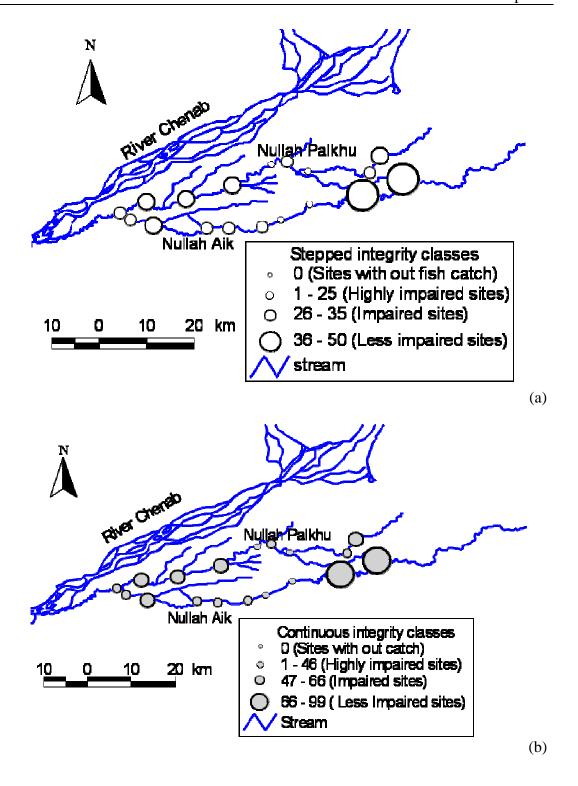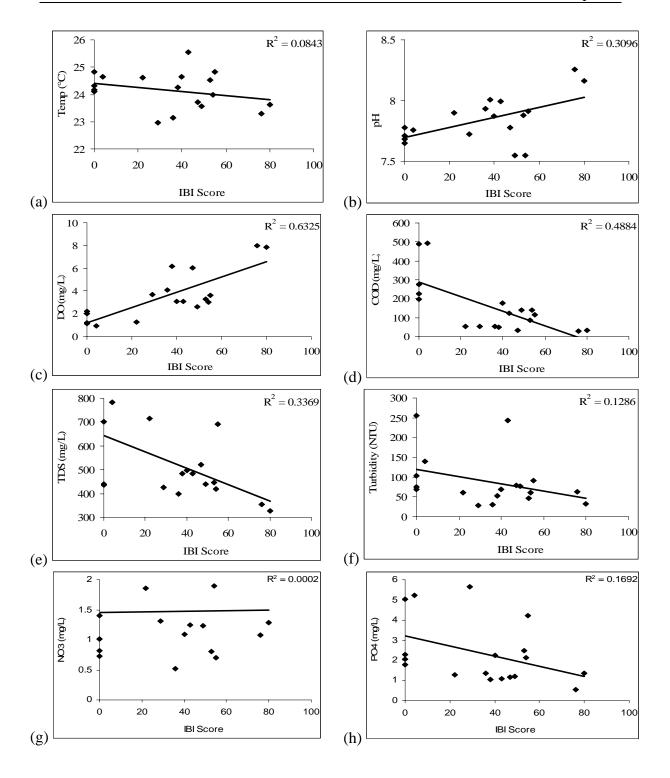
Fig. 6.5      Showing trend of stepped (a) and continuous (b) IBI scores along the longitudinal distance from upstream to downstream of Nullah Aik and Nullah Palkhu

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

(i)



(j)



(k)



(l)



(m)



(n)



(o)
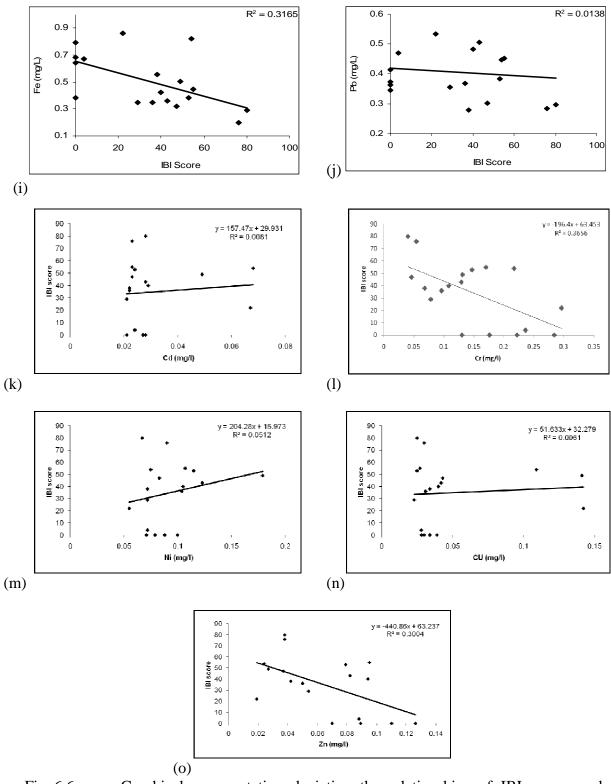
Fig. 6.6    Graphical representation depicting the relationships of IBI scores and water quality parameters (a. Temp., b. pH, c. DO, d. COD, e. TDS, f. Turbidity, g. $NO_3^-$, h. $PO_4^{3-}$, i. Fe, j. Pb, k, Cd, l, Cr, m. Ni, n. Cu and o. Zn).

