EVALUATION OF HEAVY METALS TOXICITY IN SELECTED FISH SPECIES OF RIVER KABUL

BY

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DEPARTMENT OF ZOOLOGY

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IN THE NAME OF ALLAH, MOST COMPASSIONATE, EVER MERCIFUL

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D E D I C A T I O N

Dedicated To

My loving Father Qialees Khan (Late)

My loving Mother Khaista bibi (Late)

And

My Eldest Brother Mr. Ayaz khan

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AAC: Air acetylene
ADD: Average daily doses
Kcl: Potassium chloride
Ag: Silver
As: Arsenic
Ba: Barilium
B: Boran
CDC: Complete degeneration of cillia.
Co: Cobalt
CW: Constructed wetland
CGL: Clumping of gills lamellae
Cl: Chloride
CN: Coagulative necrosis
NMA: Normal melting agarose
Cr: Chromium
Cu: Copper
DE: Degeneration of epithelium.
DC: Degenerative cillia
DMSO: Dimethylsulfoxide
DNA: Deoxyribo nucleic acid
EC: Electrical conductivity
EDTA: Ethylene diemine tetra acitic acid
ED: Epithelial disquamation
Fe: Iron
FD: Fibrillar degeneration
GAIE: Gadoon Amazai Industrial Estate
HCl: Hydrochloric acid
HD: Hydropic degeneration
Hg: Mercury
INF: Inflammation
K: Potassium
KH_2PO_4: Potassium phosphate monobasic
KPK: Khyber Pakhtonkhowa
L: Fuel-lean
LMPA: Low melting point agarose
LN: Liquefactive necrosis
Mn: Manganese
MW: Mega watt
NA: Necrotic area
Na: Sodium
NaCl: Sodium chloride
NaOH: Sodium hydroxide
NBF: Neutrally buffered formalin
NEQS: National and environmental quality standards
Ni: Nickel
CRL: Centralized Resource Laboratory
NSI: Non specific inflammation
OD: Optical density
Pb: Lead
PBS: Phosphate buffered saline
PCA: Principal component analysis
pH: Power of hydrogen
R: Fuel-rich
RDA: Recommended daily dietary allowance
Se: Selinium
SH: Spongiosis hepatis
S.S: Stainless steel
Sr: Stroncium
TA: Total alkalinity
TCS: Total comet score
TDS: Total dissoled solid
TSS: Total suspended solid
VO: Vacuolation and oedema
WHO: World health organization
WQI: Water quality indices
Zn: Zinc

PREFACE

The main objectives of the present thesis were to study physico-chemical and heavy metal contaminations in water and heavy metals accumulation and toxicity in selected fish species of River Kabul and to achieve these objectives, the thesis research work has been divided into seven chapters; each chapter is focused on specific objectives in details.They are as following.

First Chapter deals with introduction of this study. This chapter introduced the study area along with aims and objectives and justification of this study.

Second Chapter describes literature reviews that explain related studies reported by the environmental scientists in the world.

Third Chapter focuses on the physio-chemical parameters slike pH, total suspended solid (TSS), total dissolved solid (TDS), total alkalinity (TA), chloride (Cl), electrical conductivity (EC), sodium (Na) and potassium (K) and heavy metals such as zinc (Zn), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn) and mercury (Hg) in the drinking water of River Kabul. This chapter further used statistical analysis for source apportionment of the contaminations in drinking water.

Fourth Chapter describes bioaccumulation of heavy metals including zinc, nickel, chromium, copper, cadmium, lead, iron, manganese and mercury in different tissues and organs of selected fish species of River Kabul. One paper from the data of this chapter entitled; **Bioaccumulation of heavy metals in different organs of *Wallago attu* from River Kabul Khyber Pukhton Khowa, Pakistan**; has been published in *Biological Trace Element Research* international Journal.

Fifth Chapter focuses on genotoxicological impacts of heavy metals in different tissues and organs of selected fish species of River Kabul.This chapter further determined different degree of DNA damage like total comet score (TCS), comet class 0, class 1, class 2, class 3 and class 4 in order to assess the risk for human life and environmental impact.

Sixth Chapter describes histopathological impacts of heavy metals in different tissues and organs of selected fish species of River Kabul. Further more in this chapter different pathological abnormalities were investigated to assess the

pathological impacts of heavy metals as a risk for human beings. This dissertation includes the conclusions and recommendations based on personal study. References of all the chapters are given at the end.

Seventh Chapter deals with conclusions and recommendations of this research works.

ABSTRACT

The main objectives of this work were to investigate physico-chemical and heavy metal contaminations in water and heavy metals accumulation and toxicity in selected fish species of River Kabul. For this purpose water sample A (control site 3), water sample B (polluted site 4), water sample C (polluted site 1) and water sample D (polluted site 2) upstream and downstream of River Kabul were collected during low and high flow periods and analyzed for eight physico-chemical parameters (pH, TSS, TDS, TA, Cl, K, EC, Na) and nine heavy metals (Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe, Hg) and compared with water sample A and NEQS recommended limits. All the studied physico-chemical and heavy metal parameters in water samples A, B, C and D except TSS and Hg were below the NEQS proposed limits, where the values of TSS and Hg were above the NEQS recommended limits in all the water samples A, B, C and D. Thus the overall sequence of different water samples was D > C > B > A. This highlights that water sample D had higher and sample A had lower physico-chemical and heavy metal contaminations. Water samples A and B had highest TDS and lowest K for low flow and had highest TSS and lowest K for high flow seasons. Similarly water samples C and D showed highest TDS and lowest pH for low flow and showed higher TSS and lower K for high flow periods. Among heavy metals water sample A had highest Zn and lowest Pb for both low and high flow periods. Water sample B showed higher Zn and lower Hg for low flow and greater Zn and smaller Cu for high flow periods. Similarly water samples C and D had greater Zn and smaller Cr for low flow and higher Zn and lower Cu for high flow seasons, respectively.

This investigation was further aimed to determine bioaccumulation of heavy metals including Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe and Hg in intestine, skin, liver, gills and muscle of *Wallago attu, Ompok bimaculatus, Cyprinus carpio, Labeo dyocheilus and Aorichthys seenghala* of River Kabul and compared with permissible limits of RDA. Overall accumulation of metals in *Wallago attu* was highest in skin and lowest in liver, in *Aorichthys seenghala* was higher in skin and lower in intestine, in *Labeo*

dyocheilus was high in skin and low in muscle, in *Cyprinus carpio* was maximum in intestine and minimum in muscle and in *Ompok bimaculatus* was more in gills and less in muscle. Thus overall order of heavy metals concentration in different fish organs was intestine > skin > liver > gills > muscle and in different fish spcies was *Cyprinus carpio >Ompok bimaculatus >Aorichthys seenghala >Labeo dyocheilus >Wallago attu.* This shows that highest metals accumulated organ was intestine and fish was *Cyprinus carpio* while lowest metals accumulated tissue was muscle and fish was *Wallago attu*. Overall order of metals concentration in different tissues showed that Zn was the highly and Cd was the lowest accumulated metals. Comparing this result with RDA renders that in skin Zn, Ni, Cr, Cd, Pb and Hg and in muscle Ni, Cr, Cd, Pb and Hg levels were above the RDA recommended limits.

Further more the present investigation was conducted to assess genotoxicological impacts of heavy metals in various tissues and organs of selected fish species. Therefore degree of DNA damage like TCS and comet class 0, class 1, class 2, class 3 and class 4 were determined in blood, intestine, skin, gills, liver and muscle cells of *Wallago attu*, *Ompok bimaculatus*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala* of River Kabul. Overall degree of DNA damage cells in *Wallago attu* were highest in blood and lowest in muscle, in *Aorichthys seenghala* were greatest in intestine and smallest in muscle, in *Labeo dyocheilus* were more in blood and less in muscle, in *Cyprinus carpio* were high in blood and low in muscle and in *Ompok bimaculatus* were maximum in intestine and minimum in muscle. Overall trend of DNA damage cells in different tissues was intestine > blood > skin > liver > gills > muscle and in different fish species was *Cyprinus carpio >Ompok bimaculatus >Aorichthys seenghala >Labeo dyocheilus >Wallago attu*. This indicates that highest degree of DNA damage cells were found in intestine and lowest in muscle. Similarly higher frequency of DNA damage cells was observed in *Cyprinus carpio* and lowest in *Wallago attu*.

This study was further meant to investigate histopathological impacts of heavy metals in various tissues and organs like intestine, gills, liver and muscle of *Wallago*

attu, *Ompok bimaculatus*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala*. The observed lesions in intestine of selected studied fish species were degeneration of epithelium, complete degeneration of cillia, inflammation, coagulative necrosis and degenerative cillia, in gills were disquamation and distortion of secondary lamellae with epithelial cell exudate, clumping of gills lamellae, necrotic area, epithelial disquamation, non specific inflammation, vacuolation and oedema, in liver were inflammation, hydropic degeneration, coagulative necrosis, non specific inflammation, liquefactive necrosis and spongiosis hepatis and in muscle were fibrillar degeneration, inflammation, coagulative necrosis and non specific inflammation. Overall pathological abnormalities in *Wallago attu* were the highest in liver and lowest in the gills, in *Aorichthys seenghala* were more in liver and less in gills, in *Labeo dyocheilus* were maximum in gills and minimum in intestine, in *Cyprinus carpio* were higher in intestine and lower in muscle and in *Ompok bimaculatus* were greater in liver and smaller in gills. Overall order of different pathological lesions in different fish organs was liver > intestine >gills > muscle and in different fish species was *Cyprinus carpio >Ompok bimaculatus >Labeo dyocheilus >Aorichthys seenghala >Wallago attu.*This highlights that highest histopathological disorders were found in liver and lowest in muscle. Similarly highest pathological alterations were observed in *Cyprinus carpio* and lowest in *Wallago attu*.

Key Words: River Kabul, Physico-chemical parameters, Heavy metals, Bioaccumulation, Genotoxicity, Histopathology.

CHAPTER-1
GENERAL INTRODUCTION

Metals like potassium, calcium, magnesium, cobalt, copper and zinc are some of the essential metals that can induce toxicity in aquatic and other animals due to more accumulation in these organisms. Where as other heavy metals like lead, chromium, nickel and cadmium are highly toxic for the human beings if taken in low content (Angelova *et al*., 2004; Haider *et al*., 2004; Desideri *et al*., 2010). Different health problems like physiological and psychological are induced as a result of heavy metals toxicity (Flora, 2002; Liu *et al*., 2003). Heavy metals toxicities are related to its total content, specific chemical form, metal binding state and other properties like pH, organic matter and soil texture etc (Muhammad *et al*., 2011). Heavy metals are the elements, which have different chemical properties and biological functions. Heavy metals are the elements that have specific gravity. Measurement of density of a given amount of a solid substance, when it is compared to an equal amount of water is termed specific gravity (Lide, 1992). Heavy metal commonly called as trace elements that play an important role in biological systems. They become toxic due to presence of greater content (Ibok *et al*., 1989). Transformation of heavy metals in between water, soil and plants is a portion of biogeochemical cycling processes in the environment. Different factors like bed rocks weathering and erosion of ore deposits, mining, smelting, electroplating, fuel production, power transmision, intensive agriculture, waste water irrigation and sludge dumping impact this cycle (Igwe and Abia, 2006; Khan *et al*., 2008; Muhammad *et al*., 2011).

Mining activities, tailing deposit, acid mines drainage and industrial waste products can impact the ecosystem and then affect the ecological community and living things in that ecosystem (Mapanda *et al*., 2007). Heavy metals can pollute the water, which can affect the quality of both drinking and irrigation water (Krishna *et*

al., 2009). When these metals enter into the water bodies can affect the water quality and various living life both plants and animals in water (Tahiri *et al*., 2005; Antonious and Snyder, 2007). The heavy metals can enter into the human body through drinking of polluted water and consumption of contaminated food (Rattan *et al*., 2005). The environmental scientists have focused on toxicity, bioaccumulation, source identification, reclamation and management studies of heavy metals around the world (Zhou *et al*., 2008; Muhammad *et al*., 2011).

1.1 STUDY AREA DESCRIPTION

1.2 RIVER KABUL

River Kabul has its origination from the base of Unai Pass in the Paghman mountains of Afghanistan. It then flows along the northern side of the Koh-i-Sufaid range toward east; it passes through Kabul approximately 72km from its origion. Below Jalalabad, it is joined by the Kunar River (Gress well and Huxley, 1965). Hindu Kush mountains in Pakistan are the origion source of Chitral River. It enters into Afghanistan at Arandu and is joined by a branch from Nuristan, where it is called as the Kunar River. Near Jalalabad, the River Kabul is joined by the Kunar River. The River Kabul enters Pakistan into through Khyber Agency. Then it flows through the Khyber and Mohmand Agencies and finally reaches to Warsak dam. Below the dam, it is divided into three main branches known as Shah Alam, Nagoman and Adezai. The lands of Peshawar, Charsadda and Nowshera districts are irrigated through these branches of River Kabul before joining the River Indus at Kund (IUCN, 1994). About 35km below Warsak dam Shah Alam and Nagoman Rivers join each other, while the Adezi River joins River Swat. All these branches join each other 1km down stream at Akberpura. At that junction the Bara River also joins it and is known as the main Kabul River. The Kabul River joins Indus River at the Khairabad about 90km downstream of Warsak dam. The Adezai River is divided further into other branches near Larmandi village and these branches have greater volume of water than the other

two branches. The sewages from 40 villages containing 150000 populations are discharged into the River Kabul (IUCN, 1994). The Nagoman River emerges from Kabul River near Machni and receives effluent from tanneries near Nagoman and sewages from 27 villages. The River Shah Alam also emerges from Kabul River near Kander Landi at Daudzai. The Shah Alam River receives all the sewages from Peshawar and 30 villages are carried into River Kabul through Shah Alam (IUCN, 1994).

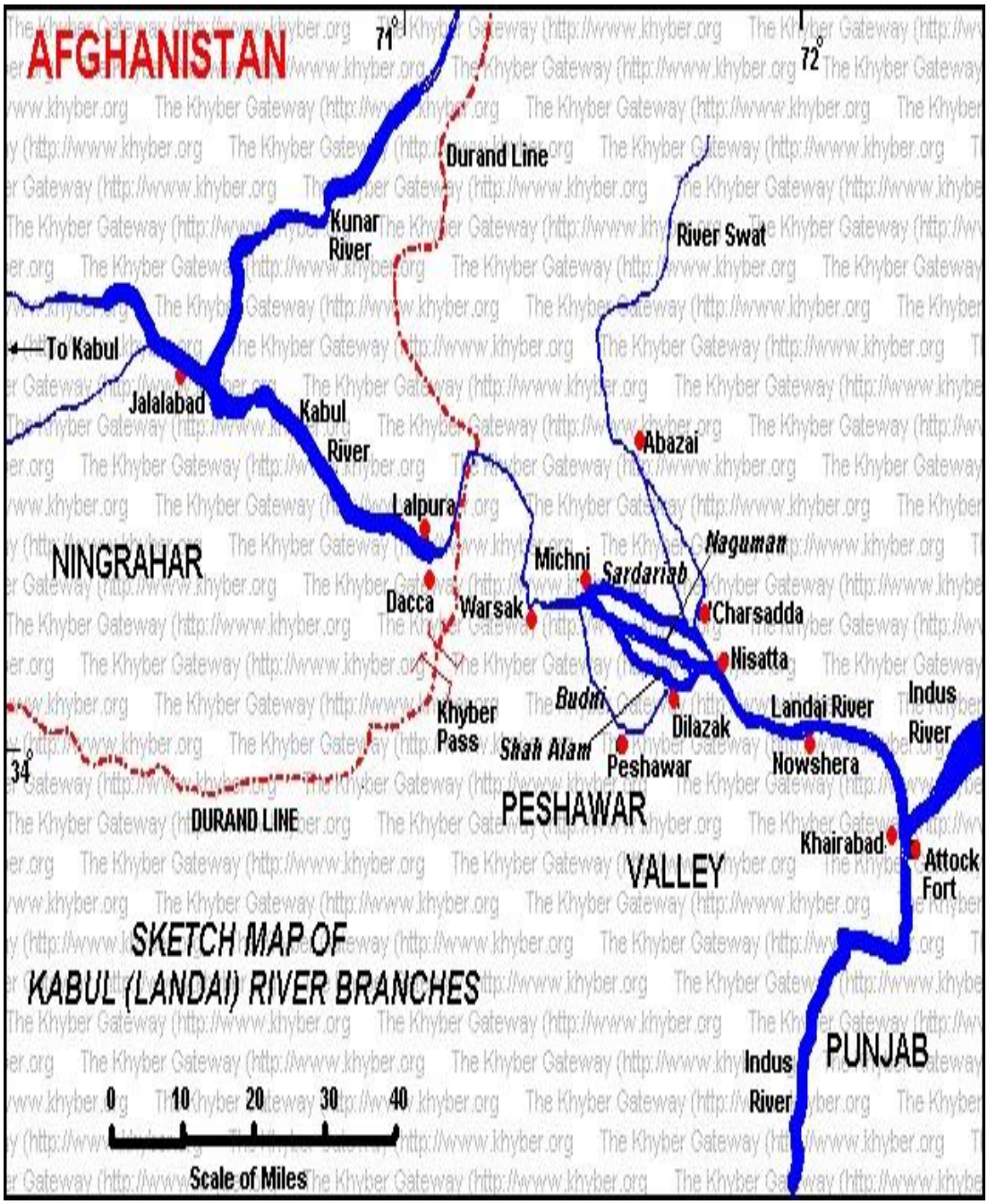


Fig. 1.1: The River Kabul and its tributaries

1.3 WARSAK DAM

It is situated on River Kabul at Warsak and is helping in both irrigation and electricity production. Its construction completed within 8 years started in 1952 and ended in 1960 with the technical and financial assistance of the Canadian Government. It is 750 feet long and 235 feet high. Its water reservoir capacity is about 26 miles and has 1000 feet storage capacity of 20,000 acre feet water. The dam can produce 240 MW (mega watt) electricity. Three canals like the gravity canal, the Kabul River canal and the Mohmand canal have been taken out. The former two canals irrigate the valley of Peshawar and the later irrigate parts of Mohmand agency. The dam is without any fish ladder and hence is an obstacle for upstream migration of the fish population especially during breeding season, which starts in spring and lasts till late summer. The reservoir inhabits almost the same fish population as found in River Kabul and is used for commercial fishing (Yousafzai, 2004).

1.4 BACKGROUND

The people, who live near the banks of the River Kabul complained for water pollution in the River Kabul. It was because of increased water pollution and fish killing in the river, skin problems in humans and maladies in animals. Many people have complained for reduction of crop production, which were irrigated from River Kabul (IUCN, 1994). Initially these complaints were taken seriously by Pakistan Council of Scientific Industrial and Research, Chemistry Department and Centre of Excellence in Physical Chemistry of Peshawar University. They verified the pollution in the River Kabul. In 1977, United Nations Industrial Development Organization investigated the impact of industrial effluents on the water quality of River Kabul (Karns, 1977). Previously it has been reported that there is a significant industrial pollution in the River Kabul and it has been recommended that all the industrial waste products should be detoxified before dumping into the River Kabul (IUCN, 1994). The number of polluting industries is increasing day by day. The scientific

community is busy in solving the pollution problem in the rivers and other water bodies. In due course the cause of the villagers was also taken up by a non-governmental organization, Pakistan Environmental Protection Foundation, Still the federal government has not taken any action against this serious matter and recently the KPK government developed a capacity to resolve this environmental problem (IUCN, 1994). In January 1992, the Sarhad Provincial Conservation Strategy program took seriously the water pollution problem in River Kabul. It is a serious environmental problem, which is needed the environmental rehabilitation program to solve this serious problem. According to the program the river should be cleaned first. It is expected that initial river clean-up action plan could be developed and there is needed more research for further identification of pollution problem in the River Kabul (IUCN, 1994).

1.5 HYDROLOGY

The River Kabul has seasonally variable monthly discharge at Warsak dam. It has an average discharge of 20,500 cusecs during low flow and high flow periods. This variation is caused due to melting of glaciars and snow on the peaks of mountains. The Chitral River as investigated has half discharge as compare to River Kabul. This is because the Chitral area is arid and has less rainfall due to glacial inputs. The tributaries in Afghanistan are also from areas of low rainfall (IUCN, 1994). River Swat is main tributary of River Kabul that joins the River Kabul before ending into River Indus. This tributary has a discharge of 22,500 cusecs, which is same to the Kabul River but there is a great seasonal variation in the Swat River discharge due to less rainfall in other seasons (IUCN, 1994). The main purpose of Warsak dam construction is to control the discharges below the dam. Most of the water is used for irrigation and some part of water is used for running of hydel plant. Several time artificial floods occurred due to release of water by the dam, which is

resulted into soil erosion. The effect of extensive canal and irrigation systems on the water quality and quantity has not been investigated (IUCN, 1994).

1.6 GEOLOGY

The Kabul River water shed is geologically complex. The lower basin of the river is occupied by the sedimentary limestone and shales, which is more in the Indus basin, while the water at the start of the main tributaries contains igneous and metamorphic rocks, which are formed by plate tectonics and mountain-building processes that are active around the edges of the sub-continent. The river channel flowing in the hills near Pakistan-Afghanistan border uplift the sedimentary rocks in this area. After passing through Warsak, the river divides into the three main distributary channels, which is helpng in the release of high volumes of bed load and suspended sediments due to low flow rates before entering the river into the plain (IUCN, 1994).

1.7 VERTEBRATES FAUNA IN THE RIVER KABUL, PAKISTAN

1.7.1 Fish Fauna

Fifty four different fish species are reported in the Kabul River and its tributaries (Rafique, 2001). Among them about thirty five are considered common. The main commercial fish species are *Tor putitora*, *Wallago attu*, *Ompok bimaculatus* and *Ompok pabda*, *Cyprinus carpio*, *Schizothorax* spp like *Schizothorax richardsonii plagiostomus*, *Schizothorax progastus labiatus*, *Schizothorax esocinus*, *Aorichthys seenghala*, *Labeo dyocheilus pakistanicus* and *Ctenopharyngodon idella*. These fish are eaten locally in the villages and in Peshawar, Charsadda, Mardan, Nowshera, Jehangera and Swabi towns. *Botia rostrata*, is the only fish that has been investigated in the River Kabul at Michni, Pakistan (Butt, 1989a; Butt and Mirza, 1981).

1.7.1.1 *Wallago Attu*

Wallago attu belongs to kingdom animalia, phylum chordata, sub phylum vertebrata, class actinopterygii, order siluriformes, family siluriformes, genus *Wallago* and species *Wallago attu.* It is locally known mulee and is a fresh water predatory fish. In natural habitat the fish is considered as predatory, demersal and potomodromous and found in rivers of Balochistan, Punjab, Sind, KPK, Azad Kashmir and Kabul Indus River system (Yousafzai *et al*., 2010a). It is widely distributed in Bangladesh, India, Pakistan, Nepal, Burma, Sri-Lanka and some other Asian countries like Thailand, Vietnam, Kampuchea, Malay Peninsula, Indonesia and Afghanistan. Its body is elongated and laterally compressed. The snout is depressed. Mouth is deeply cleft and maxilla extends behind the eyes and lower jaws. Dorsal fin is small, spineless and situated above the last half of pectoral (Roberts, 1982; Yen and Trong, 1988). It dwells both in standing and running water. Tanks, lakes, rivers, reservoirs and flood plains are the main habitat of this fish (Roberts, 1999). It usually eats insects (Sokheng *et al*, 1999) and adults depend on small fishes, shrimps and mollusks for their food. It is spawning between May to October in Cambodia, June to July in Bangladesh and Thailand and July to August in Pakistan and Nepal (Froese and Pauly, 2007). Different factors like over-exploitation, destruction of habitat, environmental contamination, toxic chemical pollution and lack of proper management are resulting into declining of *Wallago attu* population (Mijkherjee *et al*., 2002).

Fig. 1.2: Photograph showing *Wallago attu*

1.7.1.2 *Labeo dyocheilus*

Labeo dyocheilus belongs to kingdom animalia, phylum chordata, sub phylum vertebrata, class actinopterygii, order siluriformes, family cyprinidae, genus *labeo* and species *Labeo dyocheilus*. It is locally known by Torki name. This fish is omnivorous, potomodromous and benthopelagic in their feeding habit. It is distributed across the country (Yousafzai *et al*., 2010b). It is one of the most economically important and fast growing fish. Incorporation of this high valued species is not only an important step in species diversification of hill aquaculture but will also improve the socio-economic care of poor farmers in hilly region. Therefore, it is very important to get more and more information about its reproduction potential and usefulness as a candidate species for hill aquaculture (Gupta *et al*., 2013).This fish is a bottom feeder inhabiting upland streams and rivers at an elevation of 400-800m. The fish is reported to attain a length of 91.44 cm (Day, 1977). *Labeo dyocheilus* has been categorized as vulnerable species (Prasad, 1994). In the month of August and September, the matured *Labeo dyocheilus* fish started spawning. Thus, these two months are collectively called as breeding season of *Labeo dyocheilus* (Rakesh, 2013).

Fig.1.3: Photograph showing *Labeo dyocheilus*

1.7.1.3 *Cyprinus carpio*

Cyprinus carpio belongs to kingdom animalia, phylum chordata, sub phylum vertebrata, class actinopterygii, order siluriformes, family cyprinidae, genus *cyprinus* and species *Cyprinus carpio.*It is locally known as common carp and has a good position among the fish species of the country and inhabits in inland waters and is regarded as a good fish from economic point of view and breeding features. It also inhabits in lakes, ponds and deep and slow flowing rivers containing detritus and vegetation in bottom. The young fish generally prefer shallow water, while the elder ones prefer deep water for living. They also live in warm water but can not survive in high altitude lakes. It can survive in presence of less oxygen. It is considered as a good fish for culture due to its omnivorous nature, rapid growth, keeping in closed regions and tasty meat. They show maturaty between 3-4 years and spawning their eggs form April to June. They can get a length of 1 m and weight of 40 kg (Geldiay and Balık, 1988). It is a voracious omnivorous fish. Zooplankton, phytoplankton, water plants and detritus are the main food sources of this fish. Besides these sources, they also feed on small abundant organisms in the lake (Yousafzai *et al*., 2010b). *Cyprinus carpio* mainly feeds on plankton and insects (Numann, 1958).

Fig.1.4: Photograph showing *Cyprinus carpio*

1.7.1.4 *Ompok bimaculatus*

Ompok bimaculatus belongs to kingdom animalia, phylum chordata, sub phylum vertebrata, class actinopterygii, order siluriformes, family siluridae, genus *ompok* and species *Ompok bimaculatus.* It has two distinctive spots above and behind the pectoral fin base and at the caudal peduncle base. It is a very popular Asian cat fish. It is locally known as Sher mahi in Pakistan and commonly known as butter cat fish (Siraj *et al*., 2014). It is a fresh water catfish species native to India, Bangladesh, Pakistan and Myanmar. The fish has also a wide geographical distribution covering West Bengal, Bihar and North Eastern States of India as well. Open beel or wet land connected with rivers is usually considered as common habitats of this fish. *O.bimaculatus* is a higher priced, delicious and well preferred fish because of its unique Lipo-protein texture with soft bones, good taste and higher nutritional value. This species has been listed as endangered fish species in India (IUCN, 1990). In aquaculture, the *Ompok bimaculatus* did not receive much attention due to insufficiency of gravid stock for experimentation and also because of the shortage of information regarding its breeding potential, larval rearing and culture technology (Parameswaran *et al.,* 1970; CAMP, 1998). Male *Ompok bimaculatus* become matured during late March to April. Fully ripe females were observed during May to

the end of July. Breeding season extends from early June to late July (Banik *et al*., 2011).

Fig.1.5: Photograph showing *Ompok bimaculatus*

1.7.1.5. *Aorichthys seenghala*

Aorichthys seenghala locally known as Singhara in Pakistan is a cat fish, belonging to kingdom animalia, phylum chordata, sub phylum vertebrata, class Actinopterygii, order siluriformes, family Bagridae and genus *aorichthys* and species *Aorichthys seenghala*. This fish is distributed in Nepal and Banladesh, Southern Asia, Afghanistan, Pakistan, India and also found in Thailand and China (Siraj *et al*., 2014). It inhabits in fresh water, feeds on insects and commonly bottom feeder (Rahman, 2005). It usually eats insect-fry, fish-fry, fingerlings, water fleas, insects, adult-insects, tadpoles and young fish (Yadav, 1997). This species has very broad and flat head with smooth upper surface and the grayish sides. It spawns during January to April at the edge of shore about 1- 2m below the surface. It makes a sand-gravel hole near a rock about 1 m in diameter and 30 cm deep. In these holes the female lays eggs and look after their fry. Both male and female are distinguished from each other by the shape of the genital papilla. The male has pointed genital papilla and has a slender body, while the female has oval papilla and the mature female fish has a big belly containing large number of eggs. During spawning, its abdomen becomes distended.

It has reddish and prominent cloaca. Males have total length of 60-80 cm and body weight of 2.0 - 2.5 kg, while females have length between 80 and 100 cm and body weight between 3.0 and 4.5 kg (Ratanatrivong *et al*., 2008).

Fig.1.6: Photograph showing *Aorichthys seenghala*

1.8 HUMAN POPULATION

Kabul is located on the Kabul tributary from which the river gets its name. Jalalabad is the last major town located close to the confluence of the Kabul and Kunar before the river enters Pakistan. Below the Warsak dam River Kabul flows through the more populated areas of the KPK and the more populated rural areas of Pakistan. Peshawar city near to the Shah Alam branch of River Kabul has about one million populations, while the Nowshera and Charsadda towns are also located near the banks of the river. There are many Afghan refugees colonies, which are situated close to the river and its tributaries (IUCN, 1994).

1.9 PRINCIPAL USES OF RIVER KABUL

1.9.1 Irrigation

Two canals like northern and the southern canals were taken-off below the Warsak dam. The northern canal irrigates the lands of Shabqadar and Charsadda. While the southern canal irrigates the lands of Jamrud. About 5 km below the Warsak dam, a third canal was also taken off from the southrn bank of River Kabul, which

irrigates the Peshawar lands upto Akbarpura. Near Garhi Sharif there is taken off fourth canal from the south bank of Adezai River, which irrigates the lands of Charsadda district up to Agra village. Besides these large canals, the inhabitants on the bank of River Kabul have also constructed small canals for irrigating their lands during high flow season. Some times the villagers on the bank of River Kabul also utilize pump to lift water for irrigation purpose. Several canals are taken off from the Kabul and Swat Rivers, which irrigate the lands of Peshawar district.This plays a vital role in the increasing of agricultural products but also help in drainage, which result into water logging and salinization of soils. The water which, returned to the rivers composed different kinds of agricultural chemicals. The river and canals also help to provide water for washing and livestock (IUCN, 1994).

1.9.2 Fisheries in River Kabul

The entire River Kabul and its tributaries are used for commercial as well as sport fishing. Various methods of fishing are utilized such as dragnets, castnet, gillnet, long line and boats are used for commercial fishing with the proper permission of the department of fisheries of KPK, while in sport fishing rods and lines along with the above fish gears are used. The whole river is also used for fishing with dragnets, castnets, gill nets, being utilized. The main commercial species are Mullee, *Wallago attu*, Shermai, *Ompok bimacultous*, Gulfam, *Cyprinius carpio*, Swati, *Schizothorax spp*., Singhara, *Aorichthys singhara*, Torki, *Labeo dyocheilus*, Mahseer, *Tor putitora*. They are eaten by the people of Peshawar, Charsadda, Mardan and Nowshera districts (IUCN, 1994).

1.9.2.1 Importance of Fish

Fish are playing a vital role as a biological indicators for the water quality and can indicate about the new toxic chemicals, which are added into the water bodies (Powers, 1989; Bailey *et al.,* 1992). Essential and important nutrients like proteins, vitamins, minerals and Omega-3 fatty acids are found in the fish. These nutrients are

needed to human beings (Dahl *et al*., 2006). Fish is a good and easy source of protein and food for human (Olaifa *et al*., 2004). Fish has an important position as a good quality food in the human diet. The fish lipids are considered important because play a vital role against many heart and joint diseases (Shahidi and Botta, 1994). Fish contain proteins, essential fatty and amino acids, which are more essential for the growth of human beings and play a vital role in protection of the human body against different diseases (Matthew, 1992). Lipids of fish contain omega-3 fatty acids, which are necessary for the human health and growth. By making comparison with other animal lipids, the fish lipids play a significant role against cardiovascular and inflammatory abnormalities in human beings (Piclet, 1987).

1.9.3 Hunting

The river is used for sport hunting. The main hunting season starts from November when water fowls start migration from Siberia to Pakistan and India for feeding and lasts till April when emigration to Siberia for breeding along the Indus. Shooting of water fowls is a popular sport both for locals and visiting people (Yousazai *et al*., 2010a). River Kabul also provides recreation picnic spots to the people of Charsadda, Peshawar and Nowshera (Arshad *et al*., 2011).

1.9.4 Recreation

Boating, hostelling, fishing and hunting are the main recreational uses of the River Kabul. Huts and river view restaurants are present along the river bank. Fried fish is specialty of these huts and restaurants. These uses and economic benefits demand that the river must remain in a healthy" state (Yousazai *et al*., 2010a).

1.9.5 Washing and Bathing

The River Kabul is also providing washing and bathing facilities to the people who live on the banks of River Kabul (IUCN, 1994).

1.10 INDUSTRIES ALONG RIVER KABUL

According to a survey, there are working about 348 large and small scales industries in KPK. About 80 industries and industrial units out of 384 are dumping their waste products into River Kabul. They are including 4 sugar mills, 2 distilleries, 3 ghee factories, 5 textile mills, 2 woolen mills, 12 tanneries, 3 paper and board mills, 10 chemical and pharmaceutical factories, 4 match factories, 10 soap industries, 1 petroleum refinery, 10 photo laboratories, 4 paint and varnish industries and 11 rubber and plastic industries (IUCN, 1994).

1.10.1 Industries at Aman Garh Industrial Zone

Nowshera and the adjacent Aman Gar industrial zone have about 15 different types of small and large scale industries that discharg their effluents into River Kabul. They include ghee industries, Feroz son,s laboratories, colony sarhad textile industry, Adamjee chemical works, Adamjee paper board mills, petrol godowns, Nowshera engineering company limited, Nowshera DDT industry and tanneries across the railway bridge, Nowshera (Yousafzai., 2004). Unluckily all the above units are without effluents treatment facilities end up in the River Kabul through canals. These effluents have not only deteriorated the river water but also the sub-surface water of the area as well as aquatic organisms (IUCN, 1994; Khan *et al*., 1999; Akif *et al*., 2002). The villagers living at the river banks have also been complaining about the pollution in the River Kabul, which is very obvious and has often resulted into periodic fish killing. It has been reported that the dumping of industrial effluents into River Kabul are the cause of declining of the whole fish population, which are very sensitive to oxygen depletion due to the pollution in the River Kabul (Khan and Mumtaz, 1997). The textile industry at Amangarh, Nowshera are dumping their effluents into River Kabul in greater concentration than the proposed limits of NEQS. These effluents contain toxic heavy metals, high oxygen demanding wastes and appreciable amounts of sulfide. This study indicates the presence of deleterious effects of industrial pollutants (Akif *et al*., 2002).

1.11 SEWAGE AND INDUSTRIAL EFFLUENTS DISPOSAL TO RIVER KABUL

The discharging of municipal sewages and industrial effluents into the River Kabul is a wide spread activity throughout the world and particularly in the third world countries like Pakistan. The cities, towns and villages of Afghanistan, Malakand, Peshawar and Mardan, Mohmand, Khyber and Malakand agencies discharge their untreated sewages into Kabul River. The lower sections of the river pass through densely populated plains. Many industries like distilleries, paper, textile and sugar mills, tanneries and ghee factories are dumping their toxic waste products into River Kabul and make the river polluted (IUCN, 1994). In Pakistan due to lack of proper organization, supervision for industrialization and urbanization, the water pollution is a great problem. Similarly the accessible water also gets contamination due to discharging of unprocessed domestic sewages, industrial effluents and agricultural run-off (Balfours, 1987).

1.12 WATER CONTAMINATION

Water is a universal solvent that is essencial for the life on earth. The dischrge of various toxic chemicals like heavy metals into water make the life difficult in the water and on the land. Some of these heavy metals causes different acute and chronic abnormalities like skin burns and rashes, bone disorders, lungs and digestive system abnormalities in aquatic and teristrial animals (John, 1990). Pakistan is among the countries of the world that is faced with fresh water pollution in its rivers. According to ministry of environment and urban affairs, various industries dump their waste products into different water bodies. Industrial estates in Peshawar are discharging heavy metals and untreated effluents into water bodies. For instance 80000m^3 industrial effluents are discharged into River Kabul every day, which are resulted into decreasing of agricultural and fish production (Government of Pakistan Position Paper, 2010). Water pollution affects the water qualities that make the water unsuitable for drinking and irrigation purposes. Any substance, which impacts the

water quality, is called as pollutant. Organic, inorganic, suspended solids and sediments and radioactive materials are different types of water pollutants (Daniel and Edward, 1995). Water pollutants in form of heavy metals are one of the serious problems in Pakistan. There is no available clean and safe water to about 55% population. The heavy metals enter under ground water with the discharge of industrial effluents on the surface of water, which are resulted into increase in the concentration of these contaminants in the water. Greater amounts of heavy metals are the main cause of water pollution, which are attributed to metabolic and non metabolic abnormalities in both humans and animals (Lamb, 1985).

Increasing population, industrialization and urbanization are the main cuases of water pollution and water pollution is a serious threat for the humanity. Water soluble toxicants like heavy metals from industrial and municipal wastes are discharged into natural water bodies. Some serious polllutants are decomposed while others form insoluble salts which are precipitated into the sediment (Bowen, 1979). The contamination of fresh water due to heavy metals has become a serious problem for over the last few decades and not only affects water quality and made the water not suitable for drinking purposes but is also a threat to aquatic life (Canli *et al*., 1998). Leachates composed of organic and inorganic toxic chemicals, which can flow through permeable soil strata and contaminate both the surface and ground water of river, stream and lakes. The effect of such uncontrol disposal system render both the surface and the underground water and both the water systems become unsafe for human, agricultural, recreatonal uses and biotic life. Contaminated aquatic ecosystems also become a threat for human life and are therefore against the principles of sustainable development (Hanor, 1995). Not only the aquatic organism eliminated from contaminated water but they also lose their aesthetic values. Different diseases like hepatitis A, cholera, typhoid, gastro-intestinal diseases, etc are caused as a result of polluted water. There are dying a large number of people due to such diseases every year in the whole world, especially in the developing nations (Steel, 1995).

According to a report 30% illness and 40% deaths in Pakistan are the result of water born diseases (Akhtar, 1983). Similarly about 40% adult and 60% infant deaths in Pakistan are the result of water-born diseases such as typhoid, diarrhea, infective hepatitis etc. It is reported that 25% of the overall deaths occur below the age 5, mainly due to gastroenteritis caused due to water pollution (Yousafzai *et al*., 2010b). Cotaminated water not only impact aquatic organisms but also transmitte different kinds of diseases in humans (Tebbutt, 1983).

1.13 SOURCES OF HEAVY METALS

Heavy metals pollution is a serious environmental problem throughout the world. Industrial and mining activities, petroleum exploitation, processing and effluent management, atmospheric condensation and sewage disposal, earth quake, land slides, tornadoes and cyclones are the main and largest sources of heavy metals that are adding large amount of metals into the environment (Nathaniel *et al*., 2000).

1.13.1 Metals in the Environment

Analysis of the tissue of human body show the presence of most of the metallic elements in greater or less amounts. This is not surprising since the food we eat also contain a wide variety of heavy metals reflecting the distribution of these elements in the environment. The soil in which plants grow contains metals, fertilizer, sewage sludge and other materials in the course of agricultural activities. Metals are also contributed by the debries of mining and industrial waste by the dust and smoke of fossil fuel combustion and by other forms of atmospheric pollution. Water too makes its contribution to an extent related to the source of supply and the degree of pollution. The actual amount of metals found in soil sample will depend on the nature of the parent rocks, their degree of mineralization and other factors. Content of metals in the water can reflect nearby industrial activity as well as the composition of local rock and soil. In addition reticulated water may carry metalic contribution due to the composition of plumbing and containers (Reilly, 1980).

1.13.2 Metals in the Body of an Organism

The metals content of food, whether this is of animal or plant origin will depend on many factors, ranging from environmental conditions to methods of production and processing. Even in the same class of food, variations in the levels of metals may be considerable (Reilly, 1980). Within a particular food stuff or organism levels may vary between parts. Within the animals body the distribution of metals is by no means uniform. Some metals are accumulated in particular tissues and organs and in other different portions of the body. For example in 55 kg man the toxic metal like cadmium will be accumulated preferentially in kidneys (12 mg) and to much lower extent in blood (0.76 mg), where the kidney will contain 0.12 mg and blood 1.3 mg of lead. Chromium is concentrated preferentially in muscle (2.4 mg) but only reaches 0.019 mg in the kidney (Somero *et al*., 1977).

1.13.3 Essential and Non-Essential Metals

Among many metals found in the body only a small number are known to be essential for normal life. The absence of these elements will result in the appearance of characteristic pathological deficiency symptoms. Most of the other metals present are antifacts with no functional significance. The essential metals can be divided into two classes according to the amounts of each, which are required for normal function. These are macro-nutrients, which include Ca, K, Na and Mg and the micro-nutrients, which include Fe, Zn, Mn, Cu, Mo, Co, Cr, Si, Ni and Sn. Metals found in the body for, which no metabolic functions yet known are called as non essential. The non essential metals include Pb, Hg, Ag, Sn, Bi, Sn, B, Be, Li, Ga, Ti, while Ba, Ar, Sr, Cd and Va are essential elements (Reilly, 1980).

1.13.4 Role of Metals in the Body of an Organism

Metals including essential micro-nutrients and macro- nutrients work in three main ways in the body as constituent of bones and teeth as soluble salts, which control the composition of body fluids and cells and as essential adjuncts to many enzymes

and other functional proteins. The macronutrients play major roles in the body of organisms, while micro-nutrients are especially prominent in assisting enzyme function. Very few of the proteins that act as a biological catalysts can do entirely on their own. Most of the proteins need the assistance of a non-protein prosthetic group. If the prosthetic group is detachable it is known as coenzyme. The group may be an organic molecule containing trace metals or it may contain solely of a trace element. In the latter case if the metal is detachable from the protein part, it is known as an activator. Iron and copper, for example occurred in the prosthetic group of many enzymes concerned with oxidation. Zinc and manganese function as detachable activators on some enzymes involved in cellular metabolism (Reilly, 1980).

Most of the micro-nutrients have been shown to play similar enzymatic role and as a consequence the enzyme involved are often referred to as metallo- enzymes. The inorganic micro-nutrients are also found in some other body compounds including hormones and vitamins. The production and storage of insulin in the pancrease for example involved zinc. Haemoglobin, essential for transport of oxygen in the blood is an iron containing compound. Cobalt atom form part of cobalamin or vitamin B_{12} (Reilly, 1980). It is clear that some metals are essential micro-element and therefore their deficiency disturb biological functions. All the metals including essential micro-nutrients and macro-nutrients may also become toxic to aquatic organism as well as to humans, when present in excess (Under wood, 1977; Laws, 1981).

1.13.5 Heavy Metals Pollution in River Kabul

For over two decades the people living on the banks of the River Kabul are complaining about water pollution in River Kabul. These complaints are the result of the increasing signs of pollution and periodic fish killing. The river is also blamed for skin diseases in humans as well as maladies in livestock. Some people have complained for decreasing crop production in the fields irrigated from polluted River

Kabul (IUCN, 1994). Excess heavy metals in the River Kabul is a serious matter. This is the result of increased population, urbanization and expansion of natural resources, irrigation and other agricultural practices and lack of environmental regulations (Calamari and Naeve, 1994). Now a day pollution due to heavy metals is serious environmental problem in the whole world. The discharges of industrial, agricultural and commercial waste products into the water bodies affect the aquatic organisms including fish (Amal *et al*., 2012). Industrial, sewage, rivers, streams and drains are different discharges that dumped into River Kabul. By making general comparison between different kinds of effluents, rivers are including in streams and drains group (IUCN, 1994). An average discharge of 4,600 cubes is carried by Kalpani River from Mardan district into the River Kabul. It contains sewage and sugar mill waste products and indicates an evidence of organic pollution and also contains greater level of heavy metals. Generally it has poor water than the Bara River and is including in class 3. It becomes diluted, when is entered into River Kabul but impact the water quality in the immediate vicinity (IUCN, 1994).

Khazana sugar mill and sewages from Peshawar city are the major sources of heavy metals pollution in River Kabul. During low flow heavy metals loading is high in River Kabul and it is conceivable that oxygen and ammonia level became critical for fish and fish killing was seen during mill cleaning operations (IUCN, 1994). About eight major industrial units are dumping heavy metals containing effluents directly and indirectly into River Kabul. Besides these, other small industries are also dumping heavy metals to channels and finally end into the Kabul River. These heavy metals are different in nature but are most toxic than other waste products (IUCN, 1994). The greater content of heavy metals in Kabul River has an impact on flora and fauna (Enrique *et al*., 2007; Ping *et al*., 2006). Different industrial units are without effluent treatment facilities and the effluents from these units carried into River Kabul, either directly or indirectly through canals or nalla. The heavy metals have not

only deteriorated the river water but also contaminated the sub-surface water (IUCN, 1994; Khan *et al*., 1999; Akif *et al*., 2002).

River Kabul water is blamed for causing skin diseases in humans, maladies in livestock and periodic fish killing. The River Kabul water is no longer fit for drinking purposes. Its major tasks today are as carrier of domestic and industrial wastes and to provide water for irrigation (Khan *et al.,* 1985; Khan and Mumtaz, 1997). Different sources discharging their waste products into Kabul River and contaminated the surface water. Bara canal and Budni nalla carry waste products from industrial estates in Peshawar into River Kabul. However many other sources like sugar mills, distilleries, paper mills, tanneries, ghee mills and textile mills in the Charsadda and Peshawar area are also sources of heavy metals sources to Kabul River (IUCN, 1994). Due to lack of waste water treatment plants high concentration of heavy metals are discharged into Kabul River, which is most essential for both fish and agriculture. Different resources such as industries, sewage, factories, land slides and tornadoes are dumping greater amount of heavy metals into River Kabul (Khan *et al*., 2011). These industries not only produce effluents, but their wastes also composed toxic heavy metals like Pb, Cd, Cr and Hg and a large amount of organic halides (Ali *et al*., 2009). The average daily discharge of Bara canal into the River Kabul is 304 cusecs. Moreover the sewage from the Gul Bahar also joins the River Kabul (USMBA, 1989).

1.13.6 Hazards of Heavy Metals

a. Effects on Fish Fauna

Toxicological studies in fish species were initiated in 1930 for evaluation of impacts of toxic chemicals in both laboratory and field and also to know about the heavy metals, agricultural and industrial pollution in rivers, lakes and marine environment (Sprague, 1969). Heavy metals can not be changed into less harmul metals in the aquatic ecosystem. Therefore both localized and dispersed metal pollution affect the aquatic organisms like fish (Khan *et al*., 2011). Different

abnormalities like pituitary, testicular disorders and decrease in number of fry fish have investigated after exposure to heavy metals (Fernicola *et al*., 1985; Popek *et al*., 2006). High contents of heavy metals are resulted into both lethal and chronic abnormalities in fish (Kotze *et al.,* 1999). Higher level of heavy metals impact the aquatic environment and also affect the aquatic biodiversity (Farombi *et al*., 2007; Vosyliene and Jankiite, 2006; Ashraj, 2005).

Heavy metals can impact fish when enterd into rivers and other different water bodies (Bernet *et al*., 1999). Heavy metals control many body functions including enzymes involved in gene expression. Carcinogenesis, mutagenesis and teratogenesis are produced as a result of excessive intake of heavy metals in aquatic organisms (Baby *et al*., 2012). Sublethal concentration of heavy metals could be resulted into unhealthy fish. Less levels of heavy metals have no impact on the fish itself, which would not indicates any sign of illness but greater amount of heavy metals can decrease the fish populations, resulted into declining and finally leads into extinction of fish (Krishnani *et al.,* 2003; Burger and Gochfeld, 2005). Analysis of heavy metals in fish and other aquatic organisms is an important bio indicator of heavy metals pollution and their effects on aquatic organism and aquatic environment (Krishnakumar *et al*., 1994). Different disorders such as changes in sensory reception, reduced responses to normal olfactory function, reduction in swiming performance, gills purge and reproductive efficiency have appeared in the fish after exposure to toxic heavy metals (Mansour and Sidky, 2000). Fish take the essential metals from water, food or sediment for its normal metabolism. High concentration of heavy metals causes toxic effects (Tuzen, 2003). High concentration of heavy metal damage gills, liver and kidneys of fish and (Joseph *et al*., 2012).

b. Effects on Human Beings

Low heavy metal contents are necessary for enzymatic activity and many other biological processes in living things but at high concentration, the heavy metals

impact the living organisms and are toxic in nature. Due to the presence in greater concentration, the essential metals also become toxic (Bryan, 1976; Alloway and Ayres, 1993). Apart from the beneficial effects, there are some problems that take place in human beings from consumption of contaminated fish (Mozaffarian *et al*., 2000). Different abnormalities like reduction in functions of mental and central nervous system, reduction in energy levels and damage to lungs, kidneys, liver and other organs of the man are the result of heavy metal toxicity. Toxic effects of the heavy metals will take place that time, when excretory, metabolic, storage and detoxification mechanisms become shorter. Various diseases such as Alzheimer disease, Parkinson's disease, muscular dystrophy and multiple sclerosis are produced as a result of exposure of the human beings to heavy metals for long period (Joseph *et al*., 2012).

There are many reports about renal tubules impairment in the human beings as a result of heavy metals intoxication. Renal disfunction indication is investigated in children as a result of heavy metals accumulation (Friberg *et al*., 1979). Heavy metals have been shown to impair the renal tubular transport mechanism in humans (NAS, 1972). Heavy metals have also been reported that induce liver necrosis and necrosis of renal tubules of kidney and finally leading to kidney failure in humans (Davies, 1978; Baker, 1984; Langard and Norseth, 1979). Essential metals at greater contents have also lethal and sublethal toxic effects to some organisms. Low content of heavy metals impact the health of human beings. Thus essential metals also have double toxic effect (Rainbow, 2007).

Various cases of toxic effects of heavy metals on fish and fish consumers have been investigated (Bowen, 1979; Dix, 1981). Heavy metals naturally found in the earth crust that cannot be destroyed but accumulate through food chain and produce potential human health risks and ecological disturbances (Loka *et al*., 1990). Consumption of contaminated fish can produce different disorders and abnormalities

in human health (Nussey, 2000). Different disorders like changes in sensory reception, ventilation, coughs, learning impairment, loss of equilibrium, loss of reproductive efficiency and irregular metamorphosis are produced in the human beings as a result of heavy metals accumulation (Mansour and Sidky, 2000). It is a fact that many adverse disorders and abnormalities in the human beings are related to toxic heavy metals. Lung diseases, cancers, bone abnormalities, sterility and other problems in the human beings are related to these contaminants (Balfours, 1987; George, 1979). Many numbers of infectious diseases in the people are attributed to increased heavy metals pollution in the River Kabul. This is clear from the fact that about 70% illness and 30% deaths in the surroundings of River Kabul are the result of water born diseases (Rakesh *et al*., 2007; Lamb, 1985). Some metals are very dangerous to human beings. Hematological effects in both animals and humans are investigated after exposure to heavy metals. Transient increase in blood reticulocytes was investigated in the workers after drinking heavy metals containing water (Sunderman *et al*., 1988).

The presence of heavy metals in water impact health of human beings either through accumulation process or through drinking surface water. Kidney and bone damage are correlated to metals exposure. Heavy metals are also investigated as carcinogenic (Jarup *et al*., 1998; IARC, 1993). Abnormal development and neurobehavioral disorder are produced in the fetus, infants and children and elevate blood pressure in adults as a result of heavy metals (Huel beings *et al*., 1981). Different anormalities like nausea, vomiting, abdominal pain and breathing disorder in the human are the result of acute exposure to heavy metals and other disorders like obstructive lung, renal disease, fragile bones, alopecia, anemia, arthritis, learning disorders, migraines, growth impairment, osteoporosis, emphysema and cardiovascular diseases are also symptoms of chronic exposure to heavy metals (Dupler, 2001).

1.14 AIMS AND OBJECTIVES

Heavy metals pollution is a major environmental problem through out the world. Industrial and mining activities, petroleum exploitation, processing and effluent management, atmospheric condensation and sewage disposal are the common sources of heavy metals and physico-chemicals. Natural processes like earth quake, land slides, tornadoes and cyclones have also added a large amount of physico-chemical and heavy metals parameters into the water resources. In Pakistan due to the lack of adequate scientific data, proper education and environmental awareness, the industrial and mining activities, petroleum exploitation, processing and effluent management, atmospheric condensation and sewage disposal are dumping tons of heavy metals into the nearby canals and rivers without prior treatment to make them non-toxic.These toxic heavy metals impact the concerned ecosystem and food chains. Fresh water organisms are slowly declining. Especially fish population is decreasing day by day, while the most sensitive fish species became endanger or extinct. The plants and animals inhibit in such environment not only have a defective growth pattern, but are also transferred these toxic heavy metals to human beings. This contaminated water is also taken by the cattles, birds and crops and in this way toxic heavy metals are transported to human. The toxic heavy metals, which the human system in this way are likely to cause tremendous health hazards. Most of the heavy metals are non-biodegradable. Therefore it is essential to adopt preventive measures to save our environment and also the plants and animals life there in aquatic environment. In order to achieve the above objectives it is imperative to provide scientific proofs to prove the contention. The present work aims at

- To assess the pollution status of River Kabul.
- To investigate the types and levels of toxic components, both physico-chemical and heavy metals parameters in the water of River Kabul.
- To study bioaccumulation of heavy metals in selected fish species of River Kabul.

- To evaluate histopathological effects of heavy metals in selected fish species of River Kabul.
- To determine genotoxicological effects of heavy metals in selected fish species of River Kabul.

1.15 DATA INTERPRETATION AND SIGNIFICANCE

In this investigation, data was collected from water and fish and compared with national and international standards in order to assess the risk to human life and environmental impact. The data was further analyzed to know the pollution status of River Kabul, whether industries of the study area and human activities are contributing any contamination of hazardous nature to the water of River Kabul. Furthermore, possible remedies were suggested to keep the environment of target's area safe and provide a clean and healthy ecosystem for living organisms. Investigation of heavy metals accumulation in selected fish species help as bioindicators for heavy metal+s pollution in River Kabul. Part of this data has been published in journals of international repute for further dissemination to attract the national and international collaborative research projects.

CHAPTER-2
LITERATURE REVIEW

2.1 WATER ANALYSIS

Ali *et al* (2012) have studied the water samples downstream of dumping site of waste water effluent into Kabul River in the laboratory for different parameters like pH, electrical conductivity (EC), total dissolved solid (TDS), total suspended solid (TSS) and chlorides respectively. The pH, TSS, TDS, electrical conductivity (EC) and chloride showed increasing tendency in the river water.

Yousafzai *et al* (2010a) had reported that electrical conductivity increased significantly in both the downstream samples in comparison with control sample. This can be correlated to the effluent samples with high electrical conductivities. TSS, TDS, potassium, sodium, calcium and pH in downstream samples again showed an increasing tendency. This shows that all the parameters in River Kabul were in high concentration as compare to other rivers of South Asia.

Jan *et al* (2010) studied the ground water, effluents and soil for metal concentration to assess the pollution in different water bodies caused by various industries and also investigated their source, identification and distribution. A comparison was made among the metal concentration of ground water, effluents and soil of the polluted area and also compared with that of control site concentrations and WHO permissible limits. The results revealed that effluents contained higher metal levels than soil and ground water samples. Manganese and lead in water had 8.268 and 2.971 mg/L concentrations. Result indicates that the effluents had greater metal concentration than soil and ground water.

Phan *et al* (2010) studied the ground water, well water and hair samples for arsenic and other toxic heavy metals concentration and also investigated their health impacts in the Mekong River basin of Cambodia. For this purpose, the water samples

were collected from three sites like Ampil commune (control) in Kampong Cham province, Khsarch Andaet commune in Kratie province and Kampong Kong commune in Kandal Province. Among these sites, ground water results were significant for arsenic, manganese, iron and barilium content. As content in scalp hair showed positively correlations with As content in the ground water and average daily doses. This highlights that ground water is the main source of As accumulation in the people of the study area. The number of respondents that were affected by non-cancer health risks and threatened by cancer were 98.65% and 0.5%; 13.48% and 33.71% and non for Kandal province, Kratie province and Kampong Cham province. This study indicated that in future, level of As may be resulted into many health problems of arsenicosis if the problem was not taken serious.

Arian *et al* (2009) analyzed the ground water, lake water, sediment, soil and fish for accumulation of heavy metals like Zn, Ni, Cr, Cu and As and also investigated human health problems related to these metals in south-east part of the Sindh, Pakistan. As showed greater concentrations in ground water and lake water samples than the WHO permissible limits.

Kavcar *et al* (2009) investigated heavy metals and their health related problems via drinking water ingestion pathway in Province of the I˙zmir, Turkey. For this purpose water samples of drinking water were collected and studied for the concentration of heavy metals and metalloids like arsenic, nickel, chromium, cobalt, copper, manganese, lead, zinc, cadmium, and varidium. The drinking water consumption and demographics information were also collected from each sampling point. For each individual, arsenic exposure and risks were estimated. As and Ni concentrations have crossed the permissible limits in 20% and 58% respecting samples. The result highlights that As non carcinogenic risks were higher than the level of concern for 19% of the population, where as carcinogenic risks were 10-4 for 46%, and 10-6 for 90% of the population.

Krishna *et al* (2009) had studied the surface water and ground water for heavy metals concentration such as cobalt, chromium, nickel, iron, manganese, lead, zinc and arsenic in the Patancheru industrial town (India). According to the study, many chemical and pharmaceutical industries have established during last three decades and these industries dumping their waste products into surface water of rivers. Irrigation fields and surrounding land forming point and non-point are various sources for surface water and ground water contamination. In surface water, FA identified four factors having 75% of total variance and in ground water two factors having 85% of total variance. Heavy metals like cobalt, chromium and nickel surface water were related and ground water received these metals from anthropogenic and geogenic sources.Where as anthropogenic activities are the main sources of cobalt, iron, manganese, lead, zinc and arsenic for both surface and ground water.

Khan *et al* (2009) investigated the role of wetland for heavy metals removal from industrial effluents. For this purpose heavy metals like lead, cadmium, copper, iron, nickel and chromium were investigated in the industrial effluent, sediments and aquatic macrophytes of the wetland. Constructed wet lands were found more efficient for the removal of heavy metals like lead, cadmium, copper, iron, nickel and chromium. The findings also showed that the wet lands paly a significant role in the removal of some other heavy metals like cadmium, iron, and copper removal. From research it is cleared that wet land is more efficient for the removal of heavy metals from industrial effluents and other waste products like sewages.

Baig *et al* (2009) studied the ground and surface water of Jamshoro Sindh, Pakistan for physio-chemical parameters and heavy metals. Arsenic showed more content in ground as compare to surface water, where in surface water the concentration for arsenic was less. The ground water and surface water samples showed greater arsenic and physio-chemical parameters like electrical conductivity, sodium and potassium contents than WHO permissible limits for clean water. Water

logging is mainly related to Indus river irrigation system, which may be resulted into greater concentration of arsenic in the ground water. The ground water also showed higher level of iron, which is also a good source of arsenic concentration for the ground water and surface water. The result revealed that coal combustion at brick factories and power generation plants are main sources of arsenic for both ground water and surface water.

Halim *et al* (2009) investigated the arsenic concentration and distribution in the ground water of the Sherajdi khan area, Bangladesh. For this purpose ground water samples were collected from both shallow and deep tube wells and studied for the levels of arsenic and other parameters like temperature, pH, electrical conductivity, calcium, magnisum, sodium, potassium, chloride, nitrate, iron and manganese. The ground water showed greater concentration of arsenic. Further more the other studied parameters in ground water also showed higher levels. In subsurface aquifer, reducing environmental condition prevails that favors the release of As from Fe-Mn oxyhydroxides in the target aquifers.

Kazi *et al* (2009) studied the water of Manchar Lake (Pakistan) for different physio-chemical parameters, with five different monitoring sites. The water samples were collected from three significant sites named site 1, site 2 and site 3. According to the result industrial effluents, domestic sewages, agricultural runoff are the main sources for different physio-chemical parameters and saline seeps and living people, boats and fishing are the major sources of water contamination in the lake.

Manzoor *et al* (2006) studied the ground water, effluents and soil for selected heavy metals and the distribution and source identification of heavy metals in these samples were investigated through using multivariate analysis. For this purpose the ground water, effluents and soil samples were collected from three textile industries Hattar Industrial Estate, Pakistan and studied for metals. The results showed greater level of metals in these samples and the overall order was soil > effluent > water. The

result further indicated that the textile effluents were containing more level of toxic metal like chromium, which is correlated to soil and water bodies contamination. Similarly, other heavy metals such as cadmium, cobalt, iron, manganese, nickel and zinc were also observed in greater concentration in the textile effluents. The study highlights that the effluents from textile industries contaminate both the soil and ground water. The water and soil were found to be contained less concentration of heavy metals than the permissible limits.

Jonnalagadda and Mhere (2001) studied the nature, extent and contamination sources for the water of River Odzi, Zimbabwe. For this purpose the water samples were collected from 6 different points of the river for about 9 months and studied the water samples for different physical parameters like temperature, electrical conductivity, pH, total suspended solid, total dissolved solid, BOD, PO4 and NO3. There was made a comparison between the water quality of Odzi River and water quality indices at various sites. This finding indicates that upstream water quality in the river was safe and not polluted. However the water quality in plains was contaminated due to dumping of sewages and waste products from the farm lands and Mutare River that carried the seepage from abandoned mine dumps.

Khan *et al* (1999) had determined the impacts of industrial effluents on the water quality of River Kabul water at Amangarh, Nowshera and studied the water for various chemical and biochemical parameters. The water was found to high level of chloride, potassium, sodium, pH, total suspended solid and total dissolved solid.

IUCN (1994) has studied the water of River Kabul for different parameters. The concentration of sodium, chloride and potassium were within acceptable limits in the River Kabul for the fisheries and aquatic life.

Nafees and Ghulam (1991) had pointed out that different parameters were reported in the water of River Kabul. Electrical coductivity, total dissolved solid, total

suspended solid and chloride etc were higher in concentration as compare to the recommended standard for industrial effluents.

2.2 BIOACCUMULATION

Bhattacharya *et al* (2007) have investigated that some commercially edible fish accumulate heavy metals through consumption of food, water and sediment from the water ecosystem. The bioaccumulation in the fish tissues depends on the concentration of heavy metals in water. The muscle and gills tissues showed greater level of heavy metals like zinc, copper and lead than gonads and skin. Greater concentration of copper and lead were found in gill tissue, while lowest content was found in gonads. The consumption of contaminated fish impact the health of human beings.

Yilmaz and Doga (2007) had studied the muscle, liver, gills, skin and gonads of *Carasobarbus luteus* fish for bioaccumulation of heavy metals like silver, cadmium, chromium, copper, iron, nickel, lead and zinc. The gonad showed more content of heavy metals like silver, cadmium, chromium and lead were followed by the liver, gill, skin and muscle, while less content of copper, iron, nickel and zinc were found in liver followed by other tissues. The muscle of *C. luteus* had accumulated less levels than the permissible limits for human consumption. The copper concentration was very close to the permissible limits.

Fatma *et al* (2005) investigated concentration of heavy metals like Fe, Zn, Mn, Pb, Cu and Cd in several organs like muscle, gills, liver and kidneys of 0. *niloticus, T. zillii* and *C. lazera* and water from Abu Za'baal lakes. Different organs like muscle, gills, liver and kidney of *O nihiicus. T. zillii* and *C. lazera* fishes showed highest concentration of heavy metals like Fe, Zn, Mn, Pb, Cu and Cd than water.

Arvinda (2002) has investigated that accumulation of heavy metals in fish species are related to several factors like temperature, pH of water, conductivity, rainfall, hardness, salinity and also by biotic community interactions.

Chaudhari *et al* (2002) investigated that gills is the prime organ that is exposed to water pollutants and play a vital role in absorption of heavy metals from the surrounding environment. The significant decrease in total glycogen content of gill, food mantle, digestive gland and the whole body of fresh water bivalve, *Parreysia cylindrical* was due to pollution stress caused by heavy metals.

Onwumere and Oladimeji (1990) exposed *Oreochromis niloticus* to petrolume refinery effluents and reported this fish to be accumulated trace metals such as zinc, nickel, chromium, copper, cadmium, iron thousand times above the levels existing in the exposure medium, while some metals were preferentially accumulated than others.

Colburn (1993) has measured that heavy metals tend to accumulate in the air and in food chain and resulted into poisoning. The heavy metals are toxic in nature and can impact both the endocrine and reproductive systems of both terristrial and aquatic animals and has similar effect on organocholine chemicals.

Adeyeye (1993) has mentioned that level of metals in fish is an indication of heavy metal pollution of the water. Contents of different metals in the gills, skin, intestine, liver and muscle showed variation. Statistical comparison indicated that each organ had significantly different metal concentrations.

El-Ezaby (1994) had reported that heavy metals are the major part of aquatic pollutants. Since they were investigated in the ecosystem in critical concentration. Not only environmental organizations, such as EPA and UNEP but also the public communities are concerned about the possible adverse consequences of such pollutants to the aquatic biota and indirectly, to humans. Heavy metals are toxic and tend to accumulate in the body organs of aquatic organisms.

Zia and Mcdonal (1994) had exposed rainbow trout, *Oncorhynchus mykiss* to water elevated levels of heavy metal like cadmium, copper and zinc and the gills, liver and kidney were taken out and processed for heavy metals concentration. The gills concentrated metals 4-11 fold higher than other organs like liver and kidney.

Allen (1995) had investigated the influence of heavy metals like mercury, lead and cadmium by exposing the *Oreochromus aureus* fish to these heavy metals. The chronic accumulation profile was determined in the liver, brain, gill filaments, intestine, caudal muscle, spleen, trunk, kidney and gonads of *Oreocheomis aureus*. The highest concentration of Cd was found in the kidney as compare to other examined tissues. The spleen, intestine and liver also showed greater concentration of Cd. While the caudal muscle and brain contained smaller content of Cd.

Kotze (1997) had worked out that the ability of each tissue to accumulate metals can be attributed to the total amount of metal accumulation in that tissue. Further more physiological differences and the position of each tissue in the fish can also influence the bioaccumulation of a particular metal.

Brown and Gratzek (1980) had reported that there are hundred metals that have been demonstrated to be toxic to fish. Among these groups that have received the most notoriety are the synthetic organic insecticides, heavy metals, especially mercury, the polychlorinated biphenyls, ammonia and chlorine. The fish intake heavy metals from their surrounding environment by directly exposition to the metals and and other ways through which wild or cultured fish accumulate toxic materials is called food. Gills of the fish also play a vital role in the intaking of many toxic materials from their environment.

Goldstein and Weese (1999) have investigated that *Cyprinus carpio* was analyzed for bioaccumulation of heavy metals like cadmium, chromium, copper, lead, nickel, selinium and zinc. Organs like liver, muscle and whole body showed different contents. Generally, trace element contents were the greatest in liver, while

concentrations of cadmium, copper, nickel, lead and zinc in whole bodies were higher than those in muscle. Concentrations of As and Se in muscle were similar to those in the whole body. Concentration of chromium was lower in liver as compare to muscle or whole body. There was present a stronger correlations between liver and whole body concentration than those between liver and muscle concentration. But the correlation between muscle and whole body concentration was the strongest.

Kotze *et al* (1999) investigated the accumulation of heavy metal like Pb in the kidney. The physiological role of an organ helps role in accumulation of heavy metals in different organs of a fish. Behaviour and feeding habits are also other factors that play a vital role in accumulation of heavy metals in different tissues and organs of fish.

Zyadah and Chouiki (1999) studied bioaccumulation of heavy metals like copper, zinc, cadmium and lead concentration in flesh, gills and gonads of three commericial fish *Mullus barbatus*, *Merluccius merluccius* and *Boops boops*. Cu, Zn and Pb concentrations in flesh were smaller, while in gonads cadmium level was greater. Liver had maximum content of Cu than other organs. Gonads and liver had accumulated higher level of cadmium. Liver and gills showed greater concentration of Pb as compare to other organs. The organs of *Boops boops* fish had accumulated greater concentration of heavy metals as compare to others fish.

Larsson *et al* (1985) determined that the tendency of heavy metals to get accumulation in organisms is one of the important property of metals and bioavailability of trace metals is the key factor for determination of tissue concentration. The bioaccumulation of metals depend on availability of metals in fish tissues can be used as an indicator of environmental metal contamination.

Ramalingam and Rarnalingam (1982) had reported that accumulation of heavy metals may be attributed to mortality or may be the cause of many biochemical and pathological abnormalities in the aquatic organisms. Several investigations had

concerned with the effect of metals on the levels of tissue protein and lipid. Reduction in protein levels was noticed in muscle and liver of *Sarotherodon mossambicus* exposed to mercury.

2.3 GENOTOXICITY

Obiakor*et al* (2014) have investigated that micronucleus assay is a technique that applicable in aquatic organisms to evaluate the genotoxicological impacts of polluted water in both in vivo and in vitro. According to a report, gill cells are more sensitive than the hematopoietic cells for micronucleus.

Nevenka *et al* (2008) have evaluated that determination of genotoxic effects of toxic substances is necessary for comprehensive study to assess the genotoxicological impacts of pollutants like heavy metals in the aquatic organisms. Although pollutants like heavy metals can induce DNA damage in red blood cells of *Balkan loach*. They are also promising for further standardization and the use of comet assay on fish in environmental risk assessment.

Nevenka *et al* (2008) had investigated that DNA integrity in erythrocytes of *Cobitis elongate* is affected by heavy metals. A higher degree of DNA damage was seen in the blood of *Cobitis elongata* from polluted sites. The erythrocytes of specimens showed different degree of DNA damage cells.

Yingmei *et al* (2006) have studied the degree of DNA damage cells in different tissues of *hepato pancreas* of loach, *Misgurnus anguillicaudatus* fish after exposed to heavy metals like Cd, Pb and Zn. The percentage of DNA damage cells was increased with increasing exposure time to heavy metals. Therefore highest percentage of DNA damage cells was observed in the skin as compare to other tissues.

Cavas *et al* (2005a) has studied the *Cyprinus carpio, Carassius gibelio and Corydoras paleatus* for genotoxicological effects of heavy metals. Fish were exposed

to different doses of cadmium, chromium and copper. Frequency of DNA damage cells was evaluated comparatively in peripheral of liver than blood erythrocytes, gill epithelial cells. It was noticed that, fish and their tissues showed sensitivity to the heavy metal treatment.

Leonard *et al* (2004) has investigated metal toxicity. The main reason of heavy metals toxicity is related to its oxidative stress. This result provides evidences that metals are binding with nuclear proteins and DNA, which is resulted into DNA damage and also causing oxidative deterioration of biological macromolecules.

Bowden *et al* (2003) had investigated that the loaches were collected from control and polluted Sava River. The blood was collected and processed for degree of damage DNA cells. Greater degree of DNA damage cells was observed in the blood of loaches collected from the contaminated site than control site of Sava River. By making comparison, there was more variation in the tail intensity of DNA among animals. It is suggested that the tail length would be increased upto a plateau after migrating to a specific distance. The tail does not show increasing in length after reaching to specific distance.

Lee *et al* (1999) has determined that physical agents like solar radiation, x-rays and a variety of chemicals such as heavy metals are the agents that induce DNA damage cells in different types of tissues if these DNA damage cells did not repair. Then there will be produced a cascade of biological consequences in the cells, organs, animals and finally in community and population level. Reduced growth, abnormal development and reduced survival of embryos, larvae and adults are related to DNA damage cells in a variety of tissues of aquatic animals.

Steinert (1999) has investigated that the DNA damage in the cell is attributed to the impact of multiple toxic chemicals like heavy metals and environmental pollutants. However, this DNA damage is depends on both the toxicant content and

exposure time. It has been reported that different cells showed increased DNA damage after exposure to pollutants for longer time.

Mitchelmore and Chipman (1998) have investigated that different fish species were collected from control and polluted water of River Gomti. The fish from polluted portion of river showed greater degree of DNA damage cells than control site. This could be related to interaction of toxic heavy metals with DNA in polluted water. The DNA damage could be produced from DNA single-strand breaks, DNA double-strand breaks, DNA adduct formations and DNA-DNA and DNA-protein cross-links.

Pandrangi *et al* (1995) worked out that various tissues and organs were collected from differnt marine and fresh water animals and processed through comet assay to assess DNA damage cells due to various toxic chemicals like heavy metals in these organisms. Fish tissues that have been examined for DNA damage cells including gut, intestine, liver, kidney, spleen, muscle, gills and gonads. Among these tissues and organs gills and intestine showed greater frequency of DNA damage as compare to other mentioned tissues.