Factors such as stream discharge, width, depth, and substrate also influence integrity of aquatic resources and have shown correlation with IBI scores (Casatti *et al.,* 2006). Regular stream discharge rate and large width of streams generally favour higher IBI score due to presence of high diversity of intolerant and column dweller species (Oberdorff & Porchcr, 1992; Tejerina-Garro *et al.,* 2005). Stream discharge, width, depth, and substrate influence the distribution of fish species (Casatti *et al.,* 2006, Oberdorff & Porchcr, 1992). Similarly, Radwell & Kwak (2005) also highlighted that physical attributes of river system are highly influential and play major role in discriminating among rivers to assess their spatial integrity. Width of Nullah Palkhu increases toward downstream and sites located downstream showed higher IBI scores. Depth of stream and rivers also influence the overall IBI scores and is difficult to portray the exact picture of fish assemblage in deep water due to inadequacy in sampling efforts as cited by Ganasan & Hughes (1998). Thus, large sized streams with good water quality should have significantly more species than a small, poor quality stream. Sites located in upstream of Nullah Aik (reference sites) had higher IBI score and had maximum width which decreases towards downstream sites. Sites with high IBI scores were least disturbed which were characterized by complex and sensitive fish assemblages sensitive to changes in habitat quality. Sensitive species generally avoid polluted sites by relocating their niche to the sites which are relatively less disturbed (Mebane *et al.,* 2003). Based on quantitative overall IBI score, three qualitative classes were developed (impaired, moderately impaired and less impaired). Ganason & Hughes (1998) also adopted classification system for assessment of ecological integrity of aquatic habitats which comprised of three qualitative classes (poor, fair and good). The use of less number of classes has been proven more beneficial for restoration of aquatic ecosystem in comparison to use of large number of classes (Ganason & Hughes, 1998; Lyons *et al.,* 2000). Most of sites in two tributaries of Chenab river were impacted with anthropogenic stress expects sites (1 and 2) located upstream of Nullah Aik. Sites located in upstream were classified as "less impaired" integrity class on the basis of their high IBI score. These sites were also characterized with high level of DO and pH in comparison to other integrity classes. Sites with lowest IBI score placed in impaired integrity classes were located in downstream, close to Sialkot city, a major source of industrial and municipal

pollution. These sites also had high contents of toxic metal related with anthropogenic origin such as Cr, Zn, Fe, Ni, Cd, Pb and Cu and lower level of DO and high level of COD, TDS and turbidity. Ganasan & Hughes (1998) also found minimum IBI scores near sources of pollution and highest in extreme downstream sites. Water quality of Nullah Palkhu improves as it stream moves away from the midstream towards downstream to the Sialkot city in comparison to Nullah Aik. IBI scores for downstream of Nullah Aik did not improve as it move downstream, probably due to pumping of water from streams for irrigation purposes (Qadir *et al.*, 2008). Pumping of stream water for irrigation is a known practice in Pakistan because it's a cheap source for crops irrigation. Large number of water pumps was found during sampling. Upstream sites of Nullah Palkhu experience low flow rate throughout the year especially in early summer and IBI scores for these sites were in medium range (25 – 35 for stepped IBI and 45 – 65 continuous IBI) and classified into "moderately impaired" integrity class. As the streams moves away from Sialkot city towards downstream on the west, water quality gradually improves as small water channels add in before these streams fall into river Chenab. Improvement of IBI scores for sites located downstream far from Sialkot city on Nullah Palkhu was observed which could be related to better water quality and habitat conditions. Many small channels collecting water from seepage of canals join Nullah Palkhu resulting improvement of surface water which becomes favorable for re-establishment of fish assemblages.

During present study, stepped and continuous IBI criteria depicted the similar results. Stepped and continues IBI criteria were found useful for the quantification of fish biological integrity of studied streams. Stepped IBI scoring method was developed by Karr et al., (1986) and later adopted by different authors (Oberdorff & Hughes, 1992; Minns *et al*., 1994; Hughes *et al*., 1998; Toham & Teugels, 1999; McCormick *et al*., 2001) with some modification. Dauwalter & Jackson (2004) and Bozzetti & Schulz (2004) applied the continuous scoring method for the calculation of IBI scores. Mebane *et al*. (2003) used continuous IBI scoring method and indicated that it is good for graphical representation. Ganasan & Hughes (1998) applied the both traditional (stepped) IBI scoring method as developed by Karr *et al.* (1986) and continuous IBI scoring methods. The results of this study supported the use of IBI for the assessment of fish assemblage

and habitat condition. IBI is more practicable index for the assessment and monitoring of fish assemblage in small streams rather than big rivers and lakes due to sampling inadequacies (Lyons *et al*., 2000). These inadequacies are due to more depth of water in which incomplete fish sampling might have resulted. Other comprehensive and precise sampling methods such as electro-fishing should be adopted to minimize the sampling errors (Ganason & Hughes, 1998). Before application of IBI, ecological managers must consider methods of their sampling effort, selection of reference site, and richness of native and non-native species to minimize the errors to extract more reliable results (Mebane *et al.*, 2003). Habitat quality of study of streams is essential to understand the overall impact of human activities (Spence *et al*., 1999). This approach is an important step toward assessment at ecosystem level considering human and environmental factors (Joy & Death, 2004). Urbanization, ill-planed industrialization in catchment and discharge of effluent without any treatment into Nullah Aik and Nullah Palkhu is putting fish assemblage under stress and are constant threat to local streams. This study highlights the importance of an integrated effort for management and restoration of such ecosystems using an integrated approach of IBI and multivariate techniques.

## Conclusions

IBI can be used for assessment and monitoring of fish assemblage in small streams of Pakistan. The results highlighted that to study the habitat quality study of streams is essential to understand the overall impact of human activities (Spence *et al*., 1999). The results of this study supported the use of IBI as an important tool for ecological assessment at ecosystem level with consideration of human related environmental factors and IBI can be used as an indicator for fish assemblage and habitat condition. IBI was successfully developed and applied for Nullah Aik and Palkhu; tributaries of River Chenab to assess the stream health of streams at 18 selected sites to portray a wide range of anthropogenic impacts on fish assemblage. Assessment of stream health using IBI provided an appropriate approach about habitat condition and its relationships to the human activities in catchment area and can be used as indicator of freshwater ecosystem degradation. Comparative assessment of biotic conditions in streams allows along spatial gradient. Stepped and continuous IBI criteria evaluated the sites from quantitative information of IBI into qualitative integrity classes (less impaired, moderately impaired and impaired) with least error. Most of the sampling sites were included in impaired integrity class, which indicated that fish assemblage of both streams have been altered by human activities. Finally, it was concluded that IBI based on fish assemblages is the most useful criteria for the assessment of habitat quality and streams health. Urbanization, ill-planed industrialization in catchment of study area and discharge of effluent without any treatment into Nullah Aik and Nullah Palkhu had negative impact on fish assemblage and diversity. These factors are considered important contributor to wipe out the local aquatic fauna and constant threat to local streams. The study highlighted the importance of an integrated effort for management and restoration of stream ecosystems and stresses the importance to initiate concrete steps to control the indiscriminate discharge of industrial and municipal waste into the streams from further degradation.