Christopher (1994) has pointed out that blue gill sunfish, *Lepomis macrochirus* was collected from contaminated water containing heavy metals. The DNA was isolated from the blood cells and examined for DNA damage. The quantitative measures were used to determine the difference in the number of double and single strand breaks between DNA preparations. Both strand breakage was found to be greater in fish exposed to heavy metal compounds as compare to non exposed fish.

Kurelec (1993) has studied that DNA integrity is a complex intrinsic process in the organism cell. It is also affected by many external factors like exposure duration to heavy metals. Neoplasia, is a disorder that is attributed to exposure time to genotoxic chemicals in lower animals, but other genotoxic disease syndromes are also

induced as a result of exposure of the animals to toxic heavy metals and finally the extinction of species occured.

Gedik *et al* (1992) have determined that resident fish were selected as an indicator organism to assess the genotoxicological impact of polluted water in the resident fish species. For this purpose different fish species were collected from both polluted and non polluted water and processed through comet assay. Different fish species from polluted water showed greater frequency of DNA damage cells as compare to control water. The micronuclei frequency was also varied according to the season, kind of pollution and species of fish. Comet assay is more sensitive for detecting low levels of DNA damage in both fish and human beings.

Dzwonkowska and Hubner (1986) had reported that cells from the gills of the fish species were removed and used for the assay to assess DNA damage in these cells. The gills showed greater degree of DNA damage as compare to other tissues and this could be correlated to cells directly and constantly exposure of the gills to DNA damage chemicals dissolved in the water.

Hooft man and de Raat (1982) have investigated that genotoxic pollutants would not be only a threat to aquatic organisms, but also impact the aquatic ecosystem and finally humans. The aquatic environment therefore should be monitored. The micronucleus method was used in fish to assess the exposure duration to genotoxic water pollutants like heavy metals.

Latt and Allen (1977) have reported that in the sister-chromatid exchange assay there is assessment of exchange of chromosome fragments between sister-chromatids after DNA strand damage as a result of heavy metals accumulation in the aquatic organisms.

2.4 HISTOPATHOLOGY

Kaoud and Dahshan (2010) have worked out that different abnormalities like skin, liver, kidney, lung and bladder cancer, cardiovascular disease, diabetes and anemia as well as reproductive, developmental, immunological and neurological disorders are attributed to heavy metals toxicity in the human body. Mining, industrial effluents and sewages are various sources of metal contamination.

Metwally *et al* (2010) had investigated various histopathological changes like hepatocytes vacuolation, cellular swelling, nuclear degeneration and congestion of blood vessels in the liver and pathological disorders such as secondary lamellar disorganization, rupture in lamellar epithelium and epithelial lifting in gills of different fish after collection from polluted water.

Kaoud and Dahshan (2010) have studied the gills, kidney, liver, intestine and muscle tissues of *Oreochromis niloticus* from Egyptian fish farms for pathological abnormalities. Pathological disorders like mild congestion and edema of the primary lamellae. Severe edema, hyperplasia, mononuclear leukocytic infiltration, edema and congestion were seen in the gills tissue of the fish. In the liver degeneration of the hepatocytes and intravascular haemolysis in blood vessels, congestion of central vein, hemorrhages were observed. The kidney showed hydropic swelling of tubules and large glomerulus in diameter. Various pathological abnormalities such as degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis, atrophy and edema of muscle bundles as well as splitting of muscle fibers were observed in the muscular tissues. The intestine also showed different pathological changes like atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, edema and atrophy in the submucosa.

Elahee and Bhagwant (2007) have studied that gills histopathological lesions as indicators of exposure to heavy metals have previously been used in numerous laboratory and field studies around the world.

Bertin and Averbeck (2006) had investigated that heavy metals are an important toxic environmental pollutants. In humans and other animals like fish heavy metals are associated with damaging and cancer of different tissues like prostate, lungs and testes. Acute exposures of the fish to heavy metals are responsible for damage to these organs. Different changes like obstructive airway disease, emphysema, renal failure, bone disorders and immuno-suppression are related to chronic exposure to heavy metals.

Nero *et al* (2006) had studied the gills and liver of *Perca flavescens* and *Carassius auratus* fish for different pathological abnormalities after collection from polluted water. Generally gills and liver pathological data indicates that degenerative changes were the most prevalent and sensitive abnormalities seen in these fishes after exposure to elevated levels of heavy metals in polluted water.

Fatma *et al* (2005) have investigated that examination of fish gills, collected from contaminated Abu Za'baal lakes showed marked histopathological changes. Different histopathological abnormalities like proliferative, degenerative and necrotic changes in the epithelium of gills filaments and secondary lamellae, edema in secondary lamellae, separation of epithelium of the secondary lamellae from the lamellar supporting cells, dilation and congestion in the blood vessels of gills filaments, atrophy in secondary lamellae, bulging at the tips of secondary lamellae and dark deposits on the surface of gills epithelia were observed in the gills tissue.

Sabry *et al* (2005) have investigated histopathological changes in epidermis, dermis, hypodermis and underlying muscles of *Oreochromis niloticus* fish specimens collected from El-Kanater, Benha, Zefta and Talkha stations and found necrosis of epithelial and mucous cells of the epidermis, degeneration, necrosis and edema of

muscle fibers. They also revealed congestion and dilation of the dermal blood vessels together with hypodermic inflammatory signs which may extend to the underlying muscle.

Yacoub *et al* (2003) has studied several histopathological alterations in liver of different fish. These alterations including vacuolar degeneration in the hepatocytes, focal areas of necrosis, haemorrhage and haemostderin between the hepatocytes and around hepatic and hepatoportal blood vessels and dilation and congestion in hepatic and hepatoportal blood vessels. The observed degeneration in the liver may be attributed to disruption in the lysosomal membrane, which is very sensitive to toxicants as heavy metals and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of hepatocytes.

Mohammad (2003) has mentioned that different histopathological abnormalities were observed in the gills and liver of *C. gariepinus* fish after collection from El-Rahawy drain. The pathological abnormalities in gills of *C. gariepinus* could be correlated to toxicants reaction after intaking or an adaptive response to prevent the entry of the pollutants through the gill surface.

Ambrose *et al* (1994) have measured that heavy metal like zinc is known for its essential role in growth, immunity, DNA replication, body's defensive system, cell division, cell growth, wound healing and carbohydrates breaking down. High zinc intake leads to enfeeblement, stunted growth and also attributed to metabolic and pathological disorders in various organs of fish.

Orecka and Grabda (1986) have measured that heavy metals were determined in fish species like eels netted from contaminated lakes in north western Poland. Steady deterioration in the health of eels, chronic degenerative inflammation of the internal organs, non-specific anemia, aplasia or hypoplasia of the erythrocytes in fish was observed.

Laurent (1984) has evaluated that examination of fish gills collected from Abu Za'baal lakes showed marked histopathological changes. These changes are including proliferative, degenerative and necrotic changes in the epithelium of gills filaments and secondary lamellae, edema in secondary lamellae, separation of the epithelium of the secondary lamellae from the lamellar supporting cells, dilation and congestion in the blood vessels of gill filaments, atrophy in secondary lamellae, bulging at the tips of secondary lamellae and dark deposits on the surface of gills epithelia. The studied changes in the respiratory lamellar epithelium may increase the epithelial thickness, which prevent the entry of toxic metals into the blood stream.

Daoust *et al* (1984) observed pathological lesions under microscope in various tissues of fish after exposure to heavy metals like mercury and copper and different pathological abnormalities like apoptosis of lamellar epithelial cells and lamellar fusion were observed in different tissues. The latter process occurre either by simple apposition of adjacent lamellae to each other or through epithelial hypertrophy and hyperplasia

Srivastava *et al* (1979) investigated different pathological disorders and accumulation of cadmium in the fish tissues after exposure to cadmium concentration. Cadmium exposure induces pathological abnormalities like appearance of granular deposits in the liver, atrophy of the proximal renal tubules and increases in chloride cell turnover in the gills.

CHAPTER-3

PHYSICAL AND CHEMICAL PROPERTIES OF WATER COLLECTED FROM THE RIVER KABUL

3.1 INTRODUCTION

Water is necessary for the survival of both plants and animals. On average human beings consume about 2 liter of water every day. There is about 80% water on the earth surface. Out of the estimated 1011 million km^3 of the total water present on the earth, only 3340 m^3 of water is available for drinking, agriculture and domestic and industrial consumption. The rest of the water is locked up in ocean as salt water, polar ice caps and glaciers and under ground. Due to increasing industrilization and exploding, the demand of water supply is also increasing tremendously. Moreover sewage, industrial wastes and a wide array of synthetic chemical are the main causes of contamination for this limited quantity of water. The meanance of water born diseases and epidemic still threatents not only the human but also the fish population. Thus both the quality and quantity of clean water supply are essential for the welfare of mankind (Dara, 1993).

3.1.1 Physico-chemical Parameters

The water of River Kabul was analyzed for different physico-chemical parameters. The levels of pH, total suspended solid, total dissolved solid, electrical conductivity, total alkalinity, sodium, potassium and chloride were high as compare to national environmental quality standards (Yousafzai, 2004). The water of River Kabul was analyzed for different parameters. Analytical data showed that pH, total suspended solid and total dissolved solid were present in high concentration (Khan and Ullah., 1991). Previous studies have also reported a high content of total dissolved solid from different water bodies (Subramanian, 2004; Kamin, 2001; Khan and Ullah, 1991). The impacts of industrial discharges on the quality of River Kabul water at Amangarh, Nowshera was studied for various chemical and biochemical

parameters like pH, total suspended solid, electrical conductivity, alkalinity, chlorides, sulfates, sodium, potassium etc. The results indicated localized pollution within half kilometer after the confluence point where the quality of the river water was reported to have deteriorated (Akif *et al*., 2002; Khan and Mumtaz, 1997; Khan *et al*.,1999). In another finding a high level of total suspended solid has been also reported for River Swat at Mingora, Pakistan (Muhammad *et al*., 1998).

The water of different rivers such as Kerala, Cauvery, Gomti, Krishna, Godavari, Mahanadi, Narmada, Tapti, Indus, Brahamputra and Ganges was analyzed for total alkalinity and all these rivers showed different values for total alkalinity (Subramanian, 2004). The water of River Kabul has high level of total conductivity as related to the concentrations of TDS, TSS and major ions (IUCN, 1994). The water of River Kabul has greater alkalinity level, which is essential for buffering pH changes and for reduction heavy metals toxicity. The high values of alkalinity, total suspended solid and total dissolved solid all are helping in reductionof the toxicity of what would be lethal concentration of metals (IUCN, 1994).The water of ten rivers was analyzed for suspended load quantitatively and qualitatively. The Chitral and Bara rivers were found turbid most followed by River Kabul, Panjkora and Swat. Other parameters that were studied are including pH, total suspended solid, electrical conductivity, sodium and potassium, which were within the permissible limits (Sabir, 1996). The analysis of effluent sample of River Kabul indicated significant amount of total dissolved solid. The TDS was within permissible limits laid down by National Environmental Quality Standards (NEQS) for municipal and liquid industrial effluents of Pakistan (Kamin *et al*., 1985). The water of River Kabul was studied and found with high level of total alkalinity (Yousafzai, 2004).

The water of River Kabul was analyzed for total dissolved solid concentration during high and low flow seasons. The total dissolved solid had high content for both low and high flow seasons (Yousafzai, 2004). The aquatic pollution due industries in

the River Kabul is a major problem. The water of the river was analyzed for different parameters. Analytical data showed that pH, total suspended solid and total dissolved solid were high in concentration (Khan and Ullah, 1991). Silt, clay, fine particles of organic and inorganic matter, soluble organic compounds are the structural componenets of total suspended solid. Thus the concentration in River Kabul are greater but in some cases the level of total suspended solids are above desirable levels for all uses, they are mainly a natural characteristic of the river due to its catchment and discharge characteristics. Natural erosion is greater in mountainous areas and volcanic regions. The source of much of the total suspended solid is erosion of rock and soil rather than pollution (IUCN, 1994). Many people described the River Kabul as a ‘dirty river bescuse it is very turbid. This is due to the high total suspended solid (TSS) carried by the river. This is high during both low flow and high flow conditions (IUCN, 1994). Total suspended solid (TSS) values at all the sampling sites of the River Kabul including dam exceeded the NEQS value for this parameter. This could be correlated to high flooding during high flow due to snowmelt on the peaks of the surrounding hills, both in Pakistan and Afghanistan and monsoon rains during summer months, excessive deforestation, weathering, soil erosion, mining and other anthropogenic activities along the banks of the river (Yousazai *et al*., 2010a).

The River Kabul is receiving the effluents from associated industries near Nowshera. The water was taken from River Kabul and analyzed for different parameters such as electrical conductivity, alkalinity, potassium, chloride and pH. The contents of all these parameters were slightly higher in the above water than the below (Ali, 1995). The water of River Swat was analyzed and found high value for total alkalinity for River Swat at Mingora, Pakistan (Muhammad *et al*., 1998). The water of River Kabul was analyzed for alkalinity, chloride, potassium and sodium. The effluent sample discharges high quantity of chloride, magnessium, potassium and sodium both during low and high flow into the River Kabul. This discharge has caused a significant increase of chloride, potassium and sodium in down stream samples both

during low and high flows (Yousazai *et al*., 2010b). The water of different rivers such as River India, River Gomti, River Godavari, River Indus, River Brahamputra and River Ganges was studied and found that the Cl level was highest in the River Godavari and lowest in River Indus (Subramanian, 2004). Agricultural, industrial and domestic waste water are the major sources of chlorides, potassium and sodium in the River Kabul. Kitchen and human wastes are the sources of chloride in municipal sewage. Large amounts of chlorides also come from the industries (Khan, 1996; WWF, 2001).

The water of River Kabul from Warsak dam was studied for different physico-chemical parameters. The water from this site had high chloride concentration for both lowand high flow periods (Yousafzai, 2004). When the chloride content of given sewage is found too high, it may indicate the presence of industrial wastes in the water bodies (Ramakar *et al*., 2005). The water sample from Warsak dam had been taken and studied for alkalinty. The water showed high content of alkalinity (Yousafzai, 2004). Various physical, chemical and biological parameters in the water of the River Kabul at Nowshera were analyzed and found that the water of the main river was not much affected by the pollutants of the industries (Butt, 1989b).

The impacts of industrial effluents and sewages on the water quality of River Kabul was studied. The water was analyzed and found high sodium content from polluted sites of River Kabul (Yousafzai, 2004). This attempt was made for the first time in NWFP with special refrence to water pollution analysis of the industrial effluents in the River Kabul. The pH, electrical coductivity, total dissolved solid, total suspended solid and chloride etc were in higher concentration as compare to the recommended standard for industrial effluents (IUCN, 1994). The impacts of industrial effluents on Kabul River was studied. Various industrial effluents, which are ended into River Kabul revealed high concentration of pH, chloride, potassium, sodium and salinity but they all were within permissible limits (Khattak and

Rehman.,1992). The effluents of selected industries located at small Industrial Estate, Kohat Road, Peshawar were analyzed and found higher concentration of TSS, TDS, potassium and chloride and sodium in it. Higher values of these parameters are of great concern because finally these effluents are drained into River Kabul (Jan *et al*., 2002). The water of River Kabul was studied for different parameters. The concentration of sodium, chloride and potassium were within acceptable limits in the River Kabul for the maintenance of fisheries and aquatic life (IUCN, 1994).

3.1.2 Heavy Metal Parameters

Due to toxicity of heavy metals, accurate informations on their concentration in aquatic ecosystem are needed, especially from natural, uncontaminated habitats (Janssen *et al*., 2000). Analysis of heavy metals like Pb, Ni and Cu in the water of River Kabul revealed that they were below permissible limits (Nafees and Ghulam, 1991). The impacts of industrial effluents on Kabul River were studied. Various industrial effluents revealed high concentration of heavy metals like Cu, Zn, Fe, Cd, Pb and Ni. Level of most trace elements exceeded the limits for irrigation water (Khattak and Rehman., 1992). The water of the River Kabul was evaluated for different heavy metals like Cd, Cr, Ca, Pb, Fe, Mn and Zn. The Kabul River receives sewage and effluents from Peshawar through Budni nalla and Ganda vinda and disturbs it ecological status. The analysis of these effluents revealed high concentration of Cd, Cu and Fe (Nawab, 1992). The effluents of selected industries located at small Industrial Estate, Kohat Road, Peshawar was analyzed for heavy metals and found higher concentrations of Fe, Mn and Cr in it. Higher values of these parameters are of great concern because finally these effluents are drained into River Kabul (Jan *et al*., 2002). The heavy metals like Cu, Fe, Pb, Cd, Mn and Zn concentrations in the water of El-Rahawy drain and River Nile at El- Kanater El-Khyria were seasonally estimated. It was found that heavy metals concentration in water from El-Rahawy drain region were higher than those obtained from River Nile (Mohammad *et al*., 2013). Heavy metals are one of the major types of toxic pollutants

commonly present in surface water and highly toxic to marine and fresh water aquatic life (DWAF, 1996).

The chemical composition of sea and fresh water influences to a great extent the speciation of heavy metals. In river water, large proportion of metals is bound to be organic and inorganic particulate matter (Salomons and Forstner, 1984). Many studies were previously carried out on the level of heavy metals in water (El-Rafe, 1991; Abdel-Shafy *et al.*, 1995; Khallaf *et al.*, 1998; Bahnasawy, 2001; Sabae and Abdel-Satar, 2001). The water from Abu Za'baal lakes in Egypt was studied to assess the level of some heavy metals like Fe, Zn, Mn, Pb, Cu and Cd in water of this river. The heavy metals were found in high contents in the River water (Fatma *et al*., 2005). The presence of heavy metals in river, lake or any aquatic environment can impact the aquatic biodiversity and ecosystems due to their toxicity and accumulative behavioure (Heath, 1987). The heavy metals like chromium, copper, manganese and zinc are accumulated in sediments, aerobic and anaerobic bacteria in lakes, rivers and streams (Shahunthala, 1989). Metals crap market, activities of the blacksmiths by the lake, municipal wastes on the bank of the lake, agricultural activities within the catchments area of the lake and intense irrigation practices are sources of heavy metals in lake Geriyo (Jonathan and Maina, 2009).

The water of Ogun River was studied for heavy metals like lead and cadmium concentrations and found these metals were to be high above the WHO limit (Jaji *et al*., 2007). It is known that waste water and surface waters are the major sources of a multitude of chemical substances like heavy metals in the water bodies (Reifferschied and Grummt, 2000). This assumption is also sustained by the results of the chemical analysis of water and showing increased level of various heavy metals such as mercury, arsenic, copper, cadmium, chromium and manganese in municipal water at the location Ivanja Reka. Wet land ecosystems also receive composite effluents containing greater level of heavy metals, which are of the prime environmental

concern especially for the biotic components that interact with contaminated aquatic environment (Abdelmeguid *et al*., 2002; Wong, 2003; Raychaudhuri *et al*., 2008). Sharp declining of heavy metals in water and fish samples could be attributed to recycling of heavy metals in the river and their deposition into the sediments (Johnson *et al*., 2005, Tipping *et al*., 2006).

Metals have no natural elimination process in the aquatic ecosystems, so they transport from one compartment to another compartment inside the aquatic environment including the biota (Chapman, 1992). Heavy metal such as Cr content was greater during high than low flow conditions in the River Kabul. The range of Cr from 0.002 to 0.02 mg/l is considered to be acceptable standards for maintenance of fisheries and aquatic life is depending upon the valency state of the chromium. These values were greater during high flow conditions throughout the river. Three branches viz Shah Alam, Adezi and Naguman of the River Kabul showed greater level of Cr than the main river. The Naguman branch was more affected than other two branches, where high concentration was present downstream of the Akbar Tannery (IUCN, 1994). Heavy metal like Zn content compare to a standard of 0.03 mg/l for the maintenance of aquatic life was also high in the River Kabul. This is the case for the Shah Alam branch of River Kabul during high and low flows. However the levels of zinc is generally greater in the water of river during high flow (IUCN, 1994). Metals can be accumulated in bottom sediments as a result of remaining for many years. Sediments are considered the main repository of heavy metal in aquatic environment (Asaolu and Olaofe, 2005; Olowu *et al*., 2010).

Heavy metals are naturally occurring elements (Nies, 1999; Mighall *et al*., 2002) and are present deep in the earth crust (Malle, 1992). Natural erosion processes like weathering and abrasion of rocks, soils and sediments by wind and water are responsible to add heavy metals into aquatic environment. Volcanic eruptions, forest fires and aerosol formation above seas are also natural sources of heavy metals for

aquatic environment. These processes are helping in cycling of metals in the environment, in air, surface waters and soil (Mighal *et al.*, 2002). The river water survey reported three major water issues from River Kabul. They are organic water pollution in the Shah Alam, Naguman branches and lower main river and the survey found the greatest level of chromium in the three branches and the Bara River (IUCN, 1994). In the River Kabul three metals like Cr, Cu and Zn were present in content above those suitable for the maintenance of fisheries and aquatic life. The recorded levels were greater during high flow conditions due to re-suspension from sediments and possibly diffuse pollution sources (IUCN, 1994). The concentration of heavy metals such as Pb, Na, Ca and Ni were well within limits in the River Kabul for the maintenance of fisheries and aquatic life (IUCN, 1994). The heavy metal like Cu level was higher in the River Kabul during high flow than low flow conditions and were frequently above normal standards for fisheries and aquatic life. However toxicity of copper was markedly reduced in very hard water such as the Kabul River and toxic concentration was high (Anon, 1976).

3.2 MATERIALS AND METHODS

3.2.1 Study Area Description

For detail see page≠2

3.2.2 Sampling Sites

The water and fish samples were collected from different sites of River Kabul. The Kabul River enters Pakistan through Shalman in Khyber Agency. It then flows through the Khyber and Mohmand Agencies until it reaches Warsak dam constructed in 1960 without any fish ladder for upward migration of inhabitant fish. Below the dam, it is divided into three main branches are known as Shah Alam, Nagoman and Adezai and then joins the River Indus at Kund (Attock). River Swat also joins River Kabul a few km below the dam (IUCN, 1994).

There are about 81 industrial units, the heavy metals containing effluents of which are discharged directly or indirectly in the River Kabul (IUCN, 1994, Khan *et al*., 1999). Besides that sewages of Peshawar city, several large towns, large number of villages and several Afghan refuges camps ultimately drain into River Kabul.

3.2.3 Sampling Points

To evaluate the quality and quantity of physico-chemical and heavy metal paramerters at Akber pora, Nowshera and adjacent Amangarh industrial area of River Kabul, water from the following points of the main River and Warsak dam were collected (Fig.3.1).

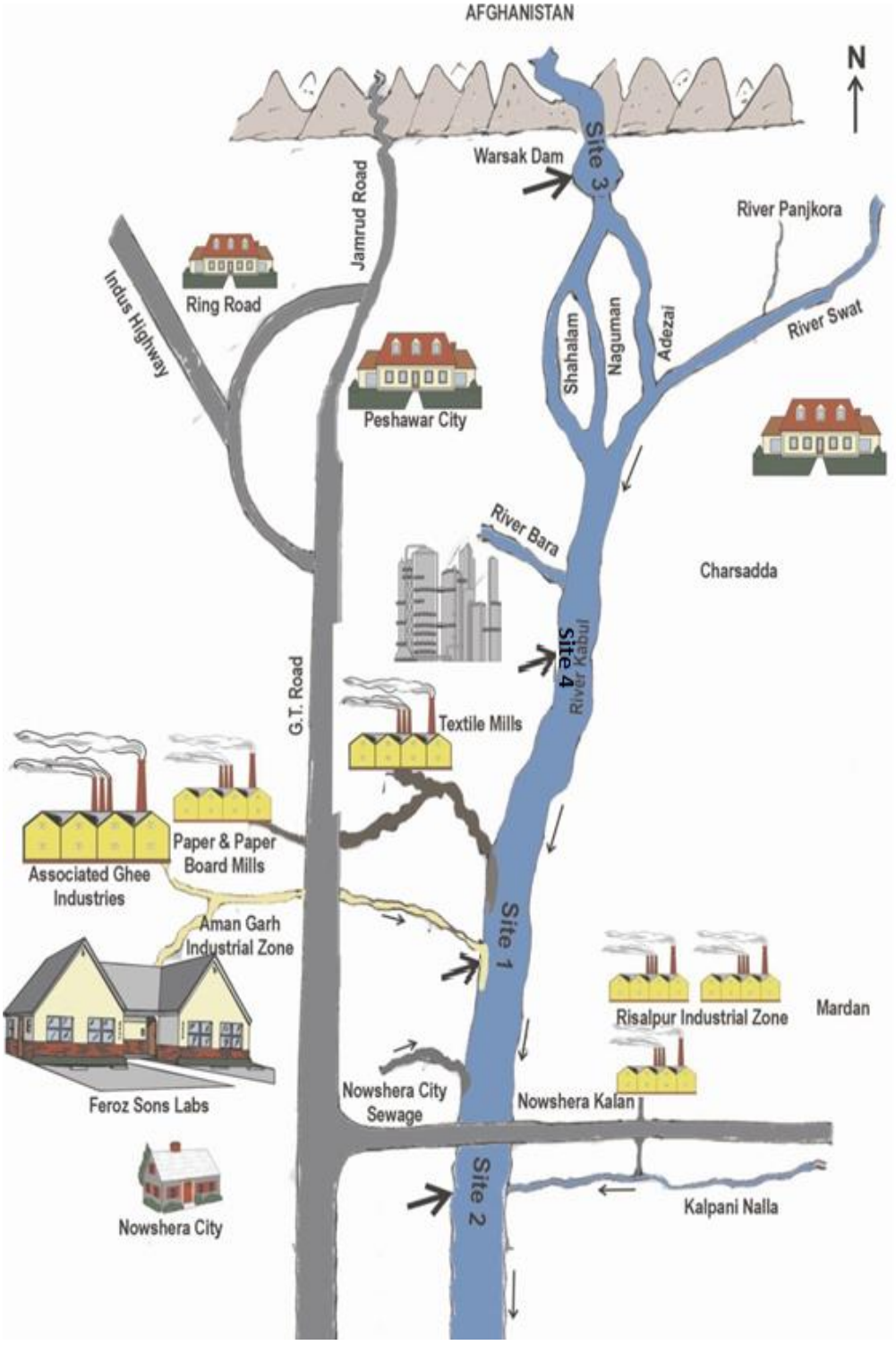


Fig 3.1: **Water sampling site 4 (water sample B), site 1 (water sample C) and site 2 (water sample D) at River Kabul (polluted samples) and site 3 (water sample A)**

3.2.4 Water sample from Warsak Dam

Water sample(A) was collected from non polluted site 3 (Warsak dam) water reservoir constructed on River Kabul in 1960, which is about 60 km upstream of the polluted part of the River and can be called safe in the sense of being far away from the dense human and industrial population. This was considered as control sample.

3.2.5 Water samples from the main River

Water samples (B) (C) and (D) were collected from polluted part of the main River at Akber Pura (site 4), Aman garh (site 1) and Nowshera (site 2). Site 4 is at the main river after city sewage discharged into it. Site 1 is at the main River after industrial effluent dumped into it and site 2 is at the main River after city sewages flows into it.

3.2.6 Collection of water samples

Water samples were collected from the sampling sites in 1000ml bottles already cleaned by vim and distilled water. At the time of sampling these bottles were also washed with the respective river water. The samples collected below the surface about 2-3 feet away from the river banks in such a way that no bobbles were allowed. Conductivity, TDS and pH for the river water were determined immediately on the spot and rest of the parameters were analyzed in the laboratory. Sampling was conducted three times a year from January 2012 to December 2014. Water samples from main river were collected once in high flow season in the month of June and two times a year in low flow season in the months of January and December to highlight the effect of water volume on the quantity and quality of water pollution.

3.2.7 Preservation of water samples

Water samples for heavy metals estimations were collected in separate 1 liter plastic bottle and were preserved with 5ml nitric acid (50%) per liter to prevent metal absorption on the inner surface of container. Samples for physico-chemical

parameters were collected in separate 1 liter plastic bottle. The water samples were then stored at 4c° in the refrigerator before analysis for various physico-chemical and heavy metal parameters.

3.2.8 Water analysis

To obtain information about the degree of contamination caused by industrial, domestic and agricultural effluents with respect to concentration of chemical, physical pollutants and heavy metals, samples were collected from three polluted sites (Akber pora, Aman garh and Nowshera) downstream and from one spot of non polluted site (Warsak dam) upstream of the River Kabul. Which is about 35 Km upstream of the polluted site B of the river and can be called safe in the sense of being far away from the dense human population and industrial activities. This was considered as control sample. The physico-chemical parameters like pH, total suspended solid (TSS), total dissolved solid (TDS), alkalinity, chloride, electrical conductivity, sodium (Na) and potassium (K) were analyzed in the water samples from both polluted and non polluted sites of River Kabul and the heavy metals such as zinc, nickel, chromium, copper, cadmium, lead, iron, manganese, mercury were determined in the water samples from both polluted and control portions of River Kabul.

3.2.9 Physico-Chemical Parameters

3.2.9.1 pH

Model; 7020- pH meter.

pH values were determined on the spot in the quickest possible time. First the pH meter was calibrated with the buffer 4 and 7. Then the pH values of the water samples from three spots of polluted sites and one from non polluted site of River Kabul were measured through pH meter.

3.2.9.2 Electrical Conductivity

Model; AGB 1000.

The instrument was calibrated with distilled water. Then conductivity of the water samples from polluted and non polluted sites of River Kabul was measured in microsiemenes per centimeter through conductivity meter.

3.2.9.3 Total Dissolved Solid (TDS)

The instrument was calibrated with distilled water. Then total dissolved solid of the water samples from polluted and non polluted sites of River Kabul was measured in milli gram per liter through TDS meter.

3.2.9.4 Total Suspended Solid (TSS)

First of all filter paper was taken and dried in oven at 103-105C^0 for an hour. After heating the filter paper kept in dessicater for moisture content absorption.The weight of filter paper was noted and then a known amount of unfiltered water from polluted and non polluted sites of River Kabul was taken in flasks and filtered through filter paper. Filter paper was again kept in the oven at 103-105C^0. The total suspended solid was calculated and reported as mg/l.

3.2.9.5 Chloride

The chloride was determined through PC multi direct spectrophotometer. Firstly filled a clean vial (24 mm) with 10ml of water samples and closed the vial with cap tightly. Then placed the vial in the sample chamber and pressed the zero key. Then removed the vial from the sample chamber and added one chloride T_1 tablet to the water sample and crushed through stirring rod and dissolved the tablet. Then another chloride T_2 tablet added to the same water sample and crushed tablet through stirring rod and dissolved the tablet. Then closed the vial with the cap tightly and swirl the vial several times until the tablet is dissolved. Then placed the vial in the sample chamber and pressed the key and waited for a reaction period of 2 munites.

After the period completion, the reading starts automatically and the result is shown in the display in mg/l chloride.

3.2.9.6 Total Alkalinity

The total alkalinity was also determined through PC multi direct spectrophotometer. Firstly filled a clean vial (24 mm) with 10ml of water samples and closed the vial with cap tightly. Then placed the vial in the sample chamber and pressed the zero key. Then removed the vial from the sample chamber and added one Alka-M- Photometer tablet straight from the foil to the water sample and crushed the tablet using a clean stirring rod and dissolved the tablet. Then closed the vial with the cap tightly and swirl the vial gently several times until the tablet is dissolved. Then placed the vial in the sample chamber and pressed the key and waited for a reaction period of two minutes. After the period completion, the reading starts automatically and the result is shown in the display in mg/l $CaCO_3$.

3.2.9.7 Sodium and Potassium

Atomic absorption Spectrophotometer (Spectra-AA-700) was used for the determination of sodium and potassium in the water samples. The same method of sample digestion and preparation was followed as adopted for the heavy metals determination. Wave lengths set were 589.0nm for soduim and 766.5nm for potassium in the presence of air acetylene flame.

3.2.10 Heavy Metals Parameters

Heavy metals like zinc, nickel, chromium, copper, cadmium, lead, manganese, iron and mercury were determined in the water samples from both polluted and control sites of River Kabul. Water sample (100ml) in the volumetric flask was acidified with 5ml nitric acid (55%) and kept on evaporated on a hot plate to about 20 ml. 5ml additional nitric acid (55%) and perchloric acid (70%) and a few drops of beads were added to prevent bumping. The mixture was then evaporated until a brown fumes change into dense white fumes. The sample was removed from hot plate,

cooled down to room temperature and diluted to 100ml volumetric flask. The solutions were then aspirated into flame Atomic Absorption Spectrophotometer (Spectra-AA-700) for determination of Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe and Hg under the following operating parameters. The flame used was air acetylene (A-AC). Standard curve were prepared and the optical density obtained were caliborated against the standard curves to know the contents of heavy metals in the water.

Table 3.1 Operating data of Atomic Absorption Spectrophotometer for determination of metals

Elements	Wave length (nm)	Flame	Working range (μgL^{-1})
Zn	213.9	AA (L)	0.4-1.6
Ni	232.0	AA (L)	3-12
Cr	357.9	AA (R)	2-8
Cu	324.7	AA (L)	2-8
Cd	228.8	AA (R)	0.5-2
Pb	217.0	AA (L)	5-20
Mn	279.5	AA (L)	1-4
Fe	248.3	AA (L)	1-4
Hg	253.7	AA (L)	100-400

Abbreviations, AA: air acetylene, R: Fuel-rich, L: Fuel-lean

3.2.11 Statistical Analysis

Statistical analysis was done by using ANOVA software for windows. Mean and standard deviation values of the data were determined. The different sets of data were analyzed for statistical differences by using student's t –test (two-tailed). a P value <0.05 was considered to show statistical significance.

3.3 RESULT AND DISCUSSION

3.3.1 Water Analysis of River Kabul

In the present study water samples from polluted sites (near Akber pura Peshawar Amangarh, Nowshera) and control site (Warsak dam) of River Kabul were taken from January 2012 to December 2014 and was studied for different Physico-

chemical and heavy metal parameters. Physico-chemical parameters include pH, total suspended solid, total dissolved solid, total alkalinity, chloride, electrical conductivity, potassium and sodium and heavy metals parameters include zinc, nickel, chromium, copper, cadmium, lead, iron, manganese and mercury.

3.3.1.1 Warsak dam upstream water from site 3 (Sample A= Control)

a. Physico-Chemical Parameters

Water samples from Warsak dam (control site 3) were analyzed for both Physico-chemical and heavy metal concentrations and showed less Physico-chemical and heavy metals contents than polluted sites 1 and 2 (Table 3.2 and Figs 3.2-3.5).

Water sample from this site of River Kabul had a pH varying between 7.1 and 7.8 with mean values of 7.3±1.7 during low and 7.3±1.0 during high flow periods. Total suspended solid (TSS) concentration was in the range of 375- 735 mg/l with mean values of 418.8±398.9 mg/l for low flow and 715.0±138.0 mg/l for high flow seasons. pH and EC values of the present study areas were higher than those reported by (Subramanian, 2004; Kamin, 2001; Khan and Ullah, 1991).Total dissolved solid of all the Warsak dam water was in the range of 298- 680 mg/l with mean values of 581.1±255.9 mg/l during low flow season and 337.0±132.2 mg/l for high flow season. The electrical conductivity in water sample from Warsak dam was ranged between 263-363µs/cm with mean values of 343.1±111.7µs/cm during low flow and 273.0±60.3µs/cm during high flow periods. The chloride ranged between 6-15 mg/l with mean values of 12.8±6.2 mg/l for low flow and 8.7±5.4 mg/l for high flow seasons.

The potassium (K) range was 1.0 -2.9 mg/l with mean values of 1.1±0.7 mg/l during low flow and 2.4±1.1mg/l during high flow periods. The sodium concentration was ranged between 3-18 mg/l with mean values of 10.8±8.5mg/l for low flow and 4.0±2.3 mg/l for high flow seasons. Similarly water from this site had total alkalinity in the range of 85-124 mg/l with mean values of 108.5±55.0 mg/l for low flow and

93.7±32.8 mg/l for high flow periods. The present results of higher TDS, TSS, EC and TA contents in water sample from this site of River Kabul agree with the previous findings (Akif *et al*., 2002; Khan and Mumtaz, 1997; Khan *et al*., 1999). On comparison with NEQS recommended values for effluents, all physico-chemical parameters except total suspended solid (TSS) were within recommended manimum limits.

Total suspended solid (TSS) had mean values of 418.8±398.9 mg/l for low flow and had 715.0±138.0 mg/l for high flow periods. Both these values were higher than NEQS recommended limit of 150 mg/l for this parameter. This shows that TSS concentration was the lowest during winter and highest during summer. The highest concentration of TSS during high flow was correlated to flooding and soil erosion in the river.The Kabul River described by many people as a 'dirty river' because it is very turbid. This could be attributed to greater level of TSS in the river, which ranged from 10 to 800 mg/l during low flow and from 340 to 1,310 mg/l during high flow conditions (IUCN, 1994). Similarly Yousafzai (2004) had also reported high TSS concentration with a mean value of 630 mg/l for high flow from the same water resources of River Kabul that verifying the validity of our findings. Comparing our study with the findings of Yousafzai (2004) and other workers showed an increasing level of TSS in water from this site in the last few years. Rest of the physico-chemical parameters like TDS, EC, TA, Cl, Na, pH and K in water from this point were below the permissible limits proposed by NEQS.

The reservoir of Warsak dam is safe and quiet fit for aquatic life. The warsak dam has been built in the tribal areas, away from the industrial activities and human population. The only parameter which exceeds the NEQS limits is TSS value, which can be correlated to excessive flooding in the River Kabul (on which Warsak dam is constructed), during summer (June, July and August). During these months snow melt occurs on the tops of surrounding hills, both in Pakistan and Afghanistan and causes

flooding in the River Kabul, moreover moon soon rains also add to flooding. Other reason can be correlated to mining activities and deforestation in the adjoining hills. As *Wallago attu, Ompok bimaculatus, Labeo dyocheilus, Cyprinus carpio* and *Aorichthys seenghala* have most of the above stated adaptations for such environment, therefore TSS can no way be a limiting factor.

Table 3.2 Physico-chemical characteristics of water sample A from Warsak dam during low (winter) and high (summer) flows during 2012-2014

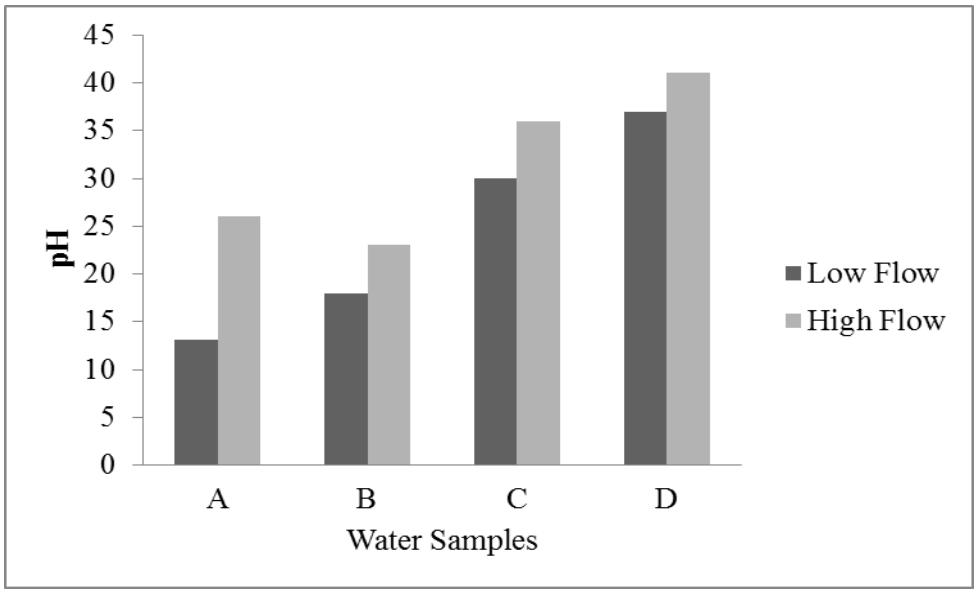
Parameters	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
pH	7.2	7.4	7.2	7.1	7.3	7.8	7.4	7.2	7.5	7.3±1.7	7.3±1.0	6-10
TSS (mg/l)	435	735	390	501	695	375	407	715	405	418.8±398.9	715.0±138.0	150
TDS (mg/l)	680	375	600	576	298	410	621	338	600	581.1±255.9	337.0±132.2	3500
EC (µs/cm)	323	273	326	363	283	359	326	263	362	343.1±111.7	273.0±60.3	NA
Cl (mg/l)	11	6	14	11	10	15	12	10	14	12.8±6.2	8.7±5.4	1000
K (mg/l)	1.0	2.1	1.3	0.9	2.4	1.6	0.8	2.9	1.2	1.1±0.7	2.4±1.1	NA
Na (mg/l)	18	8	13	9	3	11	7	5	7	10.8±8.5	4.0±2.3	NA
TA (mg/l)	119	101	115	124	85	88	119	95	86	108.5±55.0	93.7±32.8	NA

$P<0.05$

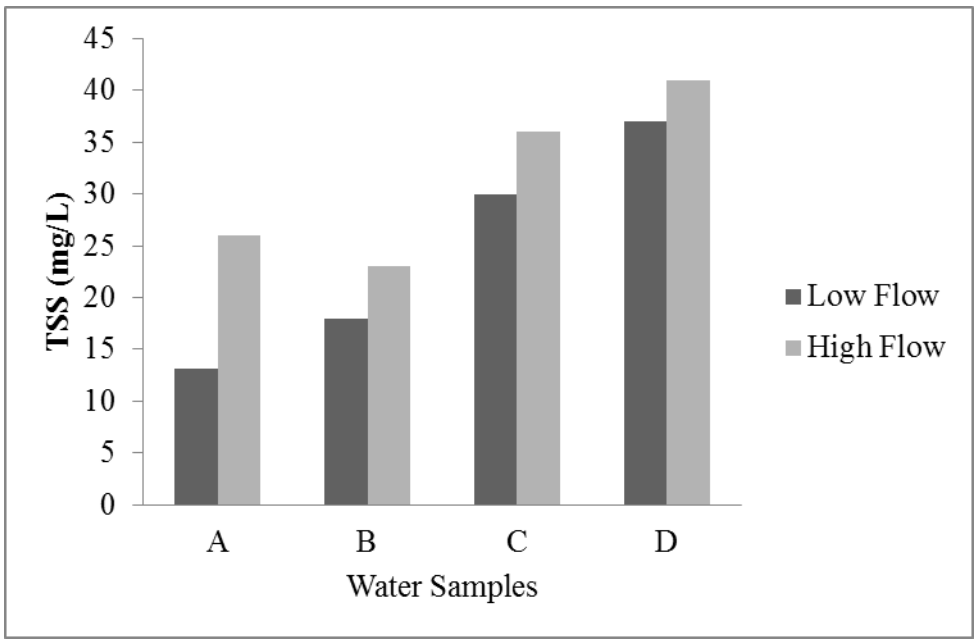
1, Mean± Standard deviation

2, NA, Not available

3, Abbrevitions: L.F, Low Flow, H.F, High Flow, TSS, Total Suspended Solid, TDS, Total Dissolved Solid, EC, Electrical Conductivity, Cl, Chloride, K, Potassium, Na, Sodium, TA, Total Alkalinity, NEQS, National Environmental Quality Standards



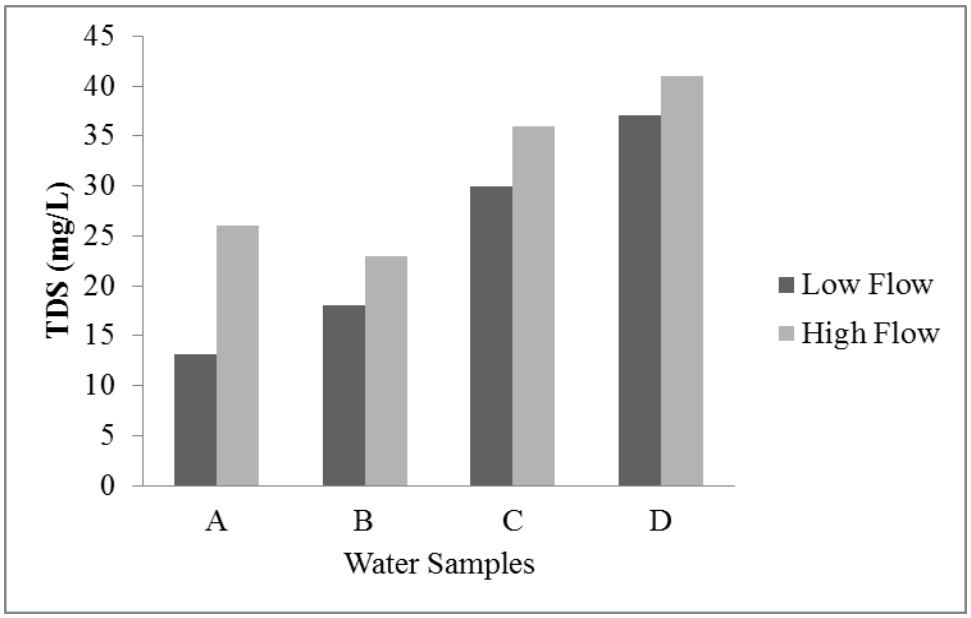
pH



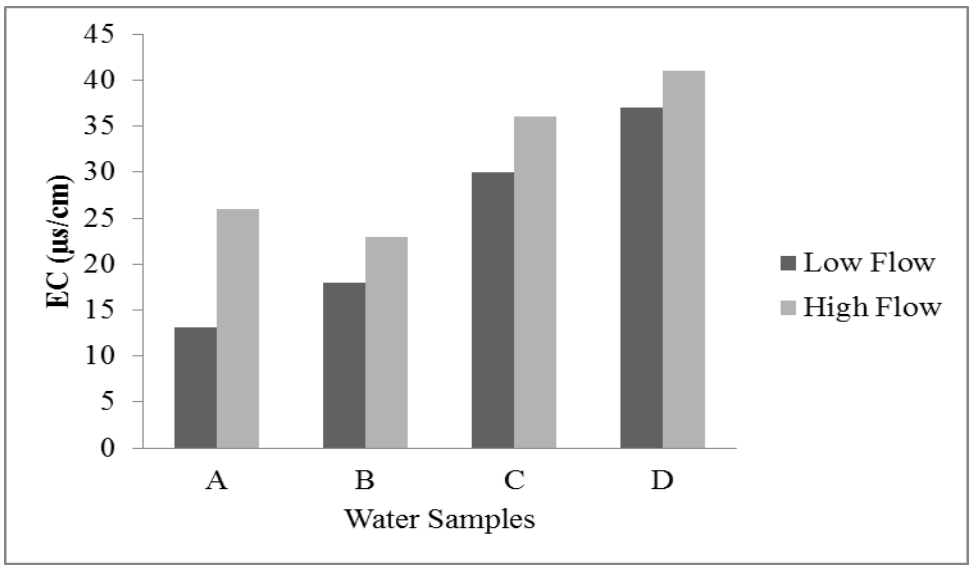
Total suspended solid (TSS)

Fig.3.2: Comparative physico-chemical parameters concentration of pH and total suspended solid (TSS) of water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



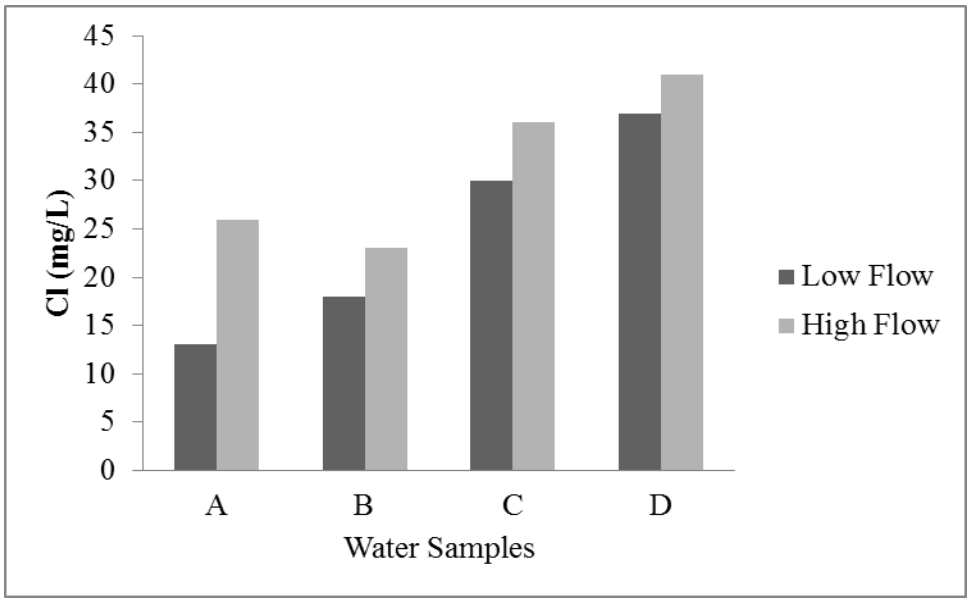
Total dissolved solid (TDS)



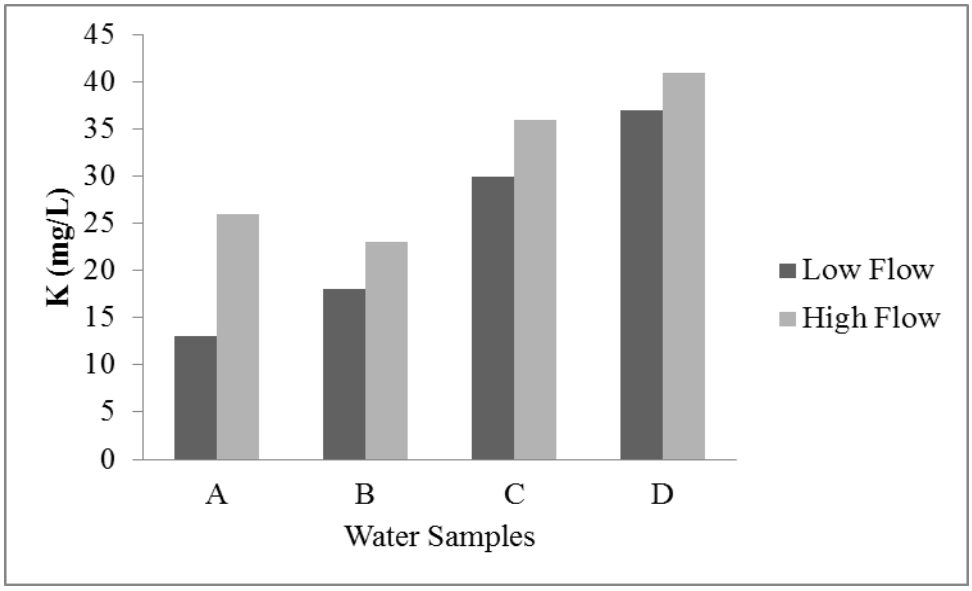
Electrical conductivity (EC)

Fig. 3.3: Comparative physico-chemical parameters concentration of total dissolved solid (TDS) and electrical conductivity (EC) of water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



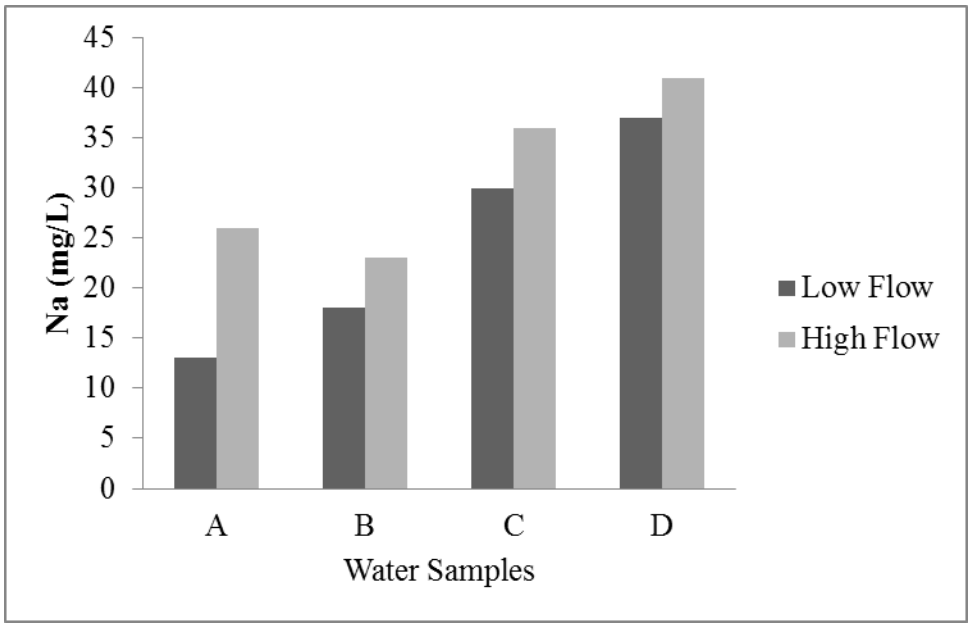
Chloride (Cl)



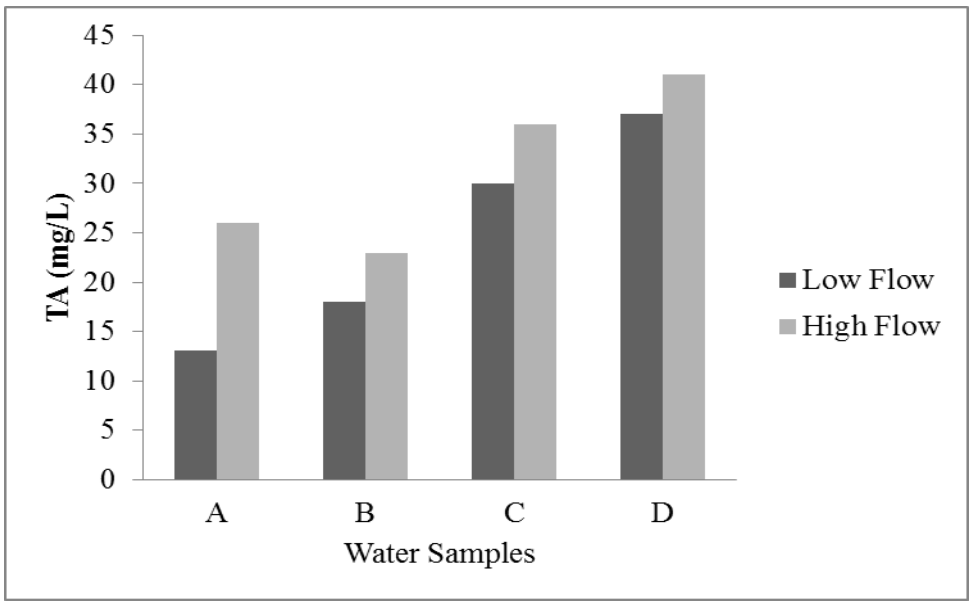
Potassium (K)

Fig.3.4: Comparative Physico-chemical parameters concentration of chloride (Cl) and potassium (K) of water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



Sodium (Na)



Total alkalinity (TA)

Fig.3.5: Comparative Physico-chemical parameters concentration of sodium (Na) and total alkalinity (TA) of water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.

b. Heavy Metals Parameters

Water samples A from control site 3 showed less concentration of heavy metals as compare to polluted sites (Table 3.3 and Figs 3.6-3.10). From amongst the heavy metal parameters, water sample A from Warsak dam had zinc in the range of 20-100 µg/l with mean values of 54.8±52.9 µg/l during low flow and 66.3±69.9 µg/l during high flow seasons. The minimum concentration recorded for nickel was 27 and maximum concentration of 65 µg/l with mean values of 33.3±14.9 µg/l for low flow and 63.3±16.8 µg/l for high flow periods. Chromium content ranged between 16 and 37 µg/l with mean values of 18.8±9.3µg/l for low and 31.3±15.5 µg/l for high flow periods. Copper concentration ranged between 2- 21 µg/l with mean values of 8.0±9.2 µg/l for low flow and 16.7±10.1µg/l for high flow seasons. The present result found greater concentration of Zn, Ni, Cr as compare to previous findings (Khattak and Rehman, 1992; Nawab, 1992; Peerzada *et al*., 1990), who reported low level of these metals from the same site of River Kabul. Similarly lead at this point had minimum range of 1 µg/l and maximum range of 12 µg/l with mean values of 3.7±3.11 µg/l for low and 8.3±6.5 µg/l for high flow seasons. The cadmium had a concentration varying between 15-31 µg/l with mean values of 16.7±6.7 µg/l for low flow and 26.7±12.8 µg/l for high flow periods. This is in agreement with the findings reported by Merian (1991). Iron had a range from 12 to 25µg/l with mean values of 15.0±7.0 µg/l for low flow and 21.0±10.5 µg/l for high flow seasons. Similarly the minimum concentration recorded for manganese was 14 and maximum concentration of 27 µg/l with mean values of 16.7±10.1µg/l for low flow and 23.3±11.5µg/l for high flow periods. Mercury content ranged between 10 and 26 µg/ l with mean values of 13.1±6.9 µg/l for low flow and 26.0±13.1µg/l for high flow periods. Heavy metals in water sample A from this point was in order of Zn>Ni>Cr>Mn>Cd>Fe>Hg>Cu>Pb for low flow and was Zn>Ni>Cr>Cd>Hg>Mn>Fe>Cu>Pb for high flow seasons. These results are in agreement with those observed by many investigators (Nafees and Ghulam, 199; Merian, 1991), who have also studied higher content of metals in water from other

resources. Many studies were previously carried out on the level of heavy metals in water (El-Rafei, 1991; Abdel-Shafy., *et al*, 1995; Khallaf., *et al.*, 1998; Radwan, 2000; Bahnasawy, 2001; Sabae and Abdel-Satar, 2001). In this study the average mean shows that levels of all these parameters were lowest during winter and highest during summer seasons. Highest concentration during summer could be correlated to increased river volume, atmospheric condensation, earth quake, land slides, tornadoes and cyclones in river. Heavy metals except Hg studied in water sample A from Warsak dam were within permissible limits laid down by National Environmental Quality Standards. All the heavy metals except Hg at this point were within permissible limits (10µg/l) lay down by NEQS. The Hg mean values for low flow and high flow periods were 13.1±6.9 µg/l and 26.0±13.1 µg/l, respectively. Therefore Warsak dam water is safe for aquatic life including fish.

Table 3.3: Heavy metals concentration of water sample A from Warsak dam during low (winter) and high (summer) flows during 2012-2014

Parameters	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec 14	L. F (n-6)	H. F (n-3)	NEQS Standards
Zn (µg/l)	24	93	89	20	100	91	60	96	45	54.8±52.9	66.3±69.9	500
Ni (µg/l)	27	60	34	30	65	37	33	65	39	33.3±14.9	63.3±16.8	1000
Cr (µg/l)	17	29	16	19	37	19	24	28	18	18.8±9.3	31.3±15.5	1000
Cu (µg/l)	2	21	13	2	17	12	4	12	15	8.0±9.2	16.7±10.1	1000
Pb (µg/l)	3	6	1	4	12	3	6	7	5	3.7±3.11	8.3±6.5	500
Cd (µg/l)	16	31	15	15	27	18	17	22	19	16.7±6.7	26.7±12.8	100
Fe (µg/l)	13	17	12	15	21	16	17	25	17	15.0±7.0	21.0±10.5	8000
Mn (µg/l)	15	19	14	17	24	16	19	27	19	16.7±10.1	23.3±11.5	1500
Hg (µg/l)	11	21	10	13	31	14	15	26	16	13.1±6.9	26.0±13.1	1018

$P < 0.0517$

1, Mean± Standard deviation

2,Abbrevitions: L.F, Low Flow, H.F, High Flow, Zn, Zinc, Ni, Nickel, Cr, Chromium, Cu, Copper, Pb, Lead, Cd Cadmium, Fe, Iron, Mn, Manganese, Hg, Mercury, NEQS. National Environmental Quality Standards

3.3.1.2 Polluted River Kabul water from site 4 receiving sewages (Water sample-B)

a. Physico-Chemical Parameters

Water samples B from polluted site 4 of River Kabul downstream to Warsak dam and upstream to confluence point was analyzed for different physico-chemical and heavy metal paremeters contents. At this point sewages from Peshawar district and effluents from Khazana sugar mills are discharged into River Kabul (Table 3.4 and Figs. 3.2-3.5).

From amongst the physico-chemical parameters, water sample B from this site had pH in the range of 7.3-8.1 with mean values of 7.9±1.5 during low flow and 7.4±1.4 during high flow periods. Total suspended solid (TSS) concentration was in the range of 467- 687 mg/l with mean value of 497.5±161.5 (mg/l) for low flow period and 601.7±261.7 mg/l for high flow period. The present result of higher TSS content in water sample from this site agree with the findings of Yousazai *et al* (2010), who had also reported high TSS values in water sample from this site of River Kabul. Similarly in a past investigation Khan *et al* (1999) have studied the impacts of industrial effluents on the water quality of River Kabul at Amangarh, Nowshera and analyzed water from this site for various chemical and biochemical parameters. They also have found high level of total suspended solid (TSS) from the same water resources. Total dissolved solid at this point had minimum range of 448 mg/l and a maximum range of 795 mg/l with mean values of 725.8±310.2 mg/l for low flow season and 478.7±139.3 mg/l for high flow season. The present result found low level of pH, TSS and TDS in water samples from this site than reported by Khan and Ullah (1991), who had reported 8.5,1230 mg/l and 2893.5 mg/L values for pH, TSS and TDS from the same water of River Kabul.

The electrical conductivity in water sample B from main river was ranged between 281- 461 µs/cm with mean values of 425.8±129.9µs/cm during low flow period and 284.3±55.0µs/cm during high flow period. The chloride of all the samples

at this point was in the range of 13-27 mg/l with mean values of 25.1±7.6 mg/l for low flow and 15.7±8.4 mg/l for high flow seasons. The potassium content ranged between 2.2 mg/l and 4.8 mg/l with mean values of 4.5±1.5 mg/l during low flow and 2.3±0.71mg/l during high flow periods. In a previous finding Ali (1991) had reported high levels of different parameters including temperature, pH, conductivity, dissolved solid, suspended solid, alkalinity, chloride and nitrate in the water of River Swat.The sodium concentration was between 16- 31 mg/l with mean values of 28.0±8.7 mg/l for low flow and 17.0±4.8 mg/l for high flow seasons. Similarly water from this site had total alkalinity in the range of 101- 262 mg/l with mean values of 185.7±142.9 mg/l for low flow and 104.7±22.5 for high flow seasons. These parameters in water sample from this site was in sequence of TDS > TSS > EC > TA > Na > Cl > pH > K for low flow and was TSS > TDS > EC > TA > Na > Cl > pH > K for high flow seasons. In this study all physico-chemical parameters in water samples from this point were below NEQS recommended values for effluents except TSS, which exceeds the NEQS recommended limit of 150 mg/l for this parameter.

The total suspended solid (TSS) had minimum value of 497.5±161.5 mg/l for low flow and maximum value of 601.7±261.7 mg/l for high flow seasons. Both these values were higher than the value of 150 mg/l proposed by NEQS for this parameter. However TSS mean value of 497.5±161.5 mg/l for low flow was higher than recorded value for the same period (418.8±398.9) for Warsak dam. While TSS mean value (601.7±261.7 mg/l) for high flow period at this point was lower than recorded value for the same period (715.0±138.0) for Warsak dam. All parameters like pH, TDS, EC, Cl, Na, K and TA were having increasing tendency on comparison with Warsak dam water samples. The high level of TSS could be correlated to mining activities, deforestation, natural process of weathering and poor agricultural practice in adjoining hills of River Swat during low flow season, which also joins River Kabul below Warsak dam. The Kabul River would be described by many people as a ‘dirty river’ in that it is very turbid. This is due to high suspended solid load (TSS) carried

by river which range between 10 to 800 mg/l under low flow condition and 340 to 1,310 mg/l under high flow condition (IUCN, 1994). In a previous study Yousazai *et al* (2010a) had also reported high TSS values at this sampling site of the River Kabul exceeded the NEQS value of 150 mg/l for this parameter. This can be correlated to high flooding during high flow due to snow melt on the peaks of surrounding hills, both in Pakistan and Afghanistan and monsoon rains during summer months, excessive deforestation, weathering, soil erosion, mining and other anthropogenic activities along the banks of river. Similarly in a past investigation Khan *et al* (1999) has also analyzed water of River Kabul and found the water sample from this site to be contained high level of total suspended solid.

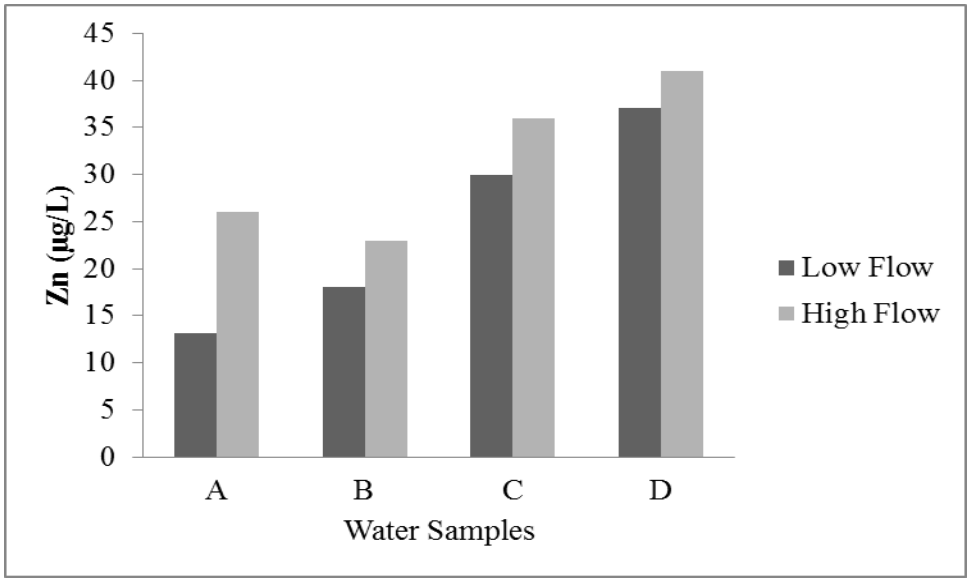
Table 3.4: Physico-chemical characteristics of water sample B from River Kabul upstream to the confluence point during low (winter) and high (summer) flows during 2012-2014

Parametes	L. F Jan. 12	H. F Jun. 12	L.F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
pH	8.0	7.6	8.1	7.8	7.5	8.0	7.8	7.3	7.6	7.9±1.5	7.4±1.4	6-10
TSS	475	601	472	507	687	527	467	517	537	497.5±161.5	601.7±261.7	150
TDS	735	495	785	635	493	615	795	448	790	725.8±310.2	478.7±139.3	3500
EC	435	287	461	395	281	401	422	285	441	425.8±129.9	284.3±55.0	NA
Cl	26	19	23	24	15	25	27	13	26	25.1±7.6	15.7±8.4	1000
K	4.5	2.5	4.8	4.8	2.3	4.3	4.1	2.2	4.8	4.5±1.5	2.3±0.7	NA
Na	29	16	27	27	18	28	31	17	26	28.0±8.7	17.0±4.8	NA
TA	262	107	252	207	101	137	149	106	107	185.7±142.9	104.7±22.5	NA

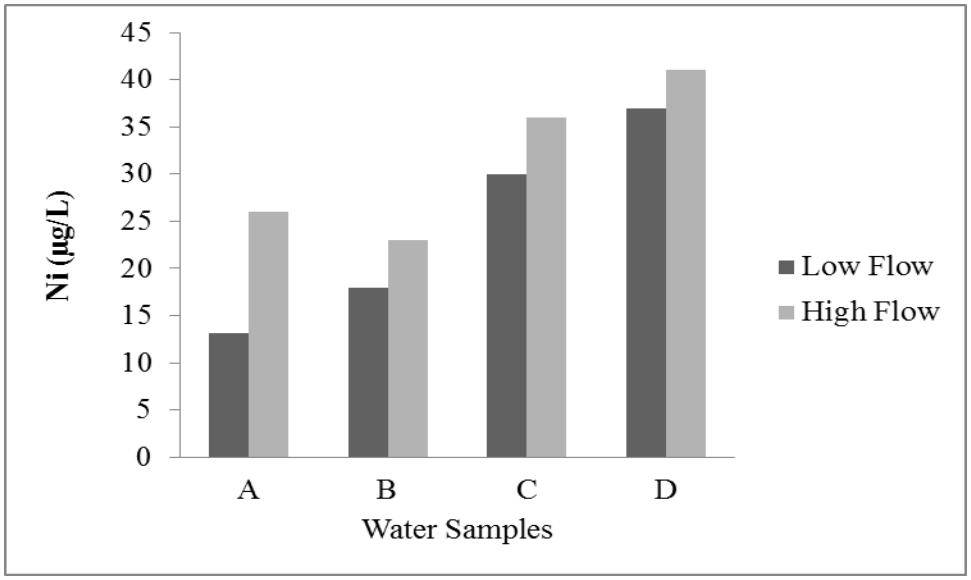
$P < 0.05$

1, Mean± Standard deviation.

2, See table. 3.2 for abbrevitions.



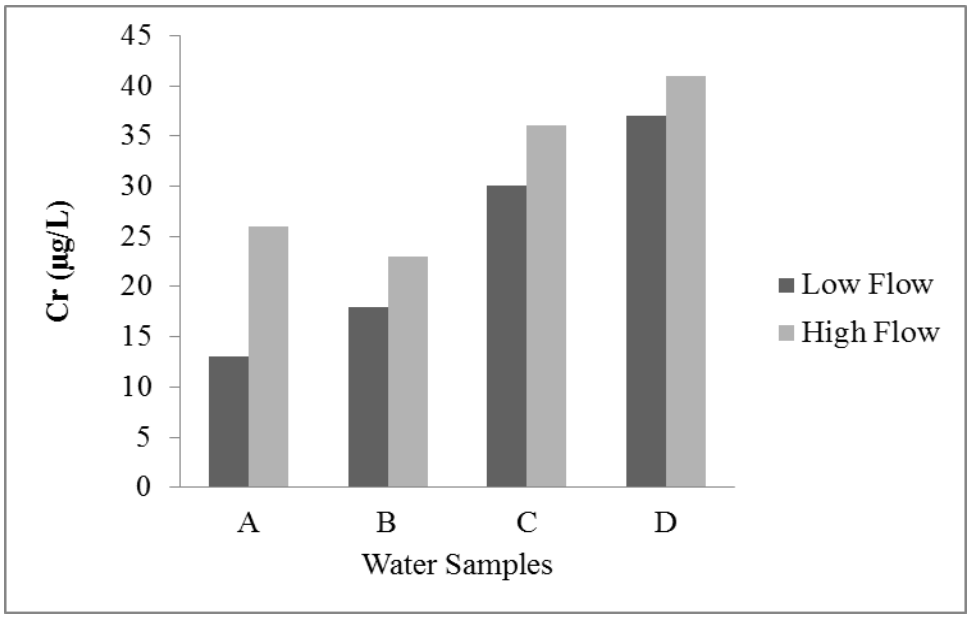
Zinc (Zn)



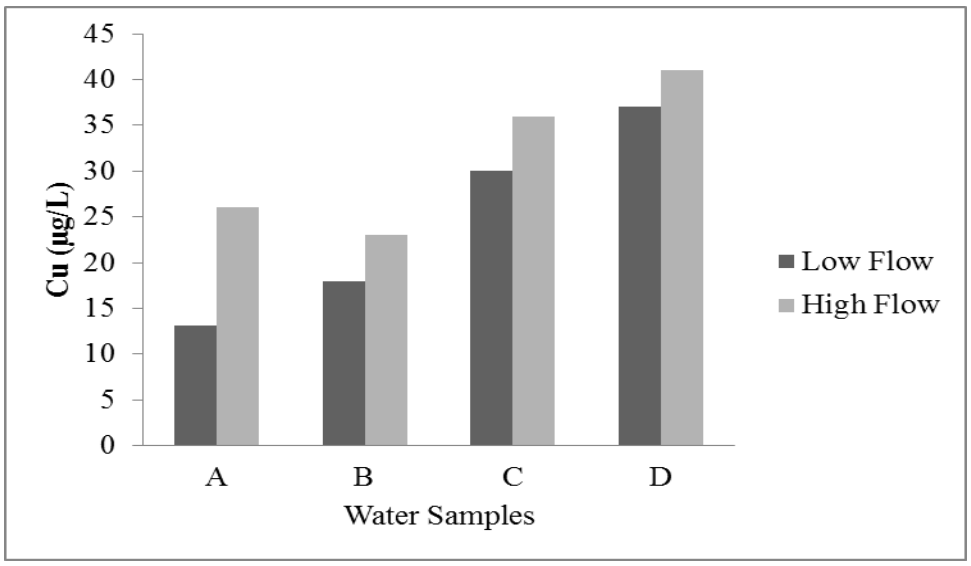
Nickel (Ni)

Fig.3.6: Comparative heavy metals concentration of zinc and nickel in water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



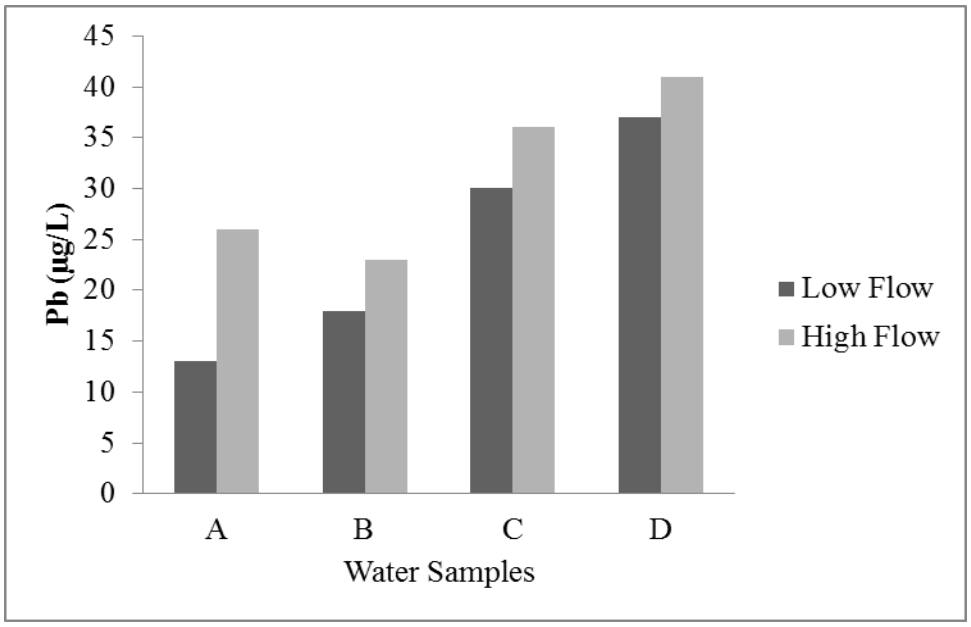
Chromium (Cr)



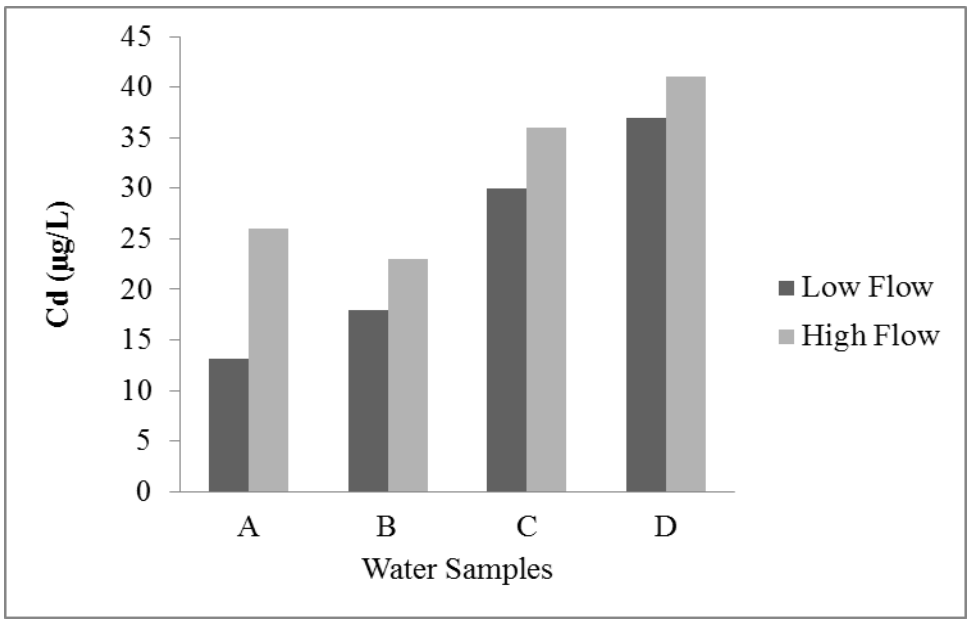
Copper (Cu)

Fig.3.7: Comparative heavy metals concentration of chromium and copper in water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



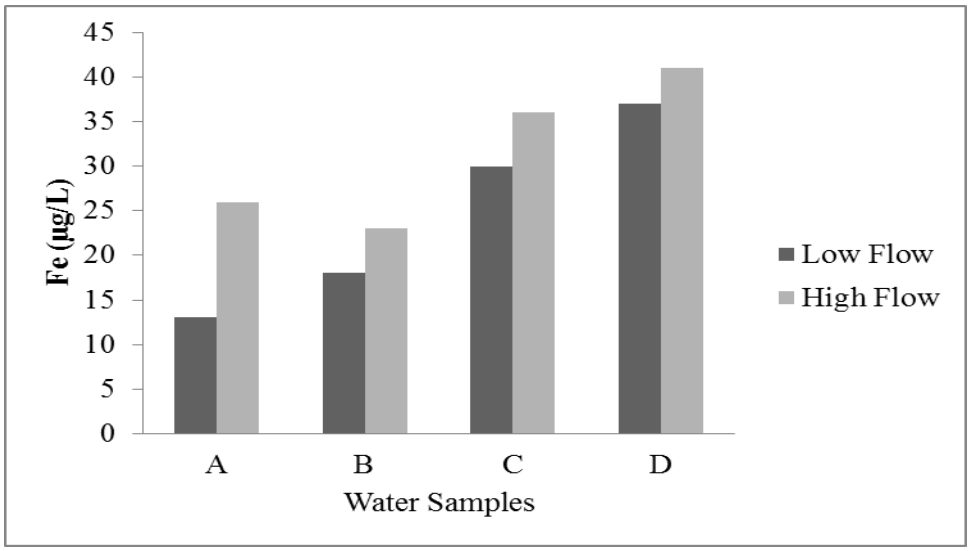
Lead (pb)



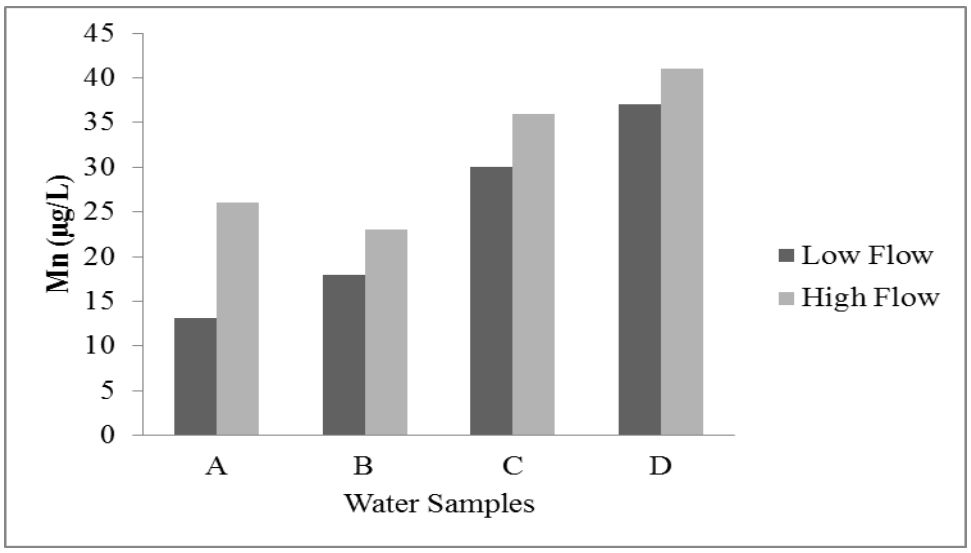
Cadmium (Cd)

Fig.3.8: Comparative heavy metals concentration of lead and cadmium in water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



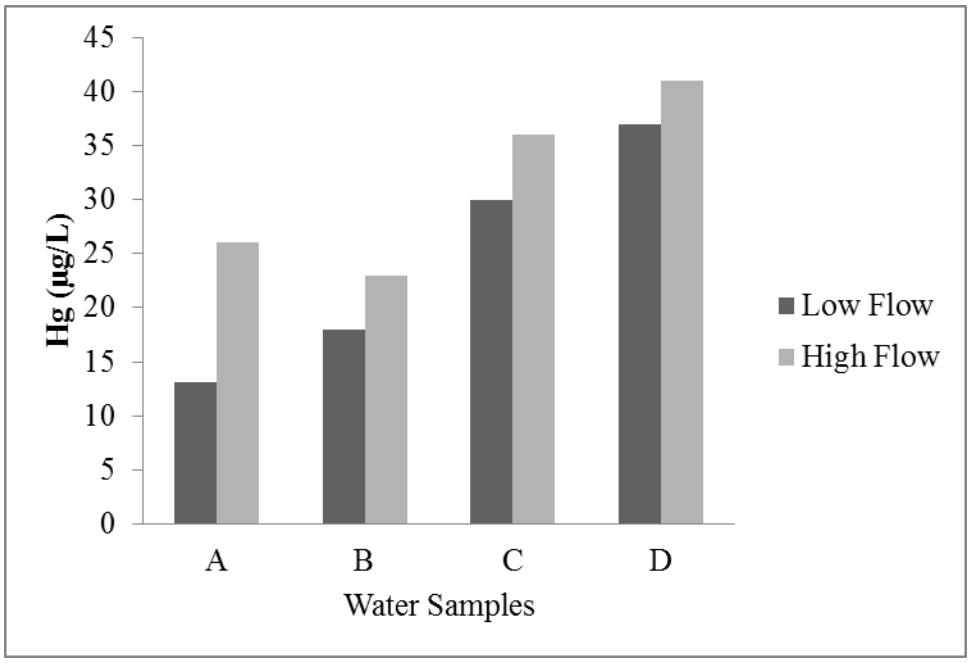
Iron (Fe)



Manganese (Mn)

Fig.3.9: Comparative heavy metals concentration of iron and manganese in water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.



Mercury (Hg)

Fig.3.10: Comparative heavy metals concentration of mercury (Hg) in water samples from Warsak dam and main River Kabul during low and high flows.

A, Warsak dam water sample. B, Upstream water sample. C, River water sample downstream to the confluence point. D, River water sample downstream to sample C and to the point where city sewage joins the river.

b. Heavy Metals Parameters

Heavy metals in water sample B from site 4 showed greater concentration than water sample A. Water sample B from this site had zinc concentration ranged between 202-227 µg/l with mean values of 211.5±45.9 µg/l for low flow and 221.3±40.9 µg/l for high flow periods (Tables 3.5 and Figs 3.6-3.10). The minimum concentration recorded for nickel was 51µg/l and maximum concentration of 61µg/l with mean values of 55.5±15.4 µg/l for low flow and 59.7±11.4 µg/l for high flow periods. The chromium of all the water samples at this site was in the range of 16-43 µg/l with mean values of 18.7±8.0 µg/l for low flow and 41.0±10.4 µg/l for high flow seasons. The copper content ranged between 13 µg/l and 28 µg/l with mean values of 21.0±15.3 µg/l during low flow and 20.3±7.6 µg/l during high flow periods. In this investigation values for Zn, Ni, Cr, Cu and Cd were higher and Fe, Mn and Pb and Hg were lower as compare to values mentioned by Shahina (2001). Similarly lead at this point had minimum concentration of 62 and maximum concentration of 169 µg/l with mean values of 65.8±16.9 µg/l during low flow and 164.3±31.9 µg/l during high flow seasons. The cadmium concentration was 49-71 µg/l with mean values 55.0±15.9 µg/l during low flow and 70.3±11.2 µg/l during high flow seasons. In a past study Nawab (1992) had studied water of the River Kabul for different heavy metals like Cd, Cr, Ca, Pb, Fe, Mn and Zn. The analysis of these effluents revealed high concentration of Cd, Cu, Fe. Iron had a range from 28-49 µg/l with mean values of 30.5±10.1µg/l for low flow and 47.0±11.2 µg/l for high flow seasons. Similarly minimum concentration recorded for manganese was 29 and maximum concentration of 51 µg/l with mean values of 35.0±12.7 µg/l for low flow and 50.0±8.1µg/l for high flow periods.

The mercury content ranged between 16 and 24 µg/l with mean values of 18.5±6.5 µg/l for low flow and 23.3±6.4 µg/l for high flow periods respectively. These results are in agreement with the findings of Nawab (1992) and Zouboulis *et al* (2004). The sequence of these parameters in water sample B was Zn> Pb> Ni> Cd> Mn> Fe> Cu> Cr >Hg during low flow and was Zn > Pb > Cd > Ni > Mn > Fe > Cr >

Hg > Cu for high flow seasons respectively. Comparing our study with the findings of above researchers showed that heavy metal and physico-chemical parameters concentrations are the main pollutants for the River Kabul and levels of these parameters have increased in River Kabul in the last few years and both heavy metal and physico-chemical parameters also showed increasing tendency in water samples from this point as compare to those with Warsak dam. The increasing in level of heavy metal and physico-chemical parameters could be attributed to effluents from factories and mills and sewages from Peshawar city. All the nine heavy metals like Zn, Ni, Cr Cu, Cd, Pb, Fe, Mn and Hg studied in the water sample B from River Kabul showed increasing tendency on comparison with water samples from Warsak dam. All heavy metals except Hg at this point were within permissible limits laid down by NEQS. The Hg mean values for low and high flow periods were 18.5±6.5 µg/l and 23.3±6.4 µg/l respectively.

As again all physico-chemical and heavy metal parameters except TSS had values within the permissible range proposed by NEQS, therefore water quality at this point seem fit for aquatic life. However all the parameters were showing increasing tendency when compared to Warsak dam water samples. This water sample point was 35 km down stream from the Warsak dam and 20 km upstream from the confluence point at Amangarh Nowshera. On down stream journey from Warsak dam, the river passes through a number of villages and the city of Peshawar. Therefore city sewage and effluents from many factories and mills and other installations in the vicinity of River Kabul tributaries also joins the river. Moreover dirty water of River Bara (IUCN, 1994) also joins the river on the downstream. Therefore it is obvious that water quality of the river would be deteriorated down stream.

Table 3.5 Heavy metals concentration of water sample B from River Kabul upstream to the confluence point during low (winter) and high (summer) flows during 2012-2014

Parametes	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
Zn	213	217	202	216	227	218	214	220	206	211.5±45.9	221.3±40.9	500
Ni	54	58	56	51	60	55	59	61	58	55.5±15.4	59.7±11.4	1000
Cr	19	39	21	16	41	20	20	43	16	18.7±8.0	41.0±10.4	1000
Cu	13	21	26	17	19	28	16	21	26	21.0±15.3	20.3±7.6	1000
Pb	62	169	66	65	164	68	64	160	70	65.8±16.9	164.3±31.9	500
Cd	49	71	54	57	69	55	59	71	56	55.0±15.9	70.3±11.2	100
Fe	31	45	33	28	47	31	32	49	28	30.5±10.1	47.0±11.2	8000
Mn	29	51	34	37	49	35	39	50	36	35.0±12.7	50.0±8.1	1500
Hg	16	24	18	20	22	20	19	24	18	18.5±6.5	23.3±6.4	10

$P < 0.05$

1, Mean± Standard deviation.

2, See table 3.3 for abbrevitions.

3.3.1.3 Polluted River Kabul water from site 1 receiving effluents (Water sample C)

a. Physico-Chemical Parameters

Water samples C from polluted site 1 down stream to the site B were taken and studied for various physico-chemical and heavy metal parameters as already mentioned to determine the extent of pollution in the River Kabul at this point. This was the site from where fish sample 1 was collected. Comparing the data from Warsak dam (control) shows that a considerable increase in pollution has occurred. Most of the parameters have increased showing signs of increased localized pollution in the river, most probably caused by effluents (Table 3.6 and Figs. 3.2-3.5).

The water sample C from this site of River Kabul had a pH ranged between 7.2 and 7.6 with mean values of 7.4±1.0 during low flow and 7.3±1.0 during high flow seasons respectively. Total suspended solid (TSS) concentration was in the range of 492- 912 mg/l with mean values of 549.7±228.7 mg/l for low flow and 881.3±409.0 mg/l for high flow periods. Total dissolved solid (TDS) of all water samples C was in the range of 530 - 965 mg/l with mean values of 830.0±482.4 mg/l and 567.3±168.8 mg/l for both low and high flow seasons. The electrical conductivity in water sample from Warsak dam was ranged between 235 and 493µs/cm with mean values of 473.7±102.0µs/cm during low flow and 245.3±69.4µs/cm during high flow periods. The present results of higher pH, TSS, TDS contents in water samples from this site agree with the findings of Adeogun (2012). On the other hand, the present data for K, total alkalinity, Na, Cl and electrical conductivity agree with those of Subramanian (2004) and Wahid and Muhammad (1992). In this study chloride concentration ranged between 23-35.6 mg/l with mean values of 32.1±12.4 mg/l for low flow and 25.0±8.1 mg/l for high flow seasons. The potassium (K) range was 5.2 - 8.8 mg/l with mean values of 8.2±2.2 mg/l during low flow and 5.8±2.2 mg/l during high flow periods. The sodium ranged between 13- 47 mg/l with mean values of

36.3±19.2 for low flow and 16.0±7.9 mg/l for high flow seasons. Similarly water sample from this site had total alkalinity in the range of 120- 271mg/l with mean values of 207.8±112.7 mg/l for low flow and 122.3±24.4 mg/l for high flow periods. These parameters in the water sample from this site were in the sequence of TDS>TSS>EC>TA>Na>Cl>K>pH for low flow and were TSS>TDS>EC>TA>Cl> Na>pH>K for high flow seasons respectively. This study found more values for TDS, TSS, EC, TA, Na, Cl, K and pH as compare to the previous investigations (Akif *et al*., 2002; Khan and Mumtaz, 1997; Khan *et al*., 1999). Previously Yousafzai (2004) had also reported high mean values for pH, TDS, EC, Cl, K, Na and TA from the same water resource of River Kabul that verifying the validity of our study. Comparing our data with the finding of Yousafzai (2004) and other mentioned studies indicates that all studied physico-chemical parameters, when compared with Warsak dam (control) are high in concentration and showing an increasing tendency in river at this point.

All the physico-chemical parameters were below the permissible limits proposed by NEQS except TSS, which exceeds the NEQS recommended limit of 150 mg/l for this parameter. TSS from this site had a minimum value of 549.7±228.7 mg/l during winter and maximum value of 881.3±409.0 mg/l during summer seasons. Both these values were higher than the value of 150 mg/l proposed by NEQS for this parameter. These two values 549.7±228.7 mg/l and 881.3±409.0 mg/l for both lower and higher flows were higher than the recorded values for the same periods (418.8±398.9, 715.0±138.0) from Warsak dam. The TSS value was highest for high flow and lowest for low seasons. This could be correlated to sewage, industrial effluents, flooding, soil erosion etc in the river at this site. All the physico-chemical parameters showed a considerable increase during low and high flow seasons in relation to Warsak dam (control). Rest of the parameters were within the NEQS range. A decrease in the pH level as compare to water sample A from Warsak dam and water sample B from the main river upstream was also observed showing deterioration of the river quality downstream. Water sample C possessed all the

parameters higher than the water sample A from Warsak dam and water sample B upstream from the main river.

Table 3.6 Physico-chemical characteristics of water sample C from River Kabul downstream to the confluence point during low (winter) and high (summer) flows during 2012-2014.

Parametes	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
pH	7.4	7.3	7.4	7.6	7.2	7.4	7.4	7.4	7.6	7.4±1.0	7.3±1.0	6-10
TSS	510	737	492	631	912	590	570	995	505	549.7±228.7	881.3±409.0	150
TDS	775	605	830	735	530	780	895	567	965	830.0±482.4	567.3±168.8	3500
EC	478	260	493	476	241	483	453	235	468	473.7±102.0	245.3±69.4	NA
Cl	34	23	27	32.6	27	34	35.3	25	30	32.1±12.4	25.0±8.1	1000
K	8.4	6.3	8.8	8.1	5.2	8.3	8.5	6.0	7.6	8.2±2.2	5.8±2.2	NA
Na	34	17	38	29	13	39	31	18	47	36.3±19.2	16.0±7.9	NA
TA	271	121	234	206	126	190	175	120	171	207.8±112.7	122.3±24.4	NA

$P < 0.05$

1, Mean± Standard deviation.

2**,** See table 3.2 for abbrevitions.

b. Heavy Metals Parameters

From amongst the heavy metals water sample C from site 1 had zinc concentration ranged between 218-234 µg/l with mean values of 226.1±39.5 µg/l for low flow and 230.3±61.4 µg/l for high flow periods (Table 3.7 and Figs 3.6-3.10). The minimum concentration recorded for nickel was 45 µg/l and maximum concentration of 49 µg/l with mean values of 46.3±10.1µg/l for low flow and 47.7±10.2 µg/l for high flow seasons. The chromium of all the water samples at this site was in the range of 16-44 µg/l with mean values of 23.0±14.5 µg/l and 43.3±8.8 µg/l for both low and high flow seasons. Copper content ranged between 15 and 32 µg/l with mean values of 23.8±17.6 µg/l during low flow and 26.0±8.3 µg/l during high flow periods. Similarly lead at this point had minimum range of 67 µg/l and maximum range of 181 µg/l with mean values of 70.8±17.5 µg/ l during low flow and 176.3±33.0 µg/l during high flow seasons. Cadmium concentration was ranged between 53-76 µg/l with mean values of 57.7±13.2 µg/l during low flow and 74.0±45.0 µg/ l during high flow seasons. The iron of all the water samples at this site was in the range of 35-61 µg/l with mean values of 40.8±15.2 µg/l and 60.0±8.9 µg/l for low and high flows seasons. These results are in agreement with previous findings as reported by (Anon, 1976; El-Ezaby, 1994; IUCN, 1994; Khan *et al*., 2011). Manganese had a range varying between 34-57 µg/l with mean values of 38.5±9.1 µg/l during winter and 55.0±12.1 µg/l during summer respectively. Mercury was in the range between 27-41 µg/l with mean values of 30.8±11.5µg/l for low flow and 36.3±15.0 µg/l for high flow periods. All the heavy metals except Hg at this point were within permissible limits laid down by NEQS.

The Hg mean values for low flow and high flow seasons were 30.8±11.5 µg/ l and 36.3±15.0 µg/ l respectively and showed an increasing tendency at this point than the Warsak dam water. The heavy metal parameters in water sample C from this site were in the order of Zn>Pb>Cd>Ni>Fe>Mn>Hg>Cu>Cr during low flow and were

Zn>Pb>Cd>Fe> Mn> Ni>Cr>Hg>Cu for high flow seasons respectively. In a previous finding Amal *et al* (2012) have investigated that pollution due to heavy metals of the aquatic environment is a serious and growing problem throughout the world. Similarly in another finding Enrique *et al* (2007) and Ping *et al* (2006) have reported that increasing number and amount of industrial, agricultural and commercial chemicals discharged into aquatic environment have led to various deleterious effects on aquatic organisms, including fish. A general comparison is made between the different types of discharge, rivers having been included within the streams and drains category. The increased load of heavy metals in Kabul River has adverse effects on flora and fauna.

All heavy metals like Zn, Ni, Cr Cu, Cd, Pb, Fe, Mn and Hg studied in the water sample C from River Kabul showed increasing tendency on comparison with water samples from Warsak dam and water sample B. All heavy metals except Hg at this point were within permissible limits laid down by NEQS. The Hg mean values for low flow and high flow periods were 30.8±11.5 µg/ l and 36.3±15.0 µg/ l respectively. All the studied heavy metals when compared with Warsak dam (control) are high in concentration and showing increasing tendency due to dumping of industrial effluents in river at this point. Zinc from this site showed high increase due to various zinc using industries present in the vicinity of River Kabul. The heavy metals contents at this point of the river were higher than the Warsak dam samples and main River upstream to the confluence point samples, showing increasing tendency due to dumping of industrial effluents into River Kabul (Table 3.7 and 3.5-3.9).

Table 3.7 Heavy metals concentration of water sample C from River Kabul downstream to the confluence point during low (winter) and high (summer) flows during 2012-2014

Parameters	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
Zn	231	239	222	229	234	227	220	218	228	226.1±39.5	230.3±61.4	500
Ni	48	49	46	45	48	46	48	48	45	46.3±10.1	47.7±10.2	1000
Cr	20	42	22	16	44	21	24	44	35	23.0±14.5	43.3±8.8	1000
Cu	15	26	29	19	24	32	18	28	30	23.8±17.6	26.0±8.3	1000
Pb	67	181	71	70	176	73	69	172	75	70.8±17.5	176.3±33.0	500
Cd	53	76	58	58	72	59	58	74	60	57.7±13.2	74.0±45.0	100
Fe	39	59	41	35	61	40	43	60	47	40.8±15.2	60.0±8.9	8000
Mn	34	57	38	39	53	40	39	55	41	38.5±9.1	55.0±12.1	1500
Hg	27	41	31	30	36	33	29	32	35	30.8±11.5	36.3±15.0	10

$P < 0.05$

1, Mean± Standard deviation.

2, See table 3.3 for abbrevitions.

3.3.1.4 Polluted River Kabul water from site 2 receiving city sewage (Water sample D)

a. Physico-Chemical Parameters

Water sample D from site 2 down stream to site 1 was taken and studied for various physico-chemical and heavy metal parameters as already mentioned. This was the site from which fish samples 2 were collected. At this point Kalpani nalla from Mardan district (IUCN, 1994) joins the River Kabul on opposite side. The quantity of water down stream of this point is considerably deteriorated (IUCN, 1994, Khan *et al.,* 1999). All physico-chemical parameters were below the permissible limits proposed by NEQS except TSS, which exceeds the NEQS recommended limit of 150 mg/l for this parameter. Water samples from this site were analyzed for different physico-chemical and heavy metal parameters to quantify the extent of pollution at this point (Table 3.8 and Figs 3.2-3.5).

Some physico-chemical parameters are components of industrial waste products, which are discharged into the River Kabul along with other industrial effluents and caused aquatic pollution. All physico-chemical parameters showed a considerable increase during low and high flow periods in relation to Warsak dam (control). The water sample D from this point had a pH in the range of 7.2-7.8 with mean values of 7.5±2.0 during low flow and 7.4±1.4 during high flow seasons. In a past study Nafees and Ghulam (1991) and IUCN (1994) have also reported higher concentration for pH in water of River Kabul above than the recommended standard for industrial effluents. Total suspended solid (TSS) was in the range of 491-989 mg/l with mean values of 543.8±189.3 mg/l for low flow period and 962.3±209.6 mg/l for high flow period. The TSS value was highest for high flow and lowest for low flow seasons. This could be correlated to sewage, industrial effluents and flooding and soil erosion in the river. In another investigation Ali (1991) had also reported a high TSS level in the water samples from River Swat. Similarly total dissolved solid (TDS) at

this point had minimum range of 565 mg/l and a maximum range of 912 mg/l with mean values of 793.1±353.4 mg/l during low flow and 615.0±201.4 mg/l during high flow seasons. The electrical conductivity in water sample D was ranged between 208-473 µs/cm with mean values of 453.0±125.1 µs/cm during low flow period and 229.7±81.1 µs/cm during high flow period. The chloride concentration of all the samples at this point was in the range of 29-45 mg/l with mean values of 39.0±14.4 mg/l for low flow and 30.7±8.2 mg/l for high flow seasons. The potassium content ranged between 21 mg/l and 40 mg/l with mean values of 75.5±65.5 mg/l during low flow and 22.3±7.0 1mg/l during high flow periods. These values were greater than the findings of other workers from the same water resources (South African Water Quality Guidelines., 1996; Akif*et al*., 2002; Khan and Mumtaz, 1997; Khan *et al*., 1999).

The sodium concentration was ranged between 16 and 31 mg/l with mean values of 28.0±8.7 mg/l for low and 17.0±4.8 mg/l for high flow seasons. Similarly the water samples from this site had total alkalinity in the range of 199-310 mg/l with mean values of 302.1±54.2 mg/l for low flow and 205.3±42.7 mg/l for high flow periods. These parameters in water sample D from this site were in the order of TDS > TSS > EC > TA > Na > Cl > K > pH for low flow and were TSS > TDS > EC > TA > Cl > Na > pH >K for high flow seasons respectively. In a past finding Yousafzai (2004) had also reported greater mean values for pH, total dissolved solid, electrical conductivity, chloride, potassium, sodium and total alkalinity from the same water resource of River Kabul verifying the validity of our study. All physico-chemical parameters in water sample D had higher concentration than the water samples A, B and C. The TSS had mean values of 543.8±189.3 mg/l for low flow and 962.3±209.6 mg/l for high flow seasons and these values were greater than the NEQS recommended limit of 150 mg/l.

Table 3.8 Physico-chemical characteristics of water sample D, downstream to C during low (winter) and high (summer) flows during 2012-2014.

Parameters	L. F Jan. 12	H. F Jun. 12	L. F Dec. 12	L. F Jan. 13	H. F Jun. 13	L. F Dec. 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
pH	7.3	7.6	7.8	7.4	7.4	7.7	7.6	7.2	7.5	7.5±2.0	7.4±1.4	6-10
TSS	575	70	500	556	928	600	491	989	541	543.8±189.3	962.3±209.6	150
TDS	810	640	865	770	565	831	571	640	912	793.1±353.4	615.0±201.4	3500
EC	463	248	458	461	233	473	400	208	463	453.0±125.1	229.7±81.1	NA
Cl	45	29	34	38	32	40	40	31	37	39.0±14.4	30.7±8.2	1000
K	9	6.1	9.3	9.2	5	9	9.6	6	9.4	9.2±1.8	5.7±2.2	NA
Na	37	22	40	40	24	37	36	21	35	75.5±65.5	22.3±7.0	NA
TA	310	205	301	300	212	293	309	199	300	302.1±54.2	205.3±42.7	NA

$P < 0.05$

1, Mean± Standard deviation.

2, See table 3.2 for abbrevitions.

b. Heavy Metals Parameters

All heavy metal parameters in water from this site 2 showed a considerable increasing tendency during low and high flow seasons in relation to control water from Warsak dam (Table 3.9 and Figs 3.6-3.10).

Among the heavy metal parameters, water sample D from this site had zinc in the range of 240- 258 µg/l with mean values of 247.7±36.4µg/l during low flow and 254.7±35.1 µg/l during high flow seasons. The minimum concentration recorded for nickel was 54 and maximum concentration of 87 µg/l with mean values of 60.0±24.2 µg/l for low flow and 84.7±16.3 µg/ l for high flow periods. The present results are in agreement with those observed by many investigators (DWAF, 1996; Salomons and Forstner, 1984), who have also studied high levels of heavy metals in water from other resources. The chromium content ranged between 17 and 37 µg/l with mean values of 29.8±20.0 µg/ l for low flow and 29.0±8.8 µg/ l for high flow periods. The copper concentration ranged varying between 17- 37 µg/l with mean values of 29.8±20.0 µg/l for low flow and 29.0±8.8 µg/ l for high flow seasons. Similarly lead at this point had minimum concentration of 71 µg/l and maximum content of 190 µg/l with mean values of 77.7±22.7 µg/l for low flow and 186.7±30.1 µg/l for high flow seasons.

The cadmium of all the samples from this site had a concentration varying between 60-84 µg/l with mean values of 65.0±14.6 µg/l for low flow and 81.3±19.0 µg/l for high flow periods. Iron had a range from 43-63 µg/l with mean values of 52.7±25.2 µg/l for low flow and 54.3±28.3 µg/l for high flow seasons. Similarly minimum concentration recorded for manganese was 39 and maximum concentration of 47µg/l with mean value of 43.7±12.7µg/l for low flow and 42.7±9.7µg/ l for high flow periods. The mercury content ranged between 34µg/l and 45µg/l with mean values of 37.3±12.5 µg/l for low flow and 41.0±14.8 µg/l for high flow periods respectively. Heavy metal parameters in water sample D from this site were in the

order of Zn>Pb>Cd>Ni>Fe>Mn>Hg>Cu>Cr during low flow and were Zn>Pb>Cd>Ni>Cd> Fe>Cr>Mn>Hg>Cu for high flow periods respectively. All heavy metals except Hg at this point were within permissible limits laid down by NEQS. The Hg mean contents for low and high flow seasons were 37.3±12.5 µg/ l and 41.0±14.8 µg/ l respectively and show an increasing tendency at this point than Warsak dam water. This study found maximum level of examined metals from this point as compare to the previous findings reported by IUCN (1994) and Merian (1991).

Heavy metals at this site showed higher concentration as compare to Warsak dam and upstream of water samples B and C and indicating increasing heavy metal pollution in down stream water. Heavy metals except Hg determined at this point were within permissible limits laid down by National Environmental Quality Standards (NEQS). But still alarming due to their bioaccumulation capability especially during low flow season when water volume shrinks. Over all result showed that industries in the vicinity are dumping effluents containing high level of TSS and Hg into the River Kabul. Comparing heavy metal contents to Warsak dam water (control) again showed a drastic increase in concentration of physico-chemical parameters both during winter and summer seasons. Similarly a further increase had also occurred in heavy metals concentration as compare to water sample C. As this sample was collected from the portion of the river, where city sewage also joins the river a little upstream, most probably this city sewage may be further increasing the heavy metal concentration and physico-chemical characteristics. The overall results show that industries in the vicinity discharging effluents and sewages containing high TSS and Hg into River Kabul especially at sites C and D. Thus both sewages and effluents causing both organic and inorganic pollution in River Kabul. The TSS and Hg in upstream from Warsak dam (water sample A) and downstream from main river (water sample B), from polluted site 1(water sample C) and polluted site 2 (water sample D) exceeds the permissible limits proposed by NEQS during both low and

high flow periods. Both physico- chemical and heavy metal parameters in downstream river water samples also showed an increasing tendency when compared with upstream samples showing both physico-chemical and heavy metal stress in down stream portion of River Kabul.

This extensive study confirmed that River Kabul has higher pollution down stream the Warsak dam and there is a localized pollution in the vicinity of Peshawar and Nowshera. Similar reports regarding River Kabul pollution have also been reported in the past findings (Akif *et al.*, 2002, IUCN, 1994, Karns, 1977, Yousafzai, 2004). Similarly in another investigation, Yılmaz *et al* (1998) reported that chromium and lead concentrations in Nilufer Stream were well above the standard limits given for the heavily polluted class of water. This pollution plug might be preventing the fish from their migration into River Indus, which definitely play a vital role in reduction in whole fish population, which are considered as clean water lover and breed in clean water. Moreover this pollution will be lethal to eggs and juvenils as compare to adults and there is the sure danger of loss of fish seed and grown up fish.

Table 3.9 Heavy metals concentration of water sample D, downstream to C during low (winter) and high (summer) flows during 2012-2014.

Paramets	L. F Jan. 12	H. F Jun. 12	L. F Dec 12	L. F Jan. 13	H. F Jun. 13	L. F Dec 13	L. F Jan. 14	H. F Jun. 14	L. F Dec. 14	L. F (n-6)	H. F (n-3)	NEQS Standards
Zn	251	258	250	248	255	240	250	251	247	247.7±36.4	254.7±35.1	500
Ni	65	84	54	69	83	54	61	87	57	60.0±24.2	84.7±16.3	1000
Cr	23	51	27	18	49	27	16	43	31	23.7±14.8	47.7±17.1	1000
Cu	17	29	36	26	27	37	26	31	37	29.8±20.0	29.0±8.8	1000
Pb	71	190	79	76	187	81	76	183	83	77.7±22.7	186.7±30.1	500
Cd	60	82	65	65	78	67	65	84	68	65.0±14.6	81.3±19.0	100
Fe	49	61	53	44	59	60	47	43	63	52.7±25.2	54.3±28.3	8000
Mn	39	44	44	43	41	46	43	43	47	43.7±12.7	42.7±9.7	1500
Hg	34	45	37	36	41	39	36	37	42	37.3±12.5	41.0±14.8	10

$P < 0.05$

1, Mean± Standard deviation.

2, See table 3.3 for abbreviations.

3.3.1.5 Conclusions and Remarks

The following tables 3.10 and 3.11 summarize physico-chemical and heavy metal parameters of different water samples collected from Warsak dam (water sample A), polluted River Kabul (water samples B, C and D) during low flow (winter) and high flow (summer).

Overall results show that industries in the vicinity are discharging effluents containing high level of TSS and Hg. Level of these parameter was exceeding the permissible limits laid down by NEQS for effluents but the remaining parameters were within permissible limits provided by NEQS. The down stream water samples B, C and D from polluted sites 3, 1 and 2 showed an increasing tendency of both physico-chemical and heavy metal parameters as when compared with water samples A showing metal stress in down stream portions of river. During low flow period in winter due to less snow melt at the top of hills, the river water volume reduces about three times, but the sewage and effluent contents remain the same. During high flow in summer, snow melts at the hills and results in flooding and hence increases the river volume, which dilutes the pollutants in the river in these months, except TSS, which exceeded the NEQS limits even in high flow months. Among heavy metals except Hg studied in the all water samples A, B, C and D were within permissible limits laid down by NEQS. The high TSS and Hg content reduces light penetration in deeper strata of river water and thus results in less growth of plankton and algae, which naturally will reduce food and consequently reduce the fish yield. Deforestation and mining activities on the hills resulted in soil erosion and hence increases TSS concentration in river water. Thus different water samples were in the trend of D> C > B > A. This shows that water sample D had highest physico-chemical and heavy metal parameters followed by water sample C, B and water sample A had lowest parameters.

The highest parameters in water sample D may be attributed to effluents and sewages discharging into River Kabul at this site. Water sample D has received effluents and sewages from Mardan and Nowshera cities and also received effluents from upstream industries of Amanghar. Therefore here at site D the pollution is high as compare to remaining studied sites of River Kabul. All investigated metals including Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg and physico-chemical parameters like TDS, TSS, EC, TA, Na, Cl, K and pH in water samples from polluted water showed increasing tendency as compare with control water from Warsak dam. The possible reasons for this tremendous increase in individual metal and physico-chemical parameter level in water of River Kabul could be correlated to mining activities in surrounding hills, agricultural activities, city sewage, industrial effluents and other anthropogenic activities.

Table 3.10 Physico-chemical and heavy metal contents of water sample-A from Warsak dam (control), water sample-B from River Kabul upstream to the confluence point, water sample C from River Kabul downstream to the confluence point and water sample-D from River Kabul downstream of C during low flow (winter) 2012-2014.

Characteristics	Parameters	Water sample A	Water sample B	Water sample C	Water sample D
Physico-chemical properties	pH	7.3±1.7	7.9±1.5	7.4±1.0	7.5±2.0
	TSS	418.8±398.9	497.5±161.5	549.7±228.7	543.8±189.3
	TDS	581.1±255.9	725.8±310.2	830.0±482.4	793.1±353.4
	EC	343.1±111.7	425.8±129.9	473.7±102.0	453.0±125.1
	Cl	12.8±6.2	25.1±7.6	32.1±12.4	39.0±14.4
	K	1.1±0.7	4.5±1.5	8.2±2.2	9.2±1.8
	Na	10.8±8.5	28.0±8.7	36.3±19.2	75.5±65.5
	TA	108.5±55.0	185.7±142.9	207.8±112.7	302.1±54.2
Heavy metals	Zn	54.8±52.9	211.5±45.9	226.1±39.5	247.7±36.4
	Ni	33.3±14.9	55.5±15.4	46.3±10.1	60.0±24.2
	Cr	18.8±9.3	18.7±8.0	23.0±14.5	23.7±14.8
	Cu	8.0±9.2	21.0±15.3	23.8±17.6	29.8±20.0
	Pb	3.7±3.11	65.8±16.9	70.8±17.5	77.7±22.7
	Cd	16.7±6.7	55.0±15.9	57.7±13.2	65.0±14.6
	Fe	15.0±7.0	30.5±10.1	40.8±15.2	52.7±25.2
	Mn	16.7±10.1	35.0±12.7	38.5±9.1	43.7±12.7
	Hg	13.1±6.9	18.5±6.5	30.8±11.5	37.3±12.5

$P < 0.05$

Mean± Standard deviation

See tables 3.2 and 3.4 for Abbreviations.

Table 3.11: Physico-chemical and heavy metal contents of water sample A from Warsak dam (control), water sample B from River Kabul upstream to the confluence point, water sample C from River Kabul downstream to the confluence point and water sample D from River Kabul downstream of C during high flow (summer) 2012-2014.

Characteristics	Parameters	Water sample A	Water sample B	Water sample C	Water sample D
Physico-chemical properties	pH	7.3±1.0	7.4±1.4	7.3±1.0	7.4±1.4
	TSS	715.0±138.0	601.7±261.7	881.3±409.0	962.3±209.6
	TDS	337.0±132.2	478.7±139.3	567.3±168.8	615.0±201.4
	EC	273.0±60.3	284.3±55.0	245.3±69.4	229.7±81.1
	Cl	8.7±5.4	15.7±8.4	25.0±8.1	30.7±8.2
	K	2.4±1.1	2.3±0.7	5.8±2.2	5.7±2.2
	Na	4.0±2.3	17.0±4.8	16.0±7.9	22.3±7.0
	TA	93.7±32.8	104.7±22.5	122.3±24.4	205.3±42.7
Heavy metals	Zn	66.3±69.9	221.3±40.9	230.3±61.4	254.7±35.1
	Ni	63.3±16.8	59.7±11.4	47.7±10.2	84.7±16.3
	Cr	31.3±15.5	41.0±10.4	43.3±8.8	47.7±17.1
	Cu	16.7±10.1	20.3±7.6	26.0±8.3	29.0±8.8
	Pb	8.3±6.5	164.3±31.9	176.3±33.0	186.7±30.1
	Cd	26.7±12.8	70.3±11.2	74.0±45.0	81.3±19.0
	Fe	21.0±10.5	47.0±11.2	60.0±8.9	54.3±28.3
	Mn	23.3±11.5	50.0±8.1	55.0±12.1	42.7±9.7
	Hg	26.0±13.1	23.3±6.4	36.3±15.0	41.0±14.8

$P < 0.05$

Mean± Standard deviation.

See tables 3.2 and 3.4 for Abbreviations

CHAPTER-4

BIOACCUMULATION OF HEAVY METALS IN SELECTED FISH SPECIES OF RIVER KABUL

4.1 INTRODUCTION

Accumulation of materials, which are not components of an organism body is termed bioaccumulation of metals (Bain, 1993). Fish accumulate metals directly from the water and indirectly by feeding on aquatic small organisms (Sasaki *et al*., 1998). Fish are the choice animals that are used as a test organism for the assessment of water pollution in the aquatic environment (Buikema *et al*., 1982). Fish are good indicators of water quality in aquatic environment and can give informations about the new toxic and dangerous chemicals, which are dumped into the aquatic environment (Powers, 1989; Bailey *et al*., 1992). Some fish are capable of bioaccumulation of metals nearly 100 times the concentration of metals in water, however it had been reported that the fish accumulate trace metals like Zn, Ni, Cr, Cu, Cd, Fe thousand times above the levels exiting in the exposure medium, while some metals were preferentially accumulated than others (Onwumere and Oladimeji, 1990). Fish acts as a bio-indicators for heavy metals pollution in the aquatic environment and can help in detection of aquatic environmental problems (Cavas and Ergen, 2005b).

The field and laboratory experimental studies indicate that heavy metals bioaccumulation in various organs and tissues of fish is attributed to heavy metals concentration in water and exposure time. Although other parameters like salinity, pH, hardness and temperature also help in metal accumulation (Jeffree *et al*, 2006; Quan *et al*., 2006; Singh *et al*., 2006). It has been investigated that problems of heavy metal accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Das and Kaviraj, 2000; Laxi, 2005; Jayakumar Paul, 2006; Kumar *et al*., 2008). Fish are considered as a good indicator of heavy metal pollution in aquatic

environment because they have various trophic levels, different size and age (Gabbianelli *et al*., 2003). Fish are the aquatic organism that can accumulate heavy metals in their tissues (Nwaedozie, 1998). Aquatic organisms can accumulate metals or pesticides directly from contaminated water or indirectly by the ingestion of contaminated feed sources (Huong *et al*., 2012). Fish has higher rate of accumulation for heavy metals than other species because of their different feeding habits. This has been shown to be especially true for cadmium (Regoli *et al*., 2002, Kavun *et al*., 2002). Aquatic organisms such as fish have capability to accumulate greater content of heavy metals in their living cells than those present in water (Forstner and Wittmann, 1981). Bioaccumulations of heavy metals adversely affect liver, muscle, kidney and other tissues of fish. They also affect metabolism, development and growth of fish (Sephar, 1976; Anadon *et al.*, 1984; Birage and Black, 1980). Heavy metals are the major industrial effluents, which along with other products from industrial operations are added into the water resources. These heavy metals are more toxic and dangerous to aquatic animals like fish (EL. Rayis and Ezzat, 1987; Dallinger *et al*, 1987; Dutton *et al*., 1988). Heavy metals have the tendency to accumulate in different tissues of fish (Buhler *et al*., 1977), which then enter into the human body through consumption of contaminated fish and can cause serious diseases in human beings (Puel *et al*., 1987).

Fish can accumulate greater amount of metals in their organs from their environment, in which metals are below the limit of detection in routine water samples (Barak and Mason, 1990). Accumulation of heavy metals in fish organs has been studied by several investigators (Abdel-Baky, 2001; Bahnasawy, 2001; EI-Ghazaly *et al.*, 1992; Gutleb *et al.*, 2002; Khallaf *et al*,, 1998; Moselhy, 1999; Heba *et al*., 2001). Fish accumulate heavy metals in their organs and tissues from contaminated environment. Different tissues and organs of fish accumulate different heavy metals with different concentrations (Jezierska and Witesta, 2001). Feeding diets, sediments and water are the sources from which the fish species mostly take

heavy metals and then heavy metals are accumulated in different tissues of the fish (McCarthy and Shugart, 1990). The fish like *C. anguillar* is accumulate heavy metals like nickel and lead, which could be correlated to the presence of nickel and lead on the surface water due to weathering of materials and soil erosion (Jonathan and Maina, 2009). Fish can store heavy metals in their liver or excretes through bile. Kidney and gills are other organ for heavy metal regulatios in fish (Nussey, 2000). The liver of fish plays a significant role in heavy metals accumulation and detoxification (Yousafzai, 2004). Several investigations have also reported that exposure duration, heavy metals concentration as well as salinity, temperature, hardness and metabolism are helping in accumulation and retaining of heavy metals in different tissues and organs of fish from their environment (Ademoroti, 1996; Cusimano *et al*., 1986; Heath, 1987; Allen, 1995; Karthikeyan *et al*., 2007). It has been shown that more heavy metals are accumulated in fish species than other animals (Adeyeye *et al*., 1996). Aquatic ecosystems are subjected to low-level and long term exposure of increasing number of new chemicals released continuously (Folmer *et al.*, 1993). Gills tissues have an active role in gas exchange, ion regulation, acid balance and waste excretion and metals accumulation, while muscle on the other hand play less role in metals bioaccumulation (Bajc *et al*., 2005; Filazi *et al*., 2003; Shukla *et al*., 2007).

Liver and gills play a significant role in metabolism and respiration process. Heavy metals accumulation in organs has been reported by many workers, which are correlated to some abnormalities in organs and tissues after exposure of the fish to heavy metals (Engelhardt *et al*., 1981; Khan, 2003). The amount of different heavy metals were determined in various tissues of two cyprinidi fish species collected from the upper, middle and lower parts of the Kor River, Iran. The purpose of the study was to find out whether these fish are suitable for human consumption or not (Ebrahimi and Taherianfard, 20 11). Heavy metal such as cadmium was analyzed in the aquatic organism like fish. This metal has a cumulative polluting effect and could

result in causing vital disorders in fish such as abnormal behaviour, locomotor abnomalities or anorexia (Woo *et al*., 1994; Bryan *et al*., 1995). Heavy metals tend to accumulate in different tissues of fish and are one of the public health concerns to both animals and humans (Asaolu and Olaofe, 2005; Olowu *et al*., 2010; Kalay *et al*., 1999; Ashraf, 2005). In recent investigation different tissues of *Synodentis budgetti* fish were studied for heavy metals bioaccumulation. The liver of *Synodentis budgetti* showed greater level of metals as compare to other tissues. The liver tissue came second for metals accumulation after gills (Joseph *et al*., 2012).

Heavy metal such as Cd is a highly toxic to aquatic organisms and accumulates in liver and kidney inducing hepatic and renal injury (Kjellstrom and Nordberg, 1985). The fish liver plays a good role in various functions like metabolism of the fish body and also help in accumulation, biotransformation and excretion of heavy metals in fish (Figueiredo *et al*., 2006). It is estimated that fish can act as front-line indicators of suspected aquatic pollutants such as heavy metals (Bailey *et al.*, 1996). The analysis of the fish liver was done. Since heavy metals have the tendency to be stored firstly in the liver as a detoxifying mechanism. Therefore the liver is a better indicator of bioaccumulation of heavy metals for environmental protection (Beder, 1990). It has been reported that *Salmo trutta* is a native fish of Spain. It is common fish species in the rivers of Spain and has a wide spread range in the fresh water ecosystem of the area ecosystem potentially polluted with heavy metals.The fish plays a vital role in biomonitring of heavy metal pollution. Heavy metals like Cu, Pb and Cd were determined in water and fish like *Salmo trutta*. Significant correlations were observed between sediment Pb concentration and Pb content in trout liver (Linde *et al*., 2002). In another study, it has been investigated that different tissues of the fish were analyzed for heavy metals accumulation. The heavy metal like copper was in the highest concentration in the liver but lowest in the muscle. The greater level of copper in the liver could be correlated to metabolic process and enzyme catalyzed reaction involving copper occurred in the liver (Gomaa *et al*., 1995;

El-Moselhy, 1999).

The water of the lake was studied for heavy metals and inorganic anions. The content of heavy metals and inorganic anions in water has variation due to the distance from the origin of the lakes, depth and seasons of the year. The flora and fauna of the deserts along with fish were analyzed for heavy metals concentration. Animals living near the lakes showed greater level of heavy metals like Pb, Hg and Cd than animals of the same species collected further away from the lakes (Saleh *et al.,* 1987). The concentration of heavy metal in the muscle tissue of edible fish collected from two selected mangrove areas was determined. The determination of the concentration of heavy metal content in the muscle tissue was made from samples of fish from Klong- dam. The concentration of heavy metals in samples was at the permissible level so it was not harmful to consumers (Monkolprasi, 1983). Heavy metals are non-biodegradable and dumped into aquatic environment through industrial effluents and city sewages. They could accumulate in aquatic organisms. Fish absorb the dissolved elements and heavy metals from surrounding enviornment, which may accumulate in different tissues (Eiman and Zamzam, 1996).

Heavy metals like Fe, Cu, Ni, Cr, Pb and Zn were studied in different tissues like muscle, skin, and gonads of *Mugil cephalus* and *Trachurus mediterraneus* fish. The fish from different study sites showed different levels of heavy metal and different stations also showed different contents of heavy metals. Two stations were much polluted due to receiving untreated domestic wastes and industrial effluents and particularly greater concentration of metals. Generally, skin and gonads had accumulated greater concentration of metals than muscle. Some metals in some tissues have crossed the permissible limits for a food source for human consumption (Yilmaz, 2003).

Water and different tissues of *Tilapia nilotica* were studied for bioaccumulation of heavy metals like Cd, Cu, Pb and Zn. The water showed greater

concentration of Pb than Cd, Cu, and Zn. These heavy metals also showed different concentrations in different parts of the fish. The visceral tissues showed the greater concentration followed by the head, while smallest level was found in the flesh of the fish (Attam *et al*., 1997). Metal contents were measured in different fish species like *Cypranus carpiocommunis*, *Cypranus carpio specularis, Cypranus* carpio *nudus* and *Salmo gairdneri*. Lead concentration was below detectable in the fish. The kidney had accumulated greater metal load. The study also reported the daily dietary intake of metals by human through fish consumption (Muralidharan *et al*., 1997). Concentration of heavy metals like cadmium, chromium, copper, nickel, lead and zinc were determined in different tissues of marine fish like *Epinephelus aerolatus, Lutjanus russelli* and *Sparus sarba*. The fish from cultured sites showed greater metal concentration than sea water and sediments. Generally tissues of all three species showed greater content of zinc and copper but nickel, lead, cadmium and chromium concentration was the smallest. Metal contents were different in various tissues, among species and among fish culture sites. Different tissues have different capacity for heavy metals accumulation. Gonads of all three fish species showed higher levels of zinc and liver seemed to be the primary organ for Cu accumulation (Wong *et al*., 2001).

The impact of heavy metals like Cd, Zn and pesticides on the rate of uptake of glucose, fructose and amino acid tryptophan by the intestine of fresh water teleost fish, *Channa punctatus and Heteropneustes fossilis* was investigated. Fish have showed decrease in the rate of transport of glucose and fructose after exposed to sub lethal concentration of Cd and Zn (Shukla *et al*., 2001). Heavy metals like Zn, Cu and Pb were studied in the muscle tissues of edible fish's sample. These metals were also analyzed in the sediment and aquatic phase to find out the degree of bioaccumulation. Different fish species showed different metals accumulation. These metals were in the order of Zn> Cu > Pb (Bhattacharya *et al*., 2001). Heavy metals like zinc, copper, cadmium, lead, chromium, nickel and cobalt were determined in sediments and three

edible fish. The findings aimed to assess heavy metals accumulation in fish inhabiting sediments characterized by varying metal bioavailability. Greater contents of zinc, cadmium and copper were found in fish than sediments. The fish of Kolleru Lake accumulated higher concentration of metals and was decleared unfit for human consumption (Sekhar *et al*., 2004).

4.2 MATERIALS AND METHODS

4.2.1 Study Area

For detail see page≠2

4.2.2 Fish Sampling Sites

Two fish samples at different times were collected from highly polluted belt of the main river. One sample (containing five different fish species) was from the area of about 3 km in length upstream Nowshera- Mardan Road Bridge to Amangarh industrial zone (site 1). It receives effluents from Amangarh Industries. The second fish sample (containing five different fish species) was taken about 4 km downstream Nowshera- Mardan Road Bridge (site 2). The sewages from Nowshera city, Mardan, Risalpur and other adjacent towns join River Kabul at this point. Both the above samples collected from sites 1 and 2 of River Kabul were considered fish samples from polluted water (tested fish sample) and were compared with third fish sample collected from non polluted Warsak dam (site 3) about 60 km upstream the polluted part of the River Kabul. This was the control fish sample. Five different fish species were selected from each polluted and non polluted part of River Kabul. Both the tested and control fish samples were compared for heavy metals to assess water pollution in River Kabul (Fig. 4.1).

4.2.3 Collection of Fish Samples

Fishing was done during late night with the help of local fishermen. The gills net (Patti) (40×6ft) with a cork line at the top rope and metal line with the ground rope

made locally of nylon was used for fishing as fish gear, with the help of four fishermen and a wooden boat usually operated a single pati. Moter driven boats were not used as the fish would be disturbed with sound from engine. Fish species were including *Wallago attu, Ompok bimaculatus, Labeo dyocheilus, Cyprinus carpio* and *Aorichthys seenghala*.

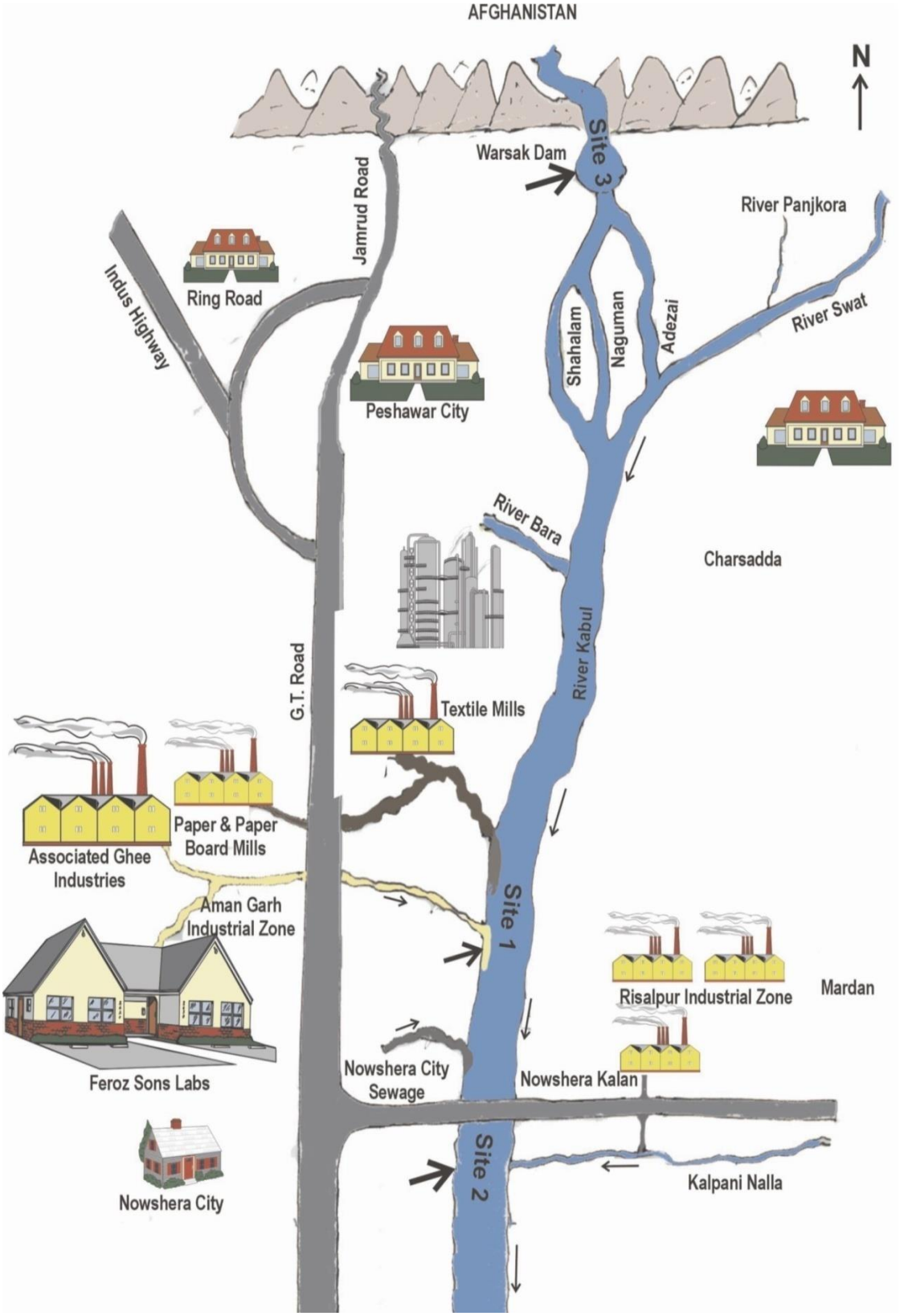


Fig 4.1 Fish sampling sites 1 and 2 at River Kabul (polluted samples) and site 3 in Warsak dam (control sample).

4.2.4 Collection and Preservation of Fish Tissues

Different fish species including *Wallago attu, Ompok bimaculatus, Labeo dyocheilus,Cyprinus carpio* and *Aorichthys seenghala* were collected from two sites of the River Kabul, downstream (site 1, Amangarh and site 2, Nowshera) and upstream (site 3, Warsak dam reservoir). The netted fish were dissected for collection of tissues like intestine, gills, skin, liver and muscle. These tissues were washed with distilled water and then shifted to marked sterilized polythene bags. Polythene bags having fish tissues were then stored in the freezer (at -20 c°) for further analysis of heavy metals accumulation.

4.2.5 Tissue Digestion

For the estimation of heavy metals, the tissues digestion was carried out in the Department of Zoology University of Peshawar. Tissues samples were thawned rinsed in distilled water and blotted with blotting paper and shifted to 100ml volumetric flasks. Before tissues transfer, all the flasks were washed with distilled water and were dried in oven at 60C˚ for a few minutes. Then the known weight of each tissue (50g) was shifted to these volumetric flasks. Samples were digested according to the methods described by Van Loon (1989). 5ml nitric acid (55%) and 1ml per chloric acid (70%) were added to each flask and the flasks then were kept for overnight. Next day a second dose of 5ml nitric acid (55%) and 4ml (70%) per chloric acid (70%) were added to each flask. The flasks were then placed on hot plate and allowed to digest at 200 to 250C° until a transparent and clear solution was obtained. After digestion samples were cooled and were diluted to 100ml with distilled water. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

4.2.6 Determination of Heavy Metals

Determination of heavy metals was done through Atomic Absorption Spectrophotometer in the Centralized Resource Laboratory (CRL) University of

Peshawar. Atomic Absorption Spectrophotometer (Spectra-AA-700) was used for determination of heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe and Hg concentrations in different tissue of each fish from both polluted and control sites of River Kabul.

Table 4.1 Operating data of Atomic Absorption Spectrophotometer for determination of metals

Elements	**Wave length (nm)**	**Flame**	**Working range (µg/m$^{L-1)}$**
Zn	213.9	AA (L)	0.4-1.6
Ni	232.0	AA (L)	3-12
Cr	357.9	AA (R)	2-8
Cu	324.7	AA (L)	2-8
Cd	228.8	AA (R)	0.5-2
Pb	217.0	AA (L)	5-20
Mn	279.5	AA (L)	1-4
Fe	248.3	AA (L)	1-4
Hg	253.7	AA (L)	100-400

Abbreviations, AA: air acetylene, R: Fuel-rich, L: Fuel-lean

4.2.7 Statistical Analysis

Statistical analysis was done by using ANOVA software for windows. Mean and standard deviation values of the data were determined. The different sets of data were analyzed for statistical differences by using student's t –test (two-tailed); a P value <0.05 was considered to show statistical significance.

4.3 RESULT AND DISCUSSION

In the present investigation heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg were determined in gills, skin, intestine, liver and muscle of different fish species like *Wallago attu*, *Aorichthys seenghala*, *Labeo dyocheilus*, *Cyprinnus carpio*, *Ompok bimaculatous* caught from site 3 (control) and site 1 and site 2 (polluted sites). Comparing our studies with the findings of other workers and Yousafzai (2004) showed that increase in levels of different heavy metals in different tissues was observed. This reflects that in past three years, a further increase in level of heavy metals has been occurred in River Kabul, which is suggestive of implementation of environmental laws and a biomonitoring progamme.

4.3.1 Bioaccumulation of Heavy Metals in Gills

Gills of five selected fish from site 3 (control site) and site 1 and site 2 (polluted sites) were taken out and processed for estimation of zinc, chromium, copper, cadmium, lead, iron, manganese and mercury. Gills of fish from site 1 and site 2 showed greater concentration as compare to those from site 3 (Table 4.2 and Figs 4.2-4.4).

From amongst heavy metals, zinc had highest concentration in gills of *Wallago attu* from sites 1 and 2 with mean values of 824.0±594.5 µg/g and 894.0±643.5µg/g and had 404.3±101.8 µg/g from site 3, *Aorichthys seenghala* from polluted sites had 499.3±477.5 µg/g and 909.0±624.7 µg/g and had 377.0±177.4 µg/g from control site, *Labeo dyocheilus* from polluted water had 1537.0±1028.5 µg/g and 1626.7±1075.2 µg/g and had 402.3±127.2 µg/g from control water, *Cyprinus carpio* from polluted site 1 and site 2 had 1416.3±1168.1 µg/g and 1497.7±1128.5 µg/g and had 392.6±81.5 µg/g from Warsak dam and gills of *Ompok bimaculatus* from polluted sites had 3496.0±5381.8 µg/g and 3633.0±5521.1 µg/g and had 360.3±142.0 µg/g from control site respectively. Mean values of zinc in different fish species followed the order: *Ompok bimaculatus* > *Labeo dyocheilus* > *Cyprinus carpio* > *Aorichthys*

seenghala > *Wallago attu*. This shows that Zn metal bioaccumulation is highest in gills of *Ompok bimaculatous* and lowest in *Wallago attu*. The present result found more Zn than those reported in previous studies (Jennings and Rainbow, 1979; Wepner *et al*., 2001). Comparing these studies with our findings reflect that River Kabul has more concentration of Zn as compare to other mentioned water resources. However, in this study gills of *Ompok bimaculatus* had accumulated higher concentration of Zn as compare to other fish species. This could be because of omnivorous nature of this fish. Being an omnivorous nature it is more exposed to metal bioaccumulation by many food chains.

Gills of *Wallago attu* from polluted sites showed more Ni mean values of 116.3±102.5 µg/g and 130.7±113.9 µg/g and showed 75.0±39.9 µg/g from site 3, *Aorichthys seenghala* from polluted sites 1 and 2 showed 113.7±86.9 µg/g and 129.3±93.5 µg/g and showed 55.7±38.4 µg/g from control site, *Labeo dyocheilus* from polluted water showed 136.7±141.7 µg/g and 160.3±209.4 µg/g and showed 41.7±19.9 µg/g from control site 3, *Cyprinus carpio* from polluted water showed 94.3±47.5 µg/g and 118.0±61.9 µg/g and showed 84.3±32.3 µg/g from site 3 and *Ompok bimaculatus* from site 1 and site 2 showed 139.7±106.0 µg/g and 163.3±124.4 µg/g and showed 49.3±23.3 µg/g from site 3 respectively. In this investigation, concentration of Ni in gills of examined fish species was higher than those reported in previous findings (Zia and Mcdonald, 1994; Ptashynski *et al*., 2002; Uluzlu *et al*., 2007**).** Nickel concentration in gills of different fish species was in the order of *Ompok bimaculatus* >*Labeo dyocheilus*>*Wallago attu*>*Aorichthys seenghala Cyprinus carpio*. This indicates that metal bioaccumulation is highest in gills of *Ompok bimaculatus* and lowest in *Cyprinus carpio*. Nickel in gills from site 2 was more than those from site 1. This could be correlated to dumping of city sewages and industrial effluents at site 2 and more exposition of fish to this metal for long period.

Chromium content in gills of *Wallago attu* from polluted sites were 555.3±418.4 µg/g and 605.0±419.9 µg/g and was 11.3±7.0 µg/g from control site, in *Aorichthys seenghala* from sites 1 and 2 were 552.7±414.1 µg/g and 610.7±441.1 µg/g and was 11.3±7.0 µg/g from site 3, in *Labeo dyocheilus* from polluted water were 680.7±413.7 µg/g and 739.0±409.2 µg/g and was 70.3±32.5 µg/g from control water, in *Cyprinus carpio* from polluted sites were 541.7±227.2 µg/g and 578.3±234.5 µg/g and was 18.0±13.6 µg/g from control site and in *Ompok bimaculatus* from sites 1 and 2 were 657.3±425.0 µg/ and 752.3±455.6 µg/g and was 40.7±45.3 µg/g from site 3 respectively. Chromium bioaccumulation in gills of different fish species was in the sequence of *Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala > Wallago attu>Cyprinus carpio.*This highlights that Cr metal bioaccumulation is highest in gills of *Ompok bimaculatus* and lowest in *Cyprinus carpio*. High concentration of Cr in gills of different fish species from River Kabul being determined in this study are in agreement with many studies that determined also high content of Cr in gills of other different fish (Avevnant- Oldewage and Marx, 2000; Olaifa *et al*., 2004). Accumulated Cr metal in this organ varied significantly depending upon fish species and sites of fish and water contamination. High metal accumulation in *Ompok bimaculatus* indicates discharge of industrial and municipal effluents into River Kabul.

Copper concenrations in gills of different fish species from polluted site 1 and site 2 are also high as compare to control site, where the recorded value for Cu was lowest. Copper concentration in gills of *Wallago attu* from both sites 1 and 2 were 105.3±89.1 µg/g and 301.0±91.7 µg/g and was of 90.7±79.3 µg/g from control site 3, in *Aorichthys seenghala* from polluted sites were 208.0±145.3 µg/g and 232.7±153.3 µg/g and was 52.7±25.9 µg/g from control site, in *Labeo dyocheilus* from sites 1 and 2 were 154.3±42.3 µg/g and 175.3±49.9 µg/g and was 57.7±33.1 µg/g from site 3, in *Cyprinus carpio* from polluted water were 148.0±103.8 µg/g and 167.0±114.8 µg/g and was 74.0±37.0 µg/g from control water and in *Ompok bimaculatus* from sites 1

and 2 were 165.0±97.6 µg/g and 216.3±146.7 µg/g and was 54.3±34.1 µg/g from site 3 respectively. Copper bioaccumulation in gills of different fish species was in the order of *Wallago attu* > *Aorichthys seenghala*> *Ompok bimaculatus* > *Labeo dyocheilus* > *Cyprinus carpio*. This revealed that metal bioaccumulation is the highest in gills of *Wallago attu* and the lowest in *Cyprinus carpio*. High concentration of Cu has also been reported in previous investigations (Clear water *et al*., 2000; Olaifa *et al*., 2004; Uluzlu *et al*., 2007; Amal *et al*., 2012). Comparing the above studies with our findings is reflecting that River Kabul has more concentration of copper as compare to other mentioned water bodies. However, in this study gills of *Wallago attu* had accumulated higher concentration of copper as compare to other fish species. This could be because of omnivorous nature, low elimination of metals from body, low metabolic rate and low detoxification mechanism of this fish.

Gills of *Wallago attu* from both polluted sites accumulated maximum concentration of cadmium with mean values of 62.7±31.1 and 79.0±58.8 and accumulated minimum mean value of 8.3±7.5 from control site, *Aorichthys seenghala* from site 1 and site 2 accumulated 58.3±87.2 µg/g and 72.7±41.5 µg/g and accumulated 5.3±3.4 µg/g from site 3, *Labeo dyocheilus* from polluted water accumulated 63.7±25.2 µg/g and 82.3±46.6 µg/g and accumulated 18.0±4.9 µg/g from Warsak dam, *Cyprinus carpio* from both sites 1 and 2 accumulated 60.7±29.4 µg/g and 74.7±40.9 µg/g and accumulated 23.7±15.5 µg/g from control site and *Ompok bimaculatus* from polluted sites 1 and 2 accumulated 69.3±43.2 µg/g and 83.0±52.2 µg/g and accumulated 53.0±49.5 µg/g from control site 3 respectively. Cadmium bioaccumulation in gills of different fish species was in the order of *Ompok bimaculatus* > *Labeo dyocheilus* > *Wallago attu*>*Cyprinus carpio*> *Aorichthys seenghala*. This indicates that metal bioaccumulation is highest in gills of *Ompok bimaculatus* and lowest in *Aorichthys seenghala*. The present results are in agreement with those observed by many investigators (Uluzlu *et al*., 2007; Farakas *et al*., 2003; Yap *et al*., 2005; Hilrny *et al*., 1985). However, in the present study gills of *Ompok*

bimaculatus from polluted sites has accumulated higher concentration of Cd as compare to fish species from control site 3.

Gills of *Wallago attu* from polluted sites had accumulated highest concentration of lead with mean values of 62.7±31.1 µg/g and 461.3±555.6 µg/g and had accumulated lowest content with mean value of 66.3±36.4 from control site, *Aorichthys seenghala* from site 1 and site 2 had accumulated 315.7±311.5 µg/g and 382.0±379.1 µg/g and had accumulated 149.3±99.0 µg/g from site 3, *Labeo dyocheilus* from polluted sites had accumulated 262.3±217.4 µg/g and 309.3±257.0 µg/g and had accumulated 16.6±13.8 µg/g from control site, *Cyprinus carpio* from polluted water had accumulated 115.3±105.9 µg/g and 133.7±125.9 µg/g and had accumulated 53.0±48.7 µg/g from Warsak dam water and *Ompok bimaculatus* from polluted water had accumulated 277.0±236.9 µg/g and 322.0±263.2 µg/g and had accumulated 21.0±17.1 µg/g from control water. Lead bioaccumulation in gills of different fish species was in the order of *Wallago attu> Aorichthys seenghala >Ompok bimaculatus > Labeo dyocheilus > Cyprinus carpio.* This shows that metal bioaccumulation was highest in gills of *Wallago attu* and lowest in *Cyprinus carpio*. In this finding, levels of Pb in gills of different fish samples were more than the concentrations reported in gills of other fish species (Barbarae, 1977; Rogers *et al*., 2003; Naghshbandi *et al*., 2007). Comparing the above studies with our finding reflecting that River Kabul has more concentration of Pb as compare to other mentioned water resources. However, in this investigation gills of *Wallago attu* has accumulated higher concentration of Pb as compare to other fish species.This could be because of large body size, exposition of this fish to metal for long period and low metabolic rate.

Gills of *Wallago attu* from polluted sites contained iron with mean values of 94.0±27.4 µg/g and 114.0±34.9 µg/g and contained 28.0±10.5 µg/g from control site, *Aorichthys seenghala* from polluted water contained 87.0±26.3 µg/g and 107.0±33.8

µg/g and contained 22.0±8.9 µg/g from control water, *Labeo dyocheilus* from polluted sites 1 and 2 contained 85.0±26.0 µg/g and 105.0±33.4 µg/g and contained18.0±8.4 µg/g from site 3, *Cyprinus carpio* from polluted sites contained 90.0±26.8 µg/g and 110.0±34.2 µg/g and contained 26.0±10.2 µg/g from control site and gills of *Ompok bimaculatus* from polluted sites contained 88.0±26.5 µg/g and 108.0±33.9 µg/g and contained 23.0±9.5 µg/g from control site respectively. The sequence of iron bioaccumulation in gills of different fish species was *Wallago attu >Cyprinus carpio >Ompok bimaculatus > Aorichthys seenghala > Labeo dyocheilus*. This reveales that metal bioaccumulation is highest in gills of *Wallago attu* and lowest in *Labeo dyocheilus*. These results are in agreement with those observed by many investigators, who have also studied different metals in fish exhibited their highest levels in gills (Olayan and Thomas, 2005; Yilmaz and Dogan, 2007; Fatma *et al*., 2005). In the present finding gills of examined fish showed maximum content of Fe as compare to other fish species of Pakistan. This is because of different feeding behaviors and exposition of these fish to Fe concentration in water for long period and the result also indicated further increase of Fe concentration in River Kabul for the last few years. Among studied metals Fe came last six in number after Ni in gills of all studied fish.