**Chapter 7**

# General Discussion

Anthropogenic activities cause multiple interlinked ecological problems of Nullah Aik and Nullah Palkhu stream system. Degradation of these streams becomes intensified after industrial revolution in the catchment area. Direct or indirect discharge of industrial effluents, municipal sewage and surface runoff are considered as the main sources of surface water pollution that have deleterious impact on the physical, chemical and biological components of Nullah Aik and Nullah Palkhu.

It is necessary to quantify the adverse impact of the multiple anthropogenic activities on ecological integrity of streams for effective management. These multiple factors are Industrialization, urbanization, atmospheric deposition and surface runoff, which affect the health status of streams and rivers (Allan, 2004; Kodarkar, 2004). Human stress is prominent in streams of those regions, where rainfall occurs with distinct seasonal pattern. This phenomenon is generally observed in temperate streams, which show wide range of variations in annual flow as well as water quality. In pristine streams, natural variations such as stream flow, rainfall and temperature occur after regular intervals and operates stream ecosystem with in natural range of variations to attain the state of equilibrium (Kauffman *et al.,* 1997) and changes in natural aquatic system can disrupt its ecological functions (Resh *et al.,* 1988), as well as structural integrity of an aquatic ecosystem (Kauffman *et al.,* 1997). Industrialization, urbanization, and intensive agriculture production are the major human activities that are responsible for deterioration and degradation of stream ecosystem (Sahasrabuddhe *et al.,* 2003). Ecological factors such as habitat degradation, barrier or abstraction for water divergence and introduction of alien species in aquatic ecosystems influence the biological state of streams and rivers (Rahel *et al*., 2008). Physical, chemical or biological characteristics have been used world wide for ecosystem management and restoration (Bundi *et al*., 2000).

Upstream of Nullah Aik showed good water as well as biological quality, whereas, middle segment receive huge volume of effluents that acts as chemical barrier, which restrict the movement of fishes from downstream to upstream. In downstream segment of Nullah Aik, a small headworks is present that is used to divert stream water for irrigation

purposes. Downstream segment of Nullah Aik also receive effluents from Begowala drain that bring effluent from tanneries of Sambrial and discharged into Nullah Aik. Therefore, presence of small headworks, Begowala drain and stream flow regulations are the main obstacle in improvement of water quality. Upstream of Nullah Palkhu experiences dry spell during summer season resulting reduction in stream flow even it may become dry and fishes get refuge in riffle and pools. This dry spell culminates with start of monsoon rainfalls. Stream fish fauna proliferate during monsoon season to re-establish the fish community in upstream of Nullah Palkhu. Higher stream flow improves water quality in downstream of Nullah Palkhu, which is favourable for higher diversity of fishes. Water logged area in surroundings of UC and MRL canals drain water into downstream of Nullah Palkhu, which resulted high stream flow. High stream flow dilutes the concentration of pollutants coming from Sialkot city and stream water quality gets improved. This improved water quality favours the organization of fish assemblage in downstream.

Spatial and temporal variations provided better understanding about the pollutants dynamics in stream water (Podgorney *et al.*, 2000). The first objective of the present study was to highlight the spatial and temporal variations in stream water quality and source identification of contaminants. Multivariate statistical techniques such as HACA, DFA and FA/PCA segregated important variables that explained most of the spatial and temporal variations and highlighted the contribution of contamination from different sources. Three classes of sampling sites viz., relatively unimpaired region (RUR), impaired region (IR) and moderately impaired region (MIR) were identified on the basis of water quality parameters of stream water using CA. RUR consisted of upstream sites, which were least contaminated and IR was highly contaminated.

Discriminant analysis highlighted most important variables, which causes variations in surface water on spatial and temporal scale. Water quality parameters identified 11 important parameters viz., stream flow, stream depth, DO, COD, TDS, $NO_3^-$, $PO_4^{3-}$, Pb (dis), Cr (dis), Mg (sus), and Ni (sus) were important to bring variations along spatial gradient. Seasonal variations were related with stream flow, temperature, EC, salinity, total hardness, Na (dis), K (dis), Ca (dis), Mg (dis), Fe (dis), Cd (dis), Cu (dis) and Na (sus). Spatial variations in water quality are the results of anthropogenic stress along the longitudinal stretch of the studied streams while, seasonal variations are mainly derived from natural factors. Low and high stream flow

are controlled by rainfall in the catchment area. Stream flow, discharge and rainfall determine the concentration of contaminants on seasonal basis. The pollutants from non-point sources are more pronounced when streams flow at full swing, while point sources showed marked variations when streams experienced lowest flow rate. Stream discharge of Nullah Aik and Nullah Palkhu become high during monsoon season, which causes dilution of contaminant concentration coming from point sources. In dry period, different pollutants like heavy metals deposited on the soil surface and process of deposition continue throughout the year. Heavy rainfall during monsoon season washed away deposited pollutants and increases the concentration in stream water. Therefore, concentration of pollutants from atmospheric deposition becomes high during monsoon season.