Gills of *Wallago attu* from polluted sites had maximum concentration of manganese with mean values of 80.0±25.3 µg/g and 97.0±32.1 µg/g and had minimum value of 34.0±11.7 µg/g from control site, *Aorichthys seenghala* from polluted sites had 73.0±24.1 µg/g and 93.0±31.5 µg/g and had 28.0±10.5 µg/g from control site, *Labeo dyocheilus* from both sites 1 and 2 had 70.0±23.7 µg/g and 90.0±31.0 µg/g and had 27.0±10.0 µg/g from control site 3, *Cyprinus carpio* from polluted site 1 and site 2 had 76.0±24 µg/g and 96.0±32.0 µg/g and had 32.0±11.3 µg/g from Warsak dam and gills of *Ompok bimaculatus* from polluted water had 71.0±23.8 µg/g and 91.0±31.1 µg/g and had 32.0±11.3 µg/g from site 3 respectively. Sequence of manganese bioaccumulation in gills of different fish species was *Wallago attu>Cyprinus carpio > Aorichthys seenghala > Ompok bimaculatus >*

Labeo dyocheilus. This highlights that manganese content was highest in gills of *Wallago attu* and was lowest in *Labeo dyocheilus*. The present result found high Fe concentration as compare to other metals in this organ. In this study, Mn concentration was higher than those reported by Rashed (2001) in gills of *Tilapia nilotica* and by Fatma *et al* (2005) in gills tisuue of *0. niloticus*, *T. zillii* and *C. lazera* collected, while was lower than those reported by Amal *et al* (2012). Comparing the present result with the findings of above mentioned workers showed further increase of Mn content in River Kabul in the last few years.

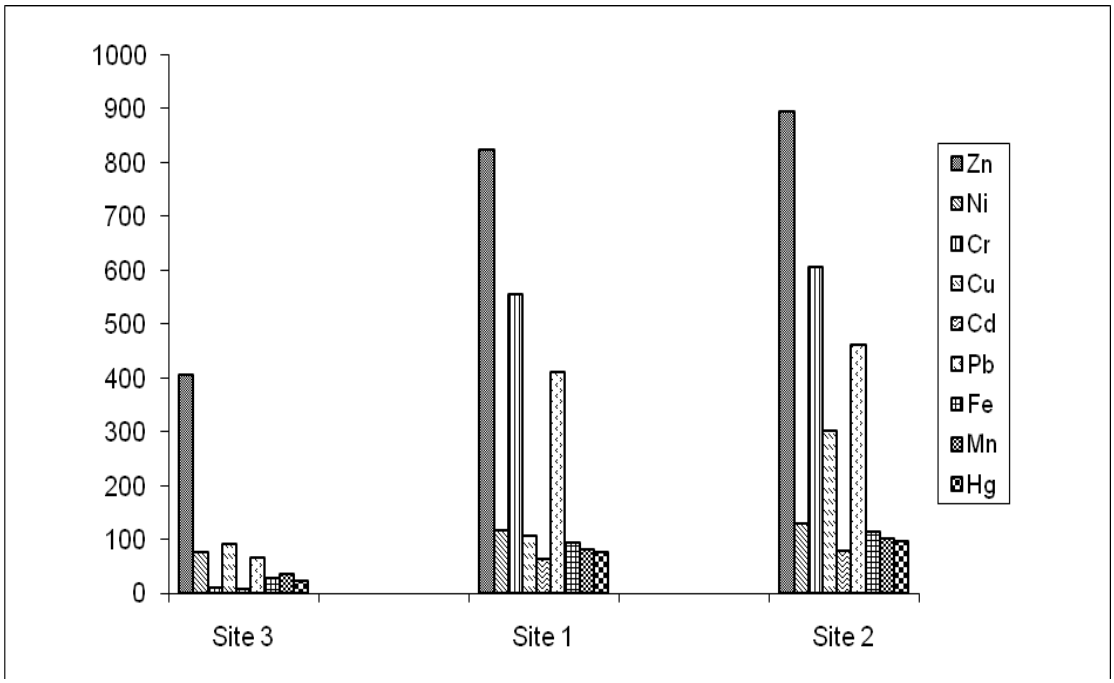
Heavy metal like mercury tend to accumulates in gills of fish. The higher level of mercury was found in gills and exceeded the allowed limit. Mercury in gills of *Wallago attu* from polluted sites were 77.0±24.8 µg/g and 97.0±32.1 µg/g and was 22.0±9.3 µg/g from control site, in *Aorichthys seenghala* from sites 1 and 2 were 69.0±21.6 and 89.0±30.0 µg/g and was 17.0±8.2 µg/g from site 3, in *Labeo dyocheilus* from both polluted sites were 69.0±21.6 and 89.0±30.0 and was 17.0±8.2 from site 3, in *Cyprinus carpio* from sites 1 and 2 were 71.0±23.8 µg/g and 91.0±31.1 µg/g and was 20.0±8.9 µg/g from site 3 and in gills of *Ompok bimaculatus* from site 1 and site 2 were 69.7±21.6 µg/g and 89.0±30.8 µg/g and was 19.0±8.7 µg/g from control site 3 respectively. Sequence of mercury accumulation in this organ was *Wallago attu> Labeo dyocheilus >Cyprinus carpio > Aorichthys seenghala > Ompok bimaculatus.* This indicates that mercury level was highest in gills of *Wallago attu* and lowest in *Ompok bimaculatus*.The present result found greater level of Hg as reported in previous findings (Azmat and Talat, 2006; Lazorchak *et al*., 2003; Masoud *et al*., 2007). Comparing the above studies with our finding revealed that increased concentration of heavy metals in tissues of different fish species from polluted sites could be correlated to exposition of fish to metals for long period and high concentration of metals in the water.

All investigated metals including Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg in gills of different fish species from polluted water showed increasing tendency as compare with those from control water. The possible reasons for this tremendous increase in the individual metal level in fish tissue could be correlated to mining activities in surrounding hills, agricultural activities, city sewage, industrial effluents and other anthrapogenic activities and also the exposure of the fish to metal for long time. In this investigation, high metal concentrations in different tisuues of different fish species also could be correlated to higher metal concentration in the water and sediments, which has not been investigated and has been reported to be potent site of metal accumulation in the natural system. This could be suggestive of a large quantity of metals uptake via the gills and the food chain because of the carnivorous, herbivoreous and omnivorous feeding habits of *Wallago attu*, *Aorichthys seenghala*, *Labeo dyocheilus*, *Cyprinus carpio* and *Ompok bimaculatous*. It has been suggested that some fish are capable of bioaccumulating of metals nearly 100 times the concentration of metals in water. Benthic vegetation also play a key role in metal bioaccumulation as most of fish including *Wallago attu*, *Aorichthys seenghala*, *Labeo dyocheilus*, *Cyprinus carpio* and *Ompok bimaculatous* especially in the early life in natural system feed on vegetation. The present data also showed that metal concentrations were highest in gills followed by skin, intestine and liver and was lowest in muscle. It is well known that gills and skin are the primary sites of metals contact and consquently their absorption. However absorption of the metals on gills surface could also be an important factor in increase in total levels of metals in gills.

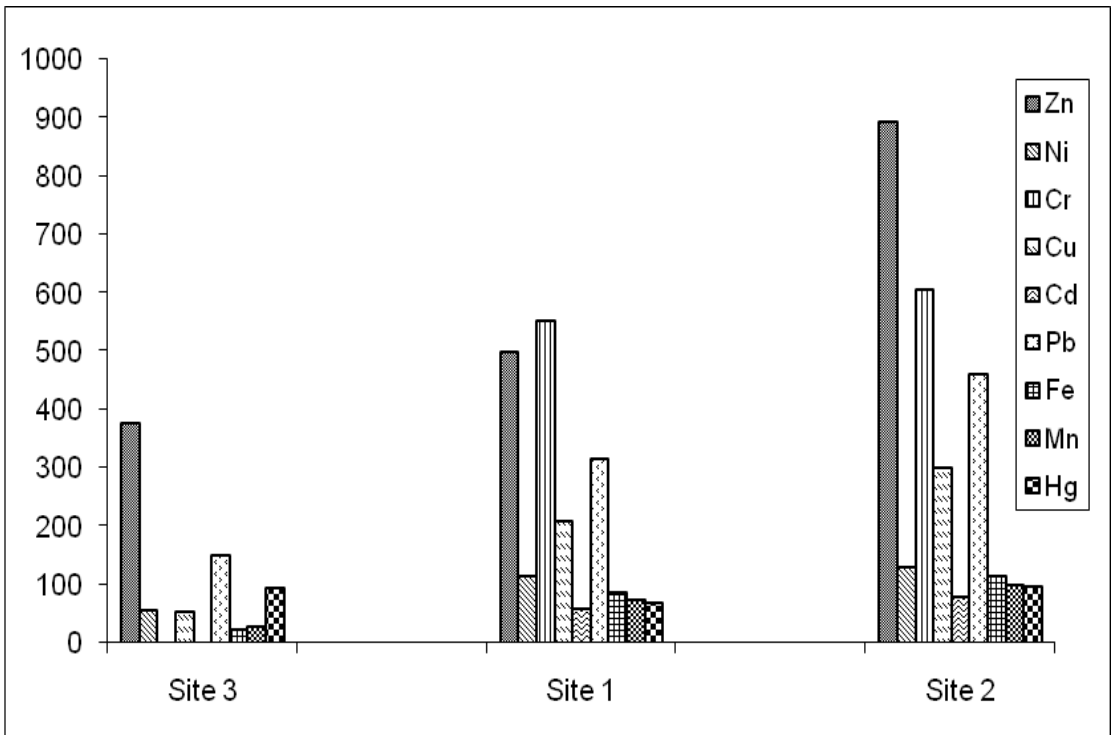
Table 4.2 Heavy metal concentrations (µg/g wet weight) in gills of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Analytes (µg/g)	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Zn	404.3±101.8	824.0±594.5	894.0±643.5
	Ni	75.0±39.9	116.3±102.5	130.7±113.9
	Cr	11.3±7.0	555.3±418.4	605.0±419.9
	Cu	90.7±79.3	105.3±89.1	301.0±91.7
	Cd	8.3±7.5	62.7±31.1	79.0±58.8
	Pb	66.3±36.4	411.7±529.4	461.3±555.6
	Fe	28.0±10.5	94.0±27.4	114.0±34.9
	Mn	34.0±11.7	80.0±25.3	100.0±32.7
	Hg	22.0±9.3	77.0±24.8	97.0±32.1
Aorichthys seenghala	Zn	377.0±177.4	499.3±477.5	909.0±624.7
	Ni	55.7±38.4	113.7±86.9	129.3±93.5
	Cr	4.6±3.2	552.7±414.1	610.7±441.1
	Cu	52.7±25.9	208.0±145.3	232.7±153.3
	Cd	5.3±3.4	58.3±87.2	72.7±41.5
	Pb	149.3±99.0	315.7±311.5	382.0±379.1
	Fe	22.0±8.9	87.0±26.3	107.0±33.8
	Mn	28.0±10.5	73.0±24.1	93.0±31.5
	Hg	93.0±8.2	**69.0±21.6**	**89.0±30.0**
Labeo dyocheilus	Zn	394.0±230.0	1537.0±1028.5	1626.7±1075.2
	Ni	41.7±19.9	136.7±141.7	160.3±209.4
	Cr	70.3±32.5	680.7±413.7	739.0±409.2
	Cu	57.7±33.1	154.3±42.3	175.3±49.9
	Cd	18.0±4.9	63.7±25.2	82.3±46.6
	Pb	16.6±13.8	262.3±217.4	309.3±257.0
	Fe	18.0±8.4	85.0±26.0	105.0±33.4
	Mn	27.0±10.0	70.0±23.7	90.0±31.0
	Hg	15.0±10.2	67.0±23.1	95.0±31.8
Cyprinus carpio	Zn	392.6±81.5	1416.3±1168.1	1497.7±1128.5
	Ni	84.3±32.3c	**94.3±47.5**	**118.0±61.9**
	Cr	18.0±13.6	541.7±227.2	578.3±234.5
	Cu	74.0±37.0	148.0±103.8	167.0±114.8
	Cd	23.7±15.5	60.7±29.4	74.7±40.9
	Pb	53.0±48.7	115.3±105.9	133.7±125.9
	Fe	26.0±10.2	90.0±26.8	110.0±34.2
	Mn	32.0±11.3	76.0±24	96.0±32.0
	Hg	20.0±8.9	71.0±23.8	91.0±31.1
Ompok bimaculatus	Zn	360.3±142.0	3496.0±5381.8	3633.0±5521.1
	Ni	49.3±23.3	139.7±106.0	163.3±124.4
	Cr	40.7±45.3	657.3±425.0	752.3±455.6
	Cu	54.3±34.1	165.0±97.6	216.3±146.7
	Cd	53.0±49.5	**69.3±43.2**	**83.0±52.2**
	Pb	21.0±17.1	277.0±236.9	322.0±263.2
	Fe	23.0±9.5	88.0±26.5	108.0±33.9
	Mn	32.0±11.3	71.0±23.8	91.0±31.1
	Hg	19.0±8.7	69.7±21.6	89.0±30.8

P<0.05, **P>0.05**. (Values in bold are non-significant)

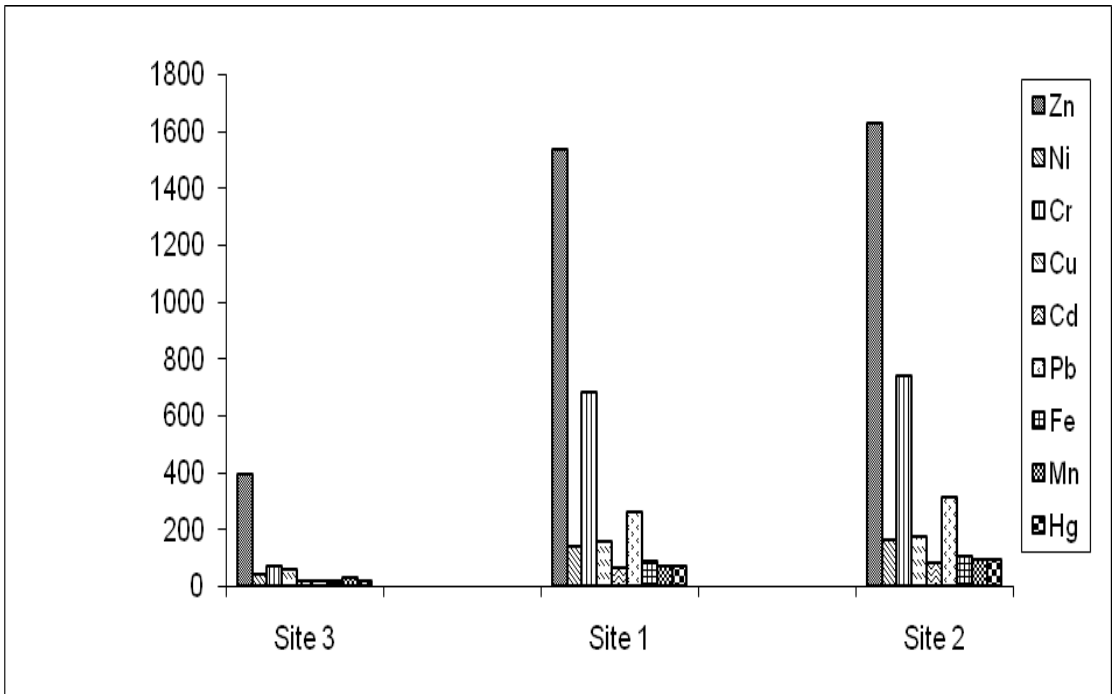


Wallago attu

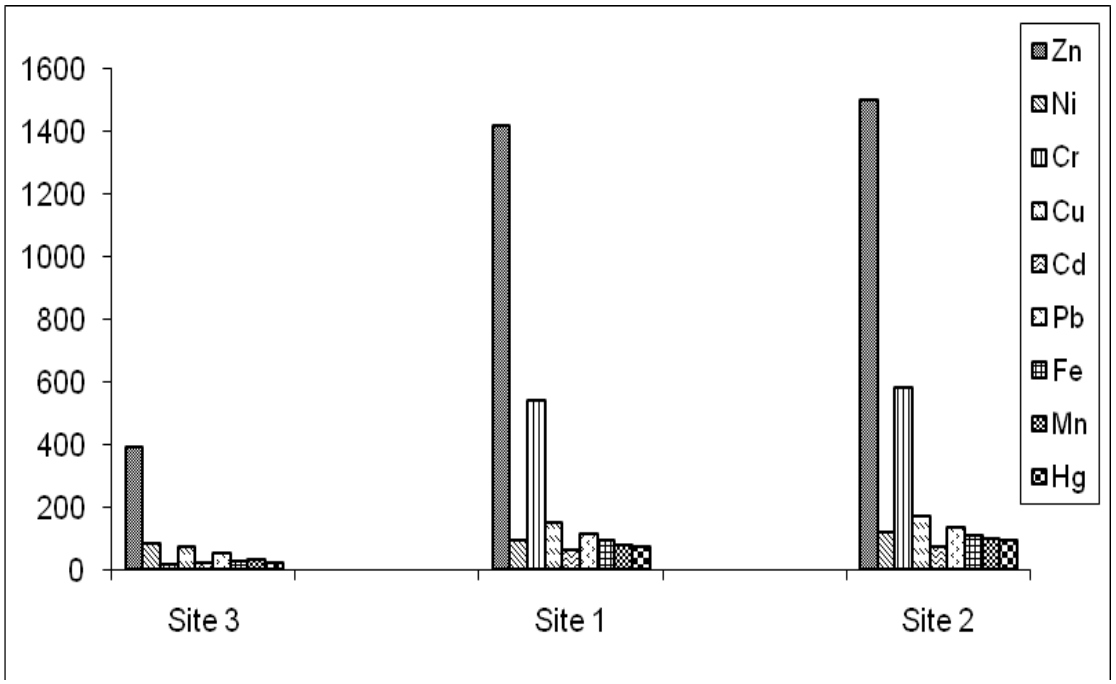


Aorichthys seenghala

Fig 4.2 **Heavy metal concentrations in gills of *Wallago attu*and *Aorichthys seenghala*netted fromsite 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

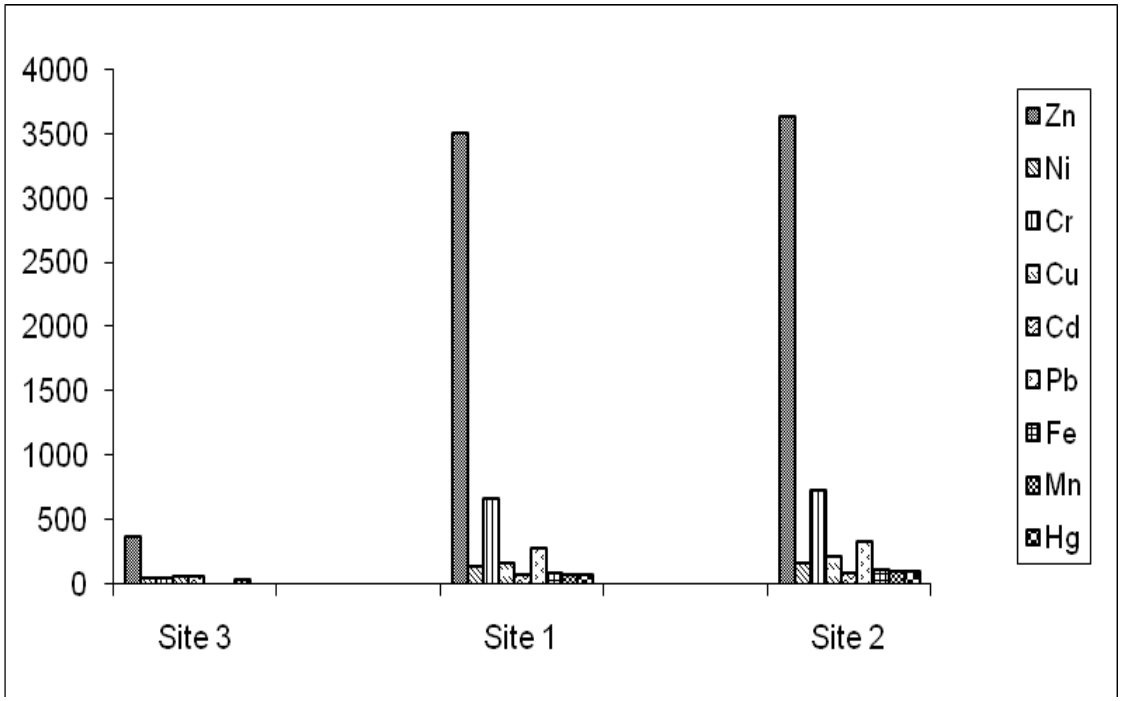


Labeo dyocheilus



Cyprinus carpio

Fig. 4.3 **Heavy metal concentrations in gills of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**



Ompok bimaculatus

Fig.4.4 **Heavy metal concentrations in gills of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents**

4.3.2 Bioaccumulation of Heavy Metals in Skin

Skin of selected different fish from site 3 (control) and site 1 and site 2 (polluted) was taken out and processed for estimation of heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg. (Table 4.3,4.4 and Figs 4.5-4.7). The skin of five different fish species from polluted sites (Site 1 and Site 2) also had accumulated greater concentatrion of metals as compare to those from control site (Site 3). Fish skin is also consumed along with muscle in most of the rural population of the world. Therefore researchers also have an emphasis on just tissues, while investigating muscle. Inhabitants living around the River Kabul also eat fish skin along with muscle. Adsorption on skin surface followed by their absorption in skin tissue by various mechanisms favours accumulation of metals in skin.When fish are exposed to elevated level of metals in aquatic environment. Fish can regulate metal concentration to a certain extent after bioaccumulation (Heath, 1991). As already stated that skin of five inhabitant fish species including *Wallago attu, Aorichthys seenghala, Labeo dyocheilus, Cyprinus carpio, Ompok bimaculatus* along with muscle cooked and consumed for being very tasty, therefore it should be compared with the U.S. Recommended Daily Dietary Allowances (RDA) supplied by 100 gm serving of fish muscle. Skin is the tissue that is exposed constantly and directly to water pollution.

Zn in skin of *Wallago attu* from polluted site 1 and site 2 showed more values of 866.3±714.8 µg/g and 921.0±527.0µg/g and showed 425.7±138.0 µg/g from site 3, *Aorichthys seenghala* from polluted sites showed 2691.0±1766.8µg/g and 2768.3±1835.4 µg/g and showed 358.7±171.2 µg/g from control site, *Labeo dyocheilus* from polluted water showed 1945.7±930.9 µg/g and 1987.0±956.7 µg/g and showed 406.3±123.7 µg/g from control water, *Cyprinus carpio* from sites 1 and 2 showed 4579.3±2585.8 µg/g and 4775.0±2578.2 µg/g and showed 442.7±150.8 µg/g from site 3 and *Ompok bimaculatus* from polluted sites showed 1255.3±610.9 µg/g and 1328.0±625.9 µg/g and showed 390.3±180.0 µg/g from control site. Overall order of zinc accumulation in this organ of different fish species was *Cyprinus carpio* >

Aorichthys seenghala> Labeo dyocheilus > Ompok bimaculatus > Wallago attu. This indicates that *Cyprinus carpio* had accumulated higher zinc level and *Wallago attu* lower content. High concentrations of Zn in this study are in agreement with the findings of previous workers (Coetzee *et al*., 2002.; Yilmaz, 2003; Honda *et al*., 1983). Comparing these values with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that zinc metal concentration in skin of *Aorichthys seenghala* and *Cyprinus carpio* were above the proposed RDA limits, which is 2600 µg/g/100g for zinc in the present finding. But in the rest of three fish zinc level was below the RDA proposed limits. Therefore *Aorichthys seenghala* and *Cyprinus carpio* from polluted sites of River Kabul are not suitable for human consumption and can prove highly toxic. In the present study skin of *Cyprinus carpio* had accumulated higher concentration of Zn as compare to other fish species. Skin is an important tissue for metal accumulation and this is also the primary site of metal contact and consequently their absorption and is second to the gills in storaging metals.

Skin of *Wallago attu* from polluted sites had more concentration of Ni with mean values of 100.3±24.0 µg/g and 113.0±27.3 µg/g and had 85.3±40.2 µg/g from reference site 3, *Aorichthys seenghala* from polluted water had 118.3±81.5 µg/g and 131.7±90.7 µg/g and had 66.3±41.0 µg/g from control water, *Labeo dyocheilus* from polluted sites had 154.7±56.7 µg/g and 175.3±61.7 µg/g and had 51.3±25.8 µg/g from control site, *Cyprinus carpio* from polluted water had 98.7±38.9 µg/g and 117.0±37.4 µg/g and had 92.3±29.9 µg/g from site 3 and *Ompok bimaculatus* from site 1 and site 2 had 138.7±84.0 µg/g and 139.7±105.5 µg/g and had 131.0±65.2 µg/g from control site 3 e(Warsak dam) respectively. Nickel accumulation order in skin of different fish species was *Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala>Cyprinus carpio>Wallago attu*. This shows that *Labeo dyocheilus* accumulated highest zinc level and *Wallago attu* accumulated lowest content. By comparison our data with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that nickel concentration in skin of all five inhabitant fish species from River Kabul

were above the RDA recommended limits, which is 10 µg/g/100g for nickel. The present finding found highest level of nickel in skin of different fish species from polluted sites. These results are agreed with the studies of those reported in previous findings (Yilmaz, 2003; Tjalve *et al*., 1988; Ptashynski *et al*., 2002). Therefore all the studied fish from polluted sites are not suitable for human consumption and proved to be highly toxic. Comparing the above mentioned studies with our finding revealed that River Kabul has more concentration of nickel as compare to other mentioned water resources. However, in this study skin of *Labeo dyocheilus* accumulated higher concentration of Ni as compare to other fish species. The skin of examined fish showed greater amount of Ni as compare to the fish of control site. This finding also indicated higher level of this metal in water of polluted sites than control water.

In this investigation chromium concentration in skin of different fish species from polluted sites was high as compare to control site. As compare to liver, gills and muscle, skin had accumulated higher content of chromium metal but this concentration was lower than the intestine from sites 1 and 2. Higher Cr concentration in skin of *Wallago attu* from both polluted sites 1 and 2 were 515.7±394.2 µg/g and 542.0±410.0 µg/g and lower content was 15.3±13.7 µg/g from control site 3, in *Aorichthys seenghala* from polluted sites were 615.3±402.5 µg/g and 656.3±403.0 µg/g and was 6.7±3.8 µg/g from control site, in *Labeo dyocheilus* from polluted sites 1 and 2 were 692.0±145.9 µg/g and 725.7±140.3 µg/g and was 76.3±45.7 µg/g from site 3, in *Cyprinus carpio* from polluted water were 459.0±120.0µg/g and 517.0±207.3 µg/g and was 46.0±20.8 µg/g from control water and in *Ompok bimaculatus* from sites 1 and 2 were 621.7±405.3 µg/g and 676.3±432.7 µg/g and was 50.3±51.0 µg/g from site 3 respectively. Chromium concentration in skin of different fish species was in the sequence of *Aorichthys seenghala*>*Labeo dyocheilus*>*Ompok bimaculatus*>*Wallago attu*>*Cyprinus carpio*. This highlights that *Aorichthys seenghala* showed highest concentration of chromium and *Cyprinus carpio* lowest content. Comparing our results with RDA maximum limits for human

consumption, it is cleared that nickel metal concentration in skin of all five inhabitant fish species from polluted sites of River Kabul were above the RDA proposed limits, which is 50-200µg/g/100g for chromium. Highest Ni concentration was observed in skin sample collected from polluted sites. Ni of the study areas was higher than those reported in skin of other fish species (Robinoson *et al*., 2004; Yousafzai, 2004).

Skin of *Wallago attu* from polluted sites contained maximum concentration of copper with mean values of 108.7±97.8 µg/g and 373.3±176.5 µg/g and contained minimum value of 96.7±90.4 µg/g from control site, *Aorichthys seenghala* from polluted sites contained 196.7±181.5 µg/g and 219.0±188.3 µg/g and contained 64.3±33.5 µg/g from control site, *Labeo dyocheilus* from both polluted sites 1 and 2 contained 198.0±146.0 µg/g and 223.3±167.4 µg/g and contained 66.7±36.9 µg/g from control site, *Cyprinus carpio* from polluted sites contained 144.0±76.2µg/g and 165.0±81.6 µg/g and contained 84.3±39.8 µg/g from site 3 and *Ompok bimaculatus* from both polluted sites 1 and 2 contained 140.7±60.3 µg/g and 166.0±62.7 µg/g and contained 62.3±38.9 µg/g from control site respectively. Copper concentration in skin of different examined fish species was in the order of *Wallago attu>Labeo dyocheilus>Aorichthys seenghala>Ompok bimaculatus>Cyprinus carpio*. This indicates that *Wallago attu* accumulated highest copper concentration and *Cyprinus carpio* lowest concentration. Comparing our findings with RDA maximum limits of Cu (in 100gm of the skin) for human consumption, it is cleared that copper metal concentration in skin of all five fish species from River Kabul were below the RDA proposed limits, which is 2000-3000 µg/g/100g for copper. In this result, values of copper were higher as compare to the values mentioned in previous studies (Yilmaz, 2003; Carvalho *et al*., 2002; Yousafzai, 2004). Comparing the other studies with our data revealed that skin of different fish accumulated greater content of metals as compare to other studied fish of the world. This high concentration in fish skin could be attributed to more Cr concentration in the water and exposition of the fish to this metal for long period.

Skin of *Wallago attu* from sites 1 and 2 accumulated highest concentration of Cd with mean values of 70.0±34.7 µg/g and 82.3±41.6 µg/g and accumulated 26.0±16.3µg/g from site 3, *Aorichthys seenghala* from polluted sites accumulated 60.7±36.9 µg/g and 77.3±46.9 µg/g and accumulated 47.7±28.3 µg/g from control site, *Labeo dyocheilus* from polluted water accumulated 68.0±16.5 µg/g and 85.7±20.3 µg/g and accumulated 22.3±8.9µg/g from control water, *Cyprinus carpio* from both sites 1 and 2 accumulated 62.0±20.4 µg/g and 72.7±24.9 µg/g and accumulated 29.3±17.0 µg/g from site 3 and *Ompok bimaculatus* from polluted water accumulated 70.7±37.1 µg/g and 83.3±44.5 µg/g and accumulated 49.3±43.9 µg/g from reference site respectively. Cadmium concentration in skin of different fish species was in the order of *Labeo dyocheilus*>*Ompok bimaculatus*>*Wallago attu* > *Aorichthys seenghala*>*Cyprinus carpio*, This revealed that *Labeo dyocheilus* had accumulated highest concentration of cadmium and *Cyprinus carpio* lowest concentration. By comparing our findings with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that cadmium concentration in skin of all five inhabitant fish species from both control and polluted sites of River Kabul was above the RDA propose limits. Which is 14 µg/g/100g for cadmium. Therefore all the studied fish from River Kabul are not suitable for human consumption. In this study content of Cd was higher than those reported by Yilmaz (2003). Comparing other findings with our study indicates that River Kabul has more concentration of Cd as compare to other mentioned water resources. However, in this study skin of *Labeo dyocheilus* had accumulated higher concentration of Cd as compare to the other fish species.

Skin of *Wallago attu* from polluted sites showed highest concentration of Pb with mean values of 669.0±619.0 µg/g and 698.3±581.9 µg/g and lowest mean value of 79.0±43.6 µg/ from control site, *Aorichthys seenghala* from polluted sites showed 281.3±355.4 µg/g and 296.0±365.1 µg/g and showed 159.3±57.8 µg/g from site, *Labeo dyocheilus* from polluted water showed 378.3±487.5 µg/g and 405.3±517.0

µg/g and showed 43.7±22.2 µg/g from Warsak dam, *Cyprinus carpio* from polluted water showed 150.0±128.3 µg/g and 185.7±163.1 µg/g and showed 60.0±17.5 µg/g from control site and *Ompok bimaculatus* from polluted sites showed 101.7±92.1 µg/g and 485.3±423.5 µg/g and showed 27.0±20.2 µg/g from control site respectively. Overall order of lead accumulation in this organ of different fish species was *Wallago attu* > *Ompok bimaculatus* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Cyprinus carpio*.This indicates that *Wallago attu* was having high accumulation of metals, while *Cyprinus carpio* accumulated less concentration. Comparing these values with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that lead concentration in skin of *Wallago attu, Ompok bimaculatus* and *Labeo dyocheilus* was above the RDA proposed limits, which is 300 µg/g/100g for lead. But in the rest of two fish the lead level was below the RDA limits. Therefore *Wallago attu, Ompok bimaculatus* and *Labeo dyocheilus* from polluted sites are not suitable for human consumption and can prove highly toxic. The present finding found highest level of nickel in skin of different fish species from polluted sites as compare to those from Warsak dam. These results are in consistent with other findings (Yilmaz, 2003; Heath, 1991; Markey, 1978; Uluzlu *et al*., 2007). In another investigation, Yousafzai (2004) had also recorded high level of lead in skin of fish, *Tor putitora caught* from the same water resources of River Kabul to verify the validity of our investigation.

Skin of *Wallago attu* from polluted sites had accumulated maximum amount of iron with mean values of 102.0±28.5 µg/g and 122.0±36.0 µg/g and had accumulated minimum values of 32.0±11.3 µg/g from controle site, *Aorichthys seenghala* from polluted sites 1 and 2 had accumulated 93.0±27.2 µg/g and 113.0±34.7 µg/g and had accumulated 24.0±9.8 µg/g from control site 3, *Labeo dyocheilus* from polluted water had accumulated 90.0±26.8 µg/g and 110.0±34.2 µg/g and had accumulated 22.0±9.3 µg/g from Warsak dam, *Cyprinus carpio* from polluted water had accumulated 97.0±27.9 µg/g and 117.0±35.3 µg/g and had accumulated 31.0±11.1 µg/g from reference site 3 and *Ompok bimaculatus* from both site 1 and site

2 had accumulated 95.0±27.5 µg/g and 115.0±35.0 µg/g and had accumulated 27.0±10.3µg/g from control site 3 respectively. These results are in agreement with the findings of Yilmaz (2003). Iron bioaccumulation in skin of different fish species was in order of *Wallago attu>Cyprinus carpio >Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus.*This shows that metal bioaccumulation is highest in *Wallago attu* and lowest in *Labeo dyocheilus*. Comparing these values with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that iron metal concentration in skin of all five inhabitant fish species was below the RDA proposed limits, which is 500-2000 µg/g/100g for iron. As compare to other investigations of the world, the present study found the Fe level high in skin tissue. By comparison with other studied tissues, the skin also showed maximum concentration of Fe.

Mn level in skin of *Wallago attu* from polluted sites were 87.0±26.3 µg/g and 107.0±33.8 µg/g and was 40.0±12.6 µg/g from control site, in *Aorichthys seenghala* from polluted sites 1 and 2 were 80.0±25.3 µg/g and 100.0±32.7 µg/g and was 33.0±11.4 µg/g from control site, in *Labeo dyocheilus* from sites 1 and 2 were 78.0±25.0 µg/g and 98.0±33.6 µg/g and was 31.0±11.1 µg/g from site 3, in *Cyprinus carpio* from polluted sites were 85.0±26.0 µg/g and 105.0±33.4 µg/g and was 38.0±12.3 µg/g from reference site and in *Ompok bimaculatus* from polluted water were 82.0±25.6 µg/g and 102.0±33.0 µg/g and was 35.0±11.3 µg/g from control water respectively. Manganese bioaccumulation in this organ of different fish was in the order of *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus*. This highlights that manganese content was highest in *Wallago attu* and lowest in *Labeo dyocheilus*. By comparing our result with RDA maximum limits (100gm of Mn in skin) for human consumption, it is cleared that manganese metal concentration in skin of all five inhabitant fish species from River Kabul was below the proposed RDA limits, which is 2500-5000 µg/g/100g for manganese. Mn of the present study was higher than those reported in skin of other fish species

(Yousuf *et al*., 2000; Mansour and Sidky, 2000). Skin from polluted sites showed high level of Mn as compare to control site of River Kabul. By comparing the present study with the above mentioned findings revealed that skin of selected fish in this study had accumulated high level of this metal than other studied fish of the world. This is because of direct and constant exposition of this tissue to Mn metal in water at polluted sites.

Skin of different fish from polluted sites 1 and 2 showed highest level of mercury as when compared with Warsak dam, where lowest concentration of mercury was found from control site 3. Hg in skin of *Wallago attu* from sites 1 and 2 showed more values of 83.0±25.8 µg/g and 103.0±33.1µg/g and showed less value of 27.0±10.3 µg/g from control site 3, *Aorichthys seenghala* from both site 1 and site 2 showed 75.0±24.4 µg/g and 95.0±31.8 µg/g and showed 21.0±9.1 µg/g from site 3, *Labeo dyocheilus* from polluted sites showed 73.0±24.1 µg/g and 93.0±31.5 µg/g and showed 18.0±8.4 µg/g from control site, *Cyprinus carpio* from polluted water showed 80.0±25.3 µg/g and 100.0±32.7 µg/g and showed 25.0±10.0 µg/g from reference site (Warsak dam) and *Ompok bimaculatus* from polluted sites showed 78.0±25.0 µg/g and 98.0±33.6 µg/g and showed 23.0±9.5 µg/g from control site respectively. These results are agreed with the findings of previous workers (Lazorchak *et al*., 2003; Bosnir *et al*., 2003; Matthew, 1992; Juresa and Blanusa, 2000). Mercury bioaccumulation in skin of different fish species was in the order of *Wallago attu*> *Cyprinus carpio*>*Ompok bimaculatus*>*Aorichthys seenghala*>*Labeo dyocheilus*. This indicates that mercury level was highest in skin of *Wallago attu* and was lowest in *Labeo dyocheilus*. Comparing these values with RDA maximum limits (in 100gm of the skin) for human consumption, it is cleared that mercury metal concentration in skin of all studied fish from polluted and reference sites was above the RDA proposed limits, which is 8µg/g/100g for mercury. Therefore these fish are not suitable for human consumption and proved to be highly toxic.

Fish skin is also consumed along with muscles in most of the rural population of the world, therefore researchers also have an emphasis on just tissues, while investigating muscle. Inhabitants living around the River Kabul also eat fish skin along with muscle. Adsorption on the skin surface followed by their absorption in the skin tissue by various mechanisms favours the accumulation of metals in skin. Comparing the present concentration of heavy metals in skin of different fish species with RDA limits showed that heavy metals such as Ni, Cd, Pb and Hg in skin were above the permissible limits proposed by RDA and the rest of metals were below the RDA proposed limits. The fish from polluted areas are not suitable for consumption. Comparing our study with the findings of Yousafzai (2004) revealed that a further increase of heavy metals in different tissues of selected fish has occurred, which reflects a further increase in the heavy metal concentration in the water i.e a further increase of heavy metals pollution in the River Kabul in the last ten years, which is suggestive for strict implementation of the environmental laws and routine biomonitoring programme.

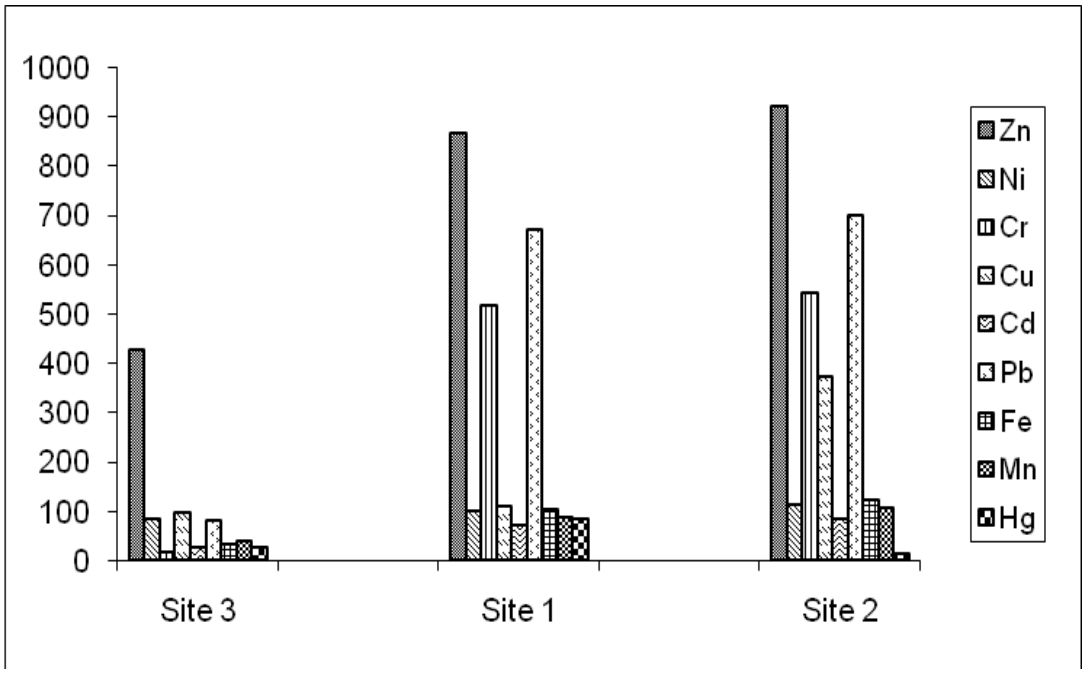
Table 4.3: Heavy metal concentrations (µg/g wet weight) in skin of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Analytes (µg/g)	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Zn	425.7±138.0	866.3±714.8	921.0±527.0
	Ni	85.3±40.2	100.3±24.0	113.0±27.3
	Cr	15.3±13.7	515.7±394.2	542.0±410.0
	Cu	96.7±90.4	108.7±97.8	373.3±176.5
	Cd	26.0±16.3	70.0±34.7	82.3±41.6
	Pb	79.0±43.6	669.0±619.0	698.3±581.9
	Fe	32.0±11.3	102.0±28.5	122.0±36.0
	Mn	40.0±12.6	87.0±26.3	107.0±33.8
	Hg	27.0±10.3	83.0±25.8	103.0±33.1
Aorichthys seenghala	Zn	358.7±171.2	2691.0±1766.8	2768.3±1835.4
	Ni	66.3±41.0	118.3±81.5	131.7±90.7
	Cr	6.7±3.8	615.3±402.5	656.3±403.0
	Cu	64.3±33.5	196.7±181.5	219.0±188.3
	Cd	47.7±28.3	60.7±36.9	77.3±46.9
	Pb	159.3±57.8	281.3±355.4	296.0±365.1
	Fe	24.0±9.8	93.0±27.2	113.0±34.7
	Mn	33.0±11.4	80.0±25.3	100.0±32.7
	Hg	21.0±9.1	75.0±24.4	95.0±31.8
Labeo dyocheilus	Zn	406.3±123.7	1945.7±930.9	1987.0±956.7
	Ni	51.3±25.8	154.7±56.7	175.3±61.7
	Cr	76.3±45.7	692.0±145.9	725.7±140.3
	Cu	66.7±36.9	198.0±146.0	223.3±167.4
	Cd	22.3±8.9	68.0±16.5	85.7±20.3
	Pb	43.7±22.2	378.3±487.5	405.3±517.0
	Fe	22.0±9.3	90.0±26.8	110.0±34.2
	Mn	31.0±11.1	78.0±25.0	98.0±33.6
	Hg	18.0±8.4	73.0±24.1	93.0±31.5
Cyprinus carpio	Zn	442.7±150.8	4579.3±2585.8	4775.0±2578.2
	Ni	92.3±29.9	**98.7±38.9**	**117.0±37.4**
	Cr	46.0±20.8	459.0±120.0	517.0±207.3
	Cu	84.3±39.8	144.0±76.2	165.0±81.6
	Cd	29.3±17.0	62.0±20.4	72.7±24.9
	Pb	60.0±17.5	150.0±128.3	185.7±163.1
	Fe	31.0±11.1	97.0±27.9	117.0±35.3
	Mn	38.0±12.3	85.0±26.0	105.0±33.4
	Hg	25.0±10.0	80.0±25.3	100.0±32.7
Ompok bimaculatus	Zn	390.3±180.0	1255.3±610.9	1328.0±625.9
	Ni	131.0±65.2	**138.7±84.0**	**139.7±105.5**
	Cr	50.3±51.0	621.7±405.3	676.3±432.7
	Cu	62.3±38.9	140.7±60.3	166.0±62.7
	Cd	49.3±43.9	70.7±37.1	83.3±44.5
	Pb	27.0±20.2	101.7±92.1	485.3±423.5
	Fe	27.0±10.3	95.0±27.5	115.0±35.0
	Mn	35.0±11.3	82.0±25.6	102.0±33.0
	Hg	23.0±9.5	78.0±25.0	98.0±33.6

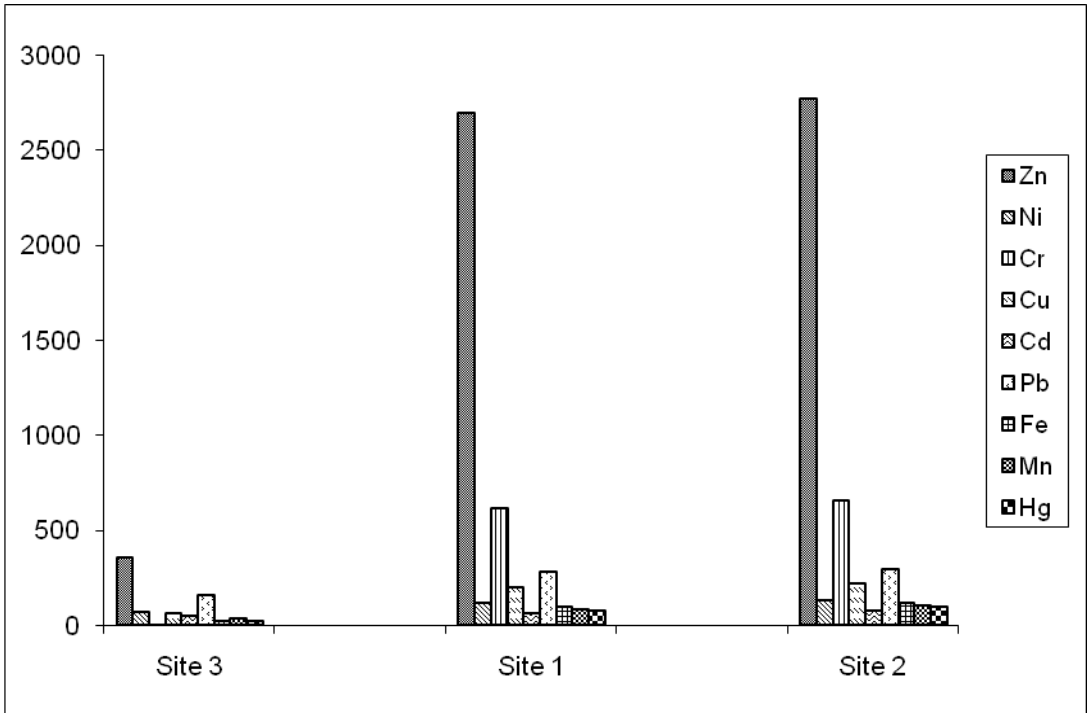
P<0.05, **P>0.05**. (Values in bold are non-significant)

Table 4.4: U.S Recommended Daily Dietary Allowance (RDA) supplied by a 100g of fish skin.

Metals	Miligram (mg)	Microgram (µg)
Cd	0.014	14
Hg	0.008	8
Pb	0.3	300
Ni	0.01	10
Zn	2.6	2600
Fe	0.5-2.0	500-2000
Cu	2.0-3.0	2000-3000
Cr	0.05-0.20	50-200
Mn	2.5-5.0	2500-5000

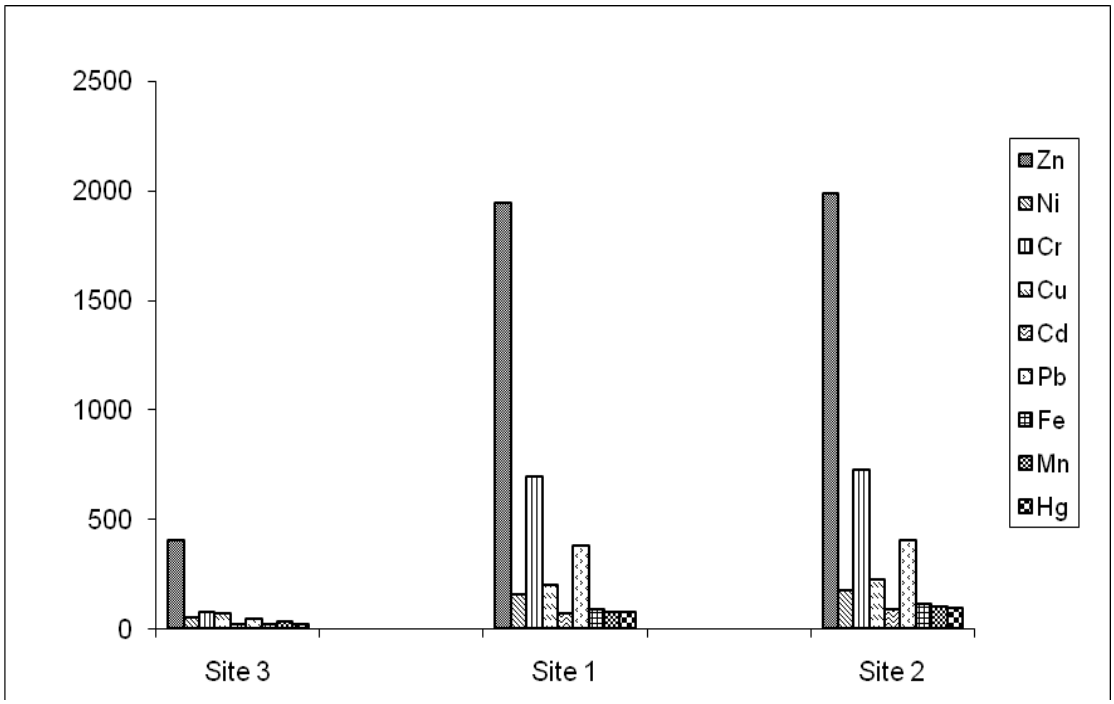


Wallago attu

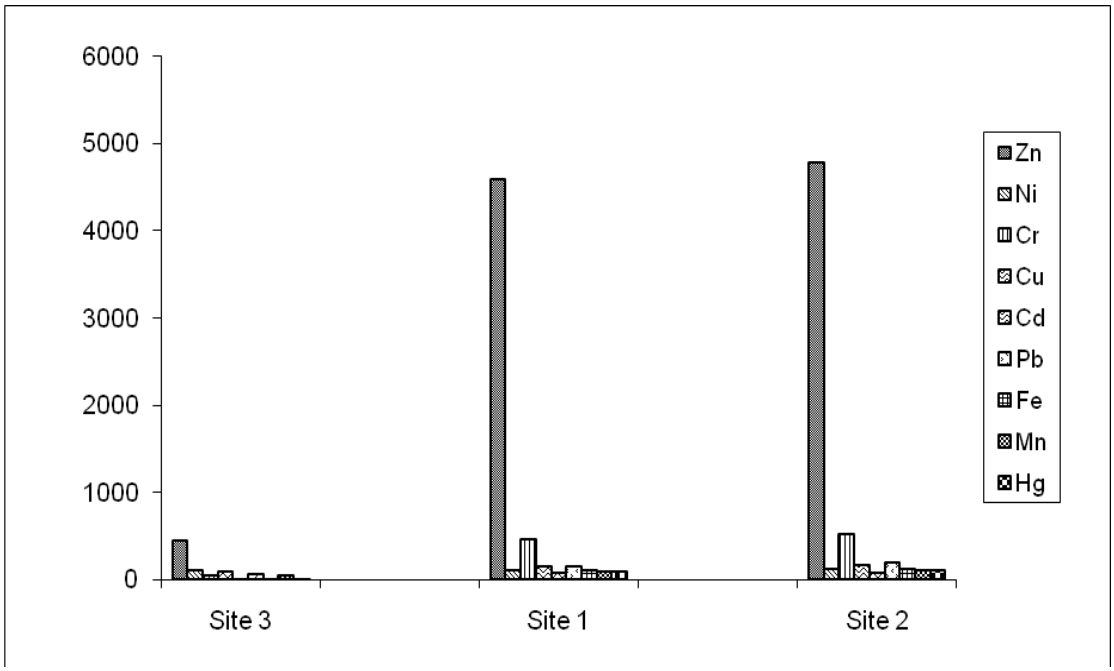


Aorichthys seenghala

Fig.4.5 **Heavy metal concentrations in skin of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

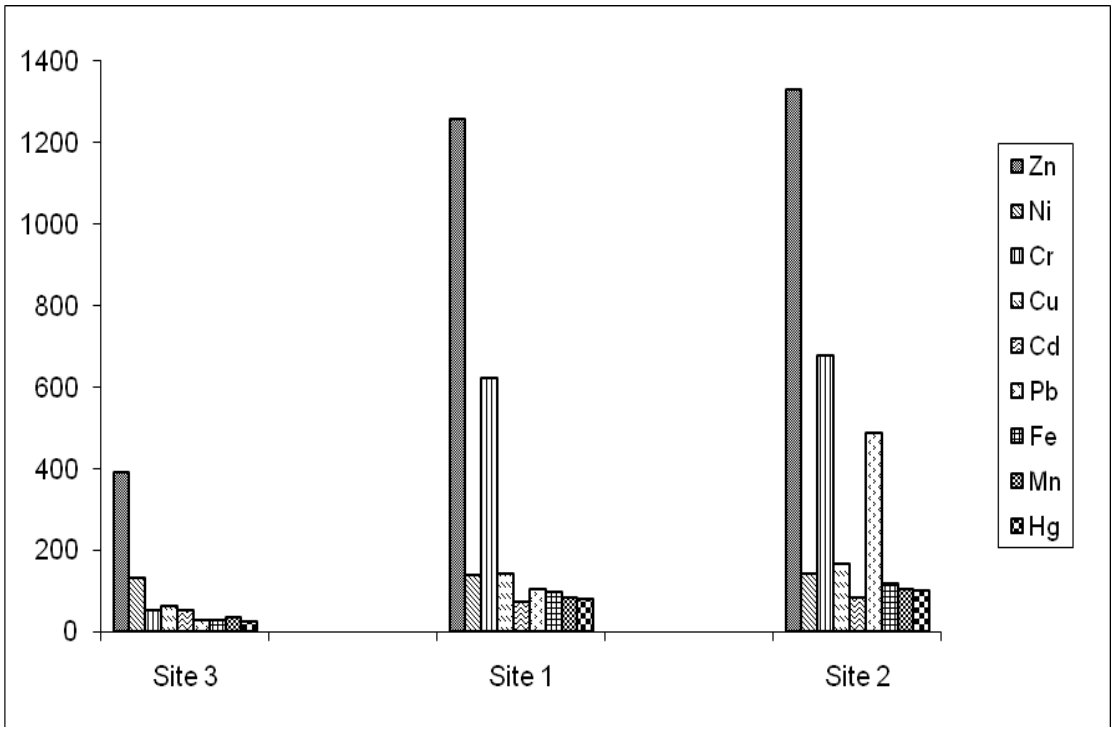


Labeo dyocheilus



Cyprinus carpio

Fig.4.6: **Heavy metal concentrations in skin of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**



Ompok bimaculatus

Fig. 4.7: **Heavy metal concentrations in skin of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

4.3.3 Bioaccumulation of Heavy Metals in Intestine

Intestine of different fish species from control site 3 and polluted sites 1 and 2 was taken out and processed for determination of Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg. Intestine of examined fish species from site 1 and site 2 (polluted) showed higher concentration of heavy metals than the fish from reference site 3 (Warsak dam). In this finding all the investigated metals in intestine of different fish from polluted water showed increasing tendency as compare to control water (Table 4.5 and Figs 4.8-4.10). The possible reasons for this tremendous increase of each metal level in this tissue could be correlated to mining activities in the surrounding hills, agricultural activities, city sewage, industrial effluents and other anthropogenic activities at polluted sites of River Kabul. Intestine is the organ in the body where the heavy metals are absorbed to the whole body and plays a vital role in the bioaccumulation of metals.

Zinc had highest concentration in intestine of *Wallago attu* from sites 1 and 2 with mean values 449.3±317.0 µg/g and 490.0±360.7 µg/g and had lowest content of Zn with mean value of 431.0±148.3 µg/g from site 3, *Aorichthys seenghala* from polluted water had 970.0±452.6 µg/g and 999.1±653.9 µg/g and had 366.0±165.2 µg/g from reference water, *Labeo dyocheilus* from polluted sites had 828.7±503.4 µg/g and 870.0±535.5 µg/g and had 412.0±123.8 µg/g from control site, *Cyprinus carpio* from polluted water had 8176.7±6563.9 µg/g and 8423.0±6561.7 µg/g and had 447.0±150.0 µg/g from control water and *Ompok bimaculatus* from polluted sites had 1016.0±798.0 µg/g and 1097.3±785.8 µg/g and had 393.7±179.98 µg/g from Warsak dam respectively. Zn bioaccumulation in intestine of different fish species was in the sequence of *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala >Labeo dyocheilus > Wallago attu*. This indicates that zinc is highest in *Cyprinus carpio* and lowest in *Labeo dyocheilus*. These results are in agreement with those observed by many investigators (Olaifa *et al*., 2004; Turkmen *et al*., 2005; Hsien

Chen and Young Chen, 1999). Comparing the above studies with our study showed enormously high concentration of Zn in our findings and reflecting that River Kabul has more concentration of Zn as compare to other mentioned water resources. This is most probably due to mining activities in the upper streches of River Kabul as is also reported by Yousafzai (2004).

Nickel in intestine of *Wallago attu* from both polluted sites contained maximum concentration with mean values of 125.0±82.9 µg/g and 139.3±88.0 µg/g and contained minimum mean value of 91.3±40.8 µg/g from control site, *Aorichthys seenghala* from polluted water contained 127.7±71.4 µg/g and 138.3±72.8 µg/g and contained 69.7±42.7 µg/g from control water, *Labeo dyocheilus* from polluted site 1 and site 2 contained 350.7±264.0 µg/g and 404.7±423.1 µg/g and contained 54.7±25.0 µg/g, *Cyprinus carpio* from polluted sites contained 103.0±28.6 µg/g and 114.3±33.3 µg/g and contained 100.0±40.1 µg/g from control site 3 and *Ompok bimaculatus* from both sites 1and 2 contained 122.3±86.2 µg/g and 144.3±90.9 µg/g and contained 136.0±67.9 µg/g from reference site respectively. The mean values of nickel in this organ of selected studied fish followed the order: *Labeo dyocheilus* > *Ompok bimaculatus* > *Wallago attu* > *Aorichthys seenghala* > *Cyprinus carpio.*This shows that nickel metal bioaccumulation is highest in intestine of *Labeo dyocheilus* and lowest in *Cyprinus carpio*. The present result of higher Ni content in intestine of *Labeo dyocheilus, Ompok bimaculatus* agree with the findings of Thielen *et al* (2004) and Ptashynski and Klaverkamp (2002). On the other hand, the present data for *Wallago attu, Aorichthys seenghala* and *Cyprinus carpio* agree with those of Demirezen and Uruc (2006) and Turkmen*et al* (2005).

The present study found higher content of Cr in intestine of examined fish from polluted sites as compare to control site. Cr level was highest in intestine as compare to other studied tissues including gills, skin, liver and muscle. Intestine of *Wallago attu* from polluted sites 1 and 2 accumulated highest concentration of Cr

with mean values of 461.3±355.4 µg/g and 471.0±360.7 µg/g and accumulated lowest concentration with mean value of 18.7±16.4 µg/g from control site 3, *Aorichthys seenghala* from sites 1 and 2 accumulated 471.0±427.1 µg/g and 506.0±457.4 µg/g and accumulated 9.0±4.9 µg/g from control site, *Labeo dyocheilus* from polluted water accumulated 849.0±396.0µg/g and 890.0±406.3µg/g and accumulated 81.7±38.2 µg/g from control water, *Cyprinus carpio* from polluted sites 1 and 2 accumulated 483.7±243.0µg/g and 513.7±245.5 µg/g and accumulated 50.0±23.1 µg/g from reference site and *Ompok bimaculatus* from polluted sites accumulated 644.7±358.1 µg/g and 701.3±378.0 µg/g and accumulated 54.3±52.0 µg/g from control site respectively. Cr bioaccumulation in intestine of different fish species was in the order of *Labeo dyocheilus*>*Ompok bimaculatus*>*Cyprinus carpio*>*Wallago attu*>*Aorichthys seenghala*. This indicates that Cr bioaccumulation is highest in *Labeo dyocheilus* and lowest in *Aorichthys seenghala*. These results are agreed with the findings of previous workers (Robinoson *et al*., 2004; Thielen *et al*., 2004; Olaifa *et al*., 2004). However, in the present study intestine of *Labeo dyocheilus* had accumulated higher concentration of Cr as compare to the other fish species from control site.

In this study intestine of *Wallago attu* from polluted sites had accumulated maximum concentration of copper with mean values of 89.0±89.4 µg/g and 377.7±274.1 µg/g and had accumulated minimum value of 82.3±83.3 µg/g from control site, *Aorichthys seenghala* from polluted sites had accumulated 157.7±99.5 µg/g and 176.0±102.5 µg/g and had accumulated 69.0±37.9 µg/g from control site, *Labeo dyocheilus* from both sites 1 and 2 had accumulated 286.3±211.7 µg/g and 314.0±224.5 µg/g and had accumulated 69.7±38.2 µg/g from reference site, *Cyprinus carpio* from polluted water had accumulated 115.0±110.7 µg/g and 136.0±125.5 µg/g and had accumulated 86.7±40.7 µg/g from control water and *Ompok bimaculatus* from both site 1 and site 2 had accumulated 160.7±114.2 µg/g and 185.3±134.0 µg/g and had accumulated 65.3±39.8 µg/g from reference site 3 respectively. Copper

bioaccumulation in intestine of different fish species was in the order of *Wallago attu >Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala>Cyprinus carpio.* This highlights that metal bioaccumulation is highest in intestine of *Wallago attu* and lowest in *Cyprinus carpio*. High levels of Cu in intestine from polluted portions of River Kabul being determined in this study are in agreement with many previous studies of those, who also examined highest accumulation of Cu in intestine of other fish species (Lundebye *et al*., 1999; Thielen *et al*., 2004; Olaifa *et al*., 2004; Dethloff *et al*., 2001). Comparing the above findings with our study is reflecting that River Kabul has more concentration of Cu as compare to other mentioned water resources of the remaining world. However the present study found the intestine of *Wallago attu* to be accumulated higher concentration of Cu as compare to other fish species from control water.

Intestine of *Wallago attu* from polluted sites contained maximum concentration of cadmium with mean values of 70.0±23.5 µg/g and 82.3±35.0 µg/g and contained minimum mean value of 29.3±21.4 µg/g from control site 3, *Aorichthys seenghala* from polluted sites 1 and 2 contained 67.0±26.9 µg/g and 80.3±37.2 µg/g and contained 50.3±29.4 µg/g from site 3, *Labeo dyocheilus* from polluted sites contained 75.7±24.0 µg/g and 90.7±28.2 µg/g and contained 25.7±10.8µg/g, *Cyprinus carpio* from both site 1 and site 2 contained 71.0±25.9 µg/g and 85.0±72.8 µg/g and contained 31.3±18.1 µg/g from site 3 and *Ompok bimaculatus* from polluted sites contained 76.7±41.0 µg/g and 92.0±42.9 µg/g and contained 52.3±45.0 µg/g from reference site respectively. In intestine of different fish species cadmium accumulation was in the order of *Labeo dyocheilus > Ompok bimaculatus > Cyprinus carpio >Wallago attu > Aorichthys seenghala.*This indicates that *Labeo dyocheilus* had accumulated highest concentration of cadmium and *Aorichthys seenghala* accumulated lowest concentration. The present study found maximum contents of Cd in intestine of studied fish from polluted water are supported by various studies (Thielen *et al*., 2004; Berntssen *et al*., 2003; Lundebyee *et al*., 1999; Fatma and

Mohamed, 2008). High concentration of Cd in intestine of *Labeo dyocheilus* could be related to greater content of this metal in the water of study area and more exposition of the fish to this metal for long period. The result also showed metal pollution in the River Kabul.

Intestine of *Wallago attu* from polluted sites had highest lead concentration with mean values of 509.7±386.1 µg/g and 801.7±199.3 µg/g and had lowest mean value of 82.7±38.4 µg/g from control site (Warsak dam), *Aorichthys seenghala* from polluted water had 347.3±340.8 µg/g and 375.3±434.5 µg/g and had 163.7±77.8 µg/g from control site, *Labeo dyocheilus* from polluted site 1 and site 2 had 577.3±480.9 µg/g and 625.0±504.0 µg/g and had 46.7±21.7 µg/g from reference site, *Cyprinus carpio* from sites 1 and 2 had 1020.7±1321.8 µg/g and 1088.3±1387.4 µg/g and had 63.3±28.8 µg/g from control site 3 and *Ompok bimaculatus* from polluted sites had 435.0±325.9 µg/g and 480.0±324.5 µg/g and had 29.7±21.5 µg/g from control site respectively. These results were higher than the finding of Pham *et al* (2007). Pb is known to be accumulated in intestine tissue of fish (Latif *et al*., 1982; Dallas and Day, 1993). The overall order of lead accumulation in this tissue was in the sequence of *Cyprinus carpio*>*Wallago attu*>*Labeo dyocheilus*>*Ompok bimaculatus*>*Aorichthys seenghala*. *Cyprinus carpio* showed highest concentration of Pb and *Aorichthys seenghala* lowest concentration. In this study intestine of *Cyprinus carpio* had accumulated higher concentration of Pb as compare to the other fish species. However Noordhuis (2002) has also recorded Pb content with mean value of 18.2 ±2.2 (ppm) in intestine of crayfish. Comparing the present results with other findings revealed that intestine had accumulated more Pb level as compare to other examined tissues.

Intestine of selected fish species from polluted sites accumulated high level of Fe as compare to those from control site. Iron level in intestine of *Wallago attu* from polluted sites 1 and 2 were 105.0±29.0 µg/g 125.0±36.5 and µg/g and was 35.0±11.8 µg/g from control site, in *Aorichthys seenghala* from polluted site 1 and site 2 were

95.0±27.5 µg/g and 115.0±35.0 µg/g and was 27.0±10,3 µg/g from control site, in *Labeo dyocheilus* from sites 1 and 2 were 92.0±27.1 µg/g and 112.0±34.5 µg/g and was 24.0±9.8 µg/g from site 3, in *Cyprinus carpio* from polluted water were 101.0±28.4 µg/g and 121.0±35.5 µg/g and was 32.0±11.3 µg/g from control water and in *Ompok bimaculatus* from polluted sites were 98.0±63.1 µg/g and 110.0±34.2 µg/g and was 29.0±10.8µg/g from control site respectively. Overall sequence of Fe accumulation in this organ of studied fish was *Wallago attu>Cyprinus carpio >Aorichthys seenghala>Labeo dyocheilus>Ompok bimaculatus.Wallago attu* showed highest concentration of Fe and *Ompok bimaculatus* lowest concentration. This study observed greater content of Fe than those reported by Fatma and Mohamed (2008) and Adeniyi *et al* (2007). Comparing this study with the findings of other workers revealed that fish can accumulate metals in their tissues. The present result found more Fe concentration in the intestine as compare to other studied tissues. Intestine from polluted sites showed more Fe content as compare to those from control site, where the recorded value for Fe was lowest at control site. The result also showed heavy metals pollution in the study areas of River Kabul.The high accumulation of this metal in the intestine may be ralated to the fact that intestine plays an important role in accumulation and absorption of metals from digested food.

Mn amount in intestine of different fish species from polluted sites was greater as compare to those from control site. Intestine of *Wallago attu* from polluted sites showed highest concentration of manganese with mean values of 91.0±27.0 µg/g and 110.0±34.2 µg/g and showed lowest mean value of 42.0±13.0 µg/g from Warsak dam, *Aorichthys seenghala* from polluted sites 1 and 2 showed 83.0±25.8 µg/g and 103.0±72.1 µg/g and showed 35.0±11.8 µg/g from site 3, *Labeo dyocheilus* from polluted water showed 80.0±25.3 µg/g and 100.0±32.7 µg/g and showed 32.0±11.3 µg/g from reference water, *Cyprinus carpio* from sites 1 and 2 showed 88.0±26.5 µg/g and 108.0±33.9 µg/g and showed 40.0±12.6 µg/g from control site and *Ompok bimaculatus* from polluted sites showed 86.0±26.2 µg/g and 106.0±33.6 µg/g and

showed 37.0±12.1 µg/g from control site. In this study, concentration of Mn was higher than those reported by Mansour and Sidky (2000) and Rashed (2001), while lower than those reported by Amal *et al* (2012). Manganese bioaccumulation in intestine of studied fish species was in the order of *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus*. This reveales that manganese content was highest in *Wallago attu* and lowest in *Labeo dyocheilus*. The present investigation found the intestine from polluted sites accumulated more Mn as compare to those from control site. The intestine also showed maximum content of Mn as compare to the above mentioned investigations. The results also showed further increase of Mn in River Kabul in the last few years.

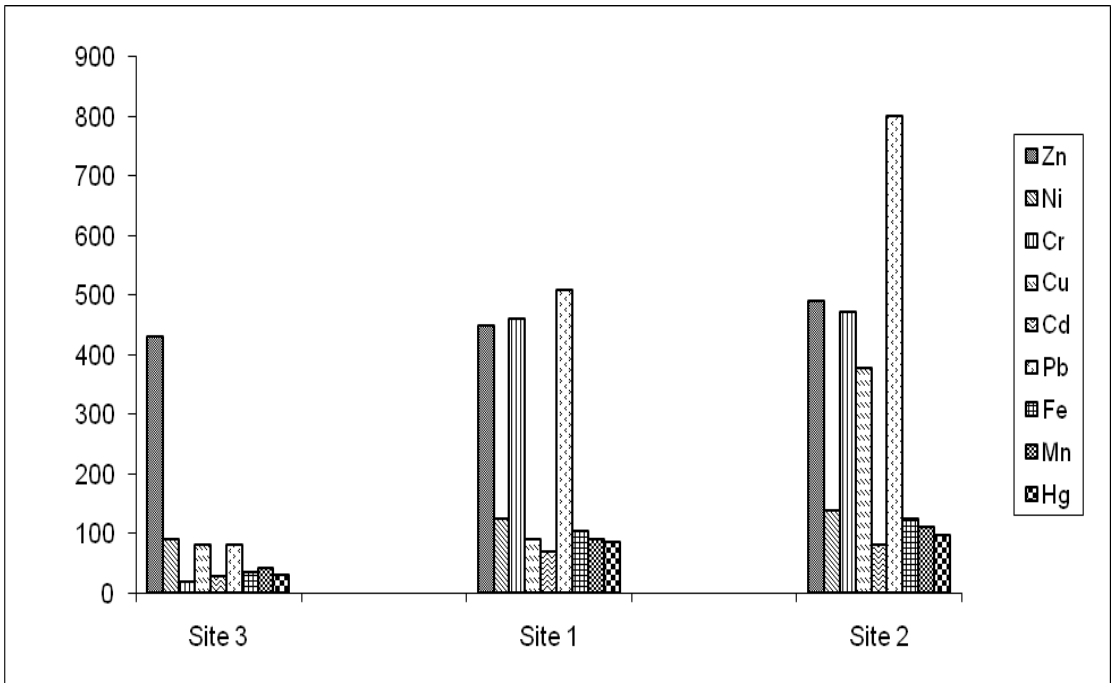
Intestine of different fish species from polluted sites showed highest level of mercury as compare with Warsak dam. Intestine of *Wallago attu* from site 1 and site 2 had more content of mercury with mean values of 83.0±25.8 µg/g and 103.0±33.1 µg/g and had less value of 27.0±10.3 µg/g from control site 3, *Aorichthys seenghala* from polluted water had 75.0±24.4 µg/g and 95.0±31.8 µg/g and had 21.0±9.1 µg/g from site 3, *Labeo dyocheilus* from polluted sites 1 and 2 had 73.0±24.1 µg/g and 93.0±31.5 µg/g and had 18.0±8.4 µg/g from control site 3, *Cyprinus carpio* from polluted sites had 80.0±25.3 µg/g and 100.0±32.7 µg/g and had 25.0±10.0 µg/g from control site and *Ompok bimaculatus* from polluted site 1 and site 2 had 78.0±25.0 µg/g and 98.0±33.6 µg/g and had 23.0±9.5 µg/g from control site respectively. This study found higher content of Hg than those reported by (Juresa and Blanusa, 2000; Bosnir *et al*., 2003). Mercury bioaccumulation in this organ of different fish species was in the sequence of *Cyprinus carpio >Aorichthys seenghala >Ompok bimaculatus >Wallago attu >Labeo dyocheilus.* This indicates that mercury level was highest in intestine of *Cyprinus carpio* and lowest in *Labeo dyocheilus*. The level of mercury was alarmingly high in intestine as compare to other examined tissues. Comparing the above findings with our study is reflecting that River Kabul has more concentration of Hg as compare to other mentioned water resources. However the present study found

the intestine of *Cyprinus carpio* to be accumulated higher concentration of Hg as compare to the other examined fish species.