FA/PCA identified six main sources of different contaminant such as (i) municipal waste (COD, EC, TDS, turbidity, $PO_4^{3-}$, $S^{2-}$, Na, K, Mg, Pb, Ni and Zn), (ii) industrial effluents (Fe, Ni, Pb, Cr and Zn), (iii) tanneries effluents (COD, EC, TDS, turbidity, $S^{2-}$, Na, Ca, Cr, Zn), (iv) agricultural runoff ($NO_3^-$, Pb, Cd, Cu and Zn), (v) urban runoff (Pb, Cd, Cu and Zn) and (iv) parent rock material (Na, K, Ca and Fe). Heavy metals contamination is related to multiple sources mainly through industrial, municipal, atmospheric deposition and surface runoff (Singh *et al*., 2005a). The impacts of human degradation on water quality were high during winter and pre monsoon season when streams flow reduced to lowest level. This reduction in stream flow makes the situation more critical from ecological as well as aesthetic view. Higher concentration of heavy metals was measured in suspended particulate matter, whereas, low concentration was present in dissolved form due to slightly alkaline pH, high salinity and water hardness level (Wright & Welboun, 2004).

Higher levels of metals in mid-stream region are largely due to tanneries effluents and municipal sewage along with waste from surgical instrument manufacturing units. Metals such as Cr, Ni and Zn were associated with point sources and showed high concentration during dry season, when stream experience low flow. The state of metals (dissolved and suspended particulate matter) is highly influenced by physiochemical water quality parameters. High concentration of heavy metals in dissolved form is considered as potential threat for aquatic organisms (Guéguen & Dominik, 2003). Among metals in dissolved form Fe, Pb, Cd, Cr and Ni were recorded higher to those of permissible levels. The high load of heavy metals in stream water is one of the major concerns for aquatic biota of the studied stream.

Second objective of the present study was the assessment of bioaccumulation patterns of heavy metals in different organs (liver, gills, kidneys and muscles) of fish species on spatial and temporal scales. Highest metal accumulation was recorded in liver followed by gills, kidneys and muscles. Significant variations of heavy metals accumulation were observed in different organs within same and among different species. The physiology of healthy fish do not allow the accumulation of metals in its organs and excrete the large amounts of heavy metals (Pourang, 1995) resulting in low accumulation of metals in fish. The results revealed that elevated concentration in various organs like liver and gills is influenced by contaminated environment with heavy metals for longer time periods (Goldstein & DeWeese, 1999). Elevated level of heavy metals reduces the growth and disturbed body physiology (Maity *et al.*, 2008). Fishes with elevated level of metals represent the degraded health of freshwater ecosystem. Feeding habits and trophic position in food chain also contributes to differences in bioaccumulation of different metals (Tawari-Fufeyin & Ekaye, 2007). Bioavailability of heavy metals is also influenced by metal form (either dissolved or suspended) in surrounding medium (Rainbow, 1995). Dissolved form of metals, which are readily available to fishes cause toxic effects on physiological equilibrium of fishes (Playle, 1998). The cumulative affect of heavy metals usually lead to acute or chronic stress and eventually causes the death of fish species (Fromm, 1980).

Accumulation of Pb and Cr in different organs of fishes was significantly different between upstream and downstream segments of streams, whereas, Fe, Cd, Ni, Cu and Zn did not show significant spatial variations in different fish species. Metal accumulation was highest at down stream of Nullah Aik and Nullah Palkhu and lowest at upstream of Nullah Aik and Nullah Palkhu. High metal concentration in fishes captured from downstream sites was due to increased concentration in ambient stream water. The mid stream and downstream sites were contaminated with heavy metals especially Pb and Cr, which were related with industrial effluent and municipal sewage discharged from tanneries and municipalities in catchment area. Concentration of Fe, Cd, Cr and Cu showed significant seasonal variations. High concentrations of heavy metals were recorded during pre monsoon season, when stream water exhibited elevated level of metals.

Significant inter-specific variations in the bioaccumulation pattern of heavy metals were recorded. Pb, Cd and Cr in muscles of *Channa punctata, Labeo rohita, Cirrhinus reba, Puntius sophore* and *Wallago attu* captured from downstream of

Nullah Aik and Nullah Palkhu exceeded the permissible level described by United States Environmental Protection Agency (1999).

Fish muscles are important for human consumption point of view (Alibabić *et al*., 2007). Metals viz., Pb, Cd and Cr were recorded above the permissible limits of heavy metals in muscles of *Labeo rohita, Puntius sophore, Channa punctata, Oreochromis niloticus* and *Wallago attu*. The concentration of Pb was reported higher in *Labeo rohita, Puntius sophore, Channa punctata* and *Wallago attu*. Higher concentration of Cd and Cr was reported in muscles of *Cirrhinus reba, Channa punctata* and *Oreochromis niloticus*.  Higher concentration of Pb and Cd can be related to non-point sources. Metals absorbed by fish through gills and digestive system while Cr is related to tanneries into stream water. High concentration of heavy metals in stream water increases the process of bioaccumulation in fish species, particularly, intolerant species that can survive in polluted water. However, further investigations are required to predict the distribution pattern of heavy metals in fish species at various trophic levels.

Third objective of present study was to describe the diversity and temporal distribution pattern of different fish assemblage in relation to stream water quality. Fish assemblage in the regular streams exhibited fewer variations in flow regime remains stable through out the year (Casatti, 2005). However, flow of intermittent streams varies from season to season through natural annual cycle that affects the fish assemblage (Grossman *et al*., 1990). Anthropogenic disturbance, which is more pronounced in studied streams, may disturb the fish assemblage (Franssen *et al*., 2006). When streams exhibited low flow along the high level of human disturbance, this unfavorable condition force the fish assemblage to move in least polluted segment of stream (Vila-Gispert *et al*., 2002). Due to pollution stress, sensitive fish species become vanished from polluted stream and their biological niche become occupied by invasive species. However, if particular segment of stream is less diverse, fish assemblage will regain during favourable season like monsoon.

A total of 24 fish species belonging to 12 families were recorded from Nullah Aik and Nullah Palkhu. Maximum diversity were recorded in upstream sites and lowest at downstream of Nullah Aik. *Channa punctata* and *Puntius sophore* were the most dominant species and found at most of the sites. Upstream of Nullah Aik showed better and least disturbed conditions that favour the proliferation of fishes. The upstream fish assemblage was comparatively stable in different seasons under natural

conditions but stream segment faces the anthropogenic stress in urban area do not support the fish life due to high degradation of surface water. As streams traverse through Sialkot city, receives a large volume of industrial effluent (especially from tanneries) and municipal sewage resulting in deterioration of water quality, which has negative impacts on fish species.

The present study concluded that municipal and industrial activities are putting fishes of stream under stress in comparison to non-point sources. Municipal and industrial effluents decrease the level of DO and increase the COD level and heavy metals. Most of the fish species cannot tolerate the low concentration of DO and high level of COD and heavy metals in stream water. In downstream sites, only those species were found, which can tolerate fluctuations in DO level and high level of COD, turbidity and heavy metals. Fish species richness increases as water quality improves in downstream. Comparatively less improvement in fish fauna in downstream of Nullah Aik was observed. This may be due to the presence of small headworks, diversion of water for irrigation and Begowala drain that bring large volume of tanneries effluents. As a result of such constraints these sites remain impaired for re-appearance of native fish fauna, however, those species, which can tolerant pollution stress may reappear. In comparison downstream sites of Nullah Palkhu, showed maximum diversity especially in post monsoon season due to improved water quality.

The fourth objective of present study was to develop and apply an index of biological integrity for quantification of ecological degradation of Aik and Palkhu streams. Two kinds of IBI scoring criteria (continuous IBI and stepped IBI) were developed. Three spatial groups of sites were obtained based on fish abundance data. This group includes least impaired sites (1 and 2), which exhibited most of the characteristics of reference conditions, impaired sites (3, 4, 5, 8, 12, 13 and 14) and moderately impaired sites (6, 7, 9, 10, 11, 15, 16, 17 and 18). Three major guilds of spatial preference groups were identified on the basis of fish associations viz., upstream guild, evenly distributed guild and downstream guild. NMDS identified the four groups of sites on the basis of habitat impairment and species richness. Based on NMDS species ordination, three groups were identified on the basis of stress tolerance and habitat impairment. Maximum IBI score was calculated from upstream sites of Nullah Aik, whereas, minimum of zero score was calculated from those sites where no fish specimen was recorded.

One of the main constrain in present study was the absence of an ideal reference site in the catchment area. Following the studies of Sudaryanti *et al*. (2001), Hart *et al*. (2001) and Tejerina-Garro *et al*. (2005) reference sites were identified on the basis of fish diversity data and subjected to cluster analysis. Two sites located in upstream of Nullah Aik showed maximum species richness and abundance and were considered as reference sites i.e., least disturbed sites. Sites in close downstream of Sialkot city showed minimum fish species richness and abundance. These sites showed highest level of contaminants in stream water. On the other hand, the sites located in far downstream of streams represent intermediate range of fish species richness and abundance. Sensitive species were recorded at reference sites. No fish species was recorded in close premises of point sources located in Sialkot city. As stream water traverse the distance toxic substances settle down in stream sediments and water quality improves. Only tolerant fish species were found in far downstream sites due to the improvement of stream conditions.

Improvement in IBI scores in downstream sites of Nullah Palkhu was evident in contrast to Nullah Aik, which was due to presence of small head works at Wain and Begowala drain discharge huge volume of tanneries effluents into Nullah Aik at Kot Zamin (Fig. 2.1) after traversing 25km. Last site located on Nullah Aik receives effluents from few tanneries located at Sialkot-Wazirabad road municipal sewage from Wazirabad city. Low IBI scores were recorded from the sites located in upstream of Nullah Palkhu as stream flow become reduced even stream become dry during pre monsoon season, when fishes refuge in small ditches and pools which restrict the proliferation of fish communities. IBI index appears quite useful for understanding and interpreting the stream health and the human impacts on aquatic ecosystem. The IBI score for each site was found to be more reliable in response to human impact on aquatic ecosystem. The results of present study supported the use of IBI for the quantitative assessment of fish assemblage and human pressure on stream health. Therefore, fish communities in various segments of a stream are the best indicators of overall biological integrity. These streams are also facing a serious infestation of exotic species in upstream and downstream sites which indicate stream ecosystem is fragile. Habitat degradation of streams provided an opportunity for the invasion of exotic fish species. Two exotic species in upstream as well downstream sites were recorded. Invasion of exotic species is another critical issue for the stream health in Nullah Aik and Nullah Palkhu.

This study presents an integrative approach about the impacts assessment of human activities on natural aquatic ecosystem of two streams. This document provides information related to impacts of human activities on water quality and fish fauna and a guideline document for future research endeavours on various aspects of Nullah Aik and Nullah Palkhu. The behaviour of contaminants was investigated on spatial and temporal basis and their impacts on the fish fauna residing in Nullah Aik and Nullah Palkhu, which was not previously studied. Human activities such as industrial, urbanization and agriculture are rapidly increasing in the catchment area of Nullah Aik and Nullah Palkhu. As a result of these activities, a huge volume of effluents containing many toxic pollutants such as heavy metals are directly or/and indirectly discharged into Nullah Aik and Nullah Palkhu, which are degrading physical, chemical and biological integrity and these effects were at the sites, which were located near the point sources with low stream flow rate. Fishes dwelling in such polluted stream segment are under stress of heavy metals, which have led to fragmentation of fishes into upstream and downstream of streams. This encourages the invasion of exotic fish species that severely change the structure and function of ecosystem and reduce the stream biodiversity. Nullah Aik and Nullah Palkhu are one of the main water sources for human use since long time and important natural source for local community but present health status indicates that it is unacceptable for human, domestic, and agricultural usage. The restoration of Nullah Aik and Nullah Palkhu is necessary not only for the well being of local area but also for aquatic integrity of River Chenab. This is the need of hour, peep into the matter of water quality deterioration and loss of fish fauna in Nullah Aik and Nullah Palkhu for restoration of polluted stream ecosystems and protection of human health at large. This alarming situation demands dire attention of concerning authorities like Govt. authorities, municipalities and environmental agencies to come and play effective role in protection, management and conservation of streams. The results of this research can be used for effective restoration of Aik-Nullah system.