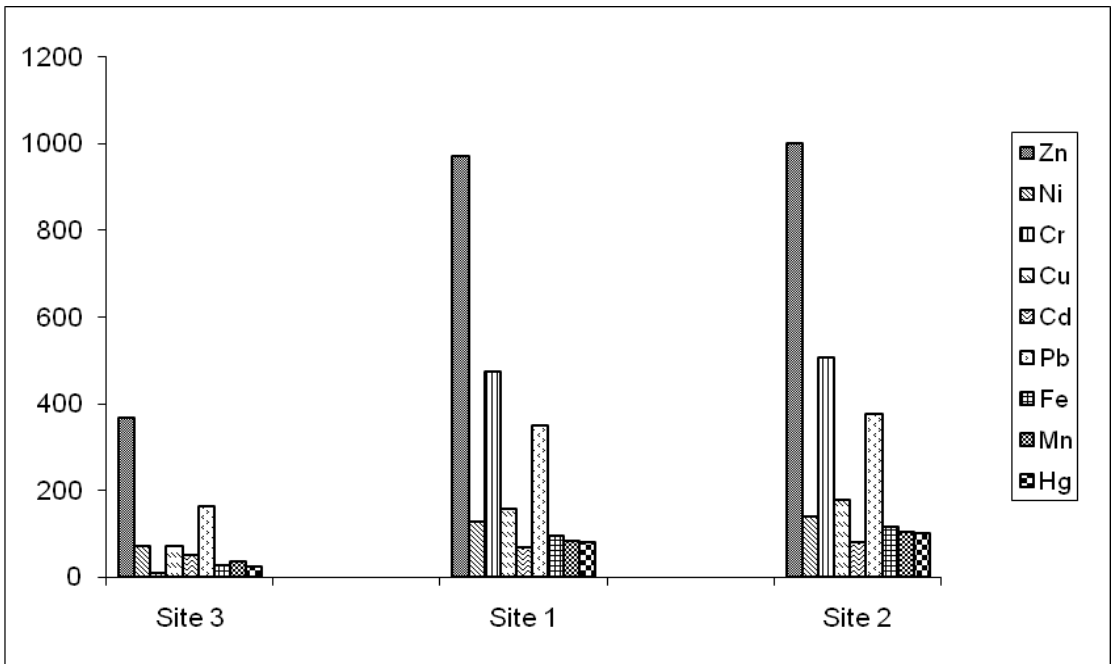
Table 4.5: Heavy metal concentrations (µg/g wet weight) in intestine of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Analytes (µg/g)	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Zn	431.0±148.3	449.3±317.0	490.0±360.7
	Ni	91.3±40.8	125.0±82.9	139.3±88.0
	Cr	18.7±16.4	461.3±355.4	471.0±360.7
	Cu	82.3±83.3	**89.0±89.4**	377.7±274.1
	Cd	29.3±21.4	70.0±23.5	82.3±35.0
	Pb	82.7±38.4	509.7±386.1	801.7±199.3
	Fe	35.0±11.8	105.0±29.0	125.0±36.5
	Mn	42.0±13.0	91.0±27.0	110.0±34.2
	Hg	30.0±10.9	85.0±26.0	97.0±32.1
Aorichthys seenghala	Zn	366.0±165.2	970.0±452.6	999.1±653.9
	Ni	69.7±42.7	127.7±71.4	138.3±72.8
	Cr	9.0±4.9	471.0±427.1	506.0±457.4
	Cu	69.0±37.9	157.7±99.5	176.0±102.5
	Cd	50.3±29.4	67.0±26.9	80.3±37.2
	Pb	163.7±77.8	347.3±340.8	375.3±434.5
	Fe	27.0±10,3	95.0±27.5	115.0±35.0
	Mn	35.0±11.8	83.0±25.8	103.0±72.1
	Hg	23.0±9.5	78.0±25.0	100.0±32.7
Labeo dyocheilus	Zn	412.0±123.8	828.7±503.4	870.0±535.5
	Ni	54.7±25.0	350.7±264.0	404.7±423.1
	Cr	81.7±38.2	849.0±396.0	890.0±406.3
	Cu	69.7±38.2	286.3±211.7	314.0±224.5
	Cd	25.7±10.8	75.7±24.0	90.7±28.2
	Pb	46.7±21.7	577.3±480.9	625.0±504.0
	Fe	24.0±9.8	92.0±27.1	112.0±34.5
	Mn	32.0±11.3	80.0±25.3	100.0±32.7
	Hg	20.0±8.9	76.0±24.7	96.0±32,0
Cyprinus carpio	Zn	447.0±150.0	8176.7±6563.9	8423.0±6561.7
	Ni	100.0±40.1	**103.0±28.6**	**114.3±33.3**
	Cr	50.0±23.1	483.7±243.0	513.7±245.5
	Cu	86.7±40.7	115.0±110.7	136.0±125.5
	Cd	31.3±18.1	71.0±25.9	85.0±72.8
	Pb	63.3±28.8	1020.7±1321.8	1088.3±1387.4
	Fe	32.0±11.3	101.0±28.4	121.0±35.5
	Mn	40.0±12.6	88.0±26.5	108.0±33.9
	Hg	27.0±10.3	83.0±25.8	103.0±72.1
Ompok bimaculatus	Zn	`393.7±179.8	1016.0±798.0	1097.3±785.8
	Ni	136.0±67.9	**122.3±86.2**	**144.3±90.9**
	Cr	54.3±52.0	644.7±358.1	701.3±378.0
	Cu	65.3±39.8	160.7±114.2	185.3±134.0
	Cd	52.3±45.0	76.7±41.0	92.0±42.9
	Pb	29.7±21.5	435.0±325.9	480.0±324.5
	Fe	29.0±10.8	98.0±63.1	110.0±34.2
	Mn	37.0±12.1	86.0±26.2	106.0±33.6
	Hg	25.0±10.0	80.0±25.3	100.0±32.7

P<0.05, **P>0.05**. (Values in bold are non-significant)

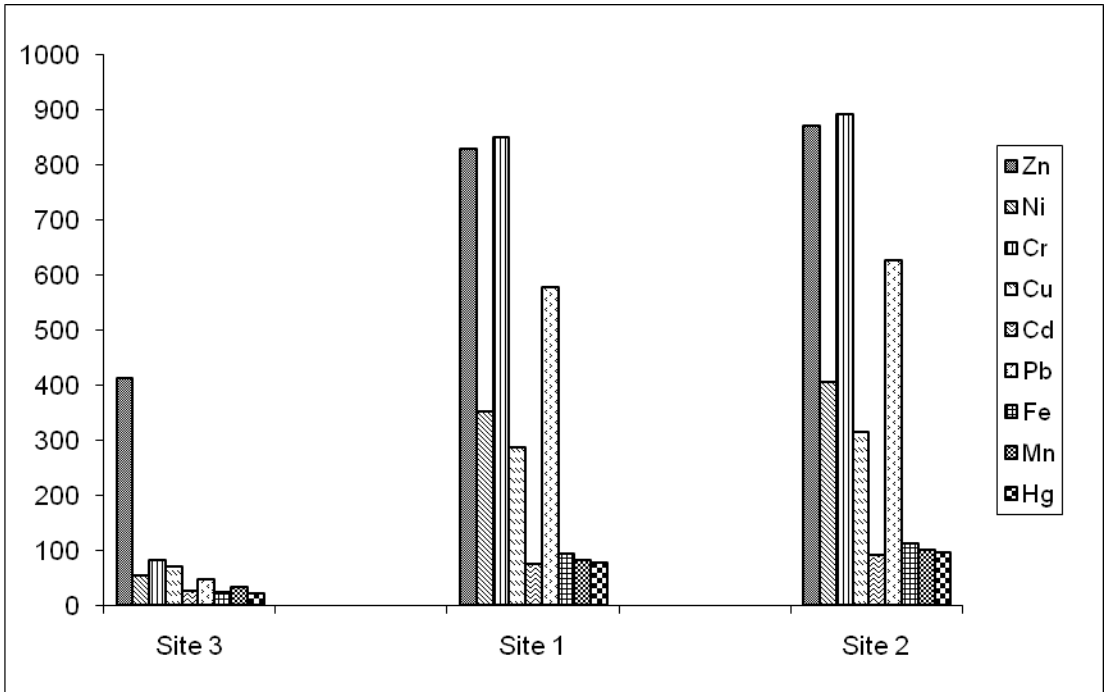


Wallago attu

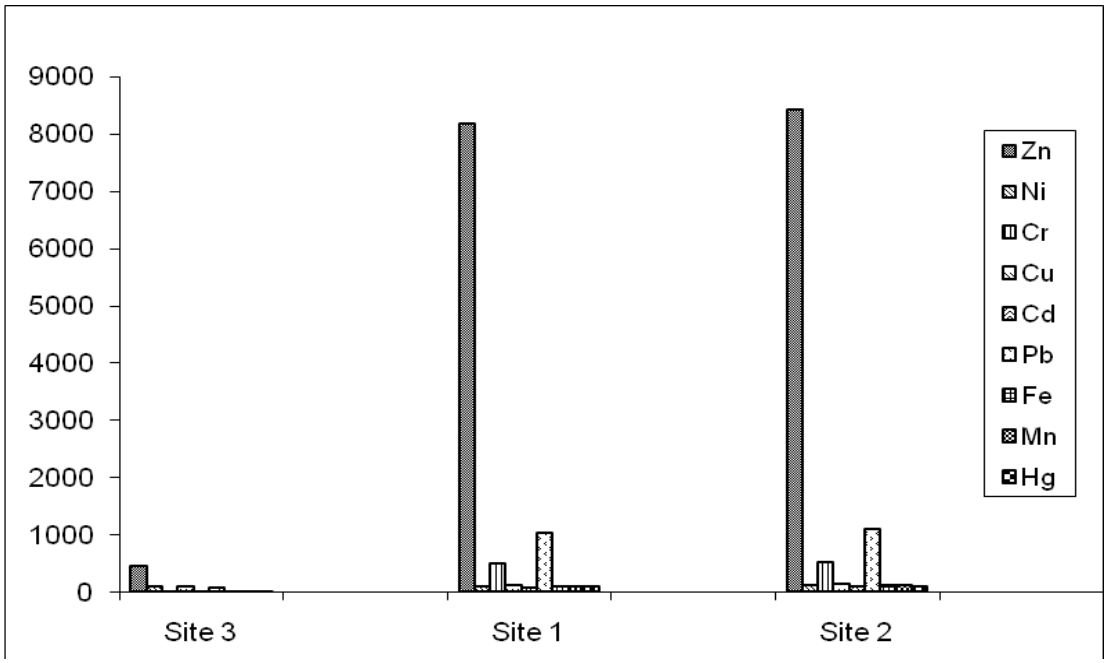


Aorichthys seenghala

Fig.4.8: **Heavy metal concentrations in intestine of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

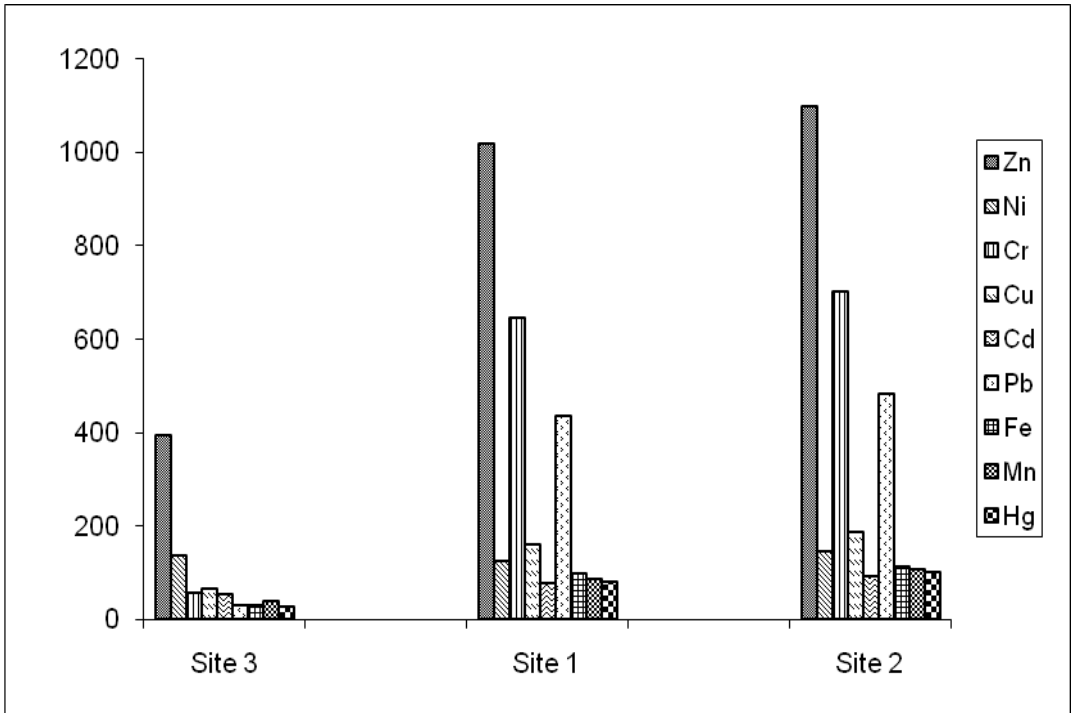


Labeo dyocheilus



Cyprinus carpio

Fig.4.9: **Heavy metal concentrations in intestine of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**



Ompok bimaculatus

Fig.4.10: Heavy metal concentrations in intestine of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

4.3.4 Bioaccumulation of Heavy Metals in Liver

Liver of five different fish from reference site 3 (Warsak dam) and polluted sites (sites 1 and 2) was collected and processed for determination of Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg. Liver of fish from site 1 and site 2 accumulated higher concentrations as compare with fish from site 3 (Table 4.6 and Figs 4.11-4.13). The present study found the liver to be accumulated less metals than intestine and skin and accumulated high metals than muscle and gills. Less accumulation of these metals in liver may be related to the fact that liver plays an important role in detoxification of metals. Liver in fish play a protective role against metal exposure by acting as a storage site and being a vital organ in the regulation of metals (Mccarter and Roch, 1983). Liver is considered as one of the major metal bioaccumulation organ (Heath, 1987; Canali and Furness, 1993). It is urged that analysis of the fish liver should has been done since metals tend to be stored in the liver as a detoxifying mechanism. The livers would therefore be a better indication of bioaccumulation of heavy metals (Sharon, 1990).

Liver of different fish from polluted sites showed highest concentration of heavy metals as compare to those from control site. Liver of *Wallago attu* from sites 1 and 2 contained highest concentration of Zn with mean values of 485.7±265.2 µg/g and 521.7±292.4 µg/g and contained lowest mean value of 413.0±145.2 µg/g from Warsak dam, *Aorichthys seenghala* from polluted sites 1 and 2 contained 485.3±288.3 µg/g and 2291.7±2789.9 µg/g and contained 355.3±167.3 µg/g from reference site 3, *Labeo dyocheilus* from polluted water contained 1096.7±1153.3 µg/g and 1181.7±1252.9 µg/g and contained 402.3±127.2 µg/g from control water, *Cyprinus carpio* from polluted sites contained 2733.0±1078.7 µg/g and 3331.0±1509.5 µg/g and contained 437.0±148.9 µg/g control site and *Ompok bimaculatus* from polluted sites contained 765.3±465.7 µg/g and 872.0±516.3 µg/g and contained 387.0±46.6 µg/g from control site. Zn bioaccumulation in liver of different fish species was in the order of *Cyprinus carpio* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Ompok bimaculatus* > *Wallago attu.* This shows that *Cyprinus carpio* had accumulated highest concentration of Zn and *Wallago attu* lowest concentration. These results are in agreement with the previous findings as reported by Rules and Osuna (2002) and

Mendez*et al* (2002). Comparing the these data with our study highlights that high amount of Zn in liver of selected fish reflecting that River Kabul has more concentration of Zn as compare to other mentioned water resources. In this investigation liver of *Cyprinus carpio* had higher concentration of Zn as compare to other fish species. This is related to omnivorous nature of this fish. Being as an omnivorous nature, it is more exposed to metal bioaccumulation by many food chains. Similarly in a past study Yousafzai (2004) has also reported high level of Zn in liver of fish, *Tor putitora* caught from the same water resources verifying the validity of our finding.

Liver of *Wallago attu* from polluted sites had maximum level of Ni with mean values of 99.3±54.8 µg/g and 116.7±61.4 µg/g and had minimum value of 79.6±43.1µg/g from control site, *Aorichthys seenghala* from polluted water had 96.7±52.2 µg/g and 129.7±56.7 µg/g and had 62.6±39.5 µg/g from control site, *Labeo dyocheilus* from both sites 1 and 2 had 105.7±68.4 µg/g and 123.7±103.2 µg/g and had 48.0±25.4 µg/g from control site 3, *Cyprinus carpio* from polluted water had 77.7±45.3 µg/g and 92.0±52.5 µg/g and had 90.6±35.3 µg/g from control water and *Ompok bimaculatus* from polluted water had 90.0±103.7 µg/g and 112.3±124.1 µg/g and had 127.3±63.9 µg/g from control water (Warsak dam) respectively. Ni bioaccumulation in liver of different studied fish was in the sequence of *Aorichthys seenghala>Labeo dyocheilus>Wallago attu>Ompok bimaculatus >Cyprinus carpio*. This indicates that Zn content was highest in liver of *Aorichthys seenghala* and was lowest in *Cyprinus carpio*. These results were higher than those reported by Ptashynski *et al* (2002) and Yousafzai (2004). In the present study liver of *Aorichthys seenghala* had accumulated higher concentration of Ni as compare to other fish species. This could be attributed to exposition of the fish to this metal for long period.

Cr content in liver of different fish species from polluted sites was greater as compare to those from control site of River Kabul. Liver of *Wallago attu* from polluted sites had maximum content of Cr with mean values of 485.0±408.1 µg/g and 525.0±422.0 µg/g and had minimum value of 12.3±12.4 µg/g from control site, *Aorichthys seenghala* from polluted water had 470.3±391.4 µg/g and 621.7±430.5 µg/g and had 4.6±4.3 µg/g from control water, *Labeo dyocheilus* from both sites 1 and 2 had 597.7±254.1 µg/g and 655.7±272.3 µg/g and had 75.3±37.2 µg/g from site 3, *Cyprinus carpio* from polluted sites had 407.3±209.0 µg/g and 505.7±233.4 µg/g and had 43.6±20.0 µg/g from site 3 and *Ompok bimaculatus* from polluted sites had

602.3±213.8 µg/g and 682.7±495.2 µg/g and had 46.3±49.1 µg/g from control water of Warsak dam respectively. Cr bioaccumulation in this organ of different fish species was in the order of *Ompok bimaculatus* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Wallago attu* > *Cyprinus carpio*. This highlights that metal bioaccumulation was highest in liver of *Ompok bimaculatus* and lowest in *Cyprinus carpio*. These results are in agreement with those observed by many investigators (Avenant and Marx, 2000; Klaassen, 1976; Gauglhoffer and Bianchi,1991). Comparing our study with the findings of Yousafzai (2004) and other investigators highlights that greater level of this metal in liver could be attributed to higher concentration of Cr in the water of River Kabul and this result also indicates that a further increase of Cr level has occurred in water of River Kabul in last few years. This could be correlated to dumping of effluents from Amanghar industries and sewages from Nowshera city and towns into River Kabul at sites 1 and 2.

Copper concentration in liver of different fish species from polluted sites was higher as compare to control site. Liver of *Wallago attu* from polluted sites had accumulated higher concentration of Cu with mean values of 148.0±50.8 µg/g and 366.0±176.2 µg/g and lower value of 134.7±56.1 µg/g from control site, *Aorichthys seenghala* from polluted sites 1 and 2 had accumulated 129.3±48.7 µg/g and 232.7±56.7 µg/g and had accumulated 58.3±30.8 µg/g from control site 3, *Labeo dyocheilus* from both sites 1 and 2 had accumulated 165.0±81.6 µg/g and 223.3±167.4 µg/g and had accumulated 63.3±34.5 µg/g from site 3, *Cyprinus carpio* from polluted water had accumulated 306.0±118.8 µg/g and 402.0±100.6 µg/g and had accumulated 81.0±41.1 µg/g from control water and *Ompok bimaculatus* from polluted sites had accumulated 141.3±135.0 µg/g and 176.0±172.9 µg/g and had accumulated 58.0±46.7 µg/g from Warsak dam respectively. Copper bioaccumulation in gills of selected fish was in the order of *Cyprinus carpio*>*Wallago attu*>*Aorichthys seenghala*>*Ompok bimaculatus*>*Labeo dyocheilus.* This indicates that metal bioaccumulation is highest in *Cyprinus carpio* and lowest in *Labeo dyocheilus*. As compare to the present investigation high copper concentrations were also found in some previous studies (Medina *et al*., 1986; Kalay *et al*., 1999; Bhattacharya *et al*., 2007; Seymour, 1994; Harris, 2000; Fernandes, 2007). In this study liver of *Labeo dyocheilus* has accumulated higher concentration of Cu as compare to other fish species. However, in a previous study Yousafzai (2004) has also reported high level of Cu in liver of

fish, *Tor putitora* caught from the same water resources to verify the validity of the present result.

Liver of *Wallago attu* from polluted sites accumulated maximum concentration of Cd with mean values of 66.3±30.1 µg/g and 76.3±32.2 µg/g and accumulated minimum content with mean value of 23.3±17.5 µg/g from site 3, *Aorichthys seenghala* from polluted water accumulated 60.7±223.0 µg/g and 76.7±46.1 µg/g and accumulated 44.3±27.7 µg/g from control water, *Labeo dyocheilus* from sites 1 and 2 accumulated 73.7±32.2 µg/g and 84.3±38.5 µg/g and accumulated 20.0±9.0 µg/g from reference site 3, *Cyprinus carpio* from polluted sites accumulated 59.3±17.0 µg/g and 70.0±19.3 µg/g and accumulated 28.3±18.4 µg/g from control site and *Ompok bimaculatus* from polluted water accumulated 117.7±124.4 µg/g and 150.3±169.7 µg/g and accumulated 58.3±24.8 µg/g from control water respectively. Cadmium bioaccumulation in liver of these fish species was in the order of *Ompok bimaculatus* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Wallago attu* > *Cyprinus carpio*. This study found more Cd in liver as compare to previous findings reported by Mcgeer *et al* (2000) and Szebedinsky *et al* (2001). High concentration of this metal in liver of *Ompok bimaculatus* may be related to more concentration of this metal in water of River Kabul.

Wallago attu from polluted sites accumulated highest concentration of Pb with mean values of 596.3±527.8 µg/g and 635.3±548.8 µg/g and accumulated lowest content with mean value of 75.6±14.9 µg/g from control site, *Aorichthys seenghala* from sites 1 and 2 accumulated 285.3±234.1 µg/g and 585.7±520.0 µg/g and accumulated 155.6±56.5 µg/g from site 3, *Labeo dyocheilus* from polluted water accumulated 353.7±467.1 µg/g and 389.0±495.2 µg/g and accumulated 39.6±21.2 µg/g from control water, *Cyprinus carpio* from polluted water accumulated 196.7±130 µg/g and 282.3±188.3 µg/g and accumulated 57.0±27.8 µg/g from control water of Warsak dam and *Ompok bimaculatus* from polluted sites accumulated 1240.3±1975.8 µg/g and 1402.0±2187.0 µg/g and accumulated 24.3±19.9 µg/g from

control site respectively. The order of lead accumulation in liver of different fish species was *Ompok bimaculatus* >*Wallago attu*>*Aorichthys seenghala* >*Labeo dyocheilus* >*Cyprinus carpio*. The Pb metal accumulation in liver of *Ompok bimaculatus* was found to be quite high in comparison with other fish species. In this study, the concentration of lead in liver of studied fish was higher than those reported by (Yousafzai, 2004; Canli and Atli, 2003; Rules and Osuna, 2002), while was lower than those reported earlier (Roesijadi and Robinson, 1994; Thielen *et al*., 2004). Comparing our result with the finding of Yousafzai (2004) and other studies indicate that liver of all the studied fish accumulated a substaincial amount of metals as compare to the other fish species of the world and the result also showed further increase of metals pollution in River Kabul in last few years.

Fe level in liver of *Wallago attu* from polluted sites were 98.0±63.1 µg/g and 118.0±35.4 µg/g and was 30.0±10.9 µg/g from control site, in *Aorichthys seenghala* from polluted sites 1 and 2 were 90.0±26.7 µg/g and 110.0±34.2 µg/g and was 22.0±9.3 µg/g from control site 3, in *Labeo dyocheilus* from polluted water were 87.0±26.3 µg/g and 107.0±33.8 µg/g and was 28.0±8.9 µg/g from control water, in *Cyprinus carpio* from sites 1 and 2 were 94.0±27.4 µg/g and 114.0±34.9 µg/g and was 28.0±10.5 µg/g from site 3 and in *Ompok bimaculatus* from polluted sites were 91.0±27.0 µg/g and 111.0±34.4 µg/g and was 22.0±9.3 µg/g from control site respectively. These results agree with the finding of Adeniyi *et al* (2007). Fe accumulation in this organ was in the sequence of *Wallago attu*>*Cyprinus carpio* >*Aorichthys seenghala*>*Ompok bimaculatus*>*Labeo dyocheilus*. This reveales that *Wallago attu* accumulated higher metal concentration and *Labeo dyocheilus* lower concentration. In this study greater concentration of Fe metal was detected in liver as compare to other organs and the results also showed that a further increase in Fe concentration in water of River Kabul has occurred due to dumping of industrial effluents from Amangarh industries and sewages from Nowshera city.

In this finding liver of different fish from polluted sites showed high manganes level as compare to those from control site. Liver of *Wallago attu* from sites 1 and 2 had more content of Mn with mean values of 4.0±25.9 µg/g and 104.0±33.3 µg/g and less concentration with mean value of 37.0±12.1 µg/g from site 3, *Aorichthys seenghala* from polluted sites had 76.0±24.7 µg/g and 96.0±32.0 µg/g and had 31.0±11.1 µg/g from control site, *Labeo dyocheilus* from polluted water had 74.0±24.3 µg/g and 94.0±31.7 µg/g and had 29.0±10.8 µg/g from control water, *Cyprinus carpio* from polluted site 1 and site 2 had 81.0±25.4 µg/g and 101.0±32.8 µg/g and had 35.0±11.8 µg/g from site 3 and *Ompok bimaculatus* from polluted sites had 78.0±25.0 µg/g and 98.0±33.6 µg/g and had 33.0±11.4 µg/g from control site respectively. Manganese content in the liver of different fish species was in the order of *Wallago attu> Cyprinus carpio >Ompok bimaculatus >Aorichthys seenghala >Labeo dyocheilus*. This highlights that manganese content was highest in the liver of *Wallago attu* and was lowest in *Labeo dyocheilus*. The values of Mn metal found by Yousuf *et al* (2000) and Rashed (2001) were lower than the values mentioned in our results. Comparing our result with the finding of above mentioned researchers highlights that greater level of Mn in liver of *Wallago attu* could be correlated to exposition of this fish to Mn for long period.

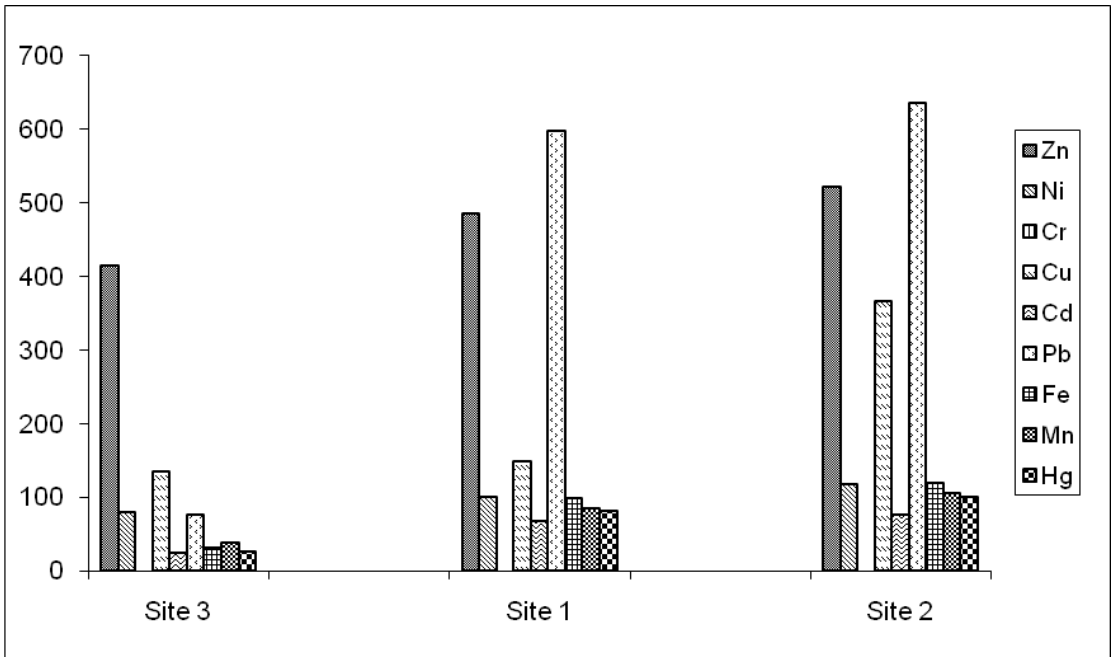
Liver of *Wallago attu* from sites 1 and 2 accumulated Hg with mean values of 80.0±25.3 µg/g and 100.0±32.7 µg/g and accumulated less level with mean value of 25.0±10.0 µg/g from site 3, *Aorichthys seenghala* from polluted sites accumulated 71.0±23.8 µg/g and 91.0±31.1 µg/g and accumulated 19.0±8.7 µg/g from reference site 3, *Labeo dyocheilus* from polluted water accumulated 70.0±23.7 µg/g and 90.0±31.0 µg/g and accumulated 16.0±8.0 µg/g from control site 3, *Cyprinus carpio* from polluted site 1 and site 2 accumulated 76.0±24.7 µg/g and 96.0±32.0 µg/g and accumulated 22.0±9.3 µg/g from control site 3 and *Ompok bimaculatus* from polluted sites 1 and 2 accumulated 74.0±24.3 µg/g and 94.0±31.7 µg/g and accumulated 21.0±9.1 µg/g from water of Warsak dam (site 3) respectively. Hg bioaccumulation

in liver of different fish species was in the order of *Wallago attu>Cyprinus carpio >Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus*. This shows that Hg metal level is highest in liver of *Wallago attu* and lowest in *Labeo dyocheilus*. These results are in accordance with those obtained by Farakas *et al* (2003), Sephar (1976), Azmat and Talat (2006), Bocher *et al* (2003). The present result found more amount of Hg in liver of exmined fish species than the previous studies (Allen, 1995; Randolph *et al*., 2004; Masoud *et al*., 2007). Comparing our finding to the studies of other workers highlights that the exmanied fish have accumulated greater content of heavy metals as compare to the rest fish species of the world. The highest concentration of metals in different organs of fish could be attributed to more exposition of fish to heavy metals for long period and high level of metals in the water and also correlated to herbivorous, carnivorous and omnivorous nature of fish.

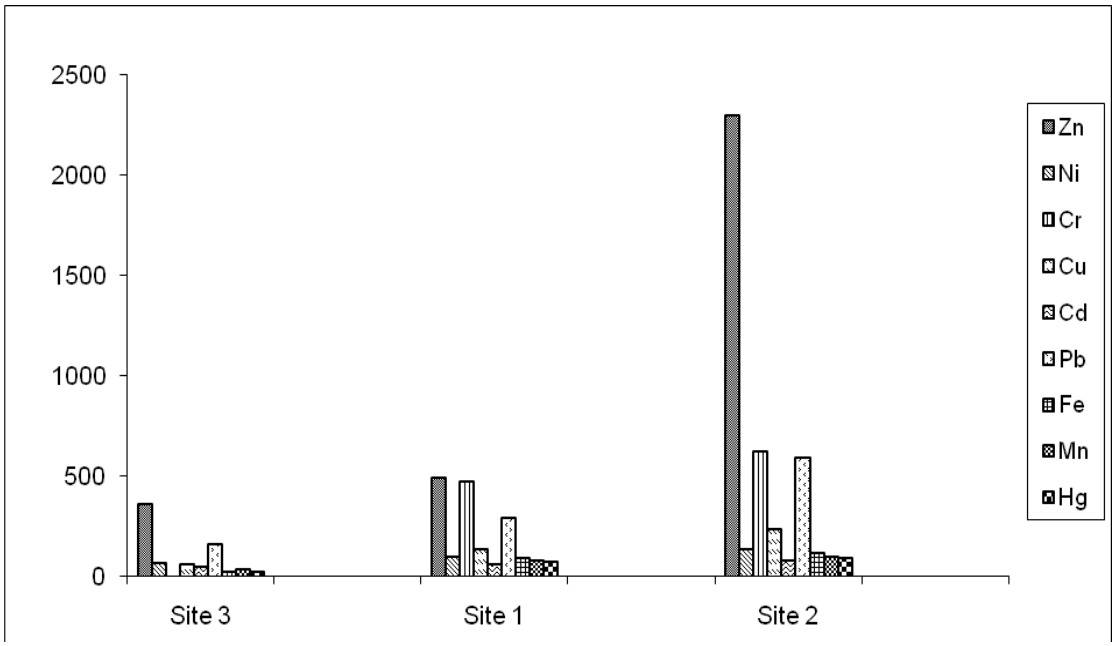
Table 4.6: Heavy metal concentrations (µg/g wet weight) in liver of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Analytes (µg/g)	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Zn	413.0±145.2	485.7±265.2	521.7±292.4
	Ni	79.6±43.1	99.3±54.8	116.7±61.4
	Cr	12.3±12.4	485.0±408.1	525.0±422.0
	Cu	134.7±56.1	148.0±50.8	366.0±176.2
	Cd	23.3±17.5	66.3±30.1	76.3±32.2
	Pb	75.6±14.9	596.3±527.8	635.3±548.8
	Fe	30.0±10.9	98.0±63.1	118.0±35.4
	Mn	37.0±12.1	84.0±25.9	104.0±33.3
	Hg	25.0±10.0	80.0±25.3	100.0±32.7
Aorichthys seenghala	Zn	355.3±167.3	485.3±288.3	2291.7±2789.9
	Ni	62.6±39.5	96.7±52.2	129.7±56.7
	Cr	4.6±4.3	470.3±391.4	621.7±430.5
	Cu	58.3±30.8	129.3±48.7	232.7±56.7
	Cd	44.3±27.7	60.7±223.0	76.7±46.1
	Pb	155.6±56.5	285.3±234.1	585.7±520.0
	Fe	22.0±9.3	90.0±26.7	110.0±34.2
	Mn	31.0±11.1	76.0±24.7	96.0±32.0
	Hg	19.0±8.7	71.0±23.8	91.0±31.1
Labeo dyocheilus	Zn	402.3±127.2	1096.7±1153.3	1181.7±1252.9
	Ni	48.0±25.4	105.7±68.4	123.7±103.2
	Cr	75.3±37.2	597.7±254.1	655.7±272.3
	Cu	63.3±34.5	165.0±81.6	223.3±167.4
	Cd	20.0±9.0	73.7±32.2	84.3±38.5
	Pb	39.6±21.2	353.7±467.1	389.0±495.2
	Fe	28.0±8.9	87.0±26.3	107.0±33.8
	Mn	29.0±10.8	74.0±24.3	94.0±31.7
	Hg	16.0±8.0	70.0±23.7	90.0±31.0
Cyprinus carpio	Zn	437.0±148.9	2733.0±1078.7	3331.0±1509.5
	Ni	90.6±35.3	77.7±45.3	92.0±52.5
	Cr	43.6±20.0	407.3±209.0	505.7±233.4
	Cu	81.0±41.1	306.0±118.8	402.0±100.6
	Cd	28.3±18.4	59.3±17.0	70.0±19.3
	Pb	57.0±27.8	196.7±130	282.3±188.3
	Fe	28.0±10.5	94.0±27.4	114.0±34.9
	Mn	35.0±11.8	81.0±25.4	101.0±32.8
	Hg	22.0±9.3	76.0±24.7	96.0±32.0
Ompok bimaculatus	Zn	387.0±46.6	765.3±465.7	872.0±516.3
	Ni	127.3±63.9	**90.0±103.7**	**112.3±124.1**
	Cr	46.3±49.1	602.3±213.8	682.7±495.2
	Cu	58.0±46.7	141.3±135.0	176.0±172.9
	Cd	58.3±24.8	117.7±124.4	150.3±169.7
	Pb	24.3±19.9	1240.3±1975.8	1402.0±2187.0
	Fe	22.0±9.3	91.0±27.0	111.0±34.4
	Mn	33.0±11.4	78.0±25.0	98.0±33.6
	Hg	21.0±9.1	74.0±24.3	94.0±31.7

P<0.05, **P>0.05**. (Values in bold are non-significant)

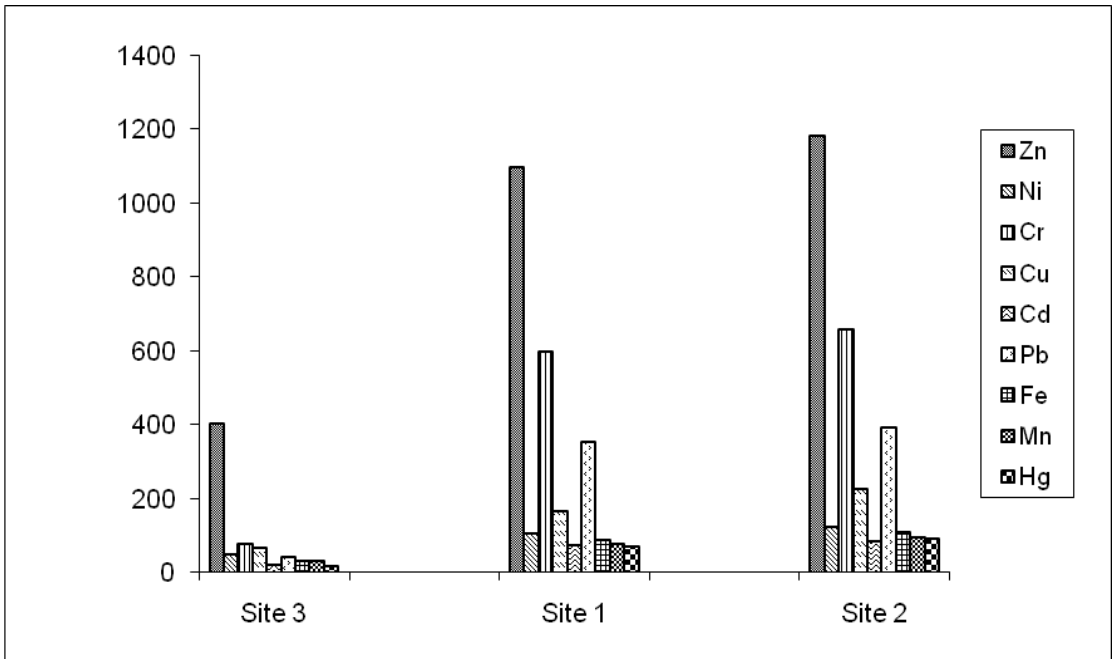


Wallago attu

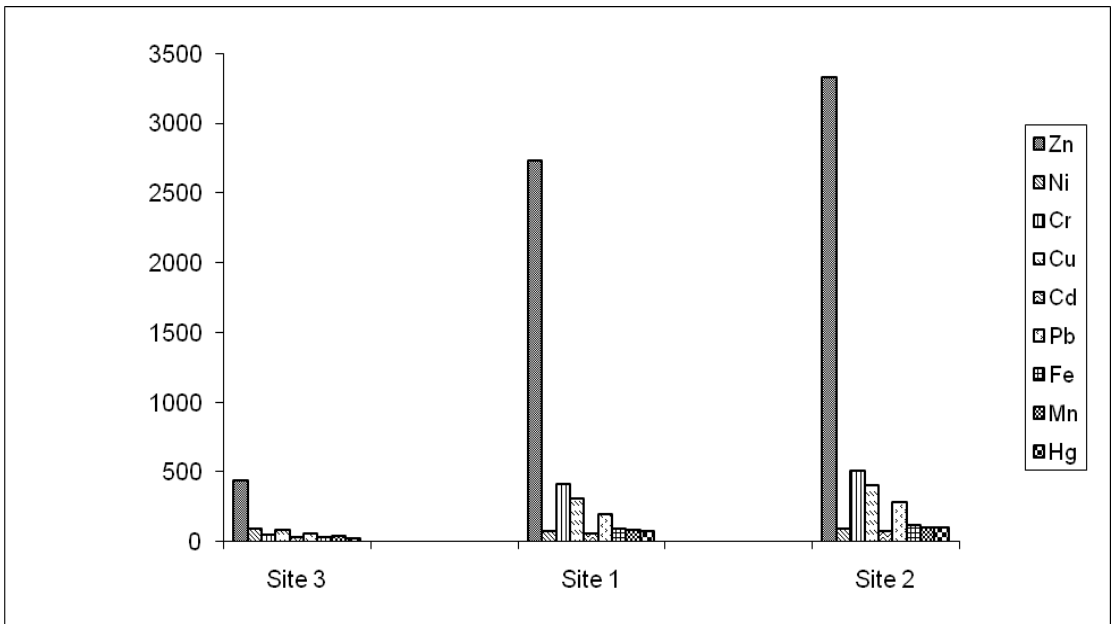


Aorichthys seenghala

Fig.4.11 **Heavy metal concentrations in liver of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

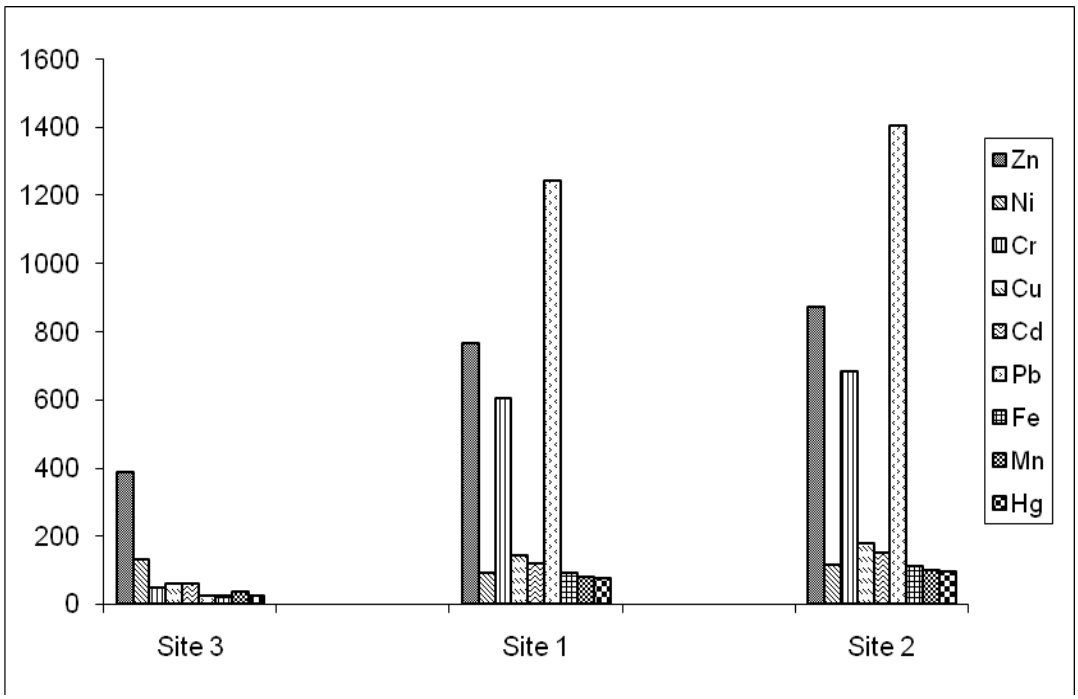


Labeo dyocheilus



Cyprinus carpio

Fig.4.12: Heavy metal concentrations in liver of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig.4.13 **Heavy metal concentrations in liver of *Ompok bimaculatus* netted from Warsak dam (Site 3) and two polluted sites (Site 1 and Site 2) of River Kabul receiving city sewages and industrial effluents.**

4.3.5 Bioaccumulation of Heavy Metals in Muscle

Muscle of five different fish species from site 3 (control) and polluted sites (sites 1 and 2) were taken out and processed for concentration of Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg. The muscle of selected fish species from site polluted 1 and site 2 showed highest content of heavy metals as compare with fish from control site 3 (Table 4.7, 4.8 and Figs 4.14-4.16).

From amongst the heavy metals more zinc concentrations were observed in muscle of *Wallago attu* from site 1 and site 2 with mean values of 582.3±290.8 µg/g and 654.0±356.7µg/g, where less value of 393.7±98.7 µg/g observed from reference site 3, in *Aorichthys seenghala* from polluted sites were observed 1119.0±738.7 µg/g and 1174.7±728.3 µg/g and observed 328.7±175.9 µg/g from site 3, in *Labeo dyocheilus* from polluted water were observed 735.0±461.3 µg/g and 888.0±495.9 µg/g and observed 384.7±105.2 µg/g from control water, in *Cyprinus carpio* from polluted site 1 and site 2 were observed 717.7±394.0 µg/g and 831.3±415.4 µg/g and observed 381.7±73.6 µg/g from control site 3 and in *Ompok bimaculatus* from polluted water were observed 791.3±347.1 µg/g and 907.0±399.1 µg/g and observed 347.7±141.4 µg/g from control water respectively. The present investigation found more Zn in muscle as reported in the previous studies by (Yilmaz, 2003; Yap *et al*., 2005). The sequence of zinc accumulation in muscle of different fish species was *Aorichthys seenghala* > *Ompok bimaculatus* > *Labeo dyocheilus* > *Cyprinus carpio* > *Wallago attu.* This shows that Zn metal concentration is highest in liver of *Aorichthys seenghala* and lowest in *Wallago attu*. Comparing our data with the RDA maximum limits (100gm muscle) for human consumption, it is cleared that Zn metal concentration in muscle of all five studied fish was below the RDA proposed limits, which is 2600 µg/g/100g for zinc. Comparing the above studies with our finding reflecting that River Kabul has more concentration of Zn as compare to other mentioned water resources. However, muscle of *Aorichthys seenghala* had accumulated higher concentration of Zn as compare to the rest of fish species.

In the present investigation Ni accumulated highly in muscle of different fish species from polluted sites as compare to those from control site (Warsak dam). Muscle of *Wallago attu* from polluted sites accumulated maximum concentration of nickel with mean values of 91.3±27.7 µg/g and 112.7±37.9 µg/g and accumulated minimum content with mean value of 69.3±36.2 µg/ from control site, *Aorichthys seenghala* from sites 1 and 2 accumulated 76.0±57.3 µg/g and100.3±80.1 µg/g and accumulated 50.7±37.8 µg/g from control site 3, *Labeo dyocheilus* from polluted sites 1 and 2 accumulated 100.0±70.1 µg/g and 123.0±82.5 µg/g and accumulated 36.7±16.2µg/g from control site, *Cyprinus carpio* from polluted water accumulated 67.3±47.0 µg/g and 80.0±52.6 µg/g and accumulated 78.3±31.1 µg/g from control water (Warsak dam) and *Ompok bimaculatus* from polluted sites accumulated 107.3±25.8 µg/g and 140.0±49.5 µg/g and accumulated 44.3±141.4 µg/g from warsak dam respectively. Nickel concentration in examined muscle of different fish species was in the order of *Ompok bimaculatus>Cyprinus carpio> Labeo dyocheilus> Wallago attu>Aorichthys seenghala.* This increased concentration of nickel in *Ompok bimaculatus* fish from polluted sites could be attributed to large size of fish, low metabolic rate and exposition of the fish to this metal for long period and high concentration of Ni in water**.** This shows that metal bioaccumulation is highest in muscle of *Ompok bimaculatous* and lowest in *Aorichthys seenghala*. Comparing our result with RDA maximum limits (100gm of the muscle) for human consumption, it is cleared that Ni metal concentration in muscle of all five studied fish species was above the RDA proposed limits, which is 10 µg/g/100g for nickel. Therefore all the fish from polluted sites of River Kabul are not suitable for human consumption and can prove highly toxic. This study found more level of Ni as compare to previous findings (Eric *et al*., 2004; Palaniappan *et al*., 2003). Comparing our results with the finding of Yousafzai (2004) showed increased amount of Ni in our study. This reflects that pollution in the River Kabul has further increased in the past three years.

Muscle of *Wallago attu* from polluted sites 1 and 2 had highest concentration of chromium with mean values of 446.0±378.0 µg/g and 539.3±191.3 µg/g and had lowest concentration with mean value of 8.7±5.4 µg/g from control site 3, *Aorichthys seenghala* from polluted water had 498.3±315.2 µg/g and 570.7±326.4 µg/g and had 3.0±2.0 µg/g from control water, *Labeo dyocheilus* from polluted sites had 518.7±170.9 µg/g and 625.7±246.3 µg/g and had 64.0±44.4 µg/g from control site, *Cyprinus carpio* from polluted sites 1 and 2 had 394.7±98.8 µg/g and 494.0±295.9 µg/g and had 14.3±9.6 µg/g from reference site 3 and *Ompok bimaculatus* from polluted water had 605.3±139.8µg/g and 708.3±426.8 µg/g and had 33.3±36.8 µg/g from control water respectively. These results are in agreement with previous studies (Olaifa *et al*., 2004; Turkmen *et al*., 2005; Demirezen and and Uruc, 2006). Cr bioaccumulation in muscle of these fish was in the sequence of *Ompok bimaculatu >Labeo dyocheilus >Aorichthys seenghala>Wallago attu>Cyprinus carpio.*This indicates that Cr metal bioaccumulation is highest in muscle of *Ompok bimaculatus* and lowest in *Cyprinus carpio*. Comparing our result with RDA maximum limits (100gm of the muscle) for human consumption, it is cleared that Cr metal concentration in muscle of all examined fish was above the RDA proposed limits, which is 50-200 µg/g/100g for Cr. Therefore all the fish from polluted sites of River Kabul are not suitable for human consumption. Comparing the above studies with our data reveales that River Kabul has more concentration of Cr as compare to other mentioned water bodies. However, in this study muscle of *Ompok bimaculatus* accumulated higher concentration of Cr as compare to other fish species. This could be correlated to exposition of the fish to this metal for long period, low detoxification mechanism and omnivorous nature of this fish. This investigation found higher values of Cr in the muscle as compare to the finding of Yousafzai (2004).

In this investigation copper content in muscle of different examined fish species from polluted water was highest as compare to those from control water of Warsak dam. Copper in muscle of *Wallago attu* from polluted sites showed higher

content with mean values of 52.0±50.1 µg/g and 286.3±97.8 µg/g and showed lower value of 41.7±40.8 µg/g from control site 3, *Aorichthys seenghala* from sites 1 and 2 showed 102.0±26.0 µg/g and 136.3±25.8 µg/g and showed 48.7±23.4 µg/g from reference site, *Labeo dyocheilus* from both polluted sites 1 and 2 showed 150.0±87.2 µg/g and 196.7±76.7 µg/g and showed 51.7±30.2 µg/g from reference water, *Cyprinus carpio* from polluted sites 1 and 2 showed 230.3±189.9 µg/g and 308.0±229.5 µg/g and showed 65.0±27.7 µg/g from site 3 and *Ompok bimaculatus* from polluted sites 1 and 2 showed 191.3±161.6 µg/g and 247.7±133.2 µg/g and showed 50.0±32.1 µg/g from Warsak dam respectively. The sequence of copper bioaccumulation in different fish species was *Cyprinus carpio>Wallago attu>Ompok bimaculatus>Labeo dyocheilus >Aorichthys seenghala.*This reveales that metal bioaccumulation is highest in *Cyprinus carpio* and lowest in *Aorichthys seenghala*. Comparing these values with RDA maximum limits (100gm of the muscle) for human consumption, it is cleared that the Cu metal concentration in muscle of all five studied fish is below the RDA limits, which is 2000-3000 µg/g/100g for copper. It is cleared that Cu metal concentration in muscle of all the examined fish is below the RDA proposed limits. This study found more level of Cu than the previous findings (Yilmaz, 2003; Yap *et al*., 2005; Marijic and Raspor, 2007; Olaifa *et al*., 2004). Comparing the findings of other workers with our data reflecting that River Kabul has more concentration of Cu as compare to other mentioned water resources. However in the present study muscle of *Cyprinus carpio* had accumulated higher concentration of Cu as compare to other fish species. This could be because of more exposition of the fish to this metal in water of River Kabul, low metabolic rate and low detoxification mechanism of this fish.

Muscle of *Wallago attu* from both polluted sites 1 and 2 accumulated the highest concentration of cadmium with mean values of 60.3±34.7 µg/g and 74.7±42.4 µg/g and accumulated 5.3±5.1 µg/g from control water, *Aorichthys seenghala* from polluted site 1 and site 2 accumulated 49.3±26.9 µg/g and 66.0±30.9 µg/g and

accumulated 4.0±2.3 µg/g from site 3, *Labeo dyocheilus* from polluted sites accumulated 64.3±27.3 µg/g and 72.3±32.8 µg/g and accumulated 15.0±4.4 µg/g from reference site 3, *Cyprinus carpio* from polluted site 1 and site 2 accumulated 46.7±16.5 µg/g and 59.0±17.7 µg/g and accumulated 20.3±12.7 µg/g from reference site 3 and *Ompok bimaculatus* from polluted sites accumulated 58.0±15.2 µg/g and 70.3±24.2 µg/g and accumulated 49.0±46.9 µg/g from Warsak dam respectively. In muscle of different fish species cadmium accumulation was in the order of *Wallago attu* > *Labeo dyocheilus* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Cyprinus carpio*. This indicates that *Wallago attu* had accumulated highest concentration of cadmium and *Cyprinus carpio* lowest concentration. Comparing these values with RDA maximum limits (100gm of the muscle) for human consumption, the Cd metal concentration in muscle of selected fish was above the RDA proposed limits, which is 14 µg/g/100g for Cd. it is cleared that Cd concentration in the muscle of all examined fish from polluted sites is above the RDA proposed limits. Therefore all the fish from polluted sites of River Kabul are not suitable for human consumption and are highly toxic. This study found maximum level of Cd as compare to the findings of Turkmen *et al* (2005) and Yap *et al* (2005). Comparing these mentioned findings with our result showed higher concentration of Cd in fish samples and reflects that River Kabul has more concentration of Cd as compare to other mentioned water bodies. In this investigation cadmium concentration in the muscle like other metals increased in both the fish samples from polluted water as compare to those from control water.

The present investigation found the muscle of examined fish species from polluted sites to be accumulated higher level of lead as compare to those from control site. Muscle of *Wallago attu* from polluted sites had highest concentration with mean values of 509.7±386.1 µg/g and 605.0±440.7 µg/g and had 61.0±34.1 µg/g from control site, *Aorichthys seenghala* from site 1 and site 2 had 296.0±305.1 µg/g and 355.7±368.6 µg/g and had 138.3±48.5 µg/g from reference site 3, *Labeo dyocheilus*

from polluted water had 454.7±395.8 µg/g and 534.0±461.5 µg/g and had 13.3±10.3 µg/g from control water, *Cyprinus carpio* from sites 1 and 2 had 184.7±246.6 µg/g and 231.3±307.6 µg/g and had 49.0±23.0 µg/g from water of Warsak dam and *Ompok bimaculatus* from polluted sites had 353.3±266 µg/g and 412.3±321.7 µg/g and had 17.3±15.3 µg/g from control site respectively. Overall order of lead accumulation in muscle of different fish species was *Wallago attu* > *Labeo dyocheilus* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Cyprinus carpio*. *Wallago attu* showed greater level of Pb and *Cyprinus carpio* showed smaller level. Comparing these values with RDA maximum limits (100gm of the muscle) for human consumption, the Pb metal concentration in muscle of three selected studied fish was above the RDA proposed limits except *Cyprinus carpio* and *Aorichthys seenghala*, where in these two fish the values for Pb were below the RDA limits. The RDA limit is 300 µg/g/100g for Pb. Therefore three fish from polluted sites of River Kabul are not suitable for human consumption and proved to be highly toxic. The previous studies also reported high concentration of Pb in muscle of other fish species (Rules and Osuna, 2002; Yilmaz, 2003; Huang *et al*., 2006; Beveridge *et al*., 1985; Honda *et al*., 1983). Comparing the above mentioned findings with the present result revealed that all the fish showed a substantial amount of Pb and the result also showed further increasing of Pb concentration in the water of River Kabul.

Muscle of different fish species from polluted sites accumulated high level of Fe as compare to those from reference site. Muscle of *Wallago attu* from sites 1 and 2 showed higher level of iron with mean values of 91.0±27.0 µg/g and 111.0±34.4 µg/g and showed lower level with mean value of 25.0±10.0 µg/g from site 3, *Aorichthys seenghala* from site 1 and site 2 showed 84.0±25.9 µg/g and 104.0±33.3 µg/g and showed 18.0±6.4 µg/g from site 3, *Labeo dyocheilus* from polluted sites showed 81.0±25.4 µg/g and 93.0±31.5 µg/g and showed 16.0±8.0 µg/g from control site 3, *Cyprinus carpio* from polluted sites showed 86.0±26.2 µg/g and 98.0±30.8 µg/g and showed 23.0±8.9 µg/g from water of Warsak dam (control) and *Ompok bimaculatus*

from both site 1 and site 2 showed 81.0±25.4 µg/g and 98.0±30.1 µg/g and showed 20.0±8.9 µg/g from site 3 respectively. The sequence of Fe accumulation in muscle of different fish species was *Wallago attu* > *Aorichthys seenghala* > *Cyprinus carpio* > *Ompok bimaculatus*>*Labeo dyocheilus*. This shows that *Wallago attu* contained highest concentration of Fe and *Ompok bimaculatus* lowest concentration. Comparing these values with RDA maximum limits (100gm of the muscle) for human consumption, the Fe metal concentration in muscle of studied fish was below the RDA limits, which is 500-2000 µg/g/100g for iron. Other workers (Carvalho *et al*., 2005; Olayan and Thomas, 2005) have also found relatively higher Fe content in muscle of different fish species. The present result found more Fe concentration in muscle tissues of examined fish species as compare to control fish from Warsak dam. Therefore this finding suggests that the Fe is considered as a good indicator of environmental pollution.

Muscle of *Wallago attu* from polluted sites contained highest concentration of manganese with mean values of 77.0±24.8 µg/g and 97.0±32.1 µg/g and contained lowest concentration of 31.0±11.0 µg/g from control site, *Aorichthys seenghala* from polluted sites 1 and 2 contained 69.0±21.4 µg/g and 89.0±30.8 µg/g and contained 24.0±9.8 µg/g from site 3, *Labeo dyocheilus* from both site 1 and site 2 contained 66.0±23.0 µg/g and 86.0±30.2 µg/g and contained 24.0±9.8 µg/g from site 3, *Cyprinus carpio* from polluted water contained 70.0±23.7 µg/g and 87.0±30.4 µg/g and contained 28.0±10.5 µg/g from control water and *Ompok bimaculatus* from polluted sites contained 67.0±23.1 µg/g and 85.0±30.1 µg/g and 28.0±10.5 µg/g from control site 3 respectively. These results are agreed to the findings of Yousuf *et al* (2000). Mn bioaccumulation in muscle of different fish species was in the order of *Wallago attu*> *Aorichthys seenghala* >*Cyprinus carpio* > *Labeo dyocheilus* > *Ompok bimaculatus*. This highlights that manganese content was highest in muscle of *Wallago attu* and lowest in *Ompok bimaculatus*. Comparing these values with RDA maximum limits (100gm of the muscle) for human consumption, the Mn metal

concentration in muscle of studied fish was below the RDA limits, which is 2500-5000 µg/g/100g for Mn. Comparing the present result with the findings of above mentioned studies revealed that in the last few years a further increase of Mn concentration has occurred in the River Kabul. Therefore muscle from polluted sites showed greater amount of Mn content as compare to Warsak dam.

Muscle of different fish species from polluted sites showed highest level of mercury as compare to Warsak dam, where lowest concentration of mercury was found from control site 3. Muscle of *Wallago attu* from polluted sites had higher concentration of mercury with mean values of 74.0±24.3 µg/g and 94.0±31.7 µg/g and had 20.0±8.9 µg/g from control water, *Aorichthys seenghala* from sites 1 and 2 had 63.0±22.4 µg/g and 83.0±29.7 µg/g and had 15.0±7.7 µg/g from site 3, *Labeo dyocheilus* from site 1 and site 2 had 63.0±22.4 µg/g and 83.0±29.7 µg/g and had 13.0±8.9 µg/g from control site 3, *Cyprinus carpio* from both polluted sites had 67.0±23.1 µg/g and 85.0±30.1 µg/g and had 6.0±8.0 µg/g from control site and *Ompok bimaculatus* from site 1 and site 2 had 65.0±22.8 µg/g and 81.0±29.3 µg/g and had 16.0±8.9 µg/g from reference site 3 respectively. Mercury bioaccumulation in the muscle of different fish species was in the order of *Wallago attu*>*Cyprinus carpio* > *Labeo dyocheilus* > *Aorichthys seenghala* >*Ompok bimaculatus*. This indicates that mercury level was highest in muscle of *Wallago attu* and lowest in *Ompok bimaculatus*. This increased concentration of mercury in muscle tissue of *Wallago attu* fish from polluted sites could be correlated to low metabolic rate, exposition of the fish to metals for long period, low elimination of metal from body and high concentration of metal in the water**.** Comparing these values with the RDA maximum limits (100gm of the muscle) for human consumption, it is cleared that Hg metal concentration in muscle of all studied fish was above the RDA limits, which is 8 µg/g/100g for Hg. Therefore all the fish from polluted sites of River Kabul are not suitable for human consumption and are highly toxic. The present results are also in agreement with the findings of previous investigations (Matthew, 1992; Masoud *et*

al., 2007; Allen, 1995). In the present finding muscle was found to be contained less concentration of all the studied metals and came last in number for metals accumulation. Muscle is the tissue that is not exposed directly to water pollution as compare to gills and skin, where these tissues are constantly and directly exposed to water pollution. Therefore the muscle showed less content of metals as compare to other studied tissues. Fish muscle is consumed in most of the rural population of the world; therefore researchers have an emphasis on just tissues. Inhabitants living around the River Kabul eat fish muscle. Absorption in muscle tissue and various mechanisms favours accumulation of metals in muscle. Comparing the present concentration of heavy metals in muscle of different fish species with RDA limits shows that heavy metals such as Ni, Cd, Pb and Hg were above the permissible limits proposed by RDA and rest of the metals are below the RDA limits. The fish from polluted areas are not suitable for consumption. Comparing our study with the finding of Yousafzai (2004) revealed that a further increase of heavy metals in different tissues have occurred, which reflects a further increase of heavy metal concentration in the water i.e a further increase of heavy metals pollution in River Kabul in last three years.

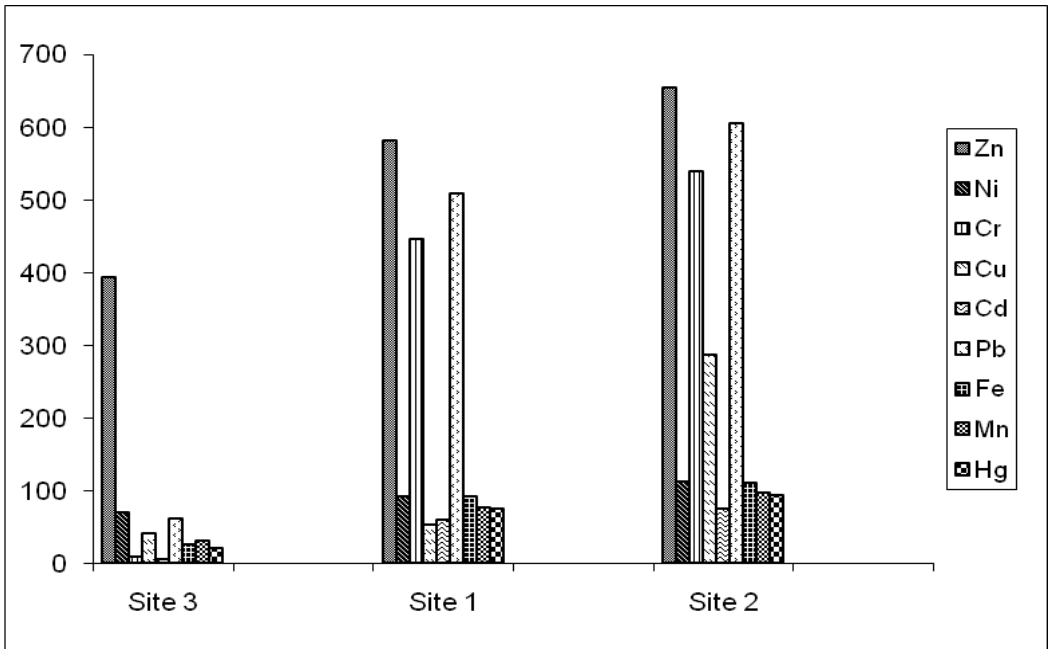
Table 4.7: Heavy metal concentrations (µg/g wet weight) in muscle of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Analytes (µg/g)	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Zn	393.7±98.7	582.3±290.8	654.0±356.7
	Ni	69.3±36.2	91.3±27.7	112.7±37.9
	Cr	8.7±5.4	446.0±378.0	539.3±191.3
	Cu	41.7±40.8	**52.0±50.1**	286.3±97.8
	Cd	5.3±5.1	60.3±34.7	74.7±42.4
	Pb	61.0±34.1	509.7±386.1	605.0±440.7
	Fe	25.0±10.0	91.0±27.0	111.0±34.4
	Mn	31.0±11.0	77.0±24.8	97.0±32.1
	Hg	20.0±8.9	74.0±24.3	94.0±31.7
Aorichthys seenghala	Zn	328.7±175.9	1119.0±738.7	1174.7±728.3
	Ni	50.7±37.8	76.0±57.3	100.3±80.1
	Cr	3.0±2.0	498.3±315.2	570.7±326.4
	Cu	48.7±23.4	102.0±26.0	136.3±25.8
	Cd	4.0±2.3	49.3±26.9	66.0±30.9
	Pb	138.3±48.5	296.0±305.1	355.7±368.6
	Fe	18.0±6.4	84.0±25.9	104.0±33.3
	Mn	24.0±9.8	69.0±21.4	89.0±30.8
	Hg	15.0±7.7	63.0±22.4	83.0±29.7
Labeo dyocheilus	Zn	384.7±105.2	735.0±461.3	888.0±495.9
	Ni	36.7±16.2	100.0±70.1	123.0±82.5
	Cr	64.0±44.4	518.7±170.9	625.7±246.3
	Cu	51.7±30.2	150.0±87.2	196.7±76.7
	Cd	15.0±4.4	64.3±27.3	72.3±32.8
	Pb	13.3±10.3	454.7±395.8	534.0±461.5
	Fe	16.0±8.0	81.0±25.4	93.0±31.5
	Mn	24.0±9.8	66.0±23.0	86.0±30.2
	Hg	13.0±8.9	63.0±22.4	83.0±29.7
Cyprinus carpio	Zn	381.7±73.6	717.7±394.0	831.3±415.4
	Ni	78.3±31.1	**67.3±47.0**	**80.0±52.6**
	Cr	14.3±9.6	394.7±98.8	494.0±295.9
	Cu	65.0±27.7	230.3±189.9	308.0±229.5
	Cd	20.3±12.7	46.7±16.5	59.0±17.7
	Pb	49.0±23.0	184.7±246.6	231.3±307.6
	Fe	23.0±8.9	86.0±26.2	98.0±30.8
	Mn	28.0±10.5	70.0±23.7	87.0±30.4
	Hg	16.0±8.0	67.0±23.1	85.0±30.1
Ompok bimaculatus	Zn	347.7±141.4	791.3±347.1	907.0±399.1
	Ni	44.3±141.4	107.3±25.8	140.0±49.5
	Cr	33.3±36.8	605.3±139.8	708.3±426.8
	Cu	50.0±32.1	191.3±161.6	247.7±133.2
	Cd	49.0±46.9	58.0±15.2	70.3±24.2
	Pb	17.3±15.3	353.3±26.6	412.3±321.7
	Fe	20.0±8.9	81.0±25.4	98.0±30.1
	Mn	28.0±10.5	67.0±23.1	85.0±30.1
	Hg	16.0±8.9	65.0±22.8	81.0±29.3

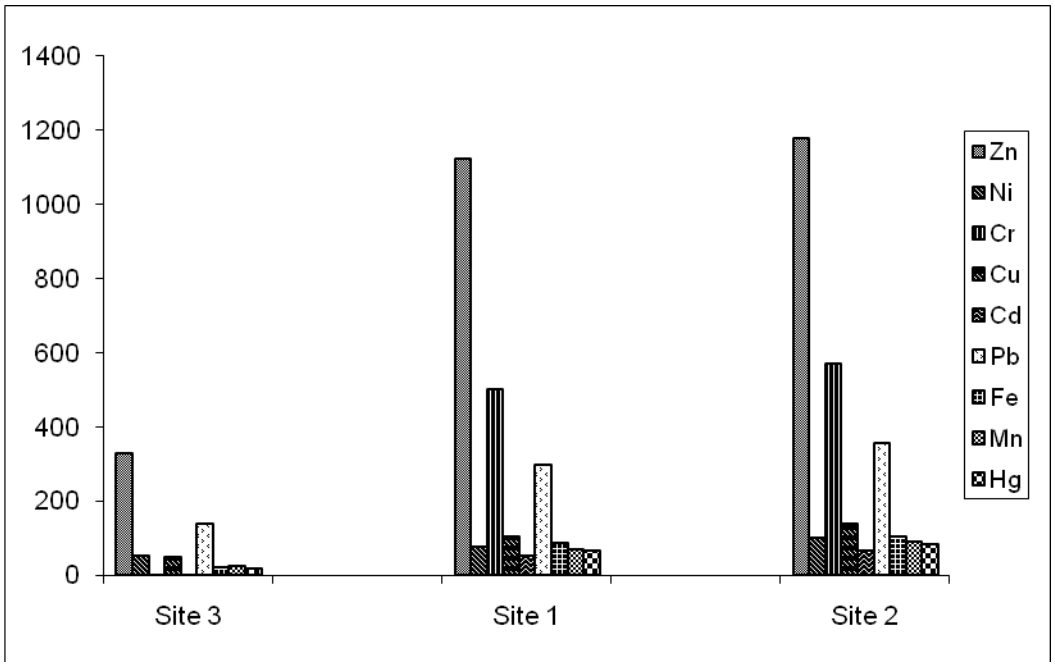
P<0.05, **P>0.05**. (Values in bold are non-significant)

Table 4.8: U.S Recommended Daily Diatary Allowance (RDA) supplied by a 100g of fish muscle.

Metals	Miligram (mg)	Microgram (µg)
Cd	0.014	14
Hg	0.008	8
Pb	0.3	300
Ni	0.01	10
Zn	2.6	2600
Fe	0.5-2.0	500-2000
Cu	2.0-3.0	2000-3000
Cr	0.05-0.20	50-200
Mn	2.5-5.0	2500-5000

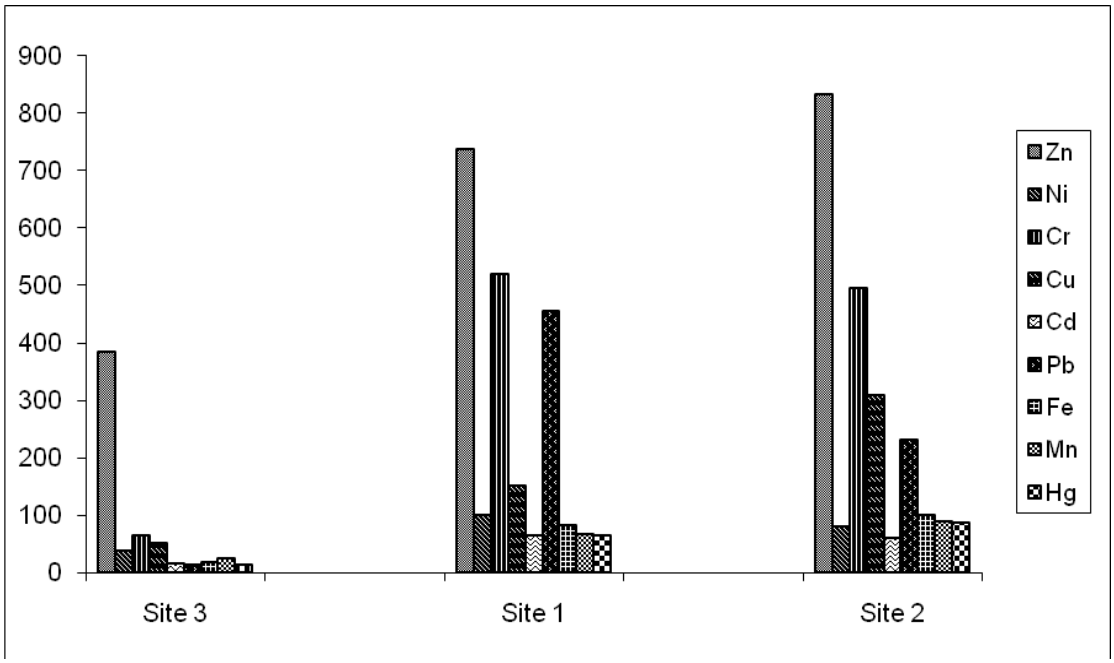


Wallago attu

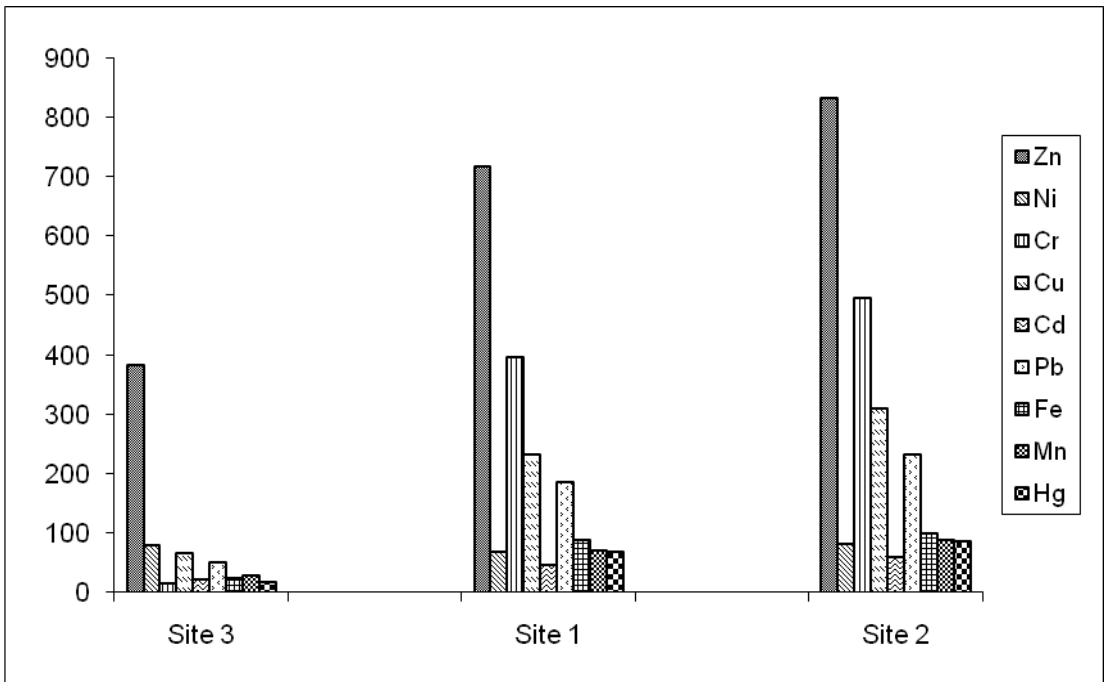


Aorichthys seenghala

Fig.4.14 **Heavy metal concentrations in muscle of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

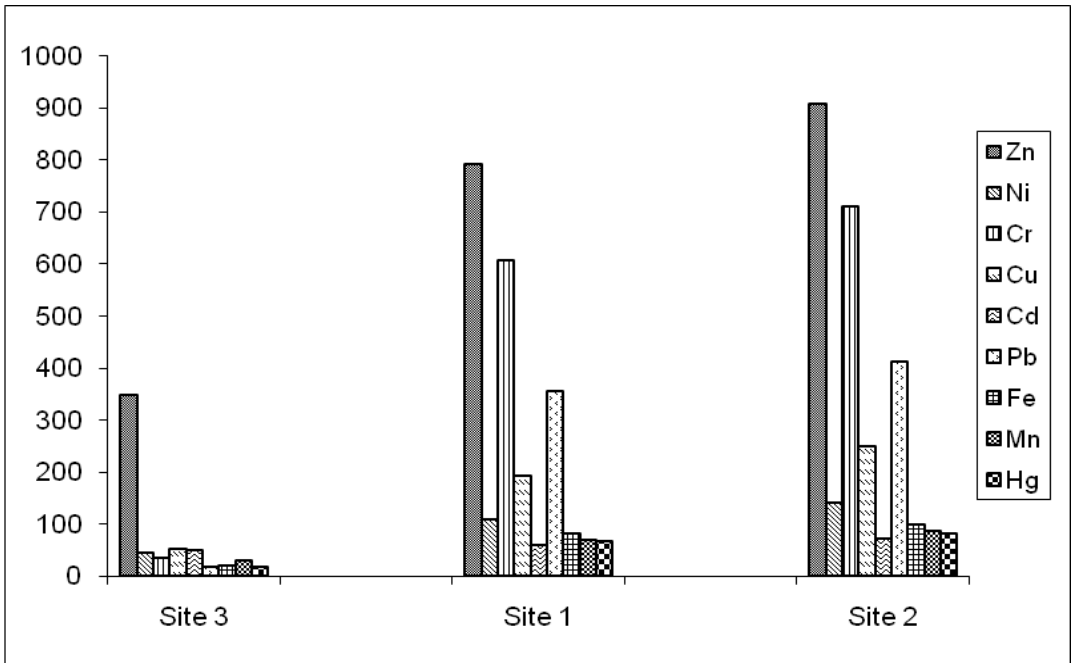


Labeo dyocheilus



Cyprinus carpio

Fig.4.15: Heavy metal concentrations in muscle of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig.4.16: Heavy metal concentrations in muscle of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

4.3.6 Sequences of Metals Bioaccumulation

The present investigation shows that all the tissues of fish accumulated a substantial amount of heavy metals. Among various tissues and fish from polluted sites different patterns of metal bioaccumulation are present. However, Zn is the highly accumulated metals, while Cd is the least one.

Zinc bioaccumulation in gills of different fish species was in the order of *Ompok bimaculatus>Labeo dyocheilus>Cyprinus carpio>Aorichthys seenghala> Wallago attu*, in skin was *Cyprinus carpio>Aorichthys seenghala>Labeo dyocheilus >Ompok bimaculatus>Wallago attu*, in intestine was *Cyprinus carpio>Ompok bimaculatus > Aorichthys seenghala > Labeo dyocheilus>Wallago attu*, in liver was *Cyprinus carpio>Aorichthys seenghala>Labeo dyocheilus>Ompok bimaculatus >Wallago attu* in muscle was *Aorichthys seenghala>Ompok bimaculatus>Labeo dyocheilus>Cyprinus carpio>Wallago attu.* Overall order of zinc concentration in different tissues was intestine > skin > gills > liver > muscle and overall order of zinc in different fish species was *Cyprinus carpio>Aorichthys seenghala>Ompok bimaculatus >Labeo dyocheilus>Wallago attu*.

Nickel concentration in gills of different fish species was in the sequence of *Ompok bimaculatus>Labeo dyocheilus>Wallago attu>Aorichthys seenghala>*

Cyprinus carpio, in skin was *Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala>Cyprinus carpio>Wallago attu*, in intestine was *Labeo dyocheilus>Ompok bimaculatus>Wallago attu>Aorichthys seenghala>Cyprinus carpio,* in liver was *Aorichthys seenghala>Labeo dyocheilus>Wallago attu>Ompok bimaculatus>Cyprinus carpio* and in muscle was *Ompok bimaculatus>Cyprinus carpio>Labeo dyocheilus>Wallago attu>Aorichthys seenghala.* Overall sequence of nickel concentration in different tissues was intestine > gills > skin > muscle > liver and overall order in different fish species was *Labeo dyocheilus>Ompok bimaculatus> Aorichthys seenghala>Wallago attu>Cyprinus carpio.*

Chromium bioaccumulation in gills of different fish species was in the order of *Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala>Wallago attu> Cyprinus carpio,* in skin was *Aorichthys seenghala>Labeo dyocheilus>Ompok bimaculatus>Wallago attu>Cyprinus carpio,* in intestine was *Labeo dyocheilus> Ompok bimaculatus>Cyprinus carpio>Aorichthys seenghala>Wallago attu,* in liver was *Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala>Wallago attu> Cyprinus carpio* and in muscle was *Ompok bimaculatus>Labeo dyocheilus> Aorichthys seenghala>Wallago attu>Cyprinus carpio.* Overall order of chromium concentration in different tissues was gills > skin > intestine > liver > muscle and overall order in different fish species was *Labeo dyocheilus>Ompok bimaculatus> Aorichthys seenghala>Wallago attu>Cyprinus carpio.*

Copper bioaccumulation in gills of different fish species was in the sequence of *Wallago attu>Aorichthys seenghala>Ompok bimaculatus>Ompok bimaculatus> Labeo dyocheilus>Cyprinus carpio*, in skin was *Wallago attu > Labeo dyocheilus > Aorichthys seenghala>Ompok bimaculatus>Cyprinus carpio,* in intestine was *Wallago attu>Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala> Cyprinus carpio,* in liver was *Cyprinus carpio>Wallago attu>Aorichthys seenghala> Ompok bimaculatus>Labeo dyocheilus* and in muscle was *Cyprinus carpio>Wallago attu>Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala*. Overall sequence of copper concentration in different tissues was liver > intestine > muscle > skin >gills and overall order in different fish species was *Wallago attu > Cyprinus carpio > Labeo dyocheilus > Aorichthys seenghala > Ompok bimaculatus.*

Cadmium bioaccumulation in gills of different fish species was in the order of *Ompok bimaculatus>Labeo dyocheilus>Wallago attu>Cyprinus carpio>Aorichthys seenghala,* in skin was *Labeo dyocheilus>Ompok bimaculatus>Wallago attu> Aorichthys seenghala>Cyprinus carpio,* in intestine was *Labeo dyocheilus>Ompok bimaculatus>Cyprinus carpio>Wallago attu>Aorichthys seenghala,* in liver was

Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala>Wallago attu> Cyprinus carpio and in muscle was *Wallago attu>Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala>Cyprinus carpio*. Overall order of cadmium concentration in different tissues was liver > intestine > skin > gills > muscle and overall order in different fish species was *Ompok bimaculatus > Labeo dyocheilus > Wallago attu > Aorichthys seenghala > Cyprinus carpio.*

Lead bioaccumulation in gills of different fish species was in the order of *Wallago attu>Aorichthys seenghala>Ompok bimaculatus>Labeo dyocheilus> Cyprinus carpio,* in skin was *Wallago attu>Ompok bimaculatus>Labeo dyocheilus> Aorichthys seenghala>Cyprinus carpio,* in intestine was *Cyprinus carpio>Wallago attu>Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala,* in liver was *Ompok bimaculatus>Wallago attu>Aorichthys seenghala>Labeo dyocheilus> Cyprinus carpio* and in muscle was *Wallago attu>Labeo dyocheilus>Ompok bimaculatus>Aorichthys seenghala>Cyprinus carpio.* Overall order of lead concentration in different tissues was intestine > liver > muscle > skin > gills and overall order of lead in different fish species was *Wallago attu > Ompok bimaculatus > Labeo dyocheilus > Aorichthys seenghala > Cyprinus carpio.*

Iron bioaccumulation in gills of different fish species was in the sequence of *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus,* in skin was *Wallago attu>Cyprinus carpio>Ompok bimaculatus> Aorichthys seenghala>Labeo dyocheilus,* in intestine was *Wallago attu>Cyprinus carpio>Aorichthys seenghala>Labeo dyocheilus>Ompok bimaculatus,* in liver was *Wallago attu>Cyprinus carpio>Aorichthys seenghala>Ompok bimaculatus>Labeo dyocheilus* and in muscle was in the sequence of *Wallago attu>Aorichthys seenghala >Cyprinus carpio>Ompok bimaculatus>Labeo dyocheilus*. Overall sequence of iron concentration in different tissues was intestine > skin > liver > gills > muscle and

overall order of iron in different fish species was *Wallago attu > Cyprinus carpio > Aorichthys seenghala > Ompok bimaculatus > Labeo dyocheilus.*

Manganese bioaccumulation in gills of different fish species was in the order of *Wallago attu>Cyprinus carpio>Aorichthys seenghala>Ompok bimaculatus> Labeo dyocheilus*, in skin was *Wallago attu>Cyprinus carpio>Ompok bimaculatus> Aorichthys seenghala>Labeo dyocheilus,* in intestine was *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus*, in liver was *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus* and in muscle was *Wallago attu>Aorichthys seenghala>Cyprinus carpio >Labeo dyocheilus>Ompok bimaculatus.* Overall order of manganese concentration in different tissues was intestine > skin > gills > liver > muscle and overall order in different fish species was *Cyprinus carpio > Aorichthys seenghala > Ompok bimaculatus > Labeo dyocheilus > Wallago attu.*

Mercury bioaccumulation in gills of different fish species was in the order of *Wallago attu>Labeo dyocheilus>Cyprinus carpio>Aorichthys seenghala>Ompok bimaculatus*, in skin was *Wallago attu>Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus*, in intestine was *Cyprinus carpio >Aorichthys seenghala>Ompok bimaculatus>Wallago attu>Labeo dyocheilus*, in liver was *Wallago attu>Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus* and in muscle was *Wallago attu>Cyprinus carpio>Labeo dyocheilus>Aorichthys seenghala>Ompok bimaculatus*. Overall order of mercury concentration in different tissues was intestine > skin > liver > gills > muscle and the overall order of mercury in different fish species was *Wallago attu > Cyprinus carpio > Ompok bimaculatus > Aorichthys seenghala > Labeo dyocheilus.*

4.3.7 Conclusions and Remarks

In the present investigation heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Mn. Fe and Hg were determined in intestine, skin, gills, liver and muscle of five different fish

species including *Wallago attu*, *Ompok bimaculatous*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala* netted from both polluted and non polluted sites of River Kabul. Overall order of heavy metals concentration in different fish organs was in the sequence of intestine>skin>liver>gills> muscle and in different fish species was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This shows that highest amount of metal bioaccumulation was found in intestine followed by skin, liver and gills while lowest concentration was observed in muscle. Similarly highest amount of metal bioaccumulation was found in *Cyprinus carpio* fish followed by *Ompok bimaculatus*, *Aorichthys seenghala*, *Labeo dyocheilus* and lowest in *Wallago attu* fish. Thus the organs level order of metal bioaccumulation in *Wallago attu* was in the sequence of skin >gills > intestine > muscle > liver, in *Aorichthys seenghala* was skin > liver > muscle > gills > intestine, in *Labeo dyocheilus* was skin > intestine >gills > liver >muscle, in *Cyprinus carpio* was intestine > skin > liver > gills > muscle and in *Ompok bimaculatus* was gills > liver > skin > intestine > muscle. Overall order of heavy metal concentrations in different tissues are as below

» in muscle was Zn>Cr>Pb>Cu>Ni>Fe>Mn>Hg>Cd.

» In intestine was Zn>Pb>Cr>Cu>Ni>Fe>Mn>Hg>Cd.

» In liver was Zn>Pb>Cr>Cu>Ni>Fe>Mn>Hg>Cd.

» In skin was Zn>Cr>Pb>Cu>Ni>Fe>Mn>Hg>Cd.

» In gills was Zn>Cr>Pb>Cu>Ni>Fe>Mn>Hg>Cd.

Thus Zn was the highly accumulated metal while Cd was the lowest. Comparing our studies with U.S Recommended Daily Dietary Allowance (RDA) render that the fish populations from polluted sites 1 and 2 of River Kabul are not suitable for human consumption. The data generated in these studies confirmed the presence of heavy metal load in River Kabul as reported in the past by many workers including

Yousafzai (2004). High heavy metal load in River Kabul which is reflective from the present studies could be one of the reason of fish decline.

CHAPTER-5

GENOTOXIC EFFECTS OF HEAVY METALS IN SELECTED FISH SPECIES OF RIVER KABUL

5.1 INTRODUCTION

Genotoxicity is a deleterious action, which affects a cell's genetic material (Smith, 1996). Heavy metals are genotoxicants that can damage DNA of a living cell (Matsumoto *et al*., 2005; Igwilo *et al*., 2006). These genotoxicants have investigated that they are related to mutations because they form strong covalent bonds with the DNA, which result into formation of DNA adducts that prevent replication of DNA and result into DNA damage (Hartwell *et al*., 2000). The comet assay is a sensitive, rapid and reliable method that can be used for the determination of DNA damage in eukaryotic and prokaryotic cells (Bajpayee *et al*., 2005). Many workers have reported that the fish erythrocytes are suitable for the comet assay, because fish blood contains 97% erythrocytes, which ensure homogenecity of cells for comet studies (Theodora *et al*., 1994). Among the various techniques so far used to assess the genotoxicity of environmental pollutants, the comet assay is sensitive, rapid technique that can be used for estimation of DNA damage in both proliferating and non-proliferating cells (Rojas *et al*., 1999). Researchers have found DNA fragmentation through comet assay before more severe abnormalities in both terrestrial and aquatic organisms. The comet assay is a sensitive technique that can be used for detecting DNA damage in a cell. It combines a biochemical approach to detect DNA strand breaks and alkali labile sites with a single-cell approach typical for cytogenetic assays (Collins *et al*., 1997, Lee and Steinert, 2003).

Comet assay is a successful method that can be used for determination of DNA damage in both laboratory and field investigations with different fish species, both from fresh and marine water (Deveaux *et al*., 1997; Belpaeme *et al*., 1998; Pandey *et al*., 2006). Therefore, fish are sentinel organisms for genotoxic studies in

aquatic environment (Landolt and Kocan, 1983). The comet assay is a new technique that is used in molecular epidemiology. It gives good results about genotoxicity which is produced as a result of diseases, therapy in clinical trials environmental or occupational exposure to toxic chemicals for short period of time and in a cost-effective manner (Faust *et al*., 2004; Bay ram *et al*., 2006). Comet assay has wide description and development. Various investigations have imposed concentration on mammalian cells for comet assay but a number have focused on marine and fresh water mussels and fish for determination of DNA damage in their hemocytes, blood cells, gill, liver and gut cells (Gertraud *et al.,* 2007). Fish are useful genetic models to find out the genotoxicological impacts of aquatic pollution (Kumar *et al*., 2009). Fish are good source for the genotoxic, mutagenic and carcinogenic studies and also used for environmental risk assessment (Ali *et al*., 2008; Banu *et al*., 2001). Fish are used for ecotoxicological studies because they help in the trophic web, in accumulation toxic chemicals and respond to low levels of genotoxic substances like heavy metals (Jha, 2008). Fish are used in testing of substances such as heavy metals, biocides, agrochemical, food additives and pharmaceuticals for genotoxicity (Brendler-Schwaab *et al*., 2005). Therefore comet assay technique is helping in investigation of genotoxicity and mutagenesis in the fish erythrocyte collected from polluted environment (Andrade *et al*., 2004). Recently increasing concern about genotoxicity in land and water has led to development of many mutagenesis test systems in bacteria, yeast, plants and animals including fish. Fish are suitable organisms for genotoxicological and ecotoxicological studies because they play different roles in the heterotrophic web, bioaccumulation of heavy metals and showed responce to mutagenic environmental pollutants like heavy metals at low concentration (Goksoyr *et al*., 1991; Cavas and Ergene-Gozukara, 2005). Fish are good model for detection of mutagencity, genotoxicity and carcinogenicity of toxic chemicals because they can accumulate, concentrate and store water pollutants like heavy metals (Al-sabti, 1995b).

Fish has slow DNA repair mechanism than mammals (Espina and Wesis, 1995). It has been investigated that DNA strand breaks was measured by the comet assay, which is acted as a biomarker of genotoxicity in fish and other aquatic species (Mitchelmore and Chipman, 1998). The researchers have focused on increasing genotoxins like heavy metals in the aquatic environment and also busy in development of sensitive biomarkers for detection of genotoxic effects of toxins in aquatic organisms (Hayashi *et al*., 1998). Environmental pollutants like heavy metals are genotoxic and can be monitored using a broad range of both in vitro and in vivo biomarker assays, but the comet assay is gaining popularity over others due to sensitivity for detecting low levels of DNA damage (Gedic *et al*., 1992). Fish are used as a good genetic model for determination of genotoxicological impacts of pollution in aquatic ecosystems (Mitchell and Kennedy, 1992) and can play vital roles in evaluation of potential risk associated with contamination in aquatic environment because they are directly exposed to chemicals and mutagens such as heavy metals received from industries, agricultural production via surface runoff or indirectly through the food chain in an ecosystem (Cavas and Ergene-Gozukara, 2005a). Therefore the fish are used as indicator for the determination of genotoxicological effects of increasing pollution and can permit early detection of aquatic environmental problems (Lopez-Barea, 1996; Van Der Oost *et al*., 2003). It is essential to investigate the level of DNA damage as determined by comet assay is not limited to the impacts of exogenous environmental pollutants only but oxidative DNA damage alone contributes to back ground levels of DNA damage and is also relevant to the secondary effects of many pollutants like heavy metals. The metabolism of several heavy metals among them can attack on DNA and leading to serious DNA damage (Livingstone, 2001, Gabbianelli *et al*., 2003, Mamaca *et al*., 2005). Fish are important indicators that are used for mutagenic or carcinogenic investigation especially in aquatic environment because they have the ability to metabolize, accumulate and store

water pollutants such as heavy metals in their different tissues and organs, which are resulted into DNA damage in tissues and organs cells (Ateeq *et al*., 2005).

Blue gill sunfish, *Lepomis macrochirus* was collected from contaminated site and the DNA was isolated from the blood cells and examined for DNA damage. These quantitative measures were used to determine the difference in the number of double and single strand breaks between DNA preparations. Both strand breakage were found to be greater in fish exposed to heavy metal compounds as compare to non exposed fish (Christopher, 1994). Heavy metals are one of the most toxic environmental metals that affect genetically various organisms like fish. High concentrations of heavy metals are resulted into DNA damage in fish (Serpi *et al*., 2006). The DNA damage due to heavy metals was determined in hepatopancreas of loach, *Misgurnus anguilli* caudatus fish. The percentage of DNA damage increased with the increased exposure time to heavy metals. Therefore the highest percentage of DNA damage was observed in the fish after exposure to heavy metals like cadmium, lead and zinc (Yingmei *et al*., 2006). Heavy metals are dangerous to living things both plants and animals because they are toxic and carcinogenic in nature and can induce different abnormalities in living organisms. In previous finding it has been investigated that heavy metals can be binded with nucleic acid by reacting in sites of the cellular DNA, which are resulted into mutations, adducts and many complexes. Metal cations were also investigated to impact DNA replication (Chang *et al*., 1996). Recent investigations have reported that heavy metals are carcinogenic and free radicals and reactive oxygen species are produced as a result of oxidative mechanism, which are attributed to DNA damage in both terrestrial and aquatic animals. Therefore heavy metals toxicity and carcinogenicity are other threats to animals in aquatic environment ecosystem and is an important concern of the scientific community (Bal and Kasprzak, 2002, Chang *et al*., 1996). Different abnormalities such as damage to plasma membranes, following binding to proteins and phospholipids, inhibition activities of sodium and potassium dependent ATP enzymes, inhibition of

transmebrane amino acid transport, enzyme inhibition, lipid peroxidation and DNA damage are attributed to heavy metal cytotoxicity in animals (Stohs and Bagchi, 1995 and Sigel, 1992). Heavy metals can enter into the cell and disturbing metabolism of the cells but some time get entry into the nucleus. There is formed ionic and coordinated bonds between heavy metals and DNA, but can not produce all the disorders seen in chromatin of cells. Hence not only the direct, but mostly indirect impacts of metals on nuclear chromatin must be essential to know about the DNA damage (Kasprzak, 1995).

Frequency of DNA damage in red blood cells of *Balkan loaches* fish was determined on the basis of tail length, tail intensity (% DNA) and tail moment. The findings indicate that *Balkan loaches* from the Sava River contain smaller degree of DNA damage cells as compare to those from reference site and therefore the comet assay is very sensitive technique for evaluation of genotoxicity. The result showed that the red blood cells in the fish showed low frequency of DNA damage at clean Kupa site, intermediate degree in the site Sava 2 and greater degree in the relatively polluted site Sava 1 (Nevenka *et al*., 2008). Recently it has been investigated genotoxic disease syndrome in the fish. The reduction of DNA integrity in red blood cells of fish affected with polluted water in their habitats (Nevenka *et al*., 2008). The genotoxic effects of environmental pollutants like heavy metals was determined in both vitro and in vivo through different assays but the comet assay has its advantages include sensitivity for detecting smaller degree of DNA damage in both the terrestrial and aquatic animals (Gedic *et al*., 1992).

It is very easy to find out degree of DNA damage cells in different tissues and organs of fish because no knowledge about metaphase and chromosome numbers are needed (Belpaeme *et al*., 1998). Some changes like strand breaks, alkali labile adducts and other modifications in the cells of aquatic animals are induced as a result of interaction of genotoxic metals with DNA, which due to enzymatic removal of

damage nucleotides can contribute to an increased level of DNA strand breaks. Overall comparison of main comet parameters determined in the red blood cells of *Balkan loach* highlights the presence of greater frequency of DNA damage in red blood cells of fish netted from the Sava River at the polluted Ivanja Reka and the result also showed genotoxicity of aquatic environment (Nevenka *et al*., 2008). The Loaches fish was collected from polluted Sava River and blood was taken out and processed for DNA damage. Synergistic effects of toxic chemicals are resulted into greater degree of DNA damage and also has an impact on immune response in loaches fish collected from the polluted water of Sava River (Nevenka *et al*., 2008). Genotoxic and carcinogenic effects of heavy metals like Ar and Cu have been determined. Both of them are attributed to higher frequency of DNA damage in aquatic animals (Reif erschied and Grummt, 2000; Gabbianelli *et al*., 2003). Genetic susceptibility, DNA repair activity, the number of alkali-labile sites, metabolic activity, antioxidant concentrations and heavy metals are different factors through, which DNA damage variability can be explained in the aquatic organisms (Mitchelmore and Chipman, 1998; Akcha *et al*., 2003; Buschini *et al*., 2004).

Variation of DNA damage was estimated between the male and female sex of fish. However studies on fish suggested controversial data on the involvement of sex in regulation of DNA damage (Devaux *et al*., 1998). In past few decades ago it has been investigated that heavy metals like Fe, Cu, Cd, Hg, Ni, Pb and Ar have the ability to generate reactive radicals, which are resulting into cellular and DNA damage in living cells of animals (Phillips, 1995). Degree of DNA damage was determined in two fish like *C. punctatus* and *M. vittatus* and a significant difference of DNA damage was found between *C. punctatus* and *M. vittatus* and the difference between baseline values of DNA damage highlights that some factors like species, age and sex may have affect these minor differences. The same differences in genotoxicity patterns were also reported for other fish species such as cyprinids (Smith, 1990; Lemos *et al*., 2001; Vigano *et al*., 2002).

Red blood and white blood cells were investigated to evaluate the degree of DNA damage due to heavy metals in aquatic organisms. The cells showed greater degree of DNA damage in the fish collected from polluted water as compare to those from non polluted water (Nagpure *et al*., 2008). It has been investigated that toxic chemicals like heavy metals have the ability to bind with DNA and resulted into greater degree of DNA damage (De Flora *et al*., 1991; Bhaskaran *et al.*, 1999), gene mutations (Maccubin *et al*., 1991) and genetic disease syndromes (Kurelec, 1993) in the aquatic organisms, particularly fish. The blood of different fish species was processed for determination of genotoxicity. The red blood cells showed high degree of DNA damage (Rajaguru *et al*., 2003). DNA damage in different tissues and organs of *C. punctatus* fish was investigated and observed a significant increase in DNA damage after different exposure times. The result showed that *C. punctatus* is more sensitive fish for evaluation the water quality in aquatic environment (Basdeo *et al*., 2012). The heavy metals like Cd and Hg are two most toxic metals, which toxicity and genotoxicity for fish have investigated (Ayllon and Garcia, 2000; Risso Fave *et al*., 2001).

5.2 METHODS AND MATERIALS

5.2.1 Study Area

For detail see page≠2

5.2.2 Fish Sampling Sites

For detail see page≠109

5.2.3 Collection of the Fish Samples

For detail see page≠109

5.2.4 Collection and Preservation of Fish Tissues

Different fish species including *Wallago attu*, *Ompok bimaculatus*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala* were collected from two sites

of the River Kabul, downstream (site 1, Amangar and site 2, Nowshera) and upstream (site 3, Warsak dam reservoir) and on the spot the blood was collected by cardiac puncture through sterilized syringe and the blood was then shifted to EDTA glass tubes to prevent blood clotting. The collected fishes were dissected for collection of tissues like intestine, gills, skin, liver and muscle were taken out. These tissues were washed with distilled water and then shifted to polythene bags and stored in the freezer (at -20 C°) for further study. For determination of genotoxicity, the tissues were grinded through grinder machine and 200µl PBS was added to the grinded tissues to obtain cell suspension. The same method was followed for genotoxicity in intestine, gills, skin, liver and muscle as adopted for the blood through comet assay.

5.2.5 Comet Assay

Comet assay was conducted according to the method described by Singh *et al* (1988) with slight modification. The comet assay technique is suitable for determination of genotoxicity in the aquatic animals due to its sensitivity (Kim and Hyun, 2006).

5.2.5.1 Preparation of different solutions for comet assay

5.2.5.2 Lysing solution (EDTA 37.2 gm, Nacl 146.1 gm and Trizma base 1.2 gm, NaOH8 gm, d.H_2O 890 mL, pH 10.0 by using concentrated Na OH or HCl,).

5.2.5.3 Final lysing solution (10 % DMSO, 1 % Trition, lysing solution).

5.2.5.4 Phosphate buffer saline (PBS) (PBS 1 packet, 1000 mL d.H_2O, pH 7.4).

5.2.5.5 Preparation of stock solutions

a. EDTA (14.89 g/200 mL d.H_2O, pH 10).

b. NaOH (200 g/500 mL d.H_2O)./

5.2.5.6 Electrophoresis buffer

For electrophoresis buffer per liter, 30 mL NaOH and 5.0 mL EDTA were added, q.s. to 1000 mL and were mixed well. The total volume was depended on our gel box capacity. The pH of the buffer was measured to be 13.

5.2.5.7 Neutralization buffer (0.4 M Tris 48.5 gm,d.H_2O 1000 mL, pH 7.5).

5.2.5.8 Staining solution (Acridine Orange, 20 μg/mL was used)

5.2.5.9 Stock solution (20mg / 20 mL, stored at room temperature and protected from the light).

5.2.5.10 Working solution (Stock solution0.4 mL, d.H_2O19.6 mL=20 μg/mL).

5.2.5.11 Preparation of 1% and 0.5 % LMPA and 1% NMA

a.1% LMPA (500 mg / 50ml PBS)

b.1 % NMA (500 mg / 50 ml mili Q H_2O)

Both the low melting point agarose (LAMP) and normal melting agarose (NMA) were boiled in microwave oven and kept in refrigerator until needed.

5.2.5.12 Preparation of base slides

For preparation of base slides, the NMA agarose was again melted briefly in microwave. After boiling the NMA was poured in caplin jar and conventional slides were kept in the caplin jor upto two-third the frosted area for a minute and then removed from the jar and placed in tray for drying. The undersides of the slides were wiped with the help of another slide to remove the agarose and were stored at room temperature until needed. The slides were labeled.

5.2.5.13 Layering of cells and LMPA on base slides

To the coated slide, added 75 μL of LMPA mixed with 10 μL of blood and suspension of grinded tissues. A cover slip was placed on it and the slide was kept on ice pack for 5 to 10 minutes. The cover slip was then removed from the slide and

added 85 µL LMPA to the slide. The cover slip was again kept on this layer and the slide was returned to the ice pack for 5 to 10 minutes.

5.2.5.14 Placing of slides in final lysing solution (Total volume 25ml**,** Tritone 250µl**,** lysing solution 22.75ml**,** DMSO 2.5ml).

The cover slip was gently removed from the third LMPA layer and the slide was kept in the glass tray and poured gently lysing solution in glass tray .The glass tray containing the slides was kept in the refrigerator for 2 hours or for overnight at 4ºC.

5.2.5.15 Electrophoresis of slides (Total volume 250ml, NaOH 7.5ml, EDTA 1.25ml, pH 13).

After 2 hours or overnight at ~4ºC, we carefully removed the slides from the lysing solution and placed the slides closely side by side on the horizontal gel box at one end. Then the horizontal gel box was gently filled with electrophoresis buffer (pH>13) and allowed the slides to remain in the electrophoresis buffer for 20 minutes to unwind the DNA. After 20 minutes the gel box was kept in refrigerator and power supply was seted at 24 Volts and 300 mill amperes current for 30 minutes time and turned on.

5.2.5.16 Neutralization of slides

After the completion of electrophoresis, the slides were removed from the gel box and washed with neutralization buffer drop by drop. The slides were let sit for about 5 minutes and again washed with neutralization buffer drop by drop and the process was repeated two more times.

5.2.5.17 Drying of slides

The slides were drained and kept for 20 minutes in cold 100% ethanol for dehydration. The slides were dried in open air for drying.

5.2.5.18 Rehydration and staining of slides

Chilled distilled water was used for rehydration of slides. The slides were kept for about 30 minutes in it and then stained with 70μL DNA specific fluorescent dye acridine orange (20 μg/ml) and kept for 5 minutes. To remove the excess of dye, the slides were dipped again in chilled distilled water. Then the slides were covered with cover slips.

5.2.5.19 Scoring of slides and visualization of DNA damage

From every slide 100 cells were randomly selected and images were taken at 400x by using fluorescent microscope (Nikon Eclipse 80 i) equipped with an excitation filter of 450-490 nm. Comet tail lengths (consisting of nuclear region and tail) were scored visually into 5 comet classes.

5.2.5.20 Comet Classes

Comet class 0 (no damage, hence no tail),

Comet class 1 (tail up to 1.5 times the diameter of the comet nucleus),

Comet class 2 (tail 1.5–2.0 times the diameter of the comet nucleus),

Comet class 3 (tail 2.0–2.5 times the diameter of the comet nucleus) and

Comet class 4 (maximally damaged with total DNA in its tail).

A final overall total comet score for all 100 cells was obtained by summing up the number of cells in each class times the class number, giving a rating between 0 (completely undamaged) and 400 (maximum damaged) (Collins, 2004) i.e.

TCS = 0(n) + 1(n) + 2(n) + 3(n) + 4(n), Where (n) indicate the number of cells in each class. One slide reader performed the overall scoring.

5.2.5.21 Statistical analysis

Statistical analysis was done by using ANOVA software for windows. Mean and standard deviation values of the data were determined. The different sets of data

were analyzed for statistical differences by using student's t –test (two-tailed); a P value <0.05 was considered to show statistical significance.

5.3 RESULTS AND DISCUSSION

The present investigation has determined genotoxicological effects of heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg in blood, intestine, skin, liver, gills and muscle of five selected fish species including *Wallago attu*, *Aorichthys seenghala*, *Cyprinus carpio, Labeo dyocheilus* and *Ompok bimaculatus* from site 1 and site 2 (polluted) of River Kabul and were compared with fish samples from site 3 (control) to estimate degree of DNA damage like total comet score (TCS) and comet classes caused by heavy metals through comet assay.

5.3.1 TCS and Comet Classes in Blood

Blood of the selected five different fish from control site 3 and polluted sites 1 and 2 was taken out and processed for estimation of degree of DNA damage like total comet score (TCS) and comet class 0, class 1, class 2, class 3 and class 4 caused by heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg. DNA damage cells observed in blood of five different fish from sites 1 and 2 were significantly higher than those observed in blood from control site 3 (Table 5.1 and Figs 5.1-5.3).

The blood of different fish species from site 1 and site 2 showed higher mean frequency of total comet score (TCS) and comet class 1, class 2, class 3 and class 4 per 100 cell as compare with those from site 3 besides comet class 0 where its value was highest in blood of studied fish species from control site 3 and was lowest from polluted sites 1 and 2. Lowest mean values of comet class 0 in blood of *Wallago attu* from polluted sites (1 and 2) were 47.0±4.0 cells and 33.3±4.5 cells and was 90.0±4.0 cells from reference sites, *in Aorichthys seenghala* from polluted sites were 30.6±3.5 cells and 20.0±4.0 cells and was 82.0±4.0 cells from site 3, in *Labeo dyocheilus* from polluted water were 38.0±4.0 cells and 26.0±3.6 cell and was 87.0±4.0 cells from control water, in *Cyprinus carpio* from polluted sites were 12.0±4.0 cells and 2.6±2.5

cells and was 71.0±4.0 cells from site 3 and in *Ompok bimaculatus* from polluted site 1 and site 2 were 21.6±1.5 cells and 14.6±3.0 cells and was 77.0±4.0 cells from site 3 respectively. The sequence of comet class 0 in blood tissues of different studied fish was *Wallago attu>Labeo dyocheilus >Aorichthys seenghala >Ompok bimaculatus > Cyprinus carpio.* This indicates that comet class 0 was greater in *Wallago attu* and lower in *Cyprinus carpio*. These results are in agreement with those observed by many investigators (Ozkan *et al*., 2011; Fagr *et al*., 2008; Al-Sabti, 1986; Dan and Nanda, 1986). Comet class 0 observed in blood of examined fish species from polluted water was significantly lower than those observed from Warsak dam. In this investigation comet class 0 in blood of selected fish species from polluted sites was lower as compare to those from control site of the River Kabul. This was correlated to less heavy metals in this tissues and the result also confirmed less heavy metals pollution in control water of Warsak dam.

The highest degree of comet class 1 observed in blood sample of *Wallago attu* from polluted site 1 and 2 were 5.0±1.0 cells and 8.0±1.0 cells and was 2.0±1.0 cells from control water, in *Aorichthys seenghala* from sites 1 and 2 were 9.3±0.5 cells and 11.0±1.0 cells and was 6.0±1.0 cells from control site 3, in *Labeo dyocheilus* from polluted water were 7.0±1.0 cells and 10.0±1.0 cells and was 4.0±1.0 cells from control water, in *Cyprinus carpio* from both sites 1 and 2 were 13.0±1.0 cells and 14.6±0.5 cells and was 9.0±1.0 cells from site 3 and in *Ompok bimaculatus* from sites 1 and were 12.0±1.0 cells and 13.0±1.0 cells and was 7.0±1.0 cells from site 3 respectively. The order of comet class 1 in this tissue of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus >Wallago attu.* This shows that comet class 1 was highest in *Cyprinus carpio* and lowest in *Wallago attu.* In this study the values for comet class 1 were lower as compare to the values mentioned by (Wirzinger *et al*., 2007; Smith., 1996). The greater degree of comet class 1 in blood of *Cyprinus carpio* is related to greater

content of heavy metals in this tissue. The result also showed metal pollution in the studied areas.

Blood of *Wallago attu* from polluted sites had highest comet class 2 with mean values of 7.0±1.0 cells and 10.0±1.0 cells and had lowest mean value of 3.0±1.0 cells from Warsak dam, *Aorichthys seenghala* from polluted sites 1 and 2 had 10.0±1.0 cells and 13.0±1.0 cells and had 5.0±1.0 cells from control site 3, *Labeo dyocheilus* from polluted sites had 9.0±1.0 cells and 12.0±1.0 cells and had 4.0±1.0 cells from control site, *Cyprinus carpio* from polluted water had 15.0±1.0 cells and 16.3±0.5 cells and had 8.0±1.0 cells from control water and *Ompok bimaculatus* from polluted sites had 12.3±0.5 cells and 15.0±1.0 cells and had 6.0±1.0 cells from reference site 3 respectively. Comet class 2 in blood having a sequence: *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus >Wallago attu.* This reveales that *Cyprinus carpio* had greater degree of comet class 2 and *Wallago attu* had smaller comet class 2.These values for comet class 2 in blood were higher than those reported by (Ozkan *et al*., 2011; Fagr *et al*., 2008). Higher degree of comet class 2 in this tissue is attributed to greater level of heavy metals in blood. Therefore blood of different fish species from polluted water showed greater degree of DNA damage cells than those from control water.

The observed mean values for comet class 3 in blood of *Wallago attu* from polluted sites were 20.0±1.0 cells and 23.0±1.5 cells and was 3.0±1.0 cells from Warsak dam, in *Aorichthys seenghala* from polluted sites were 24.0±1.0 cells and 27.0±1.0 cells and was 3.0±1.0 cells from control water, in *Labeo dyocheilus* from polluted sites 1 and 2 were 22.0±1.0 cells and 25.0±1.0 cells and was 2.0±1.0 cells from control site, in *Cyprinus carpio* from polluted water were 28.0±1.0 cells and 30.6±0.5 cells and was 6.0±1.0 cells from control site and in *Ompok bimaculatus* from sites 1 and 2 were 26.0±1.0 cells and 27.0±1.0 cells and was 5.0±1.0 cells from control site 3 respectively. The sequence of comet class 3 in this tissue was *Cyprinus*

carpio > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* >*Wallago attu.* This highlights that comet class 3 was highest in *Cyprinus carpio* and lowest in *Wallago attu.* This could be attributed to more heavy metals and low detoxification mechanism in this fish. In this study the values for comet class 3 were higher as compare to the findings of these workers (Al-Sabti, 1986; Dan and Nanda, 1986).

Blood of *Wallago attu* from both sites 1 and 2 showed high level of comet class 4 with mean values of 21.0±1.0 cells and 25.0±1.0 cells and showed low level with mean value of 2.0±1.0 cells from Warsak dam, *Aorichthys seenghala* from polluted sites showed 24.0±1.0 cells and 27.0±1.0 cells and showed 3.0±1.0 cells from control site, *Labeo dyocheilus* from polluted site 1 and site 2 showed 24.0±1.0 cells and 27.0±1.0 cells and showed 3.0±1.0 cells from control site 3, *Cyprinus carpio* from polluted water showed 32.0±1.0 cells and 35.3±0.5 cells and showed 6.0±1.0 cell from reference water and *Ompok bimaculatus* from both polluted sites 1 and 2 showed 28.0±1.0 cells and 30.3±1.5 cells and showed 5.0±1.0 cells from control site 3 respectively. The sequence of comet class 4 in blood of different fish species was *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This indicates that *Cyprinus carpio* showed maximum degree of comet class 4 while *Wallago attu* showed minimum comet class 4. These results of higher values for comet class 4 in blood agree with the findings of those reported by (Richard *et al*., 2003; Mishra and Mohanty, 2009; Li *et al*., 2011; Adams *et al*.,1989). The greater frequency of comet class 4 in blood of *Cyprinus carpio* could be related to high content of heavy metals in this tissue and exposition of the fish to heavy metals in water for long period.

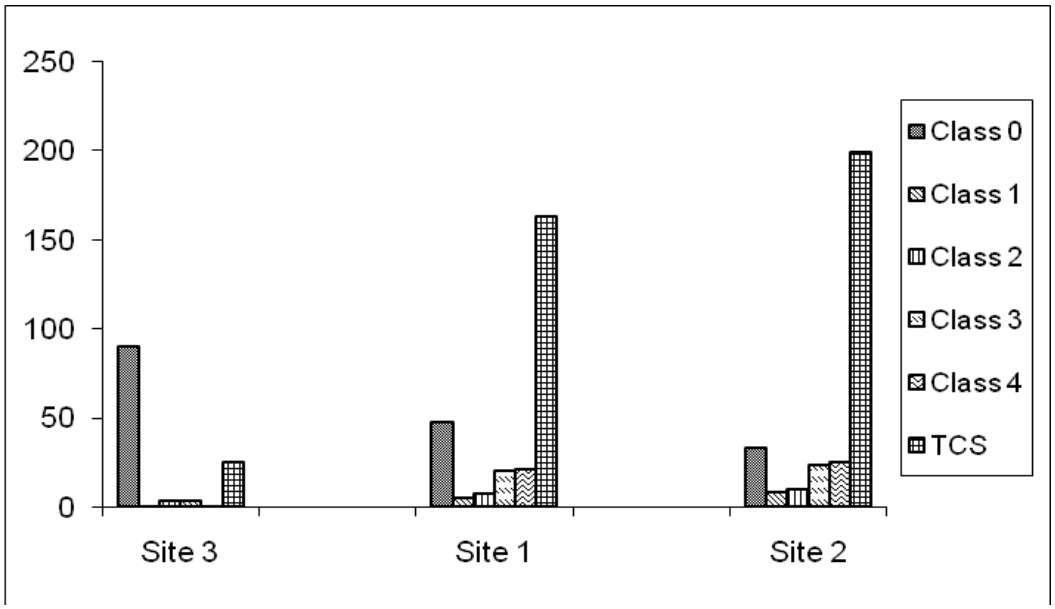
In our study degree of TCS observed in blood of studied fish species from polluted water was significantly higher than those observed from Warsak dam. Blood of *Wallago attu* from polluted sites 1 and 2 had maximum level of total comet score (TCS) with mean values of 163.0±10.0 cells and 199.0±11.5 cells and had minimum

level with mean value of 25.0±10.0 cells from control site 3, *Aorichthys seenghala* from polluted sites had 205.3±9.5 cells and 232.6±10.2 cells and had 41.0±10.0 cells from Warsak dam, *Labeo dyocheilus* from polluted water had 187.0±10.0 cells and 217.0±8.8 cells and had 30.0±10.0 cells from control water, *Cyprinus carpio* from polluted site 1 and site 2 had 255.0±10.0 cells and 279.3±4.1 cells and had 67.0±10.0 cells from reference site 3 and blood of *Ompok bimaculatus* from both sites 1 and 2 had 226.6±4.9 cells and 245.3±8.7 cells and had 54.0±10.0 cells from Warsak dam respectively. The order of TCS in different studied fish species was *Ompok bimaculatus* > *Cyprinus carpio* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This reveales that *Cyprinus carpio* had maximum TCS and *Wallago attu* had minimum. This greater degree of DNA damage cells in the blood tissue of *Cyprinus carpio* could be related to low elimination of metals from body, exposition of fish to metals for long period and more content of metals in the blood of this fish. In this study more TCS was observed than those reported in the previous findings by Christopher (1994) and Buschini *et al* (2004). In the present finding high degree of TCS was observed in blood. This is in agreement with the findings of Nevenka *et al* (2008), who had also reported greater frequency of TCS in blood of *Balkan loaches* after exposure to heavy metals. By making overall comparison, blood came second after intestine followed by skin, liver, gills and muscle for comet classes and TCS. This tissue showed more comet classes and TCS than skin, liver, gills and muscle. This is because of more heavy metals accumulation in this tissue and low detoxification mechanism of red blood cells. Overall trend of DNA damage cells in different fish species was in the order of *Cyprinus carpio*>*Ompok bimaculatus* > *Aorichthys seenghala*>*Labeo dyocheilus* >*Wallago attu* and overall order of comet classes in blood was class 4 > class 3> class 0> class 2> class 1.

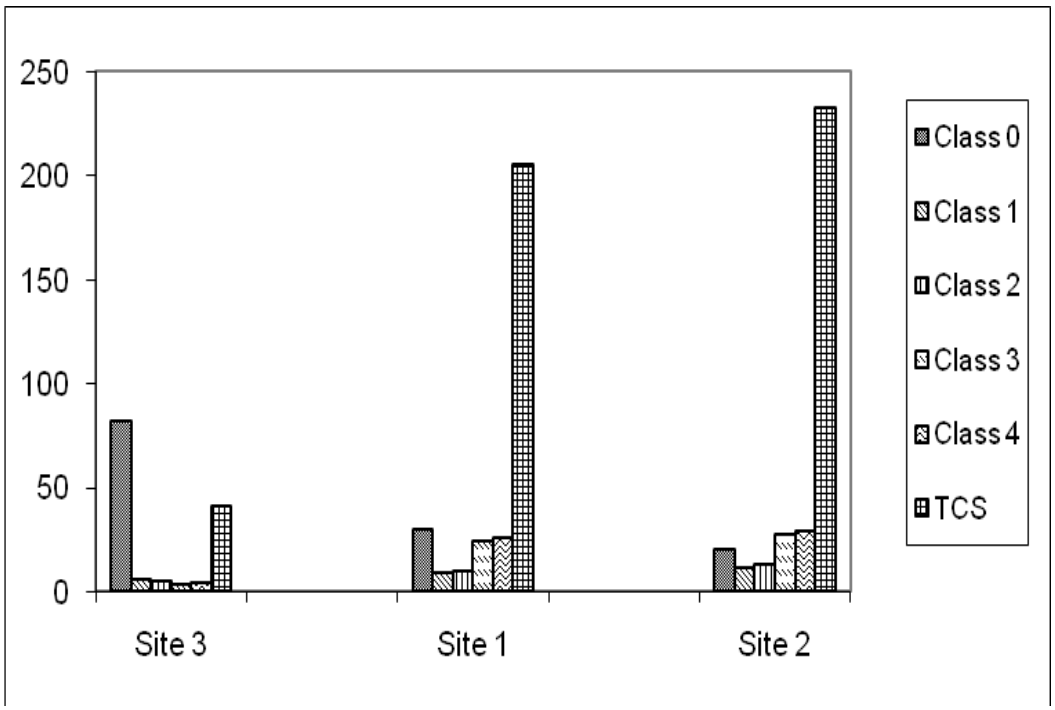
Table 5.1: Degree of total comet score (TCS) and comet classes in blood of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	90.0±4.0	47.0±4.0	33.3±4.5
	Class 1	2.0±1.0	5.0±1.0	8.0±1.0
	Class 2	3.0±1.0	7.0±1.0	10.0±1.0
	Class 3	3.0±1.0	20.0±1.0	23.0±1.5
	Class 4	2.0±1.0	21.0±1.0	25.0±1.0
	TCS	25.0±10.0	163.0±10.0	199.0±11.5
Aorichthys seenghala	Class 0	82.0±4.0	30.6±3.5	20.0±4.0
	Class 1	6.0±1.0	9.3±0.5	11.0±1.0
	Class 2	5.0±1.0	10.0±1.0	13.0±1.0
	Class 3	3.0±1.0	24.0±1.0	27.0±1.0
	Class 4	4.0±1.0	26.0±1.0	29.0±1.0
	TCS	41.0±10.0	205.3±9.5	232.6±10.2
Labeo dyocheilus	Class 0	87.0±4.0	38.0±4.0	26.0±3.6
	Class 1	4.0±1.0	7.0±1.0	10.0±1.0
	Class 2	4.0±1.0	9.0±1.0	12.0±1.0
	Class 3	2.0±1.0	22.0±1.0	25.0±1.0
	Class 4	3.0±1.0	24.0±1.0	27.0±1.0
	TCS	30.0±10.0	187.0±10.0	217.0±8.8
Cyprinus carpio	Class 0	71.0±4.0	12.0±4.0	2.6±2.5
	Class 1	9.0±1.0	13.0±1.0	14.6±0.5
	Class 2	8.0±1.0	15.0±1.0	16.3±0.5
	Class 3	6.0±1.0	28.0±1.0	30.6±0.5
	Class 4	6.0±1.0	32.0±1.0	35.3±0.5
	TCS	67.0±10.0	255.0±10.0	279.3±4.1
Ompok bimaculatus	Class 0	77.0±4.0	21.6±1.5	14.6±3.0
	Class 1	7.0±1.0	12.0±1.0	13.0±1.0
	Class 2	6.0±1.0	12.3±0.5	15.0±1.0
	Class 3	5.0±1.0	26.0±1.0	27.0±1.0
	Class 4	5.0±1.0	28.0±1.0	30.3±1.5
	TCS	54.0±10.0	226.6±4.9	245.3±8.7

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

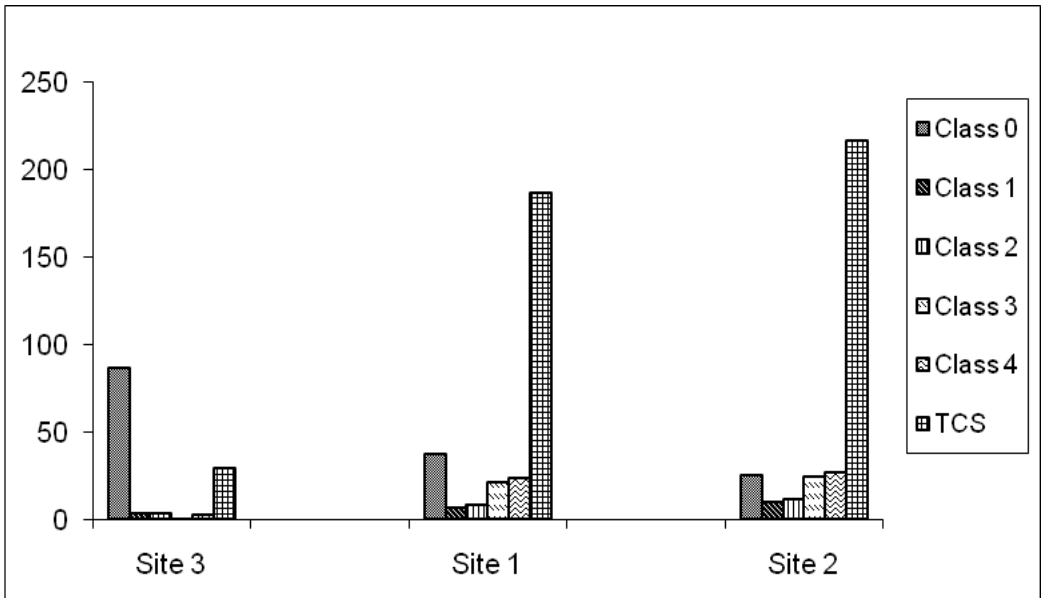


Wallago attu

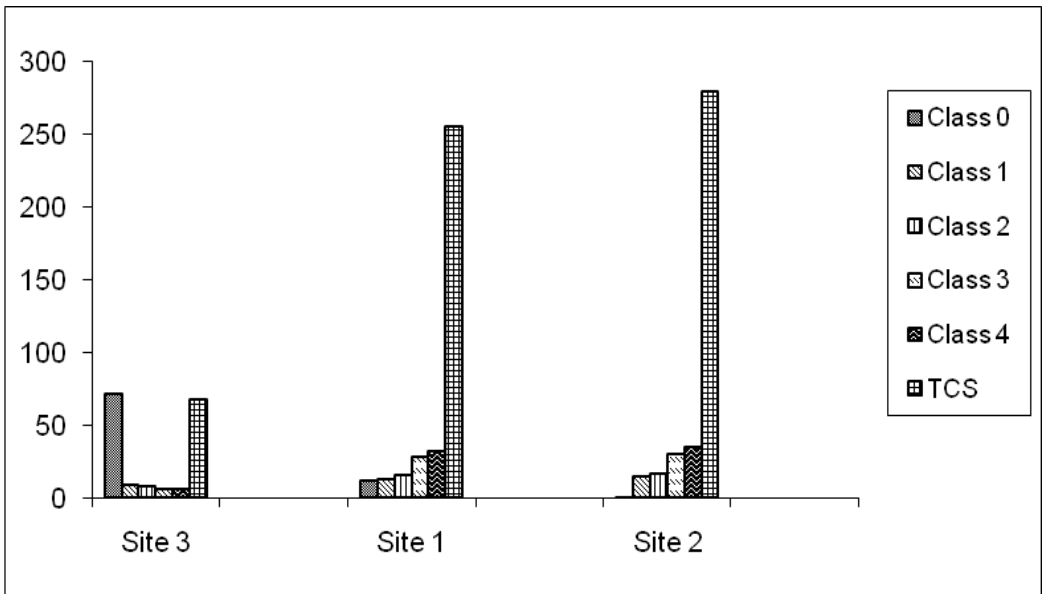


Aorichthys seenghala

Fig. 5.1: **Degree of total comet score (TCS) and comet classes in blood of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

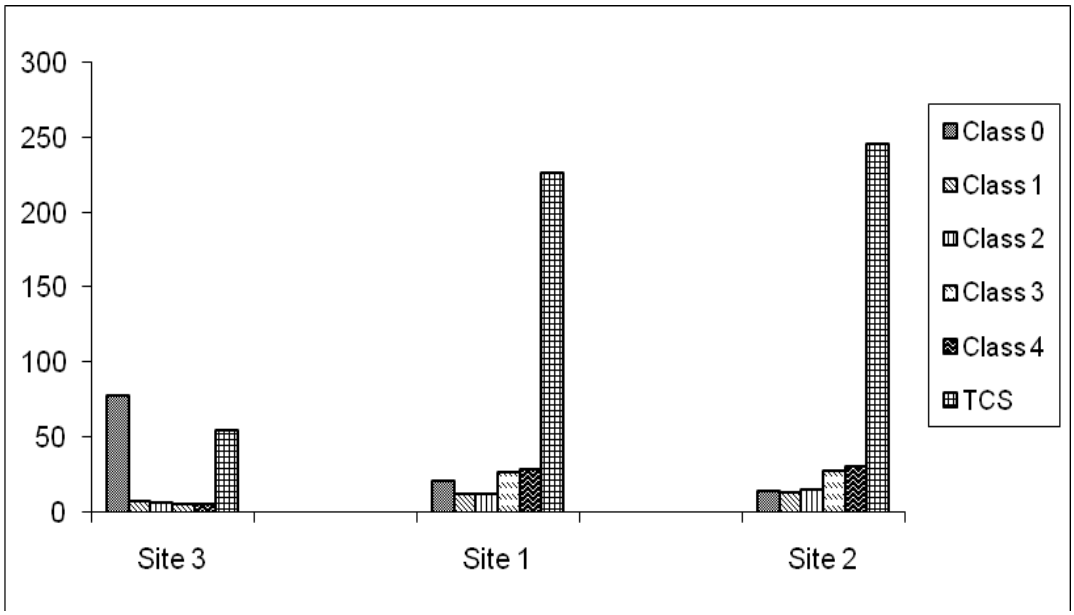


Labeo dyocheilus



Cyprinus carpio

Fig. 5.2: **Degree of total comet score (TCS) and comet classes in blood of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**



Ompok bimaculatus

Fig.5.3: **Degree of total comet score (TCS) and comet classes in blood of *Ompok bimaculatus* and netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

5.3.2 TCS and Comet Classes in Gills

Gills of the selected five different fish from Warsak dam (Site 3) and polluted sites 1 and 2 (Nowshera and Amangarh) were taken out and processed for the determination of DNA damage cells. The gills of the fish from polluted water showed higher degree of DNA damage cells as compare with those fish from Warsak dam, where lower DNA damage cells were observed (Table 5.2 and Figs 5.4-5.6).

Gills of *Wallago attu* from both polluted sites of River Kabul showed smaller frequency of comet class 0 with mean values of 55.6±4.0 cells and 43.6±4.0 cells and showed greater level with mean value of 91.6±4.0 cells from control site 3 (Warsak dam), *Aorichthys seenghala* from both sites 1 and 2 showed 42.6±4.9 cells and 30.0±2.6 cells and showed 88.0±3.6 cells from control site 3, *Labeo dyocheilus* from polluted water showed 50.6±5.8 cells and 37.0±5.0 cells and showed 89.3±4.1 cells from control water, *Cyprinus carpio* from site 1 and site 2 showed 33.0±5.5 cells and 19.3±3.0 cells and showed 85.3±40.2 cells from site 3 and *Ompok bimaculatus* from sites 1 and 2 showed 40.0±5.5 cells and 29.0±3.4 cells and showed 85.0±3.4 cells from reference site 3 respectively. The sequence of comet class 0 in different studied fish was *Wallago attu* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Ompok bimaculatus* > *Cyprinus carpio.* This highlights that comet class 0 was maximum in *Wallago attu* and minimum in *Cyprinus carpio*. In this finding degree of comet class 0 in this tissue was lower than those reported in past findings (Pandrangi *et al*., 1995; Villarini *et al*., 1998; Tice, 1995; Wilson *et al.,* 1998). The result shows that comet class 0 was lowest in gills from polluted water and highest from control water. This could be related to less metals in this tissues. Comparing our result with the findings of above mentioned studies highlights that heavy metals are toxic in nature and can induce genotoxicity in various tissues of aquatic and terrestrial animals including human beings.

The observed greater mean values for comet class 1 in gills of *Wallago attu* from polluted sites were 6.0±1.0 cells and 8.0±1.0 cells and was 3.0±1.0 cells from Warsak dam, in *Aorichthys seenghala* from polluted sites 1 and 2 were 7.0±1.0 cells and 9.3±0.5 cells and was 4.0±1.0 cells from control site 3, in *Labeo dyocheilus* from polluted water were 6.3±1.5 cells and 8.3±1.5 cells and was 3.0±1.0 cells from control water, in *Cyprinus carpio* from polluted sites were 8.3±1.5 cells and 11.3±0.5 cells and from control site was 4.0±1.0 cells and in *Ompok bimaculatus* from sites 1 and 2 were 8.0±1.0 cells and 9.0±1.0 cells and was 4.0±1.0 cells from control site 3 respectively. The order of comet class 1 in gills of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus >Wallago attu.* This indicates that *Cyprinus carpio* showed high degree of comet class 1 and *Wallago attu* low degree of comet class 1. This greater degree of comet class 1 in gills of *Cyprinus carpio* could be the cause of greater concentration of heavy metals in this tissue. The gills is the tissue that are directly and constantly exposed to heavy metals in water as compare to other organs of the fish body. These results are in agreement with the findings of those reported by (Belpaeme *et al*., 1996; Mitchelmore and Chipman, 1998; Maruya, 20000; Kim *et al.*, 2000). Comparing the present result with the findings of previous workers reveale that heavy metals are genotoxic in nature, which may be resulted into greater frequency of DNA damage cells in different tissues of the fish.

Gills of *Wallago attu* from polluted sites contained highest degree of comet class 2 with mean values of 5.0±1.0 cells and 10.0±1.0 cells and contained lowest frequency with mean value of 2.0±1.0 cells from control site, *Aorichthys seenghala* from both sites 1 and 2 contained 8.0±1.0 cells and 13.0±1.0 cells and contained 3.0±1.0 cells from reference site 3, *Labeo dyocheilus* from polluted water contained 6.3±1.5 cells and 11.6±1.5 cells and contained 3.0±1.0 cells from control water, *Cyprinus carpio* from site 1 and site 2 contained 10.3±1.5 cells and 14.3±0.5 cells and contained 5.0±1.0 cells from reference site 3 and *Ompok bimaculatus* from polluted

sites contained 8.3±1.5 cells and13.0±1.0 cells and contained 4.0±1.0 cells from control water of Warsak dam respectively. Comet class 2 in gills of different studied fish species having a sequence: *Cyprinus carpi>Ompok bimaculatus>Aorichthys seenghala > Labeo dyocheilus>Wallago attu.* This reveales that greater frequency of comet class 2 was observed in *Cyprinus carpio* and smaller in *Wallago attu.* These results are agreed with the findings of (Chandra and Khuda, 2004), who had also reported greater degree of comet class 2 in gills of *Oreochromis mossambicus* after exposure to cadmium chloride and azadirachtin.

Gills of *Wallago attu* from site 1 and site 2 showed more degree of comet class 3 with mean values of 16.0±1.0 cells and 17.3±1.5 cells and showed less degree with mean value of 2.0±1.0 cells from control site 3 (Warsak dam), *Aorichthys seenghala* from polluted sites showed 19.6±1.1 cells and 22.6±0.5 cells and showed 3.0±1.0 cells from reference site 3, *Labeo dyocheilus* from polluted water showed 17.3±1.5 cells and 19.0±1.0 cells and showed 2.6±1.5 cells from control water, *Cyprinus carpio* from site 1 and site 2 showed 23.0±1.0 cells and 26.0±1.0 cells and showed 4.0±1.0 cells from site 3 and *Ompok bimaculatus* from polluted sites showed 20.3±1.5 cells and 23.0±1.0 cells and showed 4.0±1.0 cells from Warsak dam respectively. Comet class 3 in gills of different fish species was in the order of *Cyprinus carpio > Ompok bimaculatus > Aorichthys seenghala > Labeo dyocheilus > Wallago attu.* Here again the order of comet class 3 was same to comet class 1 and 2. The present result of higher comet class 3 in gills of *Cyprinus carpio, Ompok bimaculatus* and *Aorichthys seenghala* agree with the findings of Bertin and Averbeck (2006). On the other hand, the present data for *Labeo dyocheilus* and *Wallago attu* agree with those of Cotelle and Erard (1999) and Valko *et al* (2005). In humans and other animals like fish heavy metals are associated with DNA damage. Therefore accumulation of heavy metals can induce DNA damage cells in aquatic animals like fish. The present investigation found more degree of DNA damage cells in gills of examined fish species from polluted water as compare to control fish of Warsak dam.

This could be correlated to higher concentration of heavy metals in gills of studied fish from polluted sites of River Kabul. This result also confirmed the heavy metals pollution in River Kabul.

Gills of *Wallago attu* from polluted sites showed maximum degree of comet class 4 with mean values of 17.3±1.5 cells and 21.0±1.0 cells and showed minimum mean value of 1.3±1.5 cells from control site (Warsak dam), *Aorichthys seenghala* from polluted sites 1 and 2 showed 22.3±1.5 cells and 25.0±1.0 cells and showed 2.0±1.0 cells from control site 3, *Labeo dyocheilus* from both polluted sites 1 and 2 showed 19.3±1.5 cells and 24.0±1.0 cells and showed 2.0±1.0 cells from control site 3, *Cyprinus carpio* from polluted water showed 25.3±1.5 cells and 29.0±1.0 cells and showed 4.0±1.0 cells from control water and *Ompok bimaculatus* from both site 1 and site 2 showed 23.3±1.5 cells and 26.0±1.0 cells and showed 3.0±1.0 cells from Warsak dam respectively. The order of comet class 4 in this tissue was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This order was same to comet class 1, 2 and 3. This trend shows greater degree of comet class 4 in *Cyprinus carpio* and smaller in *Wallago attu.* These results are agreed with the findings of previous investigations (Serpi *et al*., 2006; Bresler *et al*., 2001). By comparing the present result with the findings of above mentioned studies reveales that heavy metals tend to be concentrated in gills tissues and indunce greater degree of comet class 4 in gills tissues. The result also showed heavy metals pollution in the studied areas. The gills from polluted portions of River Kabul showed maximum frequency of comet class 4 as compare to control water.

Gills of *Wallago attu* from polluted sites had highest frequency of total comet score (TCS) with mean values of 133.3±11.0 cells and 164.0±10.5 cells and had lowest mean value of 18.3±11.0 cells from control site, *Aorichthys seenghala* from site 1 and 2 and had 171.3±12.0 cells and 203.3±6.6 cells and had 27.0±8.8 cells from site 3, *Labeo dyocheilus* from polluted water had 148.3±14.9 cells and

184.6±11.5 cells and had 25.0±11.1 cells from Warsak dam, *Cyprinus carpio* from polluted site 1 and site 2 had 199.0±13.6 cells and 234.0±8.5 cells and had 42.0±8.8 cells from reference site 3 and *Ompok bimaculatus* from sites 1 and 2 had 179.0±14.7 cells and 208.0±8.7 cells and had 36.0±8.7 cells from control site 3 respectively.TCS in gills of different fish species was in the sequence of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.*This indicates that TCS was highest in *Cyprinus carpio* and lowest in *Wallago attu.*This greater degree of TCS in *Cyprinus carpio* could be the result of high level of heavy metals in this fish. It is an omnivorous fish. Being an omnivorous nature, it is more exposed to heavy metals and accumulated more metals in this organ. Therefore gills of this fish showed more TCS as compare to other examined fish species. This result found more TCS than those reported by (Bresler *et al*., 2001; Ayllon and Garcia, 2000; Risso *et al*., 2001). Results of the present and previous studies demonstrated that heavy metals are genotoxic in nature and can induce genotoxicity in aquatic and terrestrial animals.

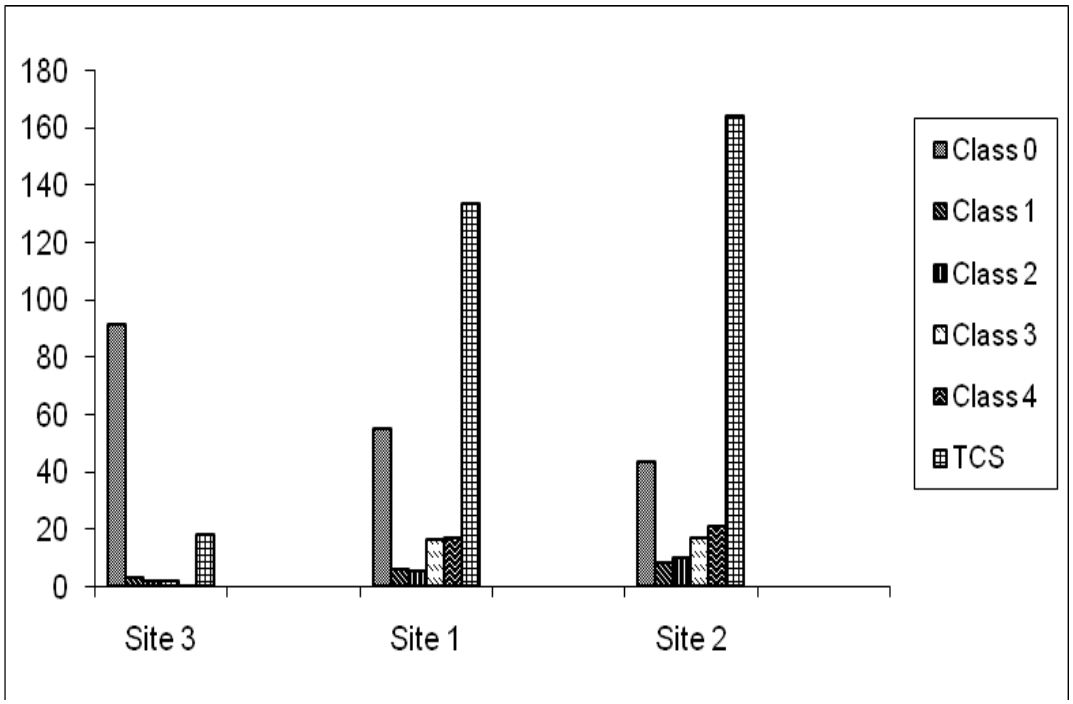
In the present study gills of the selected fish from polluted sites of River Kabul showed greater frequency of DNA damage cells as compare to those from Warsak dam. This higher degree of DNA damage cells in gills cells from polluted sites could be correlated to greater concentration of heavy metals in gills and also attributed to directly and constantly exposure of gills to heavy metals in the water. Comparing our result with the above findings shows that heavy metals are toxic and have capability to accumulate in gills, which resulted into DNA damage. By comparison the degree of DNA damage, the fish from polluted sites showed increasing tendency as compare to those from control site. All the TCS and comet class 1, 2, 3 and 4 values were highest in gills tissues of examined fish from polluted water than those from control water. Gills came last second for frequency of comet classes and TCS after intestine, blood, skin and liver and followed by muscle. Gill is the prime target organ that is directly and constantly exposed to heavy metals. The gills showed less degree of DNA

damage cells as compare to the intestine, blood, skin and liver. This could be related to low concentration of heavy metals in the gills, high elimination of heavy metals from body and strong detoxification mechanism of this tissue. Overall sequence of DNA damage cells in gills of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu* and overall order of comet classes in gills was class 0> class 4 > class 3> class 2> class 1.