## Recommendations

### For the improvement of conditions in stream and catchment area

Following are the proposed recommendations to improve the environmental conditions in catchment area and instream conditions of Nullah Aik and Nullah Palkhu:

- Concerned authorities (National, Provisional, District Govt., Environmental department etc.) should take immediate measures (such as complete ban on discharge of untreated industrial effluent and municipal sewage) for restoration of Nullah Aik and Nullah Palkhu.

- There should be proper regulations for the production and dumping of effluents produced as a result of anthropogenic activities in the catchment area.

- Environmental impact assessment should be done before the construction of any industrial unit, housing colonies and other structures.

- Heavy fines should be imposed on those authorities, industries and individuals which discharge wastes whether it is liquid or in solid form.

- Taxes should be charged from authorities; industries and individuals on the basis of pollute and pay principle.

- Stream management activities should be implemented on the priority basis in stream segments traversing in urban and industrial area.

- People residing in proximity of the Nullah Aik and Palkhu should be educated about the negative impacts of stream pollution in relation to human health and broadly at ecosystem level.

### For the improvement of stream water quality

The recommendations for the improvement of water quality of Nullah Aik and Nullah Palkhu are as under:

- Effective measures (proper legislation, implementation of environmental laws, stream management etc.) should be adapted to reduce the deleterious contributions of point sources that degrade the water quality of Nullah Aik and Nullah Palkhu especially, focusing on those water quality attributes that are contributing to major spatio-temporal variations and their concentration above the international environmental standards.

- Strict criteria should be developed and applied to treat the effluents at their generation site.

- Waste treatment plants should be established for the effective treatment of industrial and municipal waste.

- All industrial units should be encouraged to adopt the effluent treatment techniques at the source and those units which are violating and discharging their effluent without treatment should be punished.

- There should be a regular monitoring of industrial units, municipal sewage into stream water.

- People belonging to various segments of the community (stakeholders) should be encouraged to highlight the aquatic pollution problems and educate people about the deleterious effects of pollutants on aquatic ecosystem and their harmful impacts on human life.

- Guidelines of stream water should be developed not only for Nullah Aik and Palkhu but also for other streams and rivers at national level. This requires repeated experiments to establish the local water quality standards.

**For the protections and regrouping of fish assemblage**

Following recommendations are based on the information extracted from results and discussion to improve the conditions for fish assemblage in Nullah Aik and Nullah Palkhu:

- If above mentioned recommendations are followed, ultimately it will lead towards reorganization of biological communities in Nullah Aik and Palkhu.

- There should be a regular assessment of metal contents in different organs of fish species dwelling in Nullah Aik and Nullah Palkhu to educate the people about consequences consumption of contaminated fish meat.

- There should not be physical barriers like headworks to divert water for irrigation, which restrict the movement of fishes. If such structures are inevitable then there should be a fish ladder for the movement of fishes from downstream to upstream.

- The stream habitat should be managed to provide better conditions for nesting and breeding sites of fishes within streams.

**Future Thrusts**

Pakistan is among those countries, which have little or no baseline data and information for its stress stricken water bodies. The economy of Pakistan is readily growing with rapid industrialization, urbanization and extensive agriculture, which have placed the aquatic resources under immense stress. Scientific studies and investigations such as the present one is necessary for the environmental management and conservation activities. It is strongly recommended that current study should be continued in term of physiochemical and biological components of focused streams.

- Comprehensive survey should be conducted to assess the volume of effluents that are discharged through different point sources into stream system.

- The focus of future studies should be broadened to assess the status of persistent organic pollutants (POPs) along with heavy metal contamination.

- Macro-invertebrates such as aquatic crustaceans and mollusks founded at the upstream sites should be investigated as potential biological indicators on spatial and seasonal scales.

- Birds and turtles are other important group of vertebrate residing in and along the streamside could be studied as good biological indicators for the bio-magnification of heavy metals and organic pollutants.

- Fish sampling should be done with help of electro-fisher and compared with traditional netting at regular intervals conducted during post monsoon and pre monsoon seasons to find out pattern of fish assemblage.

- The IBI criteria should be applied not only for fishes but also for macro-invertebrates, birds and aquatic plants to assess the more accurate information about the degradation of an aquatic ecosystem.

- Newly developed IBI criteria for streams under focus should be applied on other streams of the same region to test its efficacy and utility.

- There should be a comprehensive study on the invasion of exotic species in natural streams and their potential impacts on the aquatic ecosystems.

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Appendix 2.1 Monthly average minimum and maximum daily temperatures (°C) Rainfall (mm) and Relative Humidity (%) at Sialkot located in the catchment of Nullah Aik and Nullah Palkhu

| Month | Min. Temperature (°C) | Max. Temperature (°C) | Precipitation (mm) | Relative Humidity (%) |
|---|---|---|---|---|
| Jan | 5 | 18 | 39.4 | 74 |
| Feb | 7 | 21.5 | 43.9 | 66.7 |
| Mar | 12 | 26 | 55.5 | 59.9 |
| Apr | 17.5 | 34 | 30.7 | 43.7 |
| May | 22 | 38 | 27.9 | 33.5 |
| Jun | 25.4 | 40 | 70.3 | 40.7 |
| Jul | 25 | 35 | 292.9 | 67.7 |
| Aug | 24.5 | 34.2 | 259.1 | 75.4 |
| Sep | 22 | 34 | 103.6 | 69.4 |
| Oct | 16 | 32 | 15 | 62.6 |
| Nov | 10 | 25.7 | 9 | 70.7 |
| Dec | 5.5 | 19.7 | 31.4 | 76.3 |