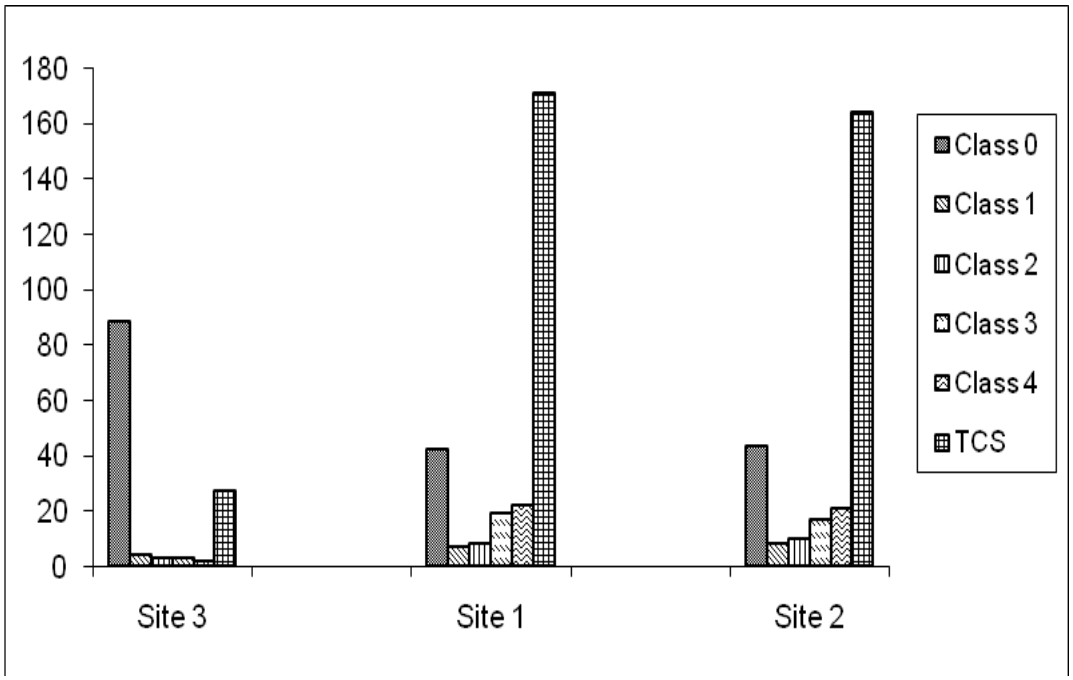
Table 5.2: Degree of total comet score (TCS) and comet classes in gills of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	91.6±4.0	55.6±4.0	43.6±4.0
	Class 1	3.0±1.0	6.0±1.0	8.0±1.0
	Class 2	2.0±1.0	5.0±1.0	10.0±1.0
	Class 3	2.0±1.0	16.0±1.0	17.3±1.5
	Class 4	1.3±1.5	17.3±1.5	21.0±1.0
	TCS	18.3±11.0	133.3±11.0	164.0±10.5
Aorichthys seenghala	Class 0	88.0±3.6	42.6±4.9	30.0±2.6
	Class 1	4.0±1.0	7.0±1.0	9.3±0.5
	Class 2	3.0±1.0	8.0±1.0	13.0±1.0
	Class 3	3.0±1.0	19.6±1.1	22.6±0.5
	Class 4	2.0±1.0	22.3±1.5	25.0±1.0
	TCS	27.0±8.8	171.3±12.0	203.3±6.6
Labeo dyocheilus	Class 0	89.3±4.1	50.6±5.8	37.0±5.0
	Class 1	3.0±1.0	6.3±1.5	8.3±1.5
	Class 2	3.0±1.0	6.3±1.5	11.6±1.5
	Class 3	2.6±1.5	17.3±1.5	19.0±1.0
	Class 4	2.0±1.0	19.3±1.5	24.0±1.0
	TCS	25.0±11.1	148.3±14.9	184.6±11.5
Cyprinus carpio	Class 0	83.0±3.6	33.0±5.5	19.3±3.0
	Class 1	4.0±1.0	8.3±1.5	11.3±0.5
	Class 2	5.0±1.0	10.3±1.5	14.3±0.5
	Class 3	4.0±1.0	23.0±1.0	26.0±1.0
	Class 4	4.0±1.0	25.3±1.5	29.0±1.0
	TCS	42.0±8.8	199.0±13.6	234.0±8.5
Ompok bimaculatus	Class 0	85.0±3.4	40.0±5.5	29.0±3.4
	Class 1	4.0±1.0	8.0±1.0	9.0±1.0
	Class 2	4.0±1.0	8.3±1.5	13.0±1.0
	Class 3	4.0±1.0	20.3±1.5	23.0±1.0
	Class 4	3.0±1.0	23.3±1.5	26.0±1.0
	TCS	36.0±8.7	179.0±14.7	208.0±8.7

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

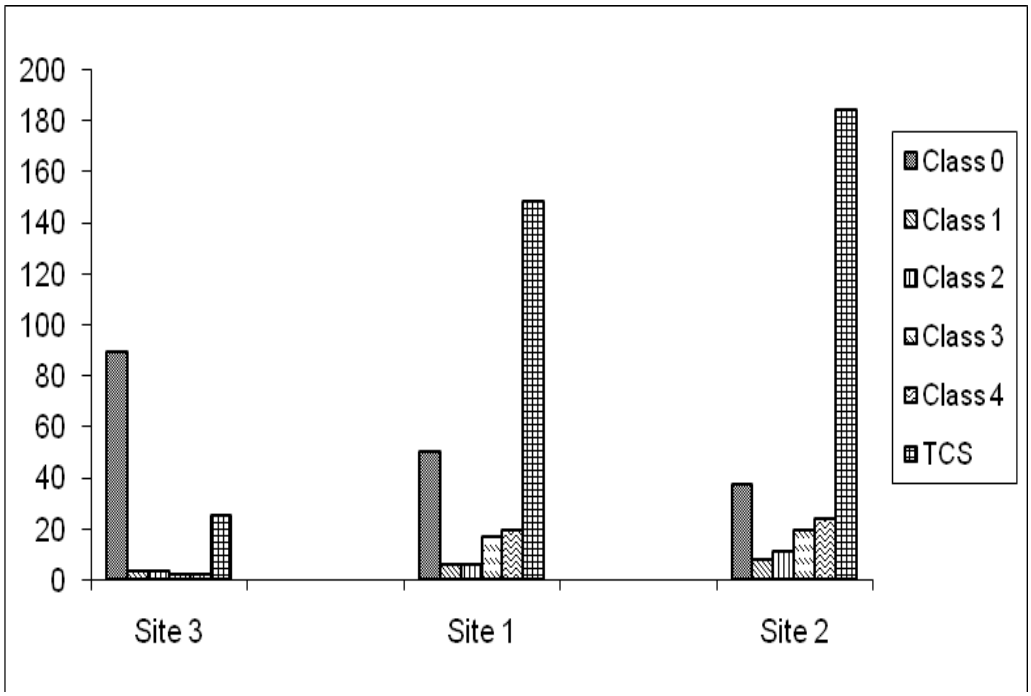


Wallago attu

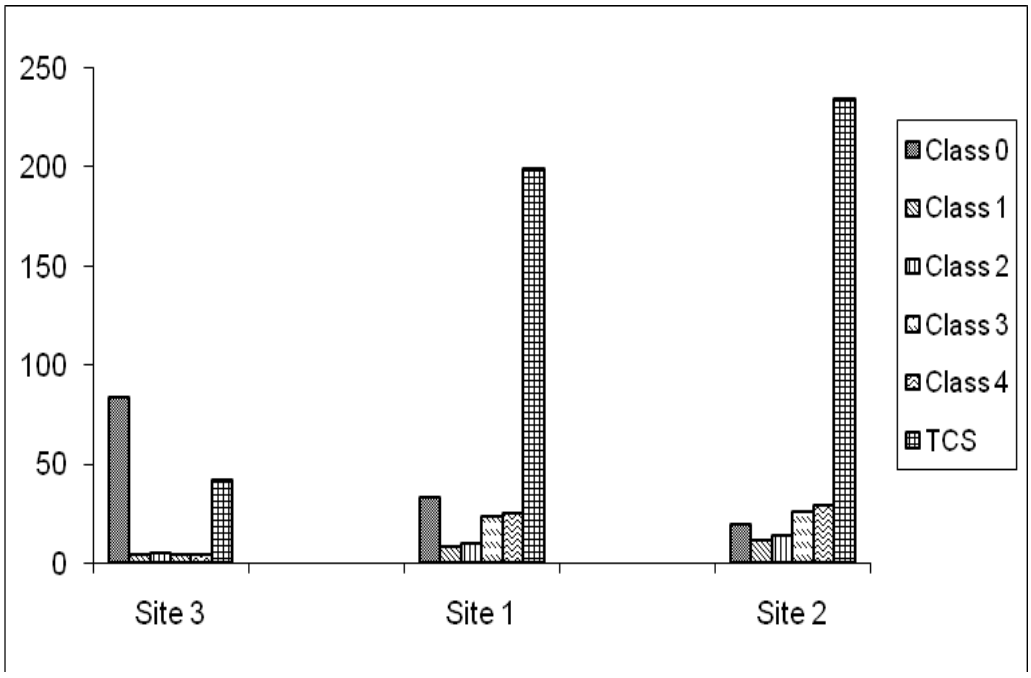


Aorichthys seenghala

Fig.5.4: **Degree of total comet score (TCS) and comet classes in gills of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

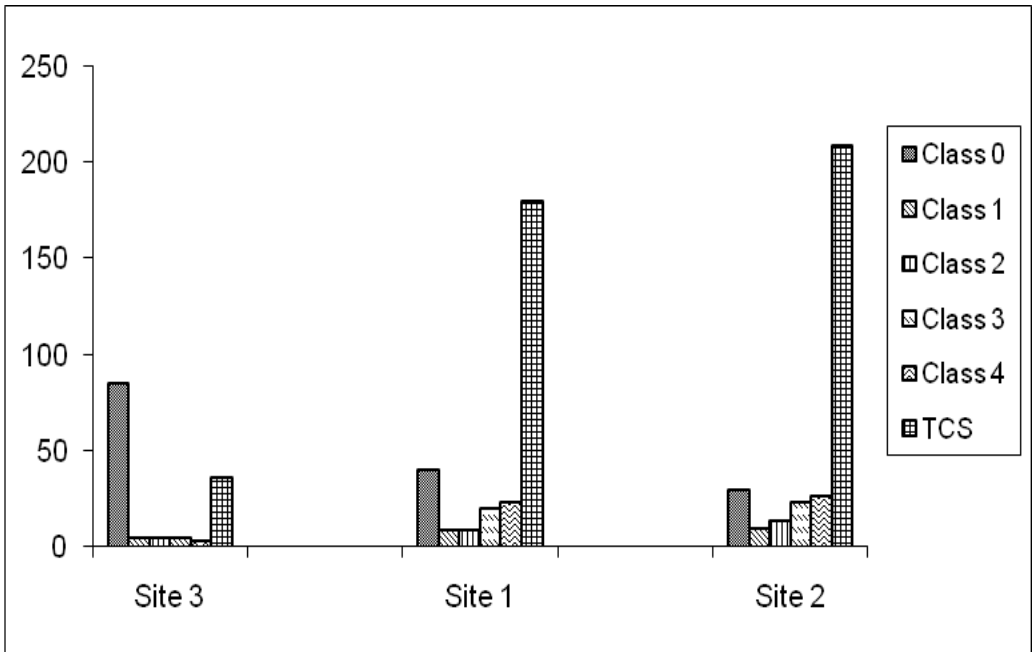


Labeo dyocheilus



Cyprinus carpio

Fig.5.5: Degree of total comet score (TCS) and comet classes in gills of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig.5.6: **Degree of total comet score (TCS) and comet classes in gills of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.**

5.3.3 TCS and Comet Classes in Skin

Skin of five selected fish species from control site 3 (Warsak dam) and polluted sites 1 and 2 were collected and processed through comet assay for estimation of total comet score (TCS) and comet classes. Skin of fish from polluted sites showed higher level of total comet score (TCS) and comet classes as compare with fish from control site 3 besides comet class 0, where mean values for comet class 0 were higher from control site as compare to polluted water (Table 5.3 and Figs 5.7-5.9).

Comet class 0 in skin of *Wallago attu* from both polluted sites were 49.3±5.5 cells and 35.6±3.2 cells and was 90.6±4.6 cells from control site, in *Aorichthys seenghala* from polluted sites were 34.0±4.0 cells and 22.3±3.0 cells and was 86.3±3.5 cells from control site, *Labeo dyocheilus* from sites 1 and 2 were 40.3±4.0 cells and 26.6±2.0 cells and was 88.0±3.4 cells from control site 3, in *Cyprinus carpio* from polluted water were 21.0±5.0 cells and 9.3±4.0 cells and was 82.3±1.5 cells from control water and in *Ompok bimaculatus* from polluted sites were 27.0±5.0 cells and 13.6±2.5 cells and was 82.0±3.6 cells from Warsak dam respectively. Comet class 0 in skin of different fish was in the order of *Wallago attu* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Ompok bimaculatus* > *Cyprinus carpio.* This highlights that *Wallago attu* was found to be contained greater mean values of comet class 0 and *Cyprinus carpio* smaller values. This data for comet class 0 was lower than those reported previously (Ali *et al.,* 2009). This organ from polluted sites of River Kabul showed low degree of comet class 0 as compare to those from control site. This greater degree of comet class 0 in this tissue from control site is because of less heavy metals concentration in skin and the result also showed less heavy metals pollution in control water of River Kabul.

Skin of *Wallago attu* from polluted sites contained maximum degree of comet class 1 with mean values of 6.3±1.0 cells and 8.6±0.5 cells and contained minimum

mean value of 3.0±1.0 cells from control site, *Aorichthys seenghala* from polluted sites 1 and 2 contained 7.0±1.0 cells and 11.3±0.5 cells and contained 3.6±1.1 cells from reference site 3, *Labeo dyocheilus* from polluted water contained 6.0±1.0 cells and 9.6±0.5 cells and contained 4.0±1.0 cells from control water, *Cyprinus carpio* from both site 1 and site 2 contained 11.0±1.0 cells and 13.0±1.0 cells and contained 5.0±1.0 cells from Warsak dam and *Ompok bimaculatus* from sites 1 and 2 contained 8.6±1.5 cells and 12.3±0.5 cells and contained 6.0±1.0 cells from site 3 respectively. The order of comet class 1 in this tissue of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Wallago attu >Labeo dyocheilus.* This indictes that comet class 1 was highest in *Cyprinus carpio* and lowest in *Labeo dyocheilus.* This result for comet class 1 was higher than the previous findings reported by Nehls and Segner (2005) and Huong *et al* (2012). Greater degree of comet class 1 in *Cyprinus carpio* could be attributed to high concentration of heavy metals in this fish. This fish has exposed to heavy metals for long period. This is also the target organ that is directly exposed to water pollution. Skin came on third no for DNA damage after intestine and blood.

Skin of *Wallago attu* from polluted sites showed highest level of comet class 2 with mean values of 7.3±1.5 cells and 11.6±0.5 cells and showed lowest mean value of 3.0±1.0 cells from Warsak dam (control), *Aorichthys seenghala* from sites 1 and 2 showed 10.0±1.0 cells and 13.3±0.5 cells and showed 4.0±1.0 cells from site 3, *Labeo dyocheilus* from polluted sites showed 8.6±1.5 cells and 12.6±0.5 cells and showed 3.0±1.0 cells from control site, *Cyprinus carpio* from polluted sites 1 and 2 showed 13.0±1.0 cells and 15.6±1.1 cells and showed 4.0±1.0 cells from control site and *Ompok bimaculatus* from polluted water showed 12.0±1.0 cells and 15.6±0.5 cells and showed 5.0±1.0 cells from control water respectively. The sequence of comet class 2 in this organ of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This shows that *Cyprinus carpio* was having higher degree of comet class 2 and *Wallago attu* was

having lower comet class 2 respectively. This result found greater frequency of comet class 2 than reported in the previous findings by Ali *et al* (2009) and Al- Sabti and Metcalfe (1995a). In the present study frequency of comet class 2 was higher significantly in skin from polluted sites as compare to Warsak dam. On the other hand skin showed higher frequency of comet class 2 than liver, muscle and gills and lower frequency than intestine and blood.

Comet class 3 in skin of *Wallago attu* from site 1 and site 2 were 17.6±1.5 cells and 21.0±2.0 cells and was 2.0±1.0 cells from site 3, in *Aorichthys seenghala* from polluted water were 23.0±1.0 cells and 25.0±1.0 cells and was 3.0±1.0 cells from control water, in *Labeo dyocheilus* from polluted sites were 21.0±1.0 cell and 24.0±1.0 cell and was 3.0±1.0 cells from control site, in *Cyprinus carpio* from site 1 and site 2 were 26.3±1.5 cells and 30.0±1.0 cells and was 4.0±1.0 cells from site 3 and in *Ompok bimaculatus* from polluted sites were 25.0±1.0 cells and 28.0±1.0 cells and was 4.0±1.0 cells from Warsak dam respectively. These results were in agreement with the study of Chipman (1998). The order of comet class 3 in the studied fish was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus > Wallago attu.* This indicates that *Cyprinus* carpio had greater degree of comet class 3 and *Wallago attu* had smaller respectively. This is because of heavy metals accumulation in this fish, which induced greater degree of comet class 3 in this fish.

Skin of *Wallago attu* from both polluted sites 1 and 2 had greater degree of comet class 4 with mean values of 19.6±1.5 cells and 23.0±1.0 cells and had 2.0±1.0 cells from Warsak dam, *Aorichthys seenghala* from polluted sites had 26.0±1.0 cells and 28.0±1.0 cells and had 3.0±1.0 cells from control site, *Labeo dyocheilus* from polluted water had 24.0±1.0 cells and 27.0±1.0 cells and had 2.0±1.0 cells from control water, *Cyprinus carpio* from polluted sites had 29.6±1.5 cells and 32.0±1.0 cells and had 4.6±0.5 cells from reference site and *Ompok bimaculatus* from both sites 1 and 2 had 27.3±1.5 cells and 30.3±0.5 cells and had 3.0±1.0 cells from site 3

respectively. The comet class 4 in skin of these fish was in the order of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu. Cyprinus carpio* was found to be contained high degree of comet class 4 and *Wallago attu* low degree of comet class 4. The values for comet class 4 were higher than the previous findings reported by Gertraud *et al* (2007). Comparing the present result with the findings of previous studies indicates that heavy metals are genotoxic in nature and indunce DNA damage cells in the aquatic and terrestrial animals.

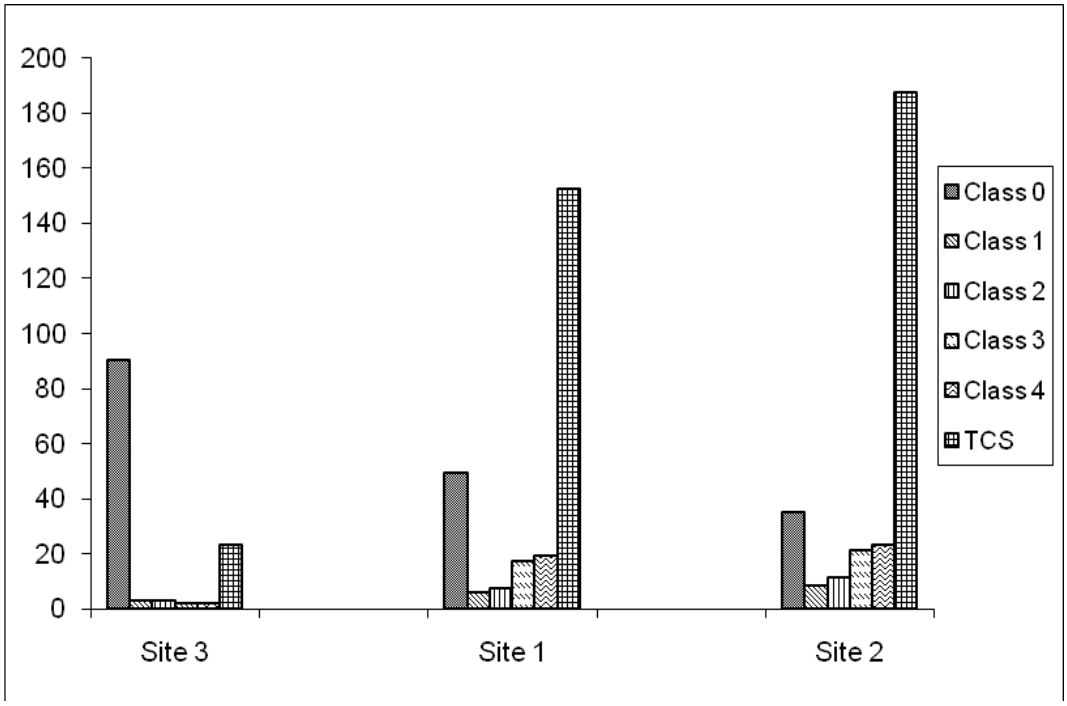
Skin of *Wallago attu* from both polluted sites 1 and 2 showed maximum frequency of TCS with mean values of 152.3±4.5 cells and 187.0±10.1 cells and showed minimum degree of TCS with mean value of 23.0±8.6 cells from control site, *Aorichthys seenghala* from polluted water showed 200.0±10.0 cells and 225.0±8.5 cells and showed 32.6±8.7 cells from Warsak dam, *Labeo dyocheilus* from sites 1 and 2 showed 182.3±9.7 cells and 215.0±6.9 cells and showed 27.0±8.8 cells from reference site, *Cyprinus carpio* from polluted sites showed 233.6±13.5 cells and 262.3±10.0 cells and showed 43.6±3.7 cells from Warsak dam and *Ompok bimaculatus* from both sites 1 and 2 showed 217.0±12.5 cells and 249.0±6.5 cells and showed 40.0±9.1 cells from site 3 respectively. The order of TCS in skin of different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* Overall order of comet classes in skin was class 4 > class 3> class 0> class 2> class 1 and TCS in different fish species was in the order of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This shows that TCS was higher in *Cyprinus carpio* and lower in *Wallago attu.* In this finding degree of DNA damage cells was higher than those reported by Yingmei *et al* (2006) and Gertraud *et al* (2007). In the present study generally frequency of DNA damage cells was higher significantly in skin from polluted sites as compare to Warsak dam. On the other hand skin cells showed higher degree of DNA damage cells than liver, muscle and gills and lower frequency than intestine and blood. The greater frequency of DNA damage cells in skin could be

attributed to more heavy metals accumulation in this tissue. The skin is laso the prime target organ like the gills that is exposed directly and constantly to heavy metals in water. Skin came third for degree of DNA damage after intestine and blood tissues. Skin showed less DNA damage cells than intestine and blood and more than liver, gills and muscle when overall comparison is made. The skin is also acting as protective layer against external agents and other chemicals. Therefore it had less degree of DNA damage cells as compare to intestine and blood. Overall order of DNA damage in different fish species was *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* Overall trend of comet classes in the skin was class 4 > class 3> class 0> class 2> class 1.

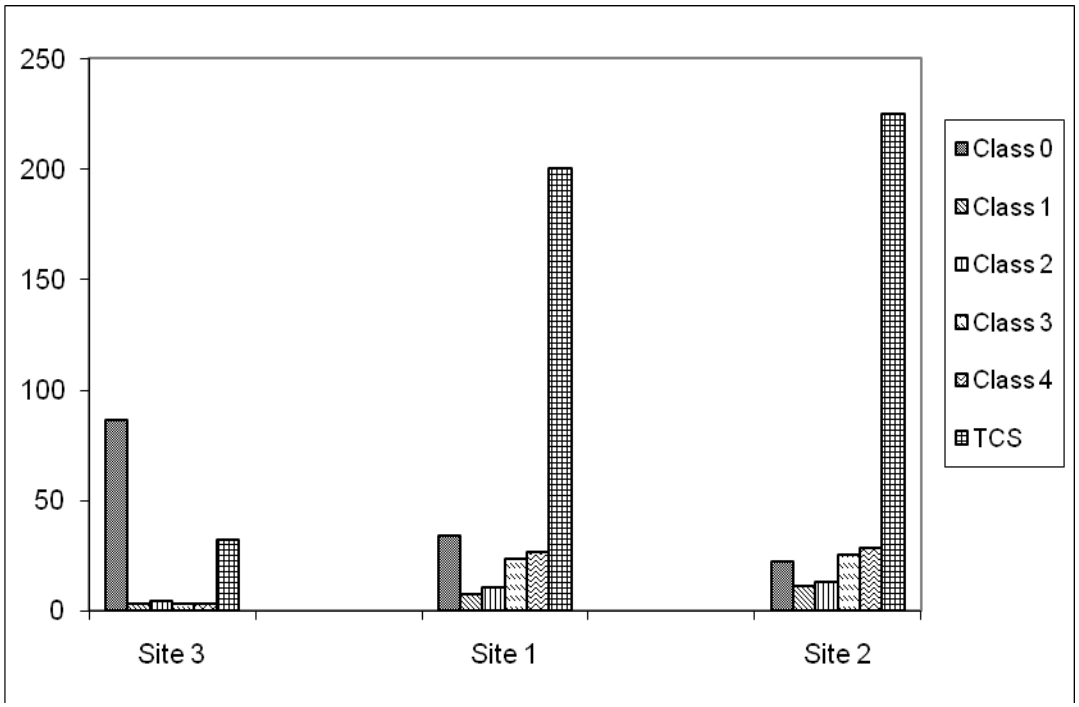
Table 5.3: Degree of total comet score (TCS) and comet classes in skin of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	90.6±4.6	49.3±5.5	35.6±3.2
	Class 1	3.0±1.0	6.3±1.0	8.6±0.5
	Class 2	3.0±1.0	7.3±1.5	11.6±0.5
	Class 3	2.0±1.0	17.6±1.5	21.0±2.0
	Class 4	2.0±1.0	19.6±1.5	23.0±1.0
	TCS	23.0±8.6	152.3±4.5	187.0±10.1
Aorichthys seenghala	Class 0	86.3±3.5	34.0±4.0	22.3±3.0
	Class 1	3.6±1.1	7.0±1.0	11.3±0.5
	Class 2	4.0±1.0	10.0±1.0	13.3±0.5
	Class 3	3.0±1.0	23.0±1.0	25.0±1.0
	Class 4	3.0±1.0	26.0±1.0	28.0±1.0
	TCS	32.6±8.7	200.0±10.0	225.0±8.5
Labeo dyocheilus	Class 0	88.0±3.4	40.3±4.0	26.6±2.0
	Class 1	4.0±1.0	6.0±1.0	9.6±0.5
	Class 2	3.0±1.0	8.6±1.5	12.6±0.5
	Class 3	3.0±1.0	21.0±1.0	24.0±1.0
	Class 4	2.0±1.0	24.0±1.0	27.0±1.0
	TCS	27.0±8.8	182.3±9.7	215.0±6.9
Cyprinus carpio	Class 0	82.3±1.5	21.0±5.0	9.3±4.0
	Class 1	5.0±1.0	11.0±1.0	13.0±1.0
	Class 2	4.0±1.0	13.0±1.0	15.6±1.1
	Class 3	4.0±1.0	26.3±1.5	30.0±1.0
	Class 4	4.6±0.5	29.6±1.5	32.0±1.0
	TCS	43.6±3.7	233.6±13.5	262.3±10.0
Ompok bimaculatus	Class 0	82.0±3.6	27.0±5.0	13.6±2.5
	Class 1	6.0±1.0	8.6±1.5	12.3±0.5
	Class 2	5.0±1.0	12.0±1.0	15.6±0.5
	Class 3	4.0±1.0	25.0±1.0	28.0±1.0
	Class 4	3.0±1.0	27.3±1.5	30.3±0.5
	TCS	40.0±9.1	217.0±12.5	249.0±6.5

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

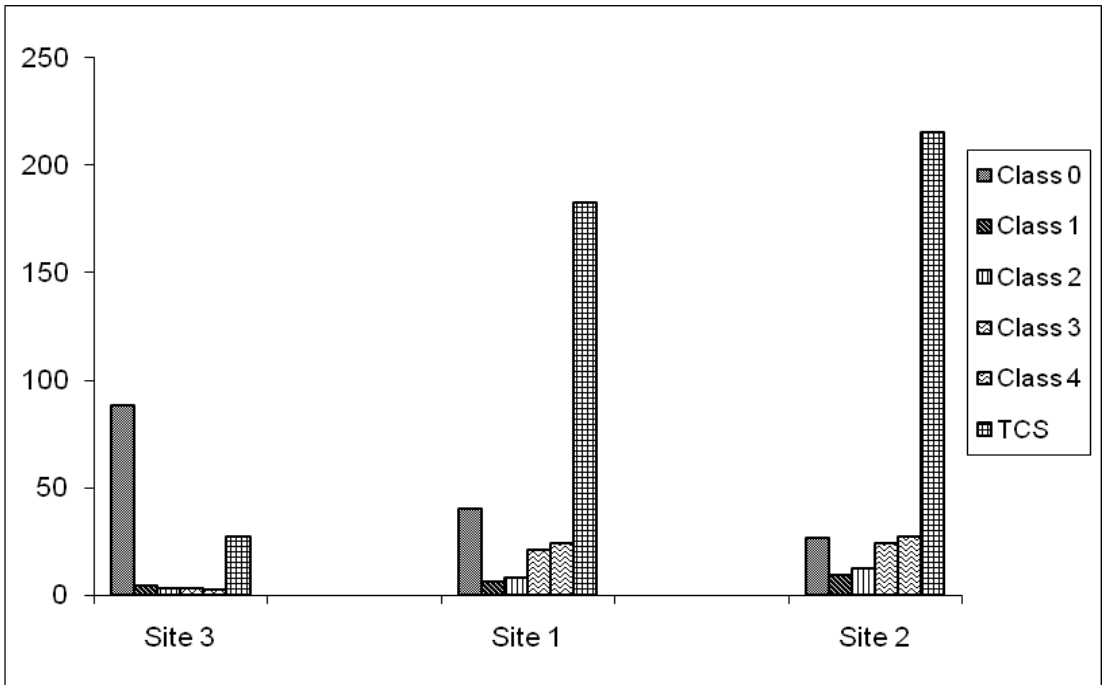


Wallago attu

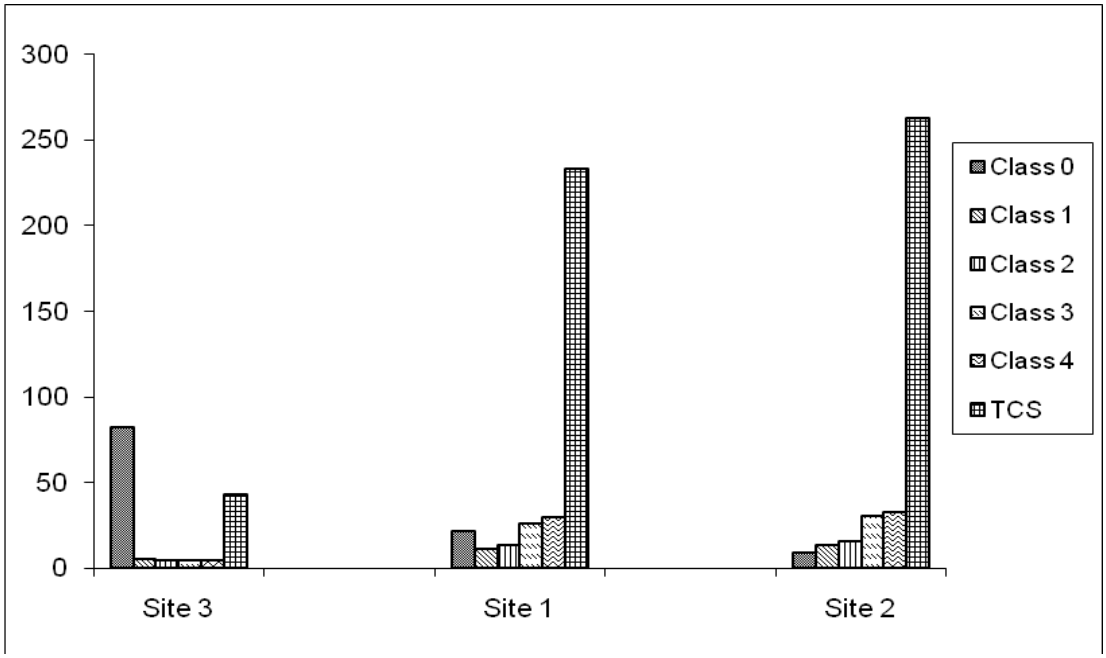


Aorichthys seenghala

Fig.5.7: Degree of total comet score (TCS) and comet classes in skin of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

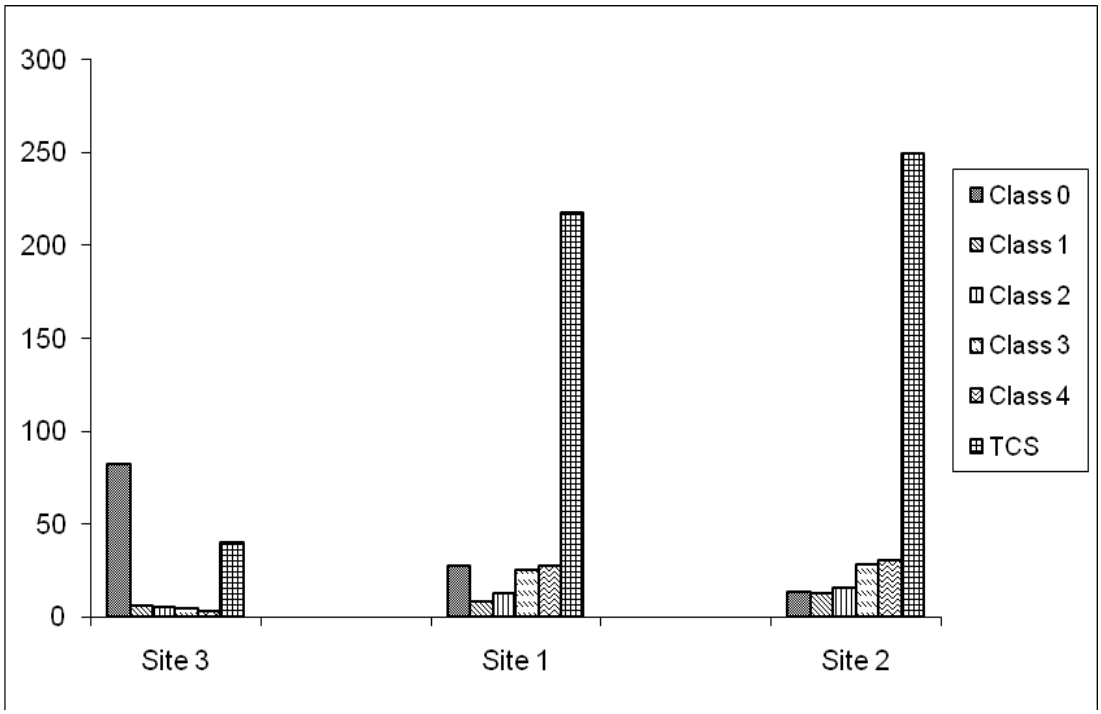


Labeo dyocheilus



Cyprinus carpio

Fig.5.8: **Degree of total comet score (TCS) and comet classes in skin of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted)of River Kabul receiving city sewages and industrial effluents.**



Ompok bimaculatus

Fig.5.9: **Degree of total comet score (TCS) and comet classes in gills of *Ompok bimaculatus* and netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents**

5.3.4 TCS and Comet Classes in Intestine

Intestine of selected fish species from polluted water and Warsak dam was processed for determination of degree of DNA damage due to accumulation of heavy metals such as Zn, Ni, Cr, Cu, Cd, Pb, Fe, Mn and Hg in it. Total comet score (TCS) and comet classes like class 0, class 1, class 2, class 3 and class 4 were found in this organ and compared with control fish species from Warsak dam. Intestine of the examined fish species from polluted sites 1 and 2 showed higher degree of TCS and comet classes as compare to control site besides comet class 0, where the lowest values of comet class 0 were found in intestine from reference site (Table 5.4 and Figs 5.10-5.12).

From amongst comet classes, intestine of *Wallago attu* from polluted sites had less degree of comet class 0 with mean values of 48.3±37 cells and 35.0±4.0 cells and had more frequency of comet class 0 with mean value of 86.6±3.7 from control site, *Aorichthys seenghala* from polluted water had 29.0±3.6 cells and 16.6±4.1cells and had 80.0±3.4 cells from reference water, *Labeo dyocheilus* from polluted sites had 39.0±3.6 cells and 27.0±4.0 cells and had 84.0±2.6 cells from Warsak dam, *Cyprinus carpio* from site 1 and site 2 had 13.0±3.6 cells and 7.3±5.0 cells and had 72.0±4.0 cells from reference site 3 and *Ompok bimaculatus* from both sites 1 and 2 had 20.0±3.5 cells and 12.0±3.6 cells and had 76.0±4.0 cells from control site respectively. Comet class 0 in this tissue of different fish species was in the order of *Wallago attu* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Ompok bimaculatus* > *Cyprinus carpio.* This highlights that more comet class 0 was observed in *Wallago attu* and less in *Cyprinus carpio.* These results are agreed with the findings of (Obiakor *et al*., 2014; Theodora is *et al*., 1994), who have also found the same values for comet class 0 in the intestine of other fish species.

Comet class 1 in intestine of *Wallago attu* from polluted water were 4.0±1.0 cells and 7.0±1.0 cells and was 4.0±1.0 cells from control water, in *Aorichthys*

seenghala from polluted sites were 8.0±1.0 cells and 11.0±1.0 cells and was 6.0±1.0 cells from control site, in *Labeo dyocheilus* from site 1 and 2 were 5.3±0.5 cells and 9.0±1.0 cells and was 5.0±1.0 cells from site 3, in *Cyprinus carpio* from polluted sites were 11.3±0.5 cells and 13.0±1.0 cells and was 8.0±1.0 cells from reference site 3 and in *Ompok bimaculatus* from polluted water were 9.6±0.5 cells and 12.0±1.0 cells and was 7.0±1.0 cells from control site respectively. The sequence of comet class 1 in this organ of different studied fish was *Cyprinus carpio>Ompok bimaculatus > Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This indicates that *Cyprinus carpio* had greater frequency of comet class 1 and *Wallago attu* had smaller frequency respectively. In the present finding the values for comet class 1 were lower as compare to the values mentioned by (Nevenka *et al*., 2008). In this study intestine from polluted water showed high degree of comet class 1 than control water. This could be related to greater concentration of heavy metals in this organ at polluted sites and less detoxification mechanism against metals.

Intestine of *Wallago attu* from polluted water contained greater degree of comet 2 with mean values of 6.6±1.0 cells and 10.0±1.0 cells and contained smaller mean value of 3.3±1.5 cells from control water, *Aorichthys seenghala* from polluted sites contained 11.0±1.0 cells and 14.0±1.0 cells and contained 5.0±1.0 cells from control site 3, *Labeo dyocheilus* from both sites 1 and 2 contained 9.0±1.0 cells and 12.0±1.0 cells and contained 4.0±1.0 cells from reference site 3, *Cyprinus carpio* from site 1 and site 2 contained 15.3±0.5 cells and 17.0±1.0 cells and contained 7.0±1.0 cells from Warsak dam and *Ompok bimaculatus* from polluted sites contained 13.0±1.0 cells and 15.0±1.0 cells and contained 6.0±1.0 cells from control site respectively. The order of comet class 2 in intestine of these fish was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This trend shows that *Cyprinus carpio* had greater frequency of comet class 2 and *Wallago attu* had smaller. The data for comet class 2 was higher than those reported by Kurelec (1993) and Pandrangi *et al* (1995). This study found highest degree of

comet class 2 in intestine of examined fish species from polluted water than those from control water. The present study reveles that heavy metals are genotoxic in nature and indunce genotoxicity in aquatic animals. The result also showed heavy metals pollution in the study areas.

Intestine of *Wallago attu* from polluted sites 1 and 2 had high degree of comet class 3 with mean values of 21.0±1.0 cells and 25.0±1.0 cells and had low mean value of 3.0±1.0 cells from Warsak dam, *Aorichthys seenghala* from polluted sites had 25.0±1.0 cells and 28.0±1.0 cells and had 4.0±1.0 cells from control site, *Labeo dyocheilus* from polluted water had 22.0±1.0 cells and 25.0±1.0 cells and had 3.0±1.0 cells from control water, *Cyprinus carpio* from polluted site 1 and site 2 had 29.0±1.0 cells and 30.0±1.0 cells and had 6.0±1.0 cells from reference site 3 and *Ompok bimaculatus* from polluted sites had 27.0±1.5 cells and 29.0±1.0 cells and had 5.0±1.0 cells from control site respectively. Comet class 3 in this organ of different fish was in the order of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* Greater degree of comet class 3 was observed in *Cyprinus carpio* and lower in *Wallago attu.* In this study more values of comet class 3 were observed than those reported by Villarini *et al* (1998) and Tice (1995) in intestine of other fish species. In this study the degree of comet class 3 in intestine of different examined fish from polluted sites was higher than the control site. This could be attributed to high contents of heavy metals in this organ from polluted water.

Comet class 4 in intestine of *Wallago attu* from both polluted sites 1 and 2 were 21.0±1.0 cells and 25.0±1.0 cells and was 3.0±1.0 cells from control site 3 (Warsak dam), in *Aorichthys seenghala* from polluted water were 27.0±1.0 cells and 30.3±1.5 cells and was 5.0±1.0 cells from control water, in *Labeo dyocheilus* from polluted sites were 24.0±1.0 cells and 27.0±1.0 cells and was 4.0±1.0 cells from control site, in *Cyprinus carpio* from polluted water were 31.3±1.5 cells and 32.3±1.5 cells and was 7.0±1.0 cells from Warsak dam and in *Ompok bimaculatus* from both

polluted sites 1 and 2 were 29.0±1.5 cells and 32.0±1.0 cells and was 6.0±1.0 cells from reference site respectively. Comet class 4 in intestine of these studied fish was in the order of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This indicates that comet class 4 was highest in *Cyprinus carpio* and lowest in *Wallago attu.* These results were higher than those reported by (Wilson *et al*., 1998; Belpaeme *et al.*, 1996). In this finding the examined fish species from polluted water showed greater frequency of comet class 4 as compare to control water. This is because of higher concentration of heavy metals in this fish and in water of River Kabul.

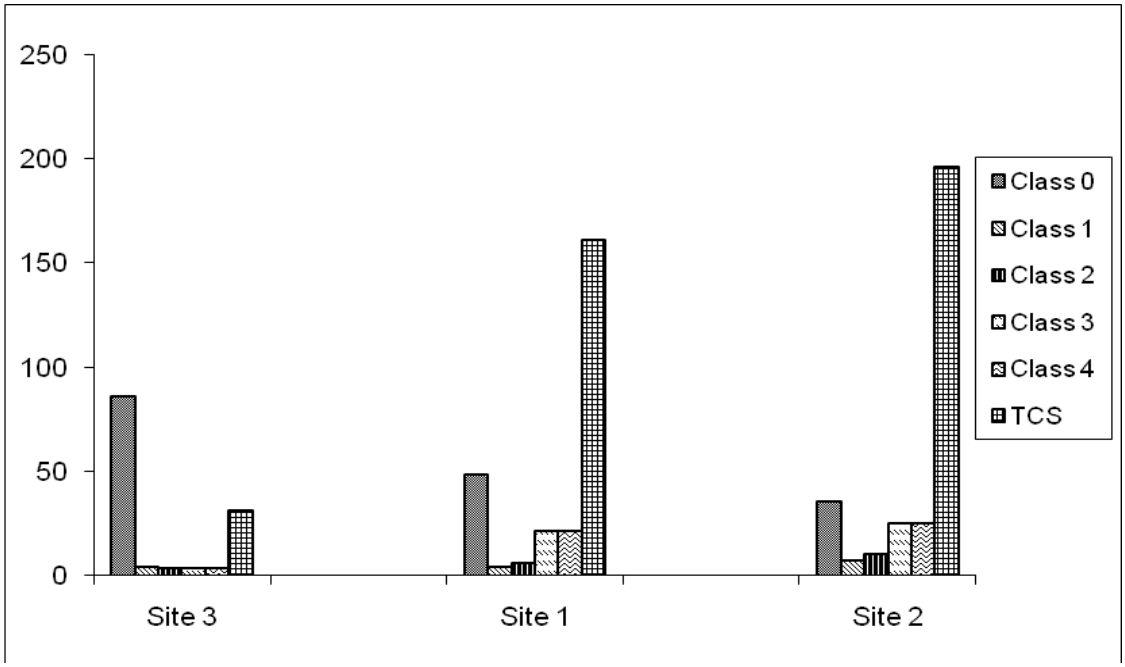
Total comet score (TCS) in intestine of *Wallago attu* from polluted water were 161.3±9.2 cells and 196.0±10.0 cells and was 31.0±9.4 cells from control site, in *Aorichthys seenghala* from site 1 and 2 were 215.3±6.8 cells and 247.6±8.3 cells and was 48.0±8.6 cells from site 3, in *Labeo dyocheilus* from polluted sites were 186.0±8.7 cells and 216.0±10.0 cells and was 38.0±7.0 cells from Warsak dam, in *Cyprinus carpio* from polluted sites were 253.3±10.6 cells and 266.3±12.0 cells and was 68.0±10.1 cells from control site and in *Ompok bimaculatus* from site 1 and site 2 were 238.6±6.4 cells and 257.0±8.7 cells and was 58.0±10.0 cells from site 3 respectively. TCS values showed a trend of *Cyprinus carpio*>*Ompok bimaculatus* > *Aorichthys seenghala*>*Labeo dyocheilus*>*Wallago attu.* This indicates that *Cyprinus carpio* had maximum degree of TCS and *Wallago attu* had minimum degree of TCS. The results of the present study are not consistent with the previous findings reported by (Cotelle and Erard, 1999; Tolga, 2008). Intestine came first for degree of DNA damage cells followed by blood, skin, liver, gills and muscle. The present study found significantly higher degree of DNA damage cells in intestine than the control sample. This could be attributed to high metal accumulation and less detoxification mechanism of this tissue and the results also showed that heavy metals are toxic in nature and can induce genotoxicity in different tissues and organs of both aquatic and terrestrial animals, which are in agreement with the past findings (Mitchelmore and

Chipman, 1998; Maruya, 20000; Kim *et al.,* 2000). High frequency of DNA damage cells in this organ may vary according to the season, kind of pollution involved and the species of fish. The result also showed heavy metals pollution in River Kabul. Overall sequence of DNA damage in different fish species was *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* Overall trend of comet classes in intestine was class 4 > class 3> class 0> class 2> class 1.

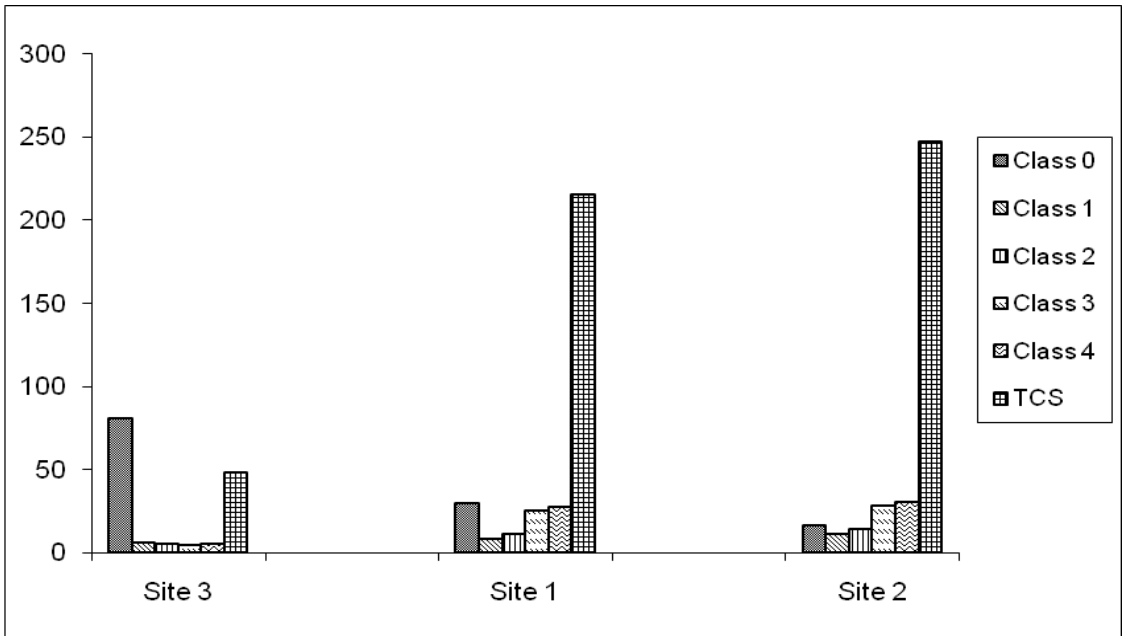
Table 5.4: Degree of total comet score (TCS) and comet classes in intestine of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	86.6±3.7	48.3±37	35.0±4.0
	Class 1	4.0±1.0	4.0±1.0	7.0±1.0
	Class 2	3.3±1.5	6.6±1.0	10.0±1.0
	Class 3	3.0±1.0	21.0±1.0	25.0±1.0
	Class 4	3.0±1.0	21.0±1.0	25.0±1.0
	TCS	31.0±9.4	161.3±9.2	196.0±10.0
Aorichthys seenghala	Class 0	80.0±3.4	29.0±3.6	16.6±4.1
	Class 1	6.0±1.0	8.0±1.0	11.0±1.0
	Class 2	5.0±1.0	11.0±1.0	14.0±1.0
	Class 3	4.0±1.0	25.0±1.0	28.0±1.0
	Class 4	5.0±1.0	27.0±1.0	30.3±1.5
	TCS	48.0±8.6	215.3±6.8	247.6±8.3
Labeo dyocheilus	Class 0	84.0±2.6	39.0±3.6	27.0±4.0
	Class 1	5.0±1.0	5.3±0.5	9.0±1.0
	Class 2	4.0±1.0	9.0±1.0	12.0±1.0
	Class 3	3.0±1.0	22.0±1.0	25.0±1.0
	Class 4	4.0±1.0	24.0±1.0	27.0±1.0
	TCS	38.0±7.0	186.0±8.7	216.0±10.0
Cyprinus carpio	Class 0	72.0±4.0	13.0±3.6	7.3±5.0
	Class 1	8.0±1.0	11.3±0.5	13.0±1.0
	Class 2	7.0±1.0	15.3±0.5	17.0±1.0
	Class 3	6.0±1.0	29.0±1.0	30.0±1.0
	Class 4	7.0±1.0	31.3±1.5	32.3±1.5
	TCS	68.0±10.1	253.3±10.6	266.3±12.0
Ompok bimaculatus	Class 0	76.0±4.0	20.0±3.5	12.0±3.6
	Class 1	7.0±1.0	9.6±0.5	12.0±1.0
	Class 2	6.0±1.0	13.0±1.0	15.0±1.0
	Class 3	5.0±1.0	27.0±1.5	29.0±1.0
	Class 4	6.0±1.0	29.0±1.5	32.0±1.0
	TCS	58.0±10.0	238.6±6.4	257.0±8.7

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

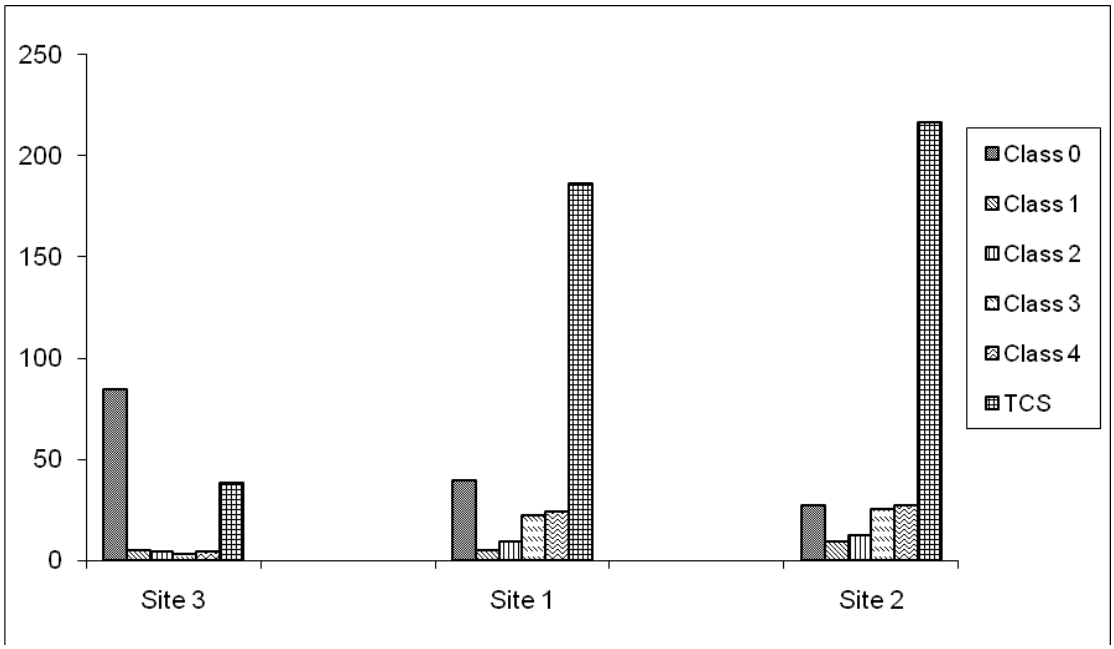


Wallago attu

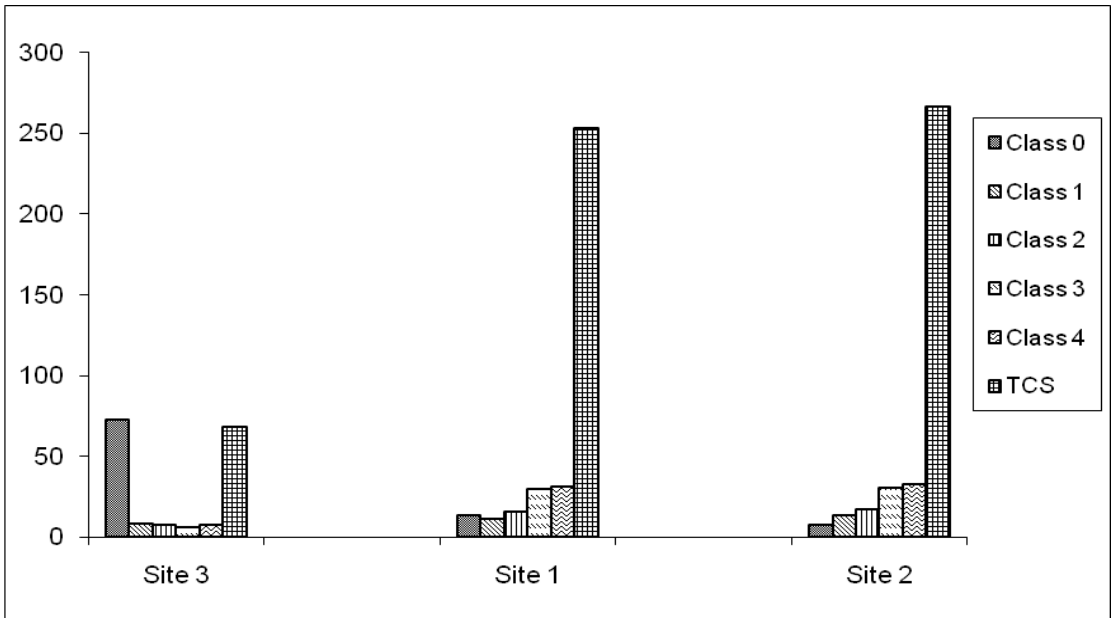


Aorichthys seenghala

Fig. 5.10: Degree of total comet score (TCS) and comet classes in intestine of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

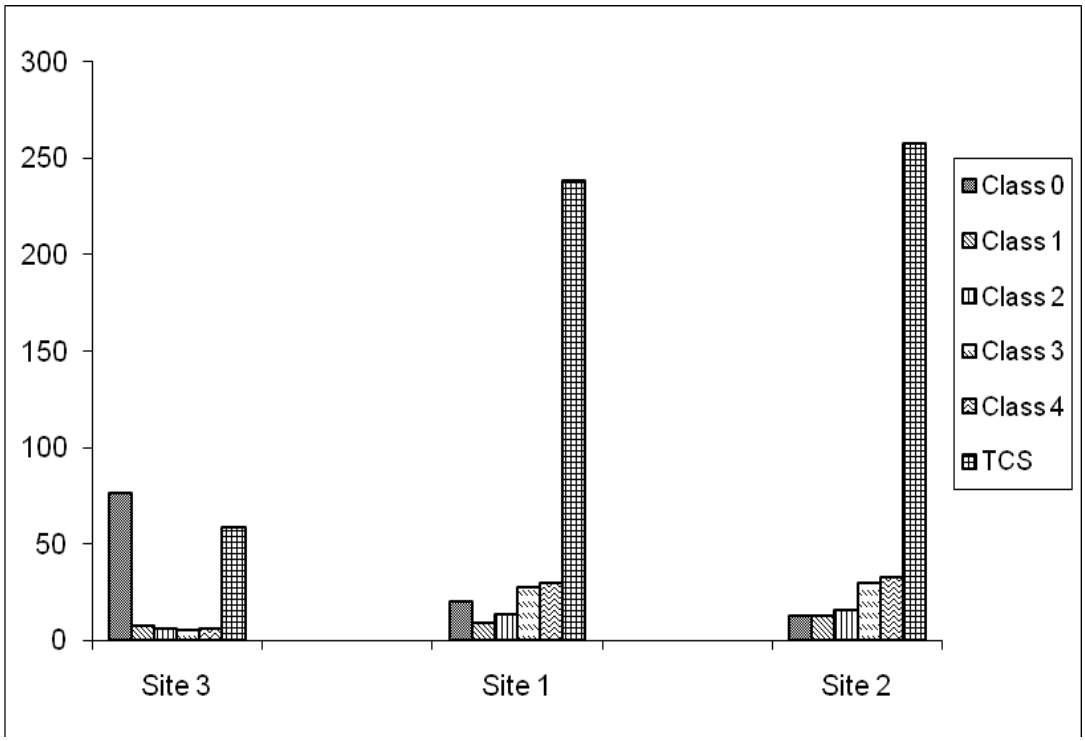


Labeo dyocheilus



Cyprinus carpio

Fig. 5.11: Degree of total comet score (TCS) and comet classes in intestine of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig. 5.12: Degree of total comet score (TCS) and comet classes in intestine of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

5.3.5 TCS and Comet Classes in Liver

All the investigated total comet score (TCS) and comet classes including class 1, class 2, class 3 and class 4 in liver of different fish species from polluted water showed increasing tendency as when compare with control water besides class 0, which showed lowest value at sites 1and 2 and highest value from control site 3. The possible reason for this tremendous increase of comet classes and total comet score in fish tissue could be correlated to highest concentration of heavy metals in tissues and also high level in the water of polluted sites 1 and 2(Table 5.5 and Figs 5.13-5.15).

Liver of *Wallago attu* from polluted water showed lowest frequency of comet class 0 with mean values of 53.0±4.3 cells and 42.0±4.5 cells and showed highest frequency with mean value of 92.0±5.0 cells from control site, *Aorichthys seenghala* from both sites 1 and 2 showed 37.6±4.1 cells and 27.3±3.0 cells and showed 89.6±3.0 cells from control site, *Labeo dyocheilus* from polluted sites showed 44.0±4.5 cells and 31.3±2.8 cells and 92.0±3.6 cells from Warsak dam, *Cyprinus carpio* from polluted sites 1 and 2 showed 33.0±5.5 cells and 20.3±3.7 cells and showed 87.6±2.0 cells from reference site 3 and *Ompok bimaculatus* from polluted sites showed 33.0±5.5 cells and 20.3±3.7 cells and showed 87.6±2.0 cells from control site respectively. The frequency of comet class 0 in liver of studied fish was in the order of *Wallago attu* > *Labeo dyocheilus* > *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala.* This indicates that comet class 0 was greatest in *Wallago attu* and smallest in *Aorichthys seenghala.* These results were higher than those reported previously (Jenssen and Ramel, 1980; Latt and Allen, 1977). All the fish from control site showed greater degree of comet class 0 as compare to polluted sites 1 and 2. This could be attributed to less heavy metals pollution at control site.

Liver of *Wallago attu* from polluted sites contained greater degree of comet class 1 with mean values of 6.0±1.0 cells and 8.3±4.5 cells and contained smaller frequency of 3.0±1.0 cells from control site, *Aorichthys seenghala* from sites 1 and 2

contained 8.0±1.0 cells and 10.0±1.5 cells and contained 3.3±0.5 cells from site 3, *Labeo dyocheilus* from polluted water contained 7.3±0.5 cells and 9.3±0.5 cells and contained 3.0±1.0 cells from control water, *Cyprinus carpio* from both sites 1 and 2 contained 9.6±1.5 cells and 12.3±0.5 cells and contained 5.0±1.0 cells from site 3 and *Ompok bimaculatus* from polluted sites contained 8.0±1.0 cells and 11.3±0.5cells and contained 4.0±1.0 cells from control site respectively. The order of comet class 1 in liver of these fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* This shows that comet class 1 was highest in *Cyprinus carpio* and lowest in *Wallago attu.* This is related to greater content of heavy metals in this fish. These results were higher than the findings of Grisolia (2002) and Al-Sabit (1994). Liver of examined fish species from polluted water had higher frequency of comet class 1 as compare to those from reference site. This could be correlated to greater concentration of heavy metals in this organ and our data also showed heavy metals pollution in River Kabul.

Liver of *Wallago attu* from polluted water had more comet class 2 with mean values of 6.3±1.5 cells and 9.0±1.0 cells and had less value of 2.0±1.0 cells from control water, *Aorichthys seenghala* from polluted sites 1 and 2 had 10.0±1.0 cells and 13.6±0.5 cells and had 3.0±1.0 cells from control site 3, *Labeo dyocheilus* from polluted sites had 8.0±1.0 cells and 12.3±0.5 cells and had 2.0±1.0 cells from control site, *Cyprinus carpio* from site 1 and site 2 had 11.6±1.5 cells and 15.0±1.0 cells and had 4.0±1.0 cells from site 3 and *Ompok bimaculatus* from polluted water had high mean values of 10.3±1.5 cells and 14.3±0.5 cells and had 3.3±0.5 cells from control water respectively. The sequence of comet class 2 in this organ of different studied fish was *Cyprinus carpio > Ompok bimaculatus > Aorichthys seenghala > Labeo dyocheilus > Wallago attu.*This highlights that *Cyprinus carpio* is having highest frequency of comet class 1 and *Wallago attu* had lowest degree of comet class 2. This data in our finding was greater than those reported in previous studies (De Flora *et al*., 1991; Bhaskaran *et al.,* 1999). Liver of the studied fish species from polluted area of

River Kabul showed greater level of comet class 2 than those from Warsak dam. This is because of greater level of heavy metals in liver and this result also showed heavy metals pollution in study area of River Kabul.

Comet class 3 in liver of *Wallago attu* from site 1 and site 2 samples were 17.0±1.0 cells and 19.3±1.5 cells and was 1.3±1.5 cells from site 3, in *Aorichthys seenghala* from polluted sites were 22.0±1.0 cells and 24.0±1.0 cells and was 2.0±1.0 cells from control site, in *Labeo dyocheilus* from polluted water were 19.3±1.5 cells and 23.0±1.0 cells and was 2.0±1.0 cells from control water, in *Cyprinus carpio* from both site 1 and site 2 were 24.6±1.5 cells and 27.3±1.5 cells and was 4.0±1.0 cell from site 3 and in *Ompok bimaculatus* from polluted sites were 23.3±1.5 cells and 26.0±1.7 cells and was 3.0±1.0 cells from Warsak dam respectively. The order of comet class 3 in liver of different studied fish was *Cyprinus carpio>Ompok bimaculatus > Aorichthys seenghala >Labeo dyocheilus >Wallago attu.* This trend shows that *Cyprinus carpio* had maximum and *Wallago attu* had minimum degree of comet class 3. These results are in agreement with those observed by many investigators in previous studies (Maccubin *et al*., 1991; Kurelec, 1993), who have also investigated the same result in the same tissue of other fish species.

Liver of *Wallago attu* from polluted sites had maximum degree of comet class 4 with mean values of 17.6±1.5 cells and 21.3±1.5 cells and had minimum frequency with mean value of 1.6±1.5 cells from control site, *Aorichthys seenghala* from polluted sites 1 and 2 had 22.3±1.5 cells and 25.0±1.0 cells and had 2.0±1.0 cells from control site, *Labeo dyocheilus* from both polluted sites had 21.3±1.5 cells and 24.0±1.0 cells and had 1.0±1.0 cells from reference site, *Cyprinus carpio* from polluted sites had 27.0±2.0 cells and 30.0±1.0 cells and had 3.6±1.1 cells from Warsak dam and *Ompok bimaculatus* from polluted water had 25.3±1.5 cells and 28.0±1.0 cells and had 2.0±1.0 cells from site 3 respectively. The sequence of comet class 4 in this organ of different examined fish was *Cyprinus carpio > Ompok*

bimaculatus > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu*. This reveales that *Cyprinus carpio* contained high degree of comet class 4 and *Wallago attu* contained low degree. These results were lower than those reported in past studies (Barry, 1992; Richard *et al*., 2003; Omar*et al*., 2012). In this study comet class 4 in liver of different examined fish species was higher than the reference site. This could be attributed to greater content and toxicity of heavy metals in this tissue.

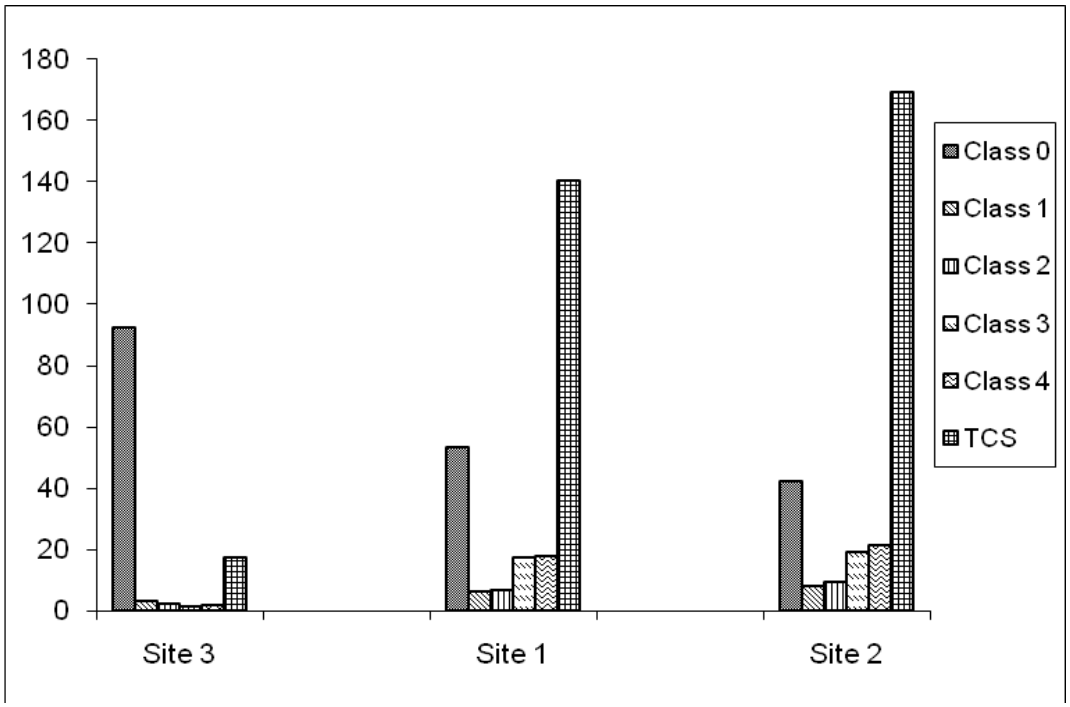
Liver of *Wallago attu* from both sites 1 and 2 had maximum frequency of total comet score (TCS) with mean values of 140.3±11.3 cells and 169.6±13.2 cells and had minimum degree of TCS with mean value of 17.6±13.5 cells from site 3, *Aorichthys seenghala* from polluted water had 183.3±11.6 cells and 209.3±8.0 cells and had 23.3±8.1 cells from Warsak dam, *Labeo dyocheilus* from polluted sites had 166.6±13.2 cells and 199.0±7.8 cells and had 17.0±9.1 cells from control site 3, *Cyprinus carpio* from polluted sites had 215.0±17.0 cells and 244.3±11.0 cells and had 39.6±7.3 cells from Warsak dam and *Ompok bimaculatus* from polluted site 1 and site 2 had 200.0±14.7 cells and 230.0±10.5 cells and had 27.6±6.6 cells from control water respectively. The order of TCS in intestine of these studied fish was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus* > *Wallago attu.* This trend indicates that *Cyprinus carpio* contained maximum and *Wallago attu* minimum frequency of TCS. This study found more TCS as compare to the previous findings (Ali *et al*., 2000; Cavas *et al*., 2005). In this study generally frequency of DNA damage cells were higher significantly in liver from polluted sites as compare to Warsak dam. On the other hand liver showed higher frequency of DNA damage cells than muscle and gills and lower frequency than intestine, blood and skin when over all comparisons is made. As compare to other studied organs liver came fourth after intestine, blood and skin for DNA damage. Liver is the organ that playing a significant role in detoxification of toxic chemicals. Therefore the liver showed less DNA damage cells as compare to intestine, blood and skin. Overall order of DNA damage in different fish species was *Cyprinus carpio* > *Ompok bimaculatus* >

Aorichthys seenghala >*Labeo dyocheilus* > *Wallago attu* and overall trend of comet classes in liver was class 4 > class 3> class 0> class 2> class 1.