Appendix 2.2 Longitude, latitude and altitude of 18 sampling sites located on Nullah
Aik and Nullah Palkhu

| Site # (locality name) | Stream | Longitude | Latitude | Altitude (m) |
|---|---|---|---|---|
| Site 1 (Umanwali) | Nullah Aik | 74° 39′ 45″ E | 32° 30′ 21″ N | 247 |
| Site 2 (Uoora) | Nullah Aik | 74° 36′ 55″ E | 32° 29′ 30″N | 245 |
| Site 3 (Pul Aik Sialkot) | Nullah Aik | 74° 31′ 50″ E | 32° 30′ 39″ N | 242 |
| Site 4 (Kotli Marlanwali) | Nullah Aik | 74° 29′ 05″ E, | 32° 27′ 26″ N | 237 |
| Site 5 (Sahikay) | Nullah Aik | 74° 25′ 03″ E | 32° 25′ 58″ N | 233 |
| Site 6 (Dellum Blaggen) | Nullah Aik | 74° 21′ 22″ E | 32° 25′ 48″N | 230 |
| Site 7 (Wain small head works) | Nullah Aik | 74° 18′ 03″ E | 32° 25′ 39″ N | 227 |
| Site 8 (Kot Zamin) | Nullah Aik | 74° 11′ 55″ E | 32° 26′ 09″ N | 222 |
| Site 9 (Ladowali Puli) | Nullah Aik | 74° 09′ 95″ E | 32° 27′ 02″ N | 219 |
| Site 10 (Chun) | Nullah Palkhu | 74° 37′ 54″ E | 32° 33′ 34″ N | 250 |
| Site 11 (Dolluwali) | Nullah Palkhu | 74° 36′ 14″ E | 32° 32′ 22″ N | 247 |
| Site 12 (Chitti Sheikhan) | Nullah Palkhu | 74° 30′ 43″ E | 32° 31′ 45″ N | 238 |
| Site 13 (Jatta) | Nullah Palkhu | 74° 27′ 24″ E | 32° 32′ 30″ N | 232 |
| Site 14 (Upper Chenab canal crossing) | Nullah Palkhu | 74° 25′ 20″ E | 32° 32′ 38″ N | 229 |
| Site 15 (Jathekay) | Nullah Palkhu | 74° 19′ 47″ E | 32° 32′ 29″ N | 227 |
| Site 16 (Kotli Khokhran) | Nullah Palkhu | 74° 16′ 40″ E | 32° 29′ 03″ N | 224 |
| Site 17 (Kot Khuda Bux) | Nullah Palkhu | 74° 12′ 30″ E | 32° 27′ 45″ N | 221 |
| Site 18 (Wazirabad) | Nullah Palkhu | 74° 07′ 08″ E | 32° 27′ 06″ N | 217 |

Appendix 2.3a  Number of collected fish specimens, Number of dissected individuals, body length and body weight of eight fish species sampled from different sampling zones located at Nullah Aik and Nullah Palkhu during postmonsoon seasons

| Species | Zone | No. of captured fish individual | No of dissected fish individual | Body Length (cm) | Body Wt.(g) |
|---|---|---|---|---|---|
| *Channa* | 1 | 8 | 2, 3 | 12.0±3.1 | 35.3±6.4 |
| *punctata* | 2 | 26 | 2, 3 | 11.3±2.9 | 31.2±6.3 |
| | 3 | 87 | 3, 3 | 16.0±4.5 | 60.05±10.45 |
| | 4 | 123 | 3, 3 | 17.3±4.9 | 48.5±10.4 |
| *Cirrihinus riba* | 1 | 24 | 3, 3 | 10.5±1.8 | 26.7±7.1 |
| | 2 | 12 | 3, 3 | 12.1±2.3 | 30.7±7.9 |
| | 4 | 15 | 3, 3 | 10.2±1.3 | 28.3±0.6 |
| *Labeo rohita* | 1 | 27 | 3, 3 | 17.4±7.4 | 83.2±9.6 |
| | 3 | 9 | 2, 3 | 19±6.1 | 88.6±6.7 |
| | 4 | 4 | 1, 2 | 20.4±4.1 | 95.4±8.9 |
| *H. fossilis* | 3 | 27 | 3, 3 | 23.5±6.4 | 70.5±7.3 |
| | 4 | 49 | 3, 3 | 25.2±7.3 | 73.7±8.9 |
| *Mystus* | 1 | 43 | 3, 3 | 15.3±2.1 | 43.2±4.6 |
| *cavasius* | 2 | 21 | 3, 3 | 13.2±1.7 | 38.1±3.7 |
| | 4 | 29 | 3, 3 | 10.2±1.3 | 28.3±0.6 |
| *O. niloticus* | 1 | 48 | 3, 3 | 12.1±1.9 | 42.1±4.2 |
| | 2 | 3 | 1, 2 | 12.6±2.1 | 44.2±6.3 |
| | 3 | 3 | 3, 3 | 11.5±1.2 | 39.1±3.8 |
| | 4 | 15 | 3, 3 | 11.4±2.7 | 37.1±5.4 |
| *Puntius* | 1 | 72 | 3, 3 | 4.3±0.5 | 8.5±0.8 |
| *sophore* | 2 | 30 | 3, 3 | 4.7±0.6 | 10.0±1.1 |
| | 3 | 38 | 3, 3 | 5.5±0.9 | 10.2±1.6 |
| | 4 | 77 | 3, 3 | 4.6±0.6 | 9.8±1.1 |
| *Wallago attu* | 1 | 11 | 3, 3 | 22.3±10.5 | 49.6±18.8 |
| | 3 | 6 | 2, 2 | 18.4±5.2 | 34.5±7.8 |
| | 4 | 16 | 2, 2 | 19.2±7.1 | 35.6±6.5 |

Appendix 2.3b Number of collected fish specimens, Number of dissected individuals, body length and body weight of eight fish species sampled from different sampling zones located at Nullah Aik and Nullah Palkhu during pretmonsoon

| Species | Zone | No. of captured fish individual | No of dissected fish individual | Body Length (cm) | Body Wt.(g) |
|---|---|---|---|---|---|
| *Channa* | | 3 | | 14.5±1.2 | 44.3±2.2 |
| *punctata* | 1 | | 1,2 | | |
| | 2 | 14 | 3,3 | 12.0±0.9 | 52.5±8.0 |
| | 3 | 21 | 3,3 | 18.0±1.5 | 103.7±11.04 |
| | 4 | 14 | 3,3 | 16.1±3.7 | 54.6±5.24 |
| *Cirrihinus* | | 70 | | 16.3±0.9 | 35.4±5.4 |
| *reba* | 1 | | 3,3 | | |
| | 2 | 4 | 2,2 | 15.5±0.9 | 30.5±6.1 |
| | 4 | 4 | 2,2 | 14.6±1.7 | 28.1±5.6 |
| *Labeo* | | 55 | | 17.4±7.4 | 83.2±9.6 |
| *rohita* | 1 | | 3,3 | | |
| *H.* | | 8 | | 27.2±6.8 | 78.4±9.3 |
| *fossiliss* | 3 | | 2,2 | | |
| | 4 | 7 | 2,2 | 25.4±4.6 | 67.9±5.6 |
| *Mystus* | | 8 | | 13.8±1.1 | 19.5±3.9 |
| *cavasius* | 1 | | 2,3 | | |
| | 2 | 5 | 2,2 | 11.8±1.3 | 17.0±1.6 |
| *O.* | | 23 | | 17.2±0.9 | 88.7±12.2 |
| *niloticus* | 1 | | 2,3 | | |
| *Puntius* | | 22 | | 11.5±1.2 | 23.0±1.8 |
| *sophore* | 1 | | 3,3 | | |
| | 2 | 12 | 3,3 | 10.7±1.5 | 24.3±2.2 |
| *W. attu* | 1 | 1 | 1 | 19.0 | 38.1 |

Appendix 6.1    Habitat structure and characteristics at sampling sites located on Nullah Aik and Nullah Palkhu

| Code | Location | Up/Down Stream | Distance between sampling sites (km) | Stream Bank vegetation | Habitat type | Habitat physical parameters | Pollution sources | Stream bed sediments (texture & colour) |
|---|---|---|---|---|---|---|---|---|
| 1 | Umranwali | Upstream Nullah Aik | 0 | Tall grasses | Running water | Clear with smell | No apparent | Sandy Pale yellow |
| 2 | Uoora | Upstream Nullah Aik | 12.56 | Tall grasses | Running water | Clear with smell | No apparent | Sandy Pale yellow |
| 3 | Pul Aik-Sialkot | Downstream Nullah Aik | 25.70 | Deciduous Trees | Running water | Turbid with effluents | Municipal and industrial | Sandy Black with organic waste |
| 4 | Kotli  Marlan | Downstream Nullah Aik | 32.65 | Deciduous Trees | Running water | Turbid with effluents | Municipal and industrial | Sandy Black with organic waste |
| 5 | Sahibkay | Downstream Nullah Aik | 36.33 | Tall grasses and deciduous Trees | Running water with marginal aquatic vegetation | Turbid with effluents | Agriculture | Sandy Black with organic waste |
| 6 | Dellum Blaggun | Downstream Nullah Aik | 43.53 | Tall grasses and deciduous Trees | Running water With marginal aquatic vegetation | Turbid with effluents | Municipal and Agriculture | Sandy Black with organic waste |
| 7 | Wain Small Head works | Downstream Nullah Aik | 47.70 | Tall grasses and deciduous Trees | Barrier for water diversion | Less turbid stagnant | Agriculture | loamy Black with organic waste |
| 8 | Kot Zamin | Downstream Nullah Aik | 59.51 | Short grasses | Running water with marginal aquatic vegetation | Turbid with tanneries effluents | Tanneries drain and Agriculture | Loamy Black with organic waste |
| 9 | Ladowali puli | Downstream Nullah Aik | 64.80 | Short grasses | Running water with marginal aquatic vegetation | Turbid with effluents | Tanneries Municipal and Agriculture | Loamy Black with organic waste |
| 10 | Chunn | Upstream Nullah Palkhu | 0 | Short grasses | Running water with ditches and pools | Turbid with silt and clay | Agriculture | Loamy Clay pale yellow |
| 11 | Dallowali | Upstream Nullah Palkhu | 5.03 | Short grasses | Running water with ditches and pools | Turbid with silt and clay | Agriculture | Loamy Clay pale yellow |
| 12 | Chiti Shaikhan | Downstream Nullah Palkhu | 17.76 | Planted trees | Sluggish water no pools formation | Turbid with heavy tanneries effluent | Tanneries Municipal and industrial | Muddy Black with organic waste |
| 13 | Jatta | Downstream Nullah Palkhu | 24.76 | Shorted grasses with scattered Eucalyptus plantation | Sluggish water no pools formation | Turbid with heavy tanneries effluent | Tanneries Municipal and industrial | Muddy Black with organic waste |
| 14 | Upper Chenab crossing | Downstream Nullah Palkhu | 28.39 | Short grasses with bushes | Running water with out pools and ditches | Turbid effluent | Municipal and Agriculture | Muddy Black with organic waste |
| 15 | Jathekay | Downstream Nullah Palkhu | 39.81 | Tall grasses with scattered trees | Running water with out pools and ditches | Less turbid water diluted with a tributary | Agriculture | Loamy grey with organic waste |
| 16 | Kotli Khokhran | Downstream Nullah Palkhu | 49.91 | Tall grasses | Running water with out pools and ditches | Less turbid water diluted with a tributary | Agriculture | Loamy grey with with organic waste |
| 17 | Kot KhdaBux | Downstream Nullah Palkhu | 54.23 | Tall grasses | Running water with pools and ditches along sides | Less turbid water diluted with a tributary | Agriculture | Loamy grey with organic waste |
| 18 | Wazirabad | Downstream Nullah Palkhu | 65.39 | Short grasses With shrubs | Running water with  pools and ditches along sides | Turbid effluent | Municipal and Agriculture | Muddy Black with organic waste |

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