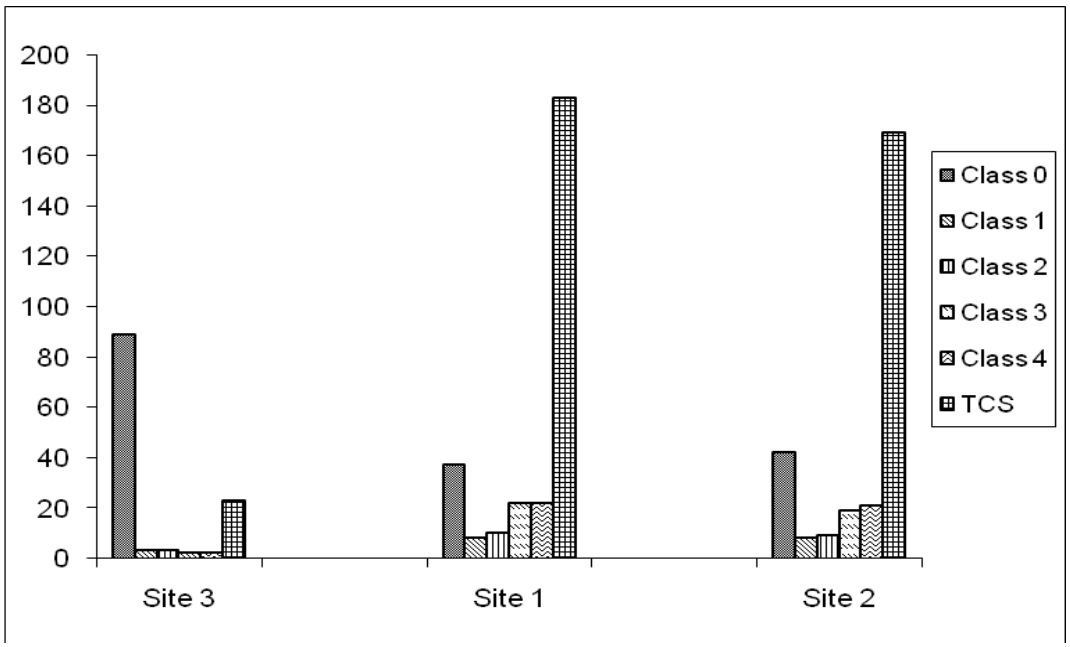
Table 5.5: Degree of total comet score (TCS) and comet classes in liver of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	92.0±5.0	53.0±4.3	42.0±4.5
	Class 1	3.0±1.0	6.0±1.0	8.3±4.5
	Class 2	2.0±1.0	6.3±1.5	9.0±1.0
	Class 3	1.3±1.5	17.0±1.0	19.3±1.5
	Class 4	1.6±1.5	17.6±1.5	21.3±1.5
	TCS	17.6±13.5	140.3±11.3	169.6±13.2
Aorichthys seenghala	Class 0	89.6±3.0	37.6±4.1	27.3±3.0
	Class 1	3.3±0.5	8.0±1.0	10.0±1.5
	Class 2	3.0±1.0	10.0±1.0	13.6±0.5
	Class 3	2.0±1.0	22.0±1.0	24.0±1.0
	Class 4	2.0±1.0	22.3±1.5	25.0±1.0
	TCS	23.3±8.1	183.3±11.6	209.3±8.0
Labeo dyocheilus	Class 0	92.0±3.6	44.0±4.5	31.3±2.8
	Class 1	3.0±1.0	7.3±0.5	9.3±0.5
	Class 2	2.0±1.0	8.0±1.0	12.3±0.5
	Class 3	2.0±1.0	19.3±1.5	23.0±1.0
	Class 4	1.0±1.0	21.3±1.5	24.0±1.0
	TCS	17.0±9.1	166.6±13.2	199.0±7.8
Cyprinus carpio	Class 0	83.3±2.8	27.0±6.5	15.3±4.0
	Class 1	5.0±1.0	9.6±1.5	12.3±0.5
	Class 2	4.0±1.0	11.6±1.5	15.0±1.0
	Class 3	4.0±1.0	24.6±1.5	27.3±1.5
	Class 4	3.6±1.1	27.0±2.0	30.0±1.0
	TCS	39.6±7.3	215.0±17.0	244.3±11.0
Ompok bimaculatus	Class 0	87.6±2.0	33.0±5.5	20.3±3.7
	Class 1	4.0±1.0	8.0±1.0	11.3±0.5
	Class 2	3.3±0.5	10.3±1.5	14.3±0.5
	Class 3	3.0±1.0	23.3±1.5	26.0±1.7
	Class 4	2.0±1.0	25.3±1.5	28.0±1.0
	TCS	27.6±6.6	200.0±14.7	230.0±10.5

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

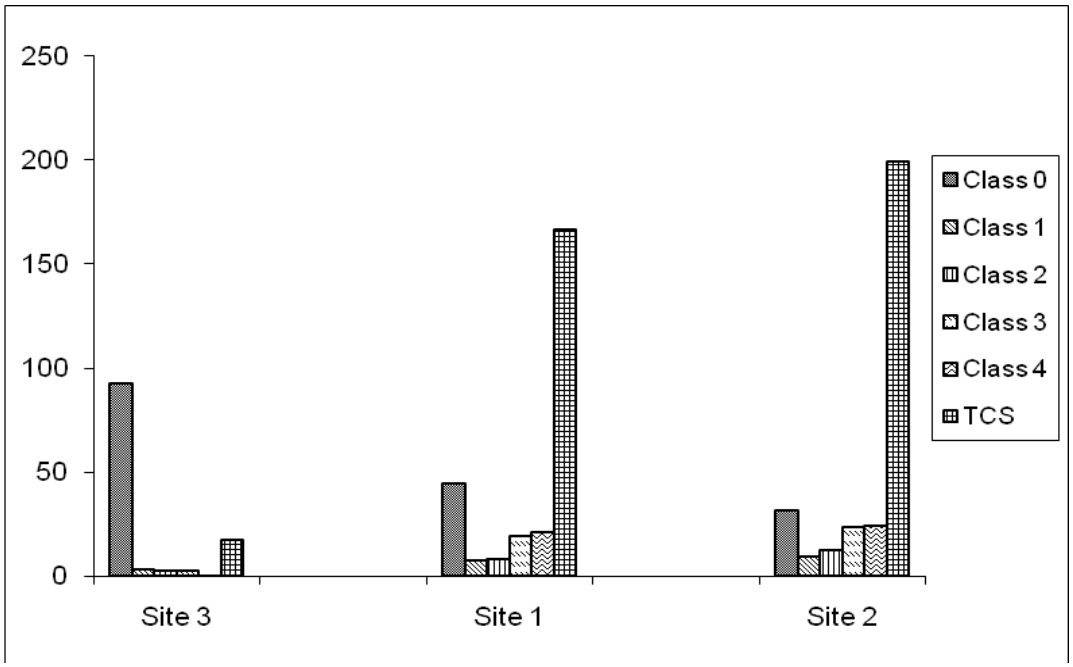


Wallago attu

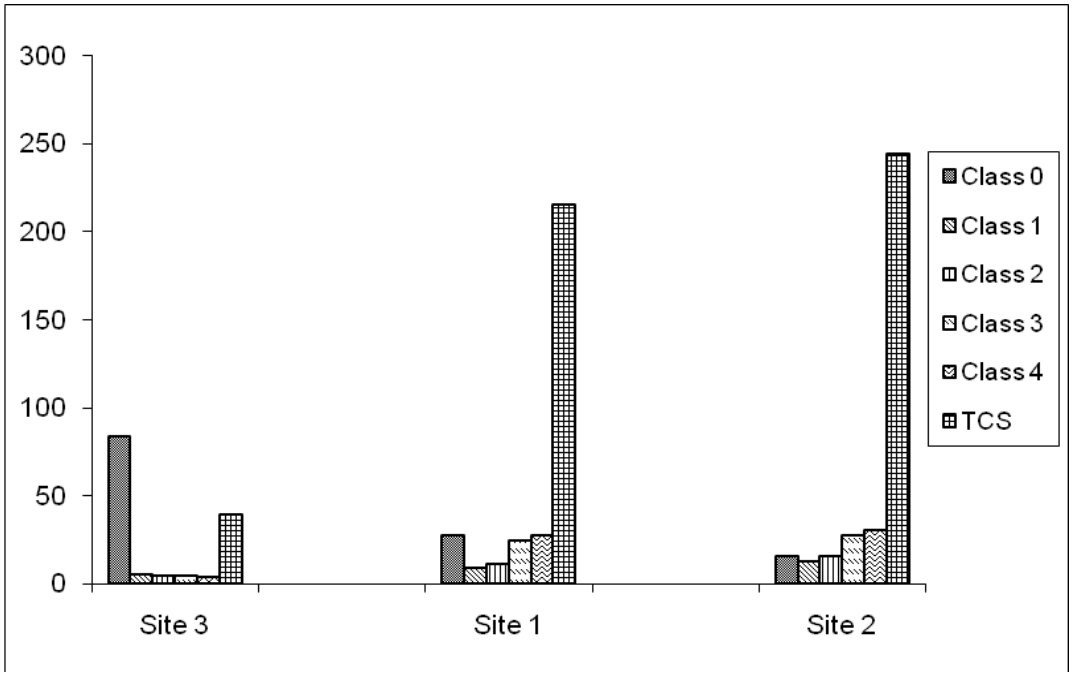


Aorichthys seenghala

Fig. 5.13: Degree of total comet score (TCS) and comet classes in liver of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

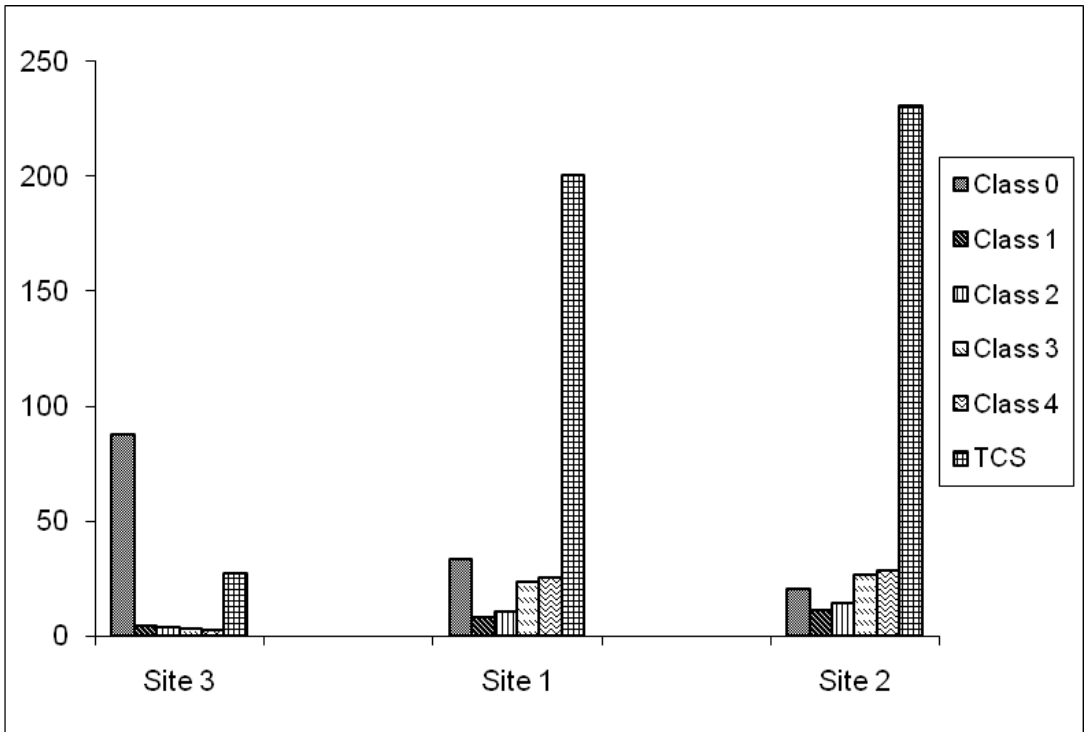


Labeo dyocheilus



Cyprinus carpio

Fig. 5.14: Degree of total comet score (TCS) and comet classes in liver of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig. 5.15: Degree of total comet score (TCS) and comet classes in liver of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

5.3.6 TCS and Comet Classes in Muscle

In the present study like blood, gills, skin, intestine and liver, the muscle of different fish species from polluted sites also had significantly more degree of total comet score (TCS) and comet classes like class 1, class 2, class 3 and class 4 as compare with control fish from Warsak dam. Comet class 0 showed highest values at control site 3 (Warsak dam) and lowest at polluted sites 1 and 2 (Table 5.6 and Figs 5.16-5.18).

Among the comet classes muscle of *Wallago attu* from polluted sites of River Kabul showed smaller frequency of comet class 0 with mean values of 58.6±4.1 cells and 50.6±4.5 cells and showed greater degree with mean value of 93.0±4.0 cells from control site, *Aorichthys seenghala* from polluted water showed 49.6±4.1 cells and 36.0±3.6 cells and showed 89.0±3.6 cells from control water, *Labeo dyocheilus* from sites 1 and 2 showed 54.6±4.1 cells and 42.6±3.2 cells and showed 90.0±5.0 cells from site 3, *Cyprinus carpio* from site 1 and site 2 showed 33.6±5.8 cells and 25.3±3.0 cells and showed 86.0±3.4 cells from control site and *Ompok bimaculatus* from both site 1 and site 2 showed 40.3±3.7 cells and 30.0±3.4 cells and showed 88.0±3.2 cells from control site 3 respectively. The order of comet class 0 in muscle of these studied fish was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus>Wallago attu.*This shows that *Cyprinus carpio* had higher and *Wallago attu* had lower degree of comet class 0. These results were in agreement with the findings reported peviously (Espina and Weiss, 1995; Bolognesi *et al*., 1996). All the examined fish species from polluted sites showed minimum comet class 0 as compare to control site 3. This could be attributed to less accumulation of heavy metals in muscle at control site 3.

Muscle of *Wallago attu* from polluted water had more comet class 1 with mean values of 5.0±1.0 cells and 7.0±1.0 cells and had less comet class 1 with mean value of 2.0±1.0 cells from control water, *Aorichthys seenghala* from polluted sites

had 7.0±1.0 cells and 9.0±1.0 cells and had 3.0±1.0 cells from control site, *Labeo dyocheilus* from sites 1 and 2 had 6.0±1.0 cells and 8.0±1.0 cells and had 2.3±1.5 cells from site 3, *Cyprinus carpio* from site 1 and site 2 had 9.6±1.1 cells and 10.0±1.0 cells and had 4.0±1.0 cells from site 3 and *Ompok bimaculatus* from polluted water had 9.0±1.0 cells and 10.3±0.5 cells and had 3.0±1.0 cells from control water respectively.The sequence of comet class 1 in this organ of different fish species was *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This indicates that *Cyprinus carpio* was found to be contained greater and *Wallago attu* smaller degree of comet class 1.These results are consisted with those observed by many other investigators (Pruski and Dixon, 2002; Pandrangi *et al.,* 1995). The present investigation found more degree of comet class 1 in this tissue from polluted water as compare to control water. This study also confirmed heavy metals pollution in the study area of River Kabul.

Muscle of *Wallago attu* from polluted water contained highest comet class 2 with mean values of 6.0±1.0 cells and 9.0±1.0 cells and contained lowest mean value of 3.0±1.0 cells from control site, *Aorichthys seenghala* from sites 1 and 2 contained 9.0±1.0 cells and 12.0±1.0 cells and contained 3.0±1.0 cells from control site 3, *Labeo dyocheilus* from polluted sites contained 6.3±1.5 cells and 10.0±1.0 cells and contained 3.0±1.0 cells from control site, *Cyprinus carpio* from polluted water contained 12.3±1.5 cells and 13.6±0.5 cells and contained 4.0±1.0 cells from control site and *Ompok bimaculatus* from polluted sites contained 11.0±1.0 cells and 13.0±1.0 cells and contained 2.6±0.5 cells from control site respectively. The order of comet class 2 in muscle of studied fish was *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This trend shows that *Cyprinus carpio* contained higher and *Wallago attu* had lower comet class 2.This could be correlated to highest content of heavy metals in this fish. This finding found more frequency of comet class 2 as compare to the findings reported by Wilson *et al* (1998) and Belpaeme *et al* (1996). Muscle from polluted sites was found to be

contained high frequency of comet class 2 than those from control site. This could be attributed to greater accumulation of heavy metals in this tissue and this result also showed heavy metals pollution in the study area.

Greater mean values of comet class 3 in muscle of *Wallago attu* from polluted water were 15.0±1.0 cells and 16.0±1.0 cells and smaller mean value was 1.0±1.0 cells from Warsak dam, in *Aorichthys seenghala* from polluted sites were 17.0±1.0 cells and 21.0±1.0 cells and was 3.0±1.0 cells from control site, in *Labeo dyocheilus* from polluted sites 1 and 2 were 19.3±4.9 cells and 19.0±1.0 cells and was 2.6±1.5 cells from control site 3, in *Cyprinus carpio* from site 1 and site 2 were 23.0±2.0 cells and 24.6±1.5 cells and was 3.0±1.0 cells from Warsak dam and in *Ompok bimaculatus* from polluted water were 19.6±1.5 cells and 23.0±1.0 cells and was 3.0±1.0 cells from control site respectively. Sequence of comet class 3 in muscle of different studied fish was *Cyprinus carpio>Ompok bimaculatus>Labeo dyocheilus > Aorichthys seenghala>Wallago attu.* This order reveales that *Cyprinus carpio* showed maximum and *Wallago attu* minimum degree of comet class 3. The greater degree of comet class 3 in this fish could be attributed to heavy metals content in this fish. This study found greater frequency of comet class 3 than those reported by Mitchelmore and Chipman (1998), Maruya (2000) and Kim *et al* (2000). The present study found more comet class 3 in muscle from polluted sites than those from control water. The present finding confirmed that heavy metals are genotoxic in nature and can induce genotoxicity in both aquatic and terrestrial animals.

Muscle of *Wallago attu* from both sites 1 and 2 showed more degree of comet class 4 with mean values of 15.3±1.5 cells and 17.3±1.5 cells and showed less frequency of comet class 4 with mean value of 1.0±1.0 cells from Warsak dam, *Aorichthys seenghala* from polluted water showed 17.3±1.5 cells and 22.0±1.0 cells and showed 2.0±1.0 cells from control water, *Labeo dyocheilus* from polluted sites showed 17.0±1.0 cells and 20.3±0.5 cells and showed 2.0±1.0 cells from control site,

Cyprinus carpio from polluted sites showed 21.3±1.5 cells and 26.3±1.5 cells and showed 3.0±1.0 cells from control site and *Ompok bimaculatus* from both polluted sites 1 and 2 showed 20.0±1.0 cells and 23.6±1.5 cells and showed 3.0±1.0 cells from site 3 respectively. Degree of comet class 4 in muscle of these studied fish was in the order of *Cyprinus carpio* > *Ompok bimaculatus* > *Aorichthys seenghala* > *Labeo dyocheilus* > *Wallago attu.* This highlights that *Cyprinus carpio* had more and *Wallago attu* had less degree of comet class 4 respectively. This is because of more heavy metals in this fish. The present result found more comet class 4 as compare to previous findings (Richard *et al*., 2003; Lee and Steinert, 2003). This study showed that comet class 4 was highest in muscle from polluted sites as compare to control water. This is related to more heavy metals concentration in this tissue and the present results also agree with the previous findings that heavy metals are genotoxic in nature.

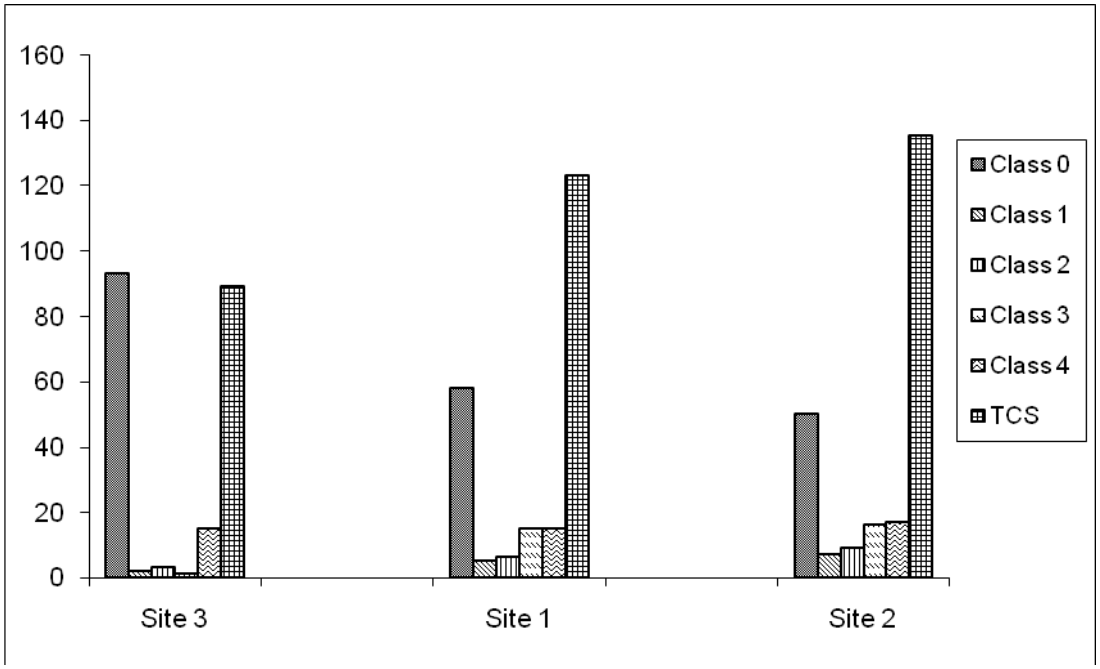
Muscle of *Wallago attu* from polluted water had highest frequency of total comet score (TCS) with mean values of 123.3±11.1 cells and 135.6±8.0 cells and had lowest degree with mean value of 15.0±1.0 cells from control site, *Aorichthys seenghala* from both sites 1 and 2 had 145.3±11.0 cells and 184.0±8.8 cells 2 and had 26.0±9.1 cells from site 3, *Labeo dyocheilus* from polluted sites had 134.6±10.5 cells and 166.3±7.5 cells and had 24.3±12.0 cells from Warsak dam, *Cyprinus carpio* from polluted water had 188.6±15.5 cells and 216.6±8.6 cells and had 33.0±8.7 cells from control water and *Ompok bimaculatus* from sites 1 and 2 had greater degree of TCS with mean values of 170.0±10.1 cells and 203.3±9.2 cells and had 29.3±8.0 cells from site 3 respectively. Frequency of total comet score in this organ of different examined fish species was in the trend of *Cyprinus carpio*>*Ompok bimaculatus* > *Aorichthys seenghala*>*Labeo dyocheilus*>*Wallago attu.* This indicates that *Cyprinus carpio* had greater and *Wallago attu* had smaller degree of TCS. This could be correlated to higher concentration of heavy metals in *Cyprinus carpio* as compare to *Wallago attu.* In this study the values of TCS were lower than those reported by Lee *et al* (1999), Shugart *et al* (1992) and Steinert (1999). In this investigation muscle

came last one in number after gills that showed least frequency of DNA damage cells. This could be correlated to less concentration of heavy metals in this tissue, high metabolic rate and strong detoxification mechanism. The present result also showed that River Kabul is a dirty river and the water and fish from the mentioned study area are not suitable for drinking and consumption purpose for human beings because the water and fish contained greater level of heavy metals that can induce different abnormalities like genotoxicity in the human beings. Overall trend of DNA damage in different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* Overall order of comet classes in muscle was class 0> class 4 >class 3> class 2> class 1 and overall trend of DNA damage cells in different tissues was intestine >blood >skin >liver > gills > muscle.

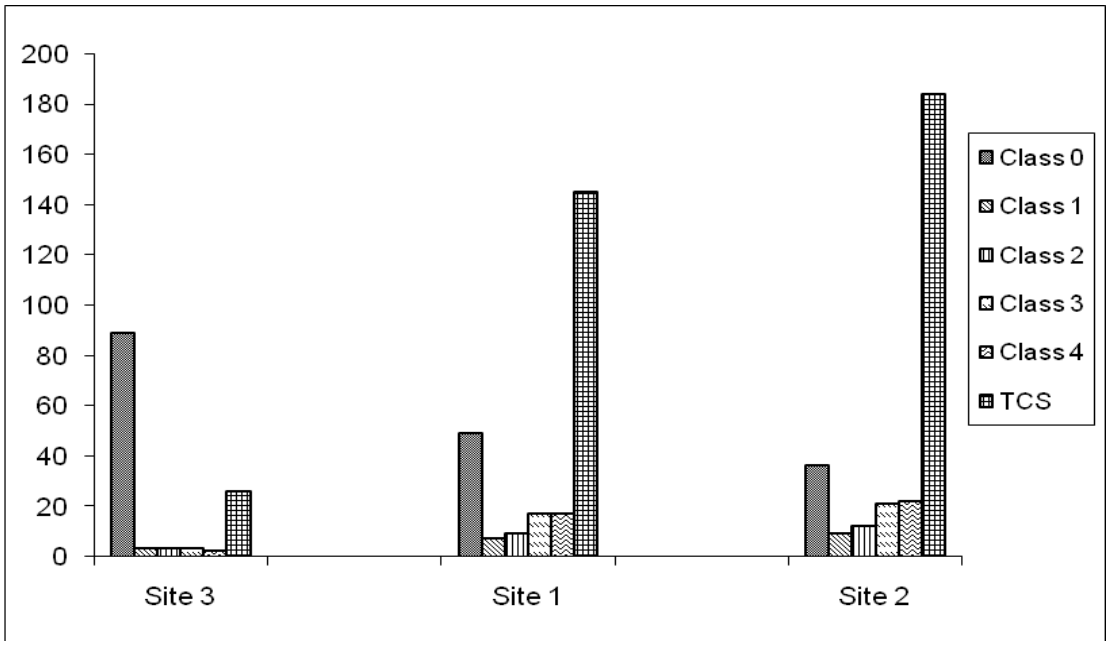
Table 5.6: Degree of total comet score (TCS) and comet classes in muscle of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	TCS and comet classes	Site 3 (n= 5)	Site 1 (n= 5)	Site 2 (n= 5)
Wallago attu	Class 0	93.0±4.0	58.6±4.1	50.6±4.5
	Class 1	2.0±1.0	5.0±1.0	7.0±1.0
	Class 2	3.0±1.0	6.0±1.0	9.0±1.0
	Class 3	1.0±1.0	15.0±1.0	16.0±1.0
	Class 4	1.0±1.0	15.3±1.5	17.3±1.5
	TCS	15.0±1.0	123.3±11.1	135.6±8.0
Aorichthys seenghala	Class 0	89.0±3.6	49.6±4.1	36.0±3.6
	Class 1	3.0±1.0	7.0±1.0	9.0±1.0
	Class 2	3.0±1.0	9.0±1.0	12.0±1.0
	Class 3	3.0±1.0	17.0±1.0	21.0±1.0
	Class 4	2.0±1.0	17.3±1.5	22.0±1.0
	TCS	26.0±9.1	145.3±11.0	184.0±8.8
Labeo dyocheilus	Class 0	90.0±5.0	54.6±4.1	42.6±3.2
	Class 1	2.3±1.5	6.0±1.0	8.0±1.0
	Class 2	3.0±1.0	6.3±1.5	10.0±1.0
	Class 3	2.6±1.5	19.3±4.9	19.0±1.0
	Class 4	2.0±1.0	17.0±1.0	20.3±0.5
	TCS	24.3±12.0	134.6±10.5	166.3±7.5
Cyprinus carpio	Class 0	86.0±3.4	33.6±5.8	25.3±3.0
	Class 1	4.0±1.0	9.6±1.1	10.0±1.0
	Class 2	4.0±1.0	12.3±1.5	13.6±0.5
	Class 3	3.0±1.0	23.0±2.0	24.6±1.5
	Class 4	3.0±1.0	21.3±1.5	26.3±1.5
	TCS	33.0±8.7	188.6±15.5	216.6±8.6
Ompok bimaculatus	Class 0	88.0±3.2	40.3±3.7	30.0±3.4
	Class 1	3.0±1.0	9.0±1.0	10.3±0.5
	Class 2	2.6±0.5	11.0±1.0	13.0±1.0
	Class 3	3.0±1.0	19.6±1.5	23.0±1.0
	Class 4	3.0±1.0	20.0±1.0	23.6±1.5
	TCS	29.3±8.0	170.0±10.1	203.3±9.2

TCS of site 1 and 2 significant ($P<0.05$) related to site 3 (control site)

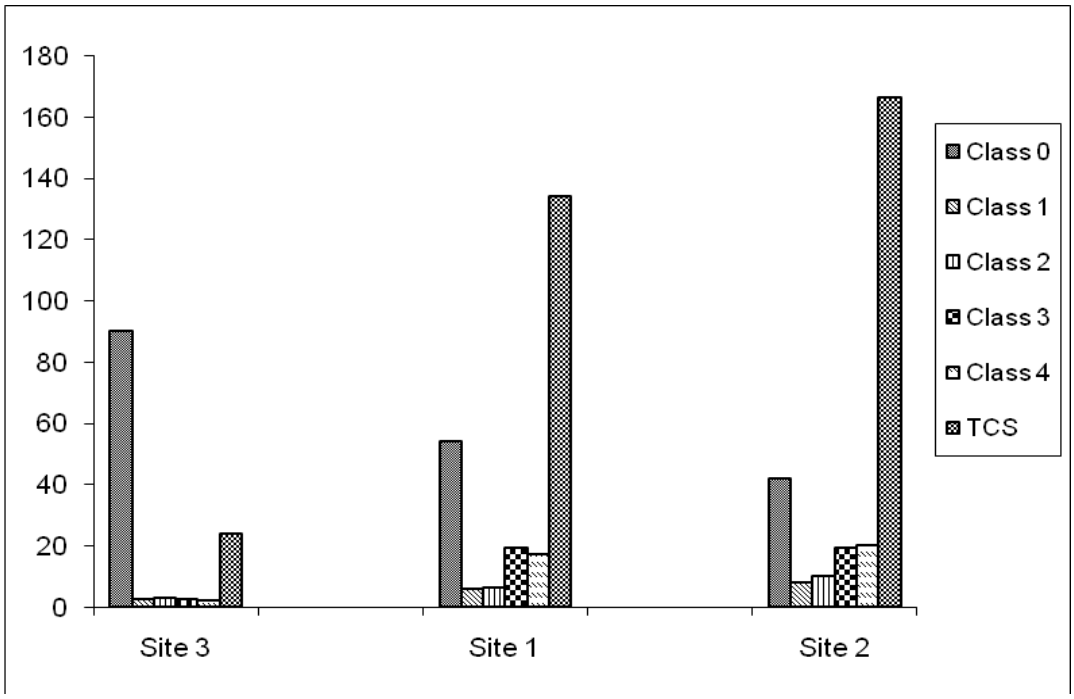


Wallago attu

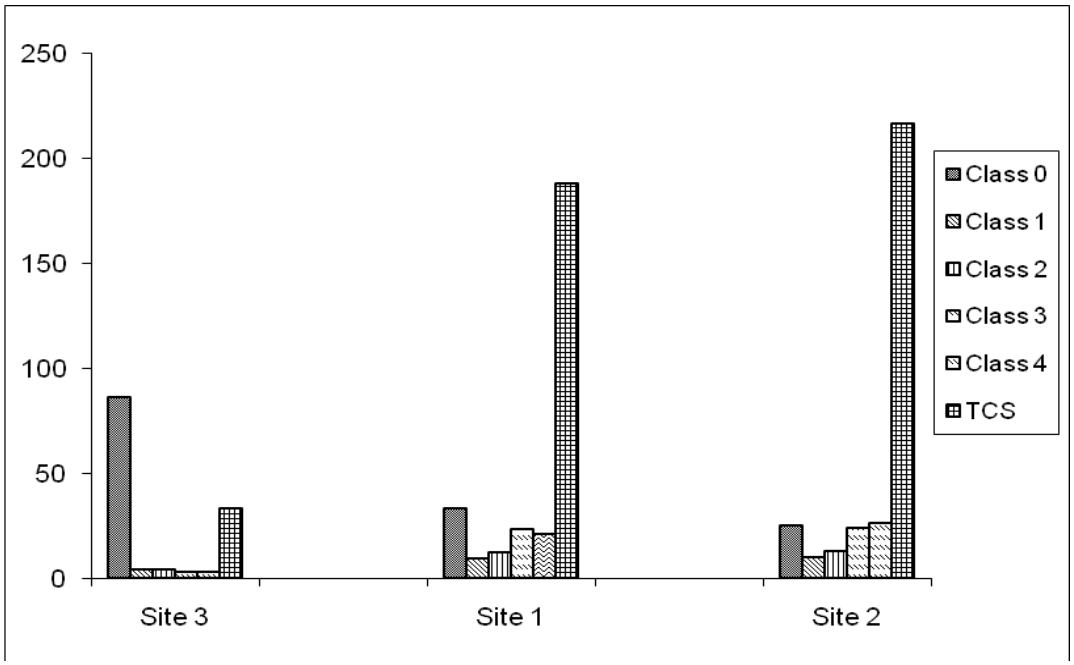


Aorichthys seenghala

Fig. 5.16: Degree of total comet score (TCS) and comet classes in muscle of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

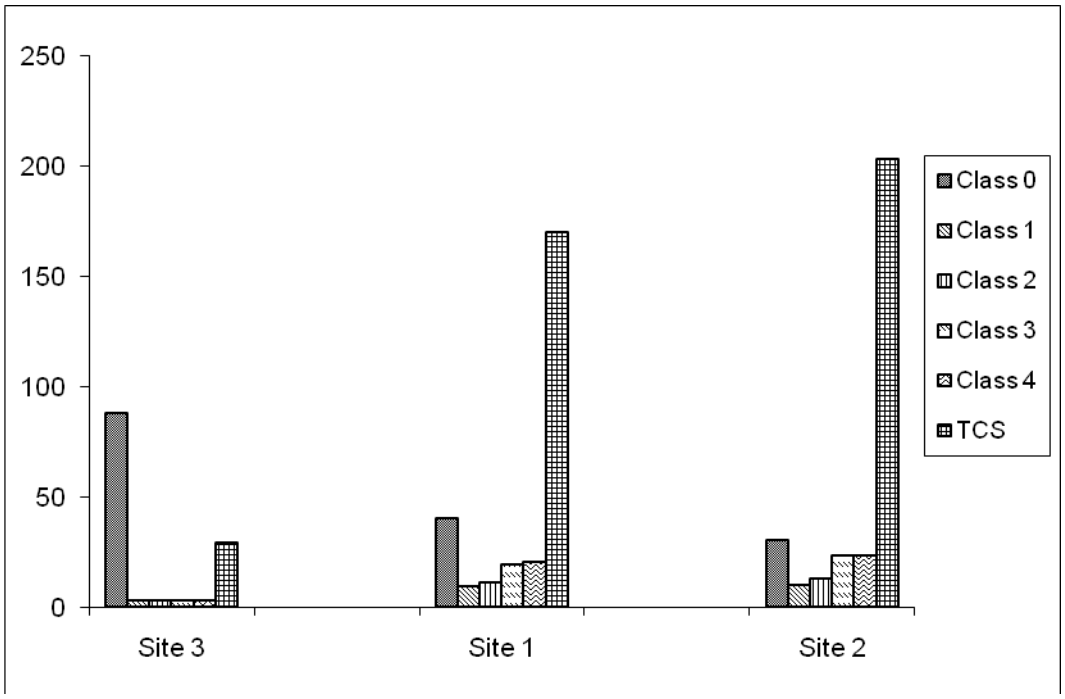


Labeo dyocheilus



Cyprinus carpio

Fig. 5.17: Degree of total comet score (TCS) and comet classes in muscle of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

Fig. 5.18: Degree of total comet score (TCS) and comet classes in muscle of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

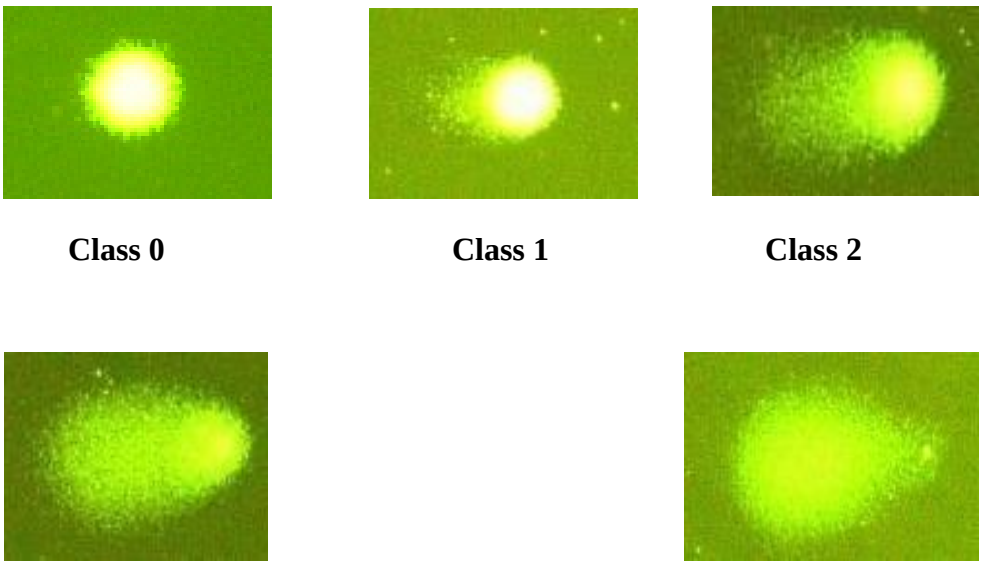


Fig. 5.19: Images showing different comet classes that are induced as a result of heavy metals accumulation in different tissues of fish.

5.3.7 Sequences of TCS and Comet Classes

The present investigations showed that all the tissues and fish have a substantial degree of DNA damage cells. Among various tissues and fish from the polluted sites different patterns of total comet score (TCS) and comet classes were found.

Comet class 0 in blood of different fish species was in the order of *Wallago attu>Labeo dyocheilus>Aorichthys seenghala>Ompok bimaculatus>Cyprinus carpio,* in gills was in *Wallago attu>Labeo dyocheilus>Aorichthys seenghala >Ompok bimaculatus>Cyprinus carpio,* in skin was *Wallago attu>Labeo dyocheilus>Aorichthys seenghala>Ompok bimaculatus> Cyprinus carpio,* in intestine was *Wallago attu>Labeo dyocheilus > Aorichthys seenghala>Ompok bimaculatus>Cyprinus carpio,* in liver was *Wallago attu>Labeo dyocheilus >Cyprinus carpio>Ompok bimaculatus > Aorichthys seenghala* and in muscle was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilu >Wallago attu.* The overall order of comet class 0 in different tissues was muscle> gills >liver> skin > intestine >blood and in different fish species was *Wallago attu>Labeo dyocheilus>Aorichthys seenghala>Ompok bimaculatus>Cyprinus carpio.*

Comet class 1 in blood of different studied fish was in the sequence of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu,* in gills was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilu>Wallago attu,* in skin was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Wallago attu>Labeo dyocheilus,* in intestine was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu,* in liver was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu* and in muscle was *Cyprinus carpio >Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* The

overall order of comet class 1 in different tissues was blood >intestine> skin>liver> gills> muscle and in different fish species was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus > Wallago attu.*

Comet class 2 in blood of different studied fish was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in gills was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu*, in skin was *Cyprinus carpio>Ompok bimaculatus> Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in intestine was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in liver was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu* and in muscle was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus>Wallago attu*. The overall order of comet class 2 in different tissues was skin>intestine>blood >liver> gills> muscle and in different fish species was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus >Wallago attu*.

Comet class 3 in blood of different examined fish was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in gills was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in skin was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus>Wallago attu*, in intestine was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu,* in liver was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala >Labeo dyocheilus >Wallago attu* and in muscle was *Cyprinus carpio>Ompok bimaculatus>Labeo dyocheilus>Aorichthys seenghala>Wallago attu.* The overall order of comet class 3 in different tissues was intestine>blood >skin>liver>gills> muscle and in different studied fish was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala >Labeo dyocheilus >Wallago attu.*

Comet class 4 in blood of different studied fish was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in gills was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in skin was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus>Wallago attu*, in intestine was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in liver was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu* and in muscle was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus>Wallago attu.* The overall order of comet class 4 in different tissues was blood >intestine>skin>liver>gills> muscle and in different fish species was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu.*

TCS in blood of different studied fish was in the order of *Ompok bimaculatus>Cyprinus carpio>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in gills was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu,* in skin was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala>Labeo dyocheilus>Wallago attu*, in intestine was *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu*, in liver was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus>Wallago attu* and in muscle was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu.* The overall order of TCS in different tissues was intestine> blood >skin>liver>gills> muscle and in different examined fish was *Cyprinus carpio>Ompok bimaculatus > Aorichthys seenghala>Labeo dyocheilus >Wallago attu.*

5.3.8 Conclusions and Remarks

In the present investigation total comet score (TCS) and comet class 0, class 1, class 2, class 3 and class 4 were determined in blood, intestine, skin, gills, liver and muscle of five different fish species including *Wallago attu*, *Ompok bimaculatus*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala* netted from both polluted and non polluted sites of River Kabul. The overall order of DNA damage cells in various organs of fish species was in the sequence of intestine >blood >skin >liver > gills > muscle and in different fish species was in the order of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu*. This shows that highest degree of DNA damage cells was found in intestine followed by blood, skin, liver and gills, while lowest frequency was found in muscle. Similarly highest frequency of DNA damage cells was observed in *Cyprinus carpio* fish followed by *Ompok bimaculatus*, *Aorichthys seenghala*, *Labeo dyocheilus* and lowest in *Wallago attu*. Thus the organs level order of DNA damage cells in *Wallago attu* was in the sequence of blood >intestine >skin >liver >gills > muscle, in *Aorichthys seenghala* was in the order of intestine>blood>skin > liver >gills>muscle, in *Labeo dyocheilus* was in the order of blood >intestine > skin >liver >gills > muscle, in *Cyprinus carpio* was in the order of blood >intestine > skin >liver >gills > muscle and in *Ompok bimaculatus* was in the order of intestine> skin >blood> liver >gills >muscle.

Overall order of comet classes in different tissues are as below

» In blood was Class 4 > Class 3> Class 0> Class 2> Class 1

» In intestine was Class 4 > Class 3> Class 0> Class 2> Class 1

» In skin was Class 4 > Class 3> Class 0> Class 2> Class 1

» In gills was Class 0> Class 4 > Class 3> Class 2> Class 1

» In liver was Class 4 > Class 3> Class 0> Class 2> Class 1

» In muscle was Class 0> Class 4 > Class 3> Class 2> Class 1

TCS in different tissues was in the order of intestine >blood >skin >liver > gills > muscle and in different fish species was in the sequence of *Cyprinus carpio>Ompok bimaculatus>Aorichthys seenghala>Labeo dyocheilus >Wallago attu.*

CHAPTER-6

HISTOPATHOPLOGIC EFFECTS OF HEAVY METALS IN SELECTED FISH SPECIES OF RIVER KABUL

6.1 INTRODUCTION

Histopathology together with other methods such as biochemical, growth, diseases and diagnostic are biomarkers used in assessing effects of both internal (feed used) and external (aquatic) environmental conditions on aquatic organisms like fish (Connell, 1976; Hinton *et al*., 1987, Segner and Braun beck, 1988). Accumulated heavy metals may lead to histopathological alterations in the tissues of fish (Monteiro *et al*., 2005). Various pathological abnormalities like apoptosis of lamellar epithelial cells and lamellar fusion were observed under microscope in different organs of the fish after exposure to the heavy metals like mercury and copper and the latter process occurred either by simple apposition of adjacent lamellae to each other or through epithelial hypertrophy and hyperplasia (Daoust *et al*., 1984). Synthesis of metallothionein is increased in fish during chronic and acute exposures to heavy metals. The metallothionein is saturated by metals and excess metal ions spill over into other cellular compartments and cause pathological lesions in different tissues of fish (Steven and Paul, 2011). Induced abnormal functioning of phagocytes, induction of respiratory burst activity and damage of tissues in fish and other animals are associated with an increase in concentration of heavy metals (Masroor *et al*., 2000). The liver histology is used as biomarker for the environmental pollution (EI. Serafy *et al*., 2009). There have been numerous reports of histopathological changes in liver of fish after exposure to a wide range of heavy metals (Au, 2004; Abdel-Moneim *et al*., 2012). The changes in fish gills are among the most commonly recognized responses to environmental stressors and are indicative of physical and chemical stress like heavy metals (Abdel-Moneim *et al*., 2012; Au, 2004; Mallat, 1985). The gills histology is used as indication of environmental pollution (EI. Serafy *et al*., 2009).

Gill histopathological lesions as indicators of exposure to a wide range of heavy metals have previously been used in numerous laboratory and field studies around the world (Au, 2004; Ribeiro *et al*., 2002; Elahee, and Bhagwant, 2007).

Histopathoiogical lesions such as degeneration and necrosis of hepatocytes as the elevation in transaminase activities may be attributed to heavy metals in the liver of fish *C. gariepinus* (Aly *et al*., 2003). Histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution (Peebua *et al*., 2008). Several pathological abnormalities have been observed in the gills, liver, kidneys and gonads of fish as exposed to agricultural, sewage and industrial pollutants (Mohamed, 2003). Pathological disorders in the gills of fish are related to specific classes of toxicants. Many researchers have investigated different pathological alterations in the gills of different fish species after exposure to heavy metals (Pacheco and Santos, 2002; Moore *et al.*, 2003). Different histopathological changes in the gills of *Fundulus heteroclitus* were observed after exposure to heavy metal like cadmium (Gardner and Yevich, 1970). Histopathological changes in the gills of *Salmo gairdneri* were observed after exposure to heavy metals (Balah *et al*., 1993). Gills are the respiratory and osmoregulatory organ of the fish. The histopathological changes of the gills due to heavy metals might impair the respiratory function of the gills by reducing respiratory surface area, resulted in hypoxia, respiratory failure problems (Alazemi *et al*., 1996; Yasser and Naser, 2011) and this badly affects the physiology of the fish body and may be resulted to the fish death (Mohamed, 2003).

The liver is particularly susceptible to damage from a variety of toxicants. Liver helps in cleaning of pollutants from the blood. Therefore it is a good indicator of aquatic environmental pollution (Soufy *et al*., 2007). The liver alterations in the *C. gariepinus* fish samples were more severe and reflecting that the water qualities of El-Rahway drain was poor. These abnorsmalities in the liver could be correlated to toxic effects of heavy metals on hepatocytes, thus liver is most important organ, which help

in detoxification of toxic chemicals (Freeman, 1983). Severals pathological disorders in the liver of *Clarias gariepinus* Cat fish were investigated as a result of heavy metals accumulation in the liver (Mohammad *et al*., 2013). Heavy metals in Elbe Rivers might cause liver damage in fish (Sorensen, 1991). The same abnormalities in the liver of *Tilapia zillii* and *Clarias gariepinus* were also investigated after exposure to heavy metals living in Nile water (Ibrahim and Mahmoud, 2005). Histopathological effects of heavy metals were reported in some fish inhabiting Bardawil lagoon. Heavy metals are the toxicants that induce histopathological abnormalities in different tissues of animals and fish (Yacoub and Abdel Satar., 2003). Histopathological alterations were seen after exposure of the lake white fish *Coregonus clupeaformis* to heavy metal like nickel (Ptashynski *et al*., 2002). The liver histopathological lesions were seen in the fish, *Clarias gariepinus* after exposure to heavy metal like Pb (Wedemeyer and Yasutake, 1978). Several pathological abnormalities in the liver of *Oreochromis niloticus* and *Tilapia zillii* fish were observed collected from the southern region of contaminated water (Mohamed, 2001).

Microscopic examination of hepatocytes and their nuclei of fish from contaminated areas exhibit histopathological changes in comparison to control ones due to contamination. Hepatocytes lose their normal boundaries (Metwally *et al*., 2010). Histopathological changes like hepatocytes vacuolation, cellular swelling, nuclear degeneration and congestion of blood vessels in the liver and pathological disorders such as secondary lamellar disorganization, rupture in lamellar epithelium and epithelial lifting in the gills of different fish were observed after collection from polluted water (Metwally *et al*., 2010). Several histopathological lesions in fish liver after exposure to heavy metals were reported and supported by many other studies deals with monitoring the fish health and environmental pollution in aquatic ecosystem (Stentford *et al*., 2003). Exposure of the fish to heavy metals like cadmium or zinc can induce histopathological conditions of the kidney and epidermis (Somasundaram, 1985), the gills (Grobler, 1989) and the liver (Morsey and

Protasowicki, 1990). Hepatocellular alterations in fish hepatic tissue after exposure to heavy metals were seen (Myers *et al*., 1998). Most previous studies have reported that exposure of fish to heavy metals is associated with structural damage of gills epithelia. Most gills changes caused by pollutants in the fish like epithelial lifting and inflammatory response of the tissue were seen in the gills of the fish (Metwally *et al*., 2010).

It has been observed that lifting and peeling of the lamellar epithelium and rupture of capillaries are common lesions in gills of *Senegal sole* fish after exposure to heavy metals (Arellano *et al*., 1999). Same changes were also seen in other fish species after exposure to heavy metals like copper, zinc and mercury (Krishnani *et al*.,2003). Pathological alterations like strand scission, depurination, cross linking and base modifications were observed after exposure of experimental fish to carcinogenic metals (Bal and Kasprzak, 2002). Heavy metals accumulation in fish could be resulted to high mortality rate and biochemical and histological abnormalities in different tissues of the fish (Ramalingam and Rarnalingam, 1982). Several pathological disorders were seen in gills, liver and kidneys of the studied fish collected from Abu Za'baall akes in Egypt (Fatima *et al*., 2005). Several pathological abnormalities were also observed in the muscle, gills. liver and kidneys of *Archosargus probatocephalus* as a result of exposure to copper (Cardeilhac *et al*,, 1979), in *Tijapia niloiica* exposed to lead acetate, mercuric chloride and cadmium chloride (Balah *et al*., 1993), in *Cyprinion mhaknsis* exposed lo copper (Ghazaly *et al*., 1994), in *Macropsobrycon uruguayanae* exposed to cadmium (Randi *et al*., 1996) and in *Salmo trutta* exposed to iron sulphate (Dalzell and Macfarlane, 1999). Pathological diorders were seen in the kidneys of *Heteropnettstes fossilis* after exposure to heavy metals like mercury and cadmium (Bano and Hassan, 1990), in *Cyprinus carpio* exposed to cadmium (Singhal and Jain, 1997) and in *rainbow trout* exposed to Cd (Iliopoulou and Kotsanis, 2001).

The gills of three studied fish collected from Abu Za'baal lakes showed different pathological disorders like proliferative changes in the epithelium of gills filaments and secondary lamellae and degenerative and necrotic changes in gills filaments and secondary lamellae. Besides these changes inflammatory cells infiltration was also noticed among the proliferated epithelial cells. Moreover, in the gills of *O. nilolicus* histopathological alterations such as severe curling of secondary lamellae, severe atrophy of secondary lamellae and dilation and congestion in the blood vessels of gills filaments were also seen. Collapse of the epithelium of gills filaments, bulging with blood at the tips of lamellae and dark deposits were seen on the surface of gills epithelia (Fatima *et al*., 2005). The pathological alterations such as dilation of the lamellar blood vessels and the presence of edematous fluid in the secondary lamellae may be related to increased permeability induced as a result of exposure of the fish to toxic metals for long period (Balah *et al*., 1993).The edematous fluid separated the respiratory epithelium from the underlying tissues and led to its disquamation as well as necrosis. The dark deposits seen on the surface of gills epithelia were most probably related to heavy metals (Peuranen *et al*., 1994). It has been investigated that the kinetics of heavy metal uptake and metallothionein synthesis are both to be taken into account, where the pathological effects would appear when the rate of metal uptake exceeds the rate of metallothionein synthesis (Mccarter *et al*., 1982).

The histopathological changes observed in present investigation after exposure to lethal and sublethal content of mercury chloride and copper chloride in the liver, muscle and intestine of test fish *Channa gachua* (Deore and Wagh, 2012). The pathological lesions in different tissues of aquatic organisms confirmed that exposure of aquatic organisms to heavy metals may be resulted into histological and pathological disorders, as reported already in previous findings by (Damek and Sawicka, 2003; Zhang *et al.,* 2005; Martin-Diaz *et al*., 2006; Raldua *et al*., 2007).

The histological structure is not altered even in experiments in which fish were fed heavy metals containing food (Andreozzi *et al*., 1994). Histopathological alterations were seen in tissues of *Anguilla anguilla* after exposure to heavy metal like copper (Grosell *et al*., 1996), in liver of *Rutilus rubiliohridanus* (Roganovic *et al*.,1998), in hepatic capillaries in *Barbus meridionalis* (Roganovic *et al*., 2003), in liver of *Cyprinus carpio* on exposure to copper sulphate (Varanka *et al*., 2001). Different histopathological changes in epidermis, dermis, hypodermis and underlying muscle of *Oreochromis niloticus* fish specimens collected from El-Kanater, Benha, Zefta and Talkha stations were investigated and found necrosis of epithelial and mucous cells of the epidermis, degeneration, necrosis and edema of muscle fibers. They also revealed congestion and dilation of the dermal blood vessels together with hypodermic inflammatory signs which may extend to underlying muscle (Sabry *et al*., 2005). Several histopathological alterations were studied in the liver of different fish. These alterations including vacuolar degeneration in the hepatocytes, focal areas of necrosis, haemorrhage and haemostderin between the hepatocytes and around hepatic and hepatoportal blood vessels and dilation and congestion in hepatic and hepatoportal blood vessels. The observed degeneration in the liver may be attributed to disruption in the lysosomal membrane, which is very sensitive to toxicants as heavy metals and thus their enzymes released and caused degeneration and vacuolation of cytoplasm of hepatocytes (Yacoub *et al*., 2003).

It has been investigated that heavy metal like zinc is known for its essential role in growth, immunity, DNA replication, body's defensive system, cell division, cell growth, wound healing and the breakdown of carbohydrates. High zinc intake leads to enfeeblement, retardation of growth and may bring about metabolic and pathological changes in various organs in fish (Ambrose *et al*., 1994). Different heavy metals were determined in fish species like eels caught from polluted lakes in north western Poland. Steady deterioration in the health of eels, chronic degenerative inflammation of the internal organs, non-specific anemia, aplasia or hypoplasia of the

red blood cells in fish were observed (Orecka and Grabda, 1986). Examination of the gills of the fish collected from Abu Za'baal lakes showed marked histopathological changes. These changes are including proliferative, degenerative and necrotic changes in the epithelium of gills filaments and secondary lamellae, edema in secondary lamellae, separation of the epithelium of the secondary lamellae from the lamellar supporting cells, dilation and congestion in the blood vessels of gills filaments, atrophy in secondary lamellae, bulging at the tips of secondary lamellae and dark deposits on the surface of gills epithelia. The observed proliferative changes in the respiratory lamellar epithelium may increase the epithelial thickness which retard or prevent the entry of toxic metals into the blood stream (Laurent, 1984).

6.2 METHODS AND MATERIALS

6.2.1 Study Area

For detail see page≠2

6.2.2 Fish Sampling Sites

For detail see page≠109

6.2.3 Collection of Fish Samples

For detail see page≠109

6.2.4 Collection and Preservation of Fish Tissues

For detail see page≠188

6.2.5 Procedure

6.2.6 Preparation of Solutions for Tissue Processing

The following different solutions for tissues processing were prepared according the method described by Prophet *et al* (1992).

6.2.7 Preparation of Fixative Solution

10% neutrally buffered formalin (NBF) was used as a fixative and was prepared by mixing 10% formalin with PBS.

6.2.8 Preparation of PBS (Potassium chloride 0.2 gm, sodium phosphate dibasic dehydrate 1.44 gm, potassium phosphate monobasic 0.24 gm, d.H_2O 1000 ml, pH 7.4)

6.2.9 Preparation of 10% NBF (37% formaldehyde 270.27 ml, PBS1000 mL).

6.2.10 Preparation of Different Ethanol Solutions

6.2.10.1 50% Ethanol solution (ethanol 250 ml, d.H_2O 250 ml).

6.2.10.2 70% Ethanol solution (ethanol 350 ml, d.H_2O 150 ml).

6.2.10.3 80% Ethanol solution (ethanol 400 ml, d.H_2O 100 ml).

6.2.10.4 90% Ethanol solution (ethanol 450 ml, d.H_2O 50 ml).

6.2.10.5 95% Ethanol solution (ethanol 950 ml, d.H_2O50 mL).

6.2.11 Preparation of Alcohol-Xylene Solution

(Distilled alcohol 50 mL, 100% xylene 50 mL).

6.2.12 Preparation of Xylene-Paraffin Solution

(Paraffin 50 mL, 100% xylene 50 mL).

6.2.13 Preparation of Different Solutions for Staining

6.2.13.1 Mayer's Albumin

Equal quantities of both fresh egg albumin and glycerol was mixed. Few drops of formaldehyde 37% were also added and then stored at 4^oC.

6.2.13.2 Harris Hematoxylin Stain

(Hematoxylin powder 5.0 gm, mercuric oxid 2.5 gm, potassium alum 100 gm, absolute ethanol 50 mL, glacial acetic acid 40 mL, d. H_2O1000 mL)**.**

6.2.13.3 Eosin Stain

Working solution was prepared from eosin and phloxine-B stock solutions.

6.2.13.4 Eosin-Y Stock Solution (Eosin-Y powder 1gm, d.H_2O 100mL, few drops of 37% formaldehyde).

6.2.13.5 Phloxine-B Stock Solution (Phloxine-B 1g, d.H_2O 100 ml, few drops of 37% formaldehyde).

6.2.13.6 Eosin-Phloxine Working Solution (Eosin Y stock solution 100 ml, phloxine-B stock solution 10 ml,95% ethanol 780 mL, Glacial acetic acid 4 mL).

6.2.13.7 1% Acid-Alcohol Solution (Fuming HCl5ml, 70% ethanol 500 mL).

6.2.13.8 1000mL Ammonia Solution (Ammonium hydroxide 2 ml, d. H_2O 998 mL).

6.2.14 Tissue Processing

The tissues were processed according to the protocol of Prophet *et al* (1992).

6.2.15 Tissues Fixation

Weighted tissues of liver, intestine, muscle and gills were kept in 10% NBF for 48 hours.

6.2.16 Tissues Dehydration

Tissues (80gm) were cut into a small pieces and kept in tissue cassettes. These cassettes were then kept in a series of ethanol solutions (30%, 50%, 70%, 80%, 90%, 95%).To accelerate dehydration, the tissues were kept on hot plate magnetic stirrer and adjusted the temperature between 55-57^oC. The tissues were dehydrated according to the following schedule.

6.2.16.1 70% Ethanol solution for 1 hour.

6.2.16.2 80% Ethanol solution for 1 hour.

6.2.16.3 90% Ethanol solution for 1 hour.

6.2.16.4 Two changes each of 100% ethanol solution for 1 hour.

6.2.17 Clearing of Tissues

Tissues were transferred to clearing solution (50% Xylene, 50% ethanol) and kept on hot plate magnetic stirrer and adjusted the temperature between 45-47^{o}C. The cleared tissues were finally treated twice with 100% Xylene.

6.2.18 Paraffin Infiltration of Tissues

The tissues were infiltrated with melted paraffin wax. First tissues were incubated at 62^{o}C with combined solution of 50% xylen and molten paraffin wax for one hour and then kept twice for I hour each in paraffin wax.

6.2.19 Embedding of Tissues

Tissues were transferred to stainless steel (S.S). Melted paraffin was poured into moulds and tissues were placed with heated forceps in it. Warm tissue cassettes were placed on the surface of paraffin. The paraffin wax moulds were kept in freezer till solidified. After solidification the tissue wax blocks were removed.

6.2.20 Sectioning of Tissues

The tissues were sectioned through a rotary microtome. The sections were adjusted to 12µm thickness. The section ribbon was removed from blade edge through forceps and the shiny side of the sectioned tissue was kept in water bath containing clean water at a temperature of 10 degree below the melting point of wax. Then the tissues were mounted on microscopic slides coated with Mayer's albumin and cooled at ambient temperature and placed in staining slide tray. The staining slide tray was then placed in oven at 58^{o}C for 10-15 minutes.

6.2.21 Staining of Tissues

The tissues were stained with hematoxylin and eosin in staining glass jars. Before staining wax were removed and then rehydrated by xylene and then gradual ethanol solution and finally with 100 % ethanol. For hemotoxyline staining, tissue

slides were kept in it for 5 min and then genetly washed with running tap water. The same tissue slides were then kept in eosin-phloxine solution for 8 min and washed gently with running tap water. Stained tissues were covered with candabolsm and then placed cover slips on it.

6.2.22 Observation of Tissues under Microscope

The dried stained tissues were observed under a compound microscope using all magnification (40X, 100X, 400X). Photomicrographs of tissues sections were obtained on a digital camera connected to the microscope. Photomicrographs of low and higher resolutions were taken.

6.2.23 Statistical Analysis

Statistical analysis was done by using ANOVA software for Windows. Percentage values of pathological disorders were determined through the following formula

$$\frac{\text{No of fish in which pathological abnormalities were observed}}{\text{Total no of fish species studied for pathological disorders}} \times 100$$

6.3 RESULT AND DISCUSSION

The present study was aimed to assess the histopathological effects of heavy metals in intestine, liver, gills and muscle of *Wallago attu, Aorichthys seenghala, Cyprinus carpio, Labeo dyocheilus* and *Ompok bimaculatus* from polluted sites of River Kabul and compared with fish samples from reference site (Warsak dam). Histopathological changes associated with heavy metals in fish have been studied by many authors. But no histopathological studies have been carried out on the fish of River Kabul. Therefore, for the first time this study was conducted to determine pathological abnormalities due to heavy metals accumulation in selected fish species of River Kabul. Metal contamination of aquatic ecosystems has long been recognized as a serious pollution problem. When fish are exposed to elevated levels of metals in a

polluted aquatic ecosystem. They tend to take directly these metals up from their environment, which are associated to pathological abnormalities in aquatic animals like fish.

6.3.1 Histopathological Lesions in Intestine

Intestine of different fish species from polluted water showed highest percentage of histopathological conditions as compare to the fish species from control water, where the histopathological lesions were lowest. The lesions which were observed in intestine of selected studied fish species including degeneration of epithelium, complete degeneration of cillia, inflammatiom, coagulative necrosis and degenerative cillia (Table 6.1 and Figs 6.1-6.8).

More percentage of pathological disorders was observed in intestine of *Wallago attu* from polluted water as compare to control water. The intestine of *Wallago attu* from polluted sites showed 46 % and 53 % degeneration of epithelium and showed 7% from control site 3, showed 45 % and 50 % complete degeneration of cillia from polluted sites 1 and 2 and showed 9 % from reference site respectively. The intestine of this fish showed maximum percentage of degeneration of epithelium and minimum percentage of complete degeneration of cillia. These results were same to the findings of Fatima and Mohamed (2008), who had also observed the same pathological abnormalities in intestine of *Tilapia nilotica* and *Tilapia galilea* after exposure to heavy metals. In this study the histological changes in intestine of *Wallago attu* could be attributed to greater concentration and toxicity of heavy metals in this tissue. The result also confirmed heavy metals pollution in the studied area of River Kabul.

Three histopathological abnormalities including inflammation, coagulative necrosis and degenerative cillia were observed in intestine of *Aorichthys seenghala* from both polluted and control water of River Kabul. The observed percentage of histopathological disorder like inflammation in intestine of *Aorichthys seenghala*

from polluted sites 1 and 2 were 43 % and 49 % and was 14 % from reference site, for coagulative necrosis were 40 % and 50 % from polluted water and was 9% from control water and for degenerative cillia were 42 % and 59 % from polluted sites and was 10 % from control site respectively. In this tissue the percentage of degenerative cillia was highest and was lowest for inflammation. In a previous study Orecka and Grabda (1986) have also observed different pathological disorders like chronic degenerative inflammation, non specific anemia, aplasia or hypoplasia of the erythrocytes in intestine of eel fish after collection from polluted water. The greater percentage of different pathological disorders in intestine of *Aorichthys seenghala* could be related to greater content of heavy metals in this fish, low detoxification mechanism and low elimination of metals from the fish body and toxicity of heavy metals. This is a carnivorous fish and being as a carnivorous nature it is more exposed to heavy metals in the water. Due to high concentration and toxicity of heavy metals, the fish from polluted water showed more percentage of pathological disorders as compare to control water. The present result also confirmed heavy metals pollution in River Kabul.

Like other examined fish such as *Labeo dyocheilus* from polluted sites 1 and 2 also showed greater percentage of various pathological abnormalities as compare to control site 3. Intestine of *Labeo dyocheilus* from polluted sites 1 and 2 had 48 % and 55% complete degeneration of cillia and had 11 % from control site 3, had 40% and 46% inflammation from polluted water and had 12 % from control water respectively. High concentration of heavy metal such as copper may badly damage gills, adversely affect the liver, intestine and kidneys of fish or cause some neurological damage (Rask *et al*., 1990). The present investigation found more pathological disorders in intestine of *Labeo dyocheilus* from polluted sites of River Kabul as compare to control site. This could be related to higher concentration of heavy metals in intestine of this fish from polluted sites.The present result also showed that heavy metals are

toxic in nature and can induce various pathological abnormalities in living organisms especially fish.

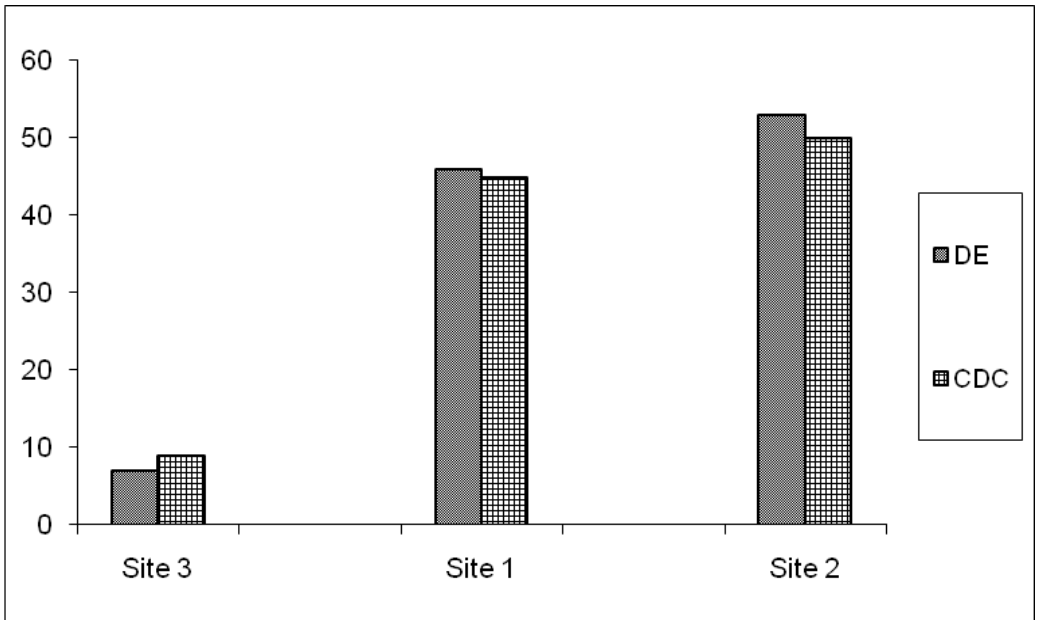
Intestine of *Labeo dyocheilus* from polluted water had greater percentage of pathological disorders as compare to control water. Four histopathological conditions including complete degeneration of cillia, inflammation, coagulative necrosis and degenerative cillia were observed in intestine of *Cyprinus carpio* from both polluted and control sites. In this study the observed histopathological changes in intestine of *Labeo dyocheilus* are in agreement with those observed by Masroor *et al* (2000), who have also observed the same pathological disorders to the present result. The intestine of *Cyprinus carpio* from polluted site 1 and site 2 showed 55% and 61 complete degeneration of cillia, 49% and 55% inflammation, 51% and 59% coagulative necrosis and showed 49% and 60% degenerative cillia and showed 14% complete degeneration of cillia, 15% inflammation, 13% coagulative necrosis and 14% degenerative cillia from control site 3 respectively. In this tissue greater percentage was observed for complete degeneration of cillia and lower for inflammation. Chronic exposure of the fish to heavy metals are responsible for disorders like obstructive airway disease, emphysema, irreversible renal failure, bone disorders and immuno suppression and some pathological lesions in fish (Bertin and Averbeck, 2006). Comparing our data with the findings of other workers indicates that heavy metals are toxic and can induce pathological disorders in aquatic organisms.

Histopathological abnormalities like inflammation, coagulative necrosis and degenerative cillia were observed in intestine of *Ompok bimaculatus* from polluted and control sites of River Kabul. *Ompok bimaculatus* from polluted sites showed 47% and 51% inflammation, showed 43% and 53% coagulative necrosis and showed 45% and 63% degenerative cillia and from reference site showed 14% inflammation, 11% coagulative necrosis and 12 % degenerative cillia respectively. In intestine of this fish more percentage was observed for degenerative cillia and less for inflammation. The

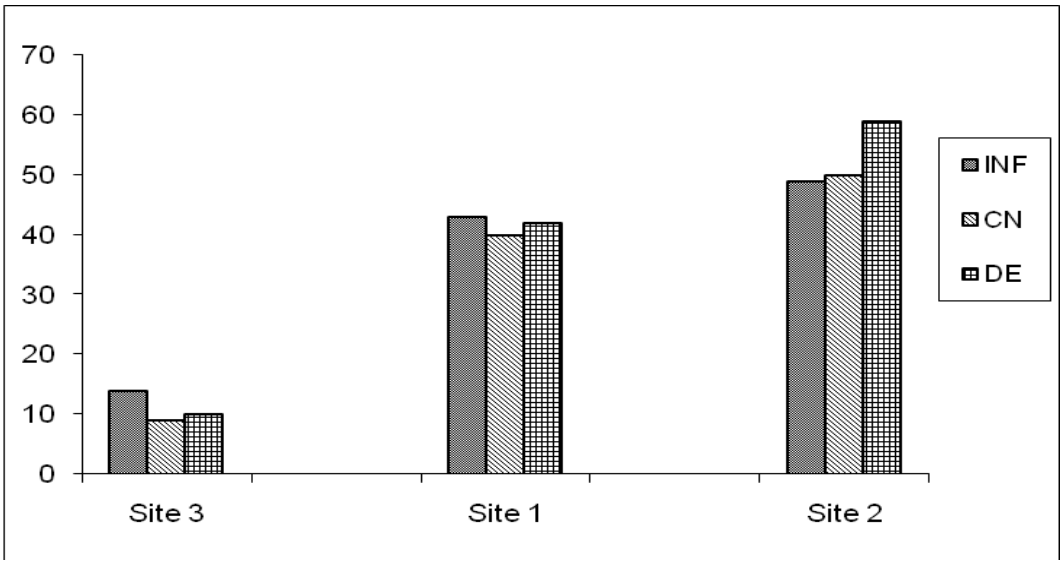
present result of higher percentag of pathological disorders in this tissue of *Ompok bimaculatus* agree with the findings of Uran *et al* (2009). Results of the present and previous studies (Orecka and Grabda, 1986; Ebrahimi and Taherian, 2009; Masroor *et al*., 2000) have demonstrated that heavy metals are toxic in nature and could be associated to pathological abnormalities in both the animals from aquatic and terrestrial environments. The observed percentage of pathological lesions in intestine was in the order of inflammation >degenerative cillia >complete degeneration of cillia > coagulative necrosis>degeneration of epithelium and in different fish species was in the sequence of *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala > Wallago attu>Labeo dyocheilus.*This indicates that greater percentage of pathological disorders was observed in *Cyprinus carpio* and smaller in *Labeo dyocheilus.*The highest degree of lesions in *Cyprinus carpio* could be correlated to greater content of heavy metals in this fish, low elimination of metals from fish body, low metabolic rate and exposion of this fish to heavy metals for long period. Comparing the above findings with our result confirmed that heavy metals are toxic and induce patholgical changes in the external and internal organs of aquatic organisms like fish. In our finding more histopathological lesions were found in the intestine of different fish species from polluted sites. More histopathological abnormalities could be attributed to high concentration of heavy metals in this organ. This result also showed heavy metals pollution in the down stream portions of River Kabul. The overall result indicates that intestine came second after liver followed by gills and muscle for pathological abnormalities.

Table 6.1: Histopathological lesions (%) in intestine of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Lesions	Site 3 (%)	Site 1 (%)	Site 2 (%)
Wallago attu	Degeneration of epithelium	7	46	53
	Complete degeneration of cillia	9	45	50
Aorichthys seenghala	Inflammation	14	43	49
	Coagulative necrosis	9	40	50
	Degenerative cillia	10	42	59
Labeo dyocheilus	Complete degeneration of cillia	11	48	55
	Inflammation	12	40	46
Cyprinus carpio	Complete degeneration of cillia	14	55	61
	Inflammation	15	49	55
	Coagulative necrosis	13	51	59
	Degenerative cillia	14	49	60
Ompok bimaculatus	Inflammation	14	47	51
	Coagulative necrosis	11	43	53
	Degenerative cillia	12	45	63



Wallago attu

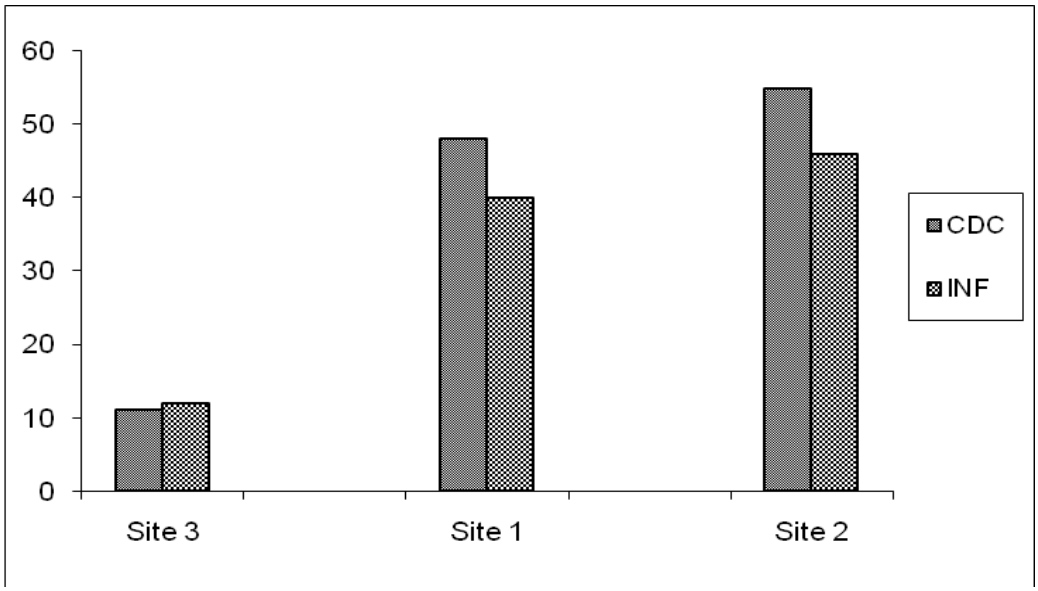


Aorichthys seenghala

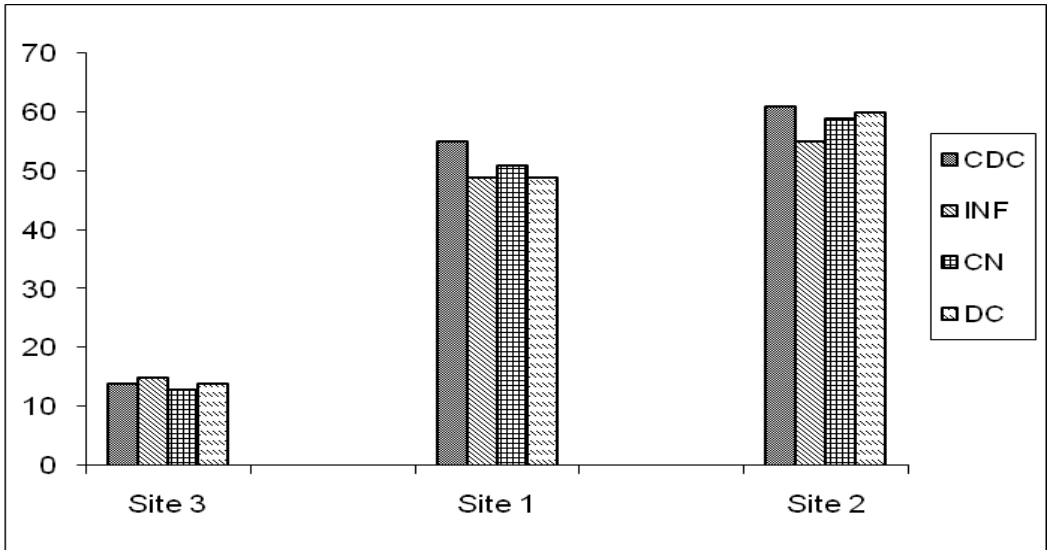
DE: Degeneration of epithelium. CDC: Complete degeneration of cillia.

INF: Inflammation. CN: Coagulative necrosis

Fig.6.1: Histopathological lesions (%) in intestine of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Labeo dyocheilus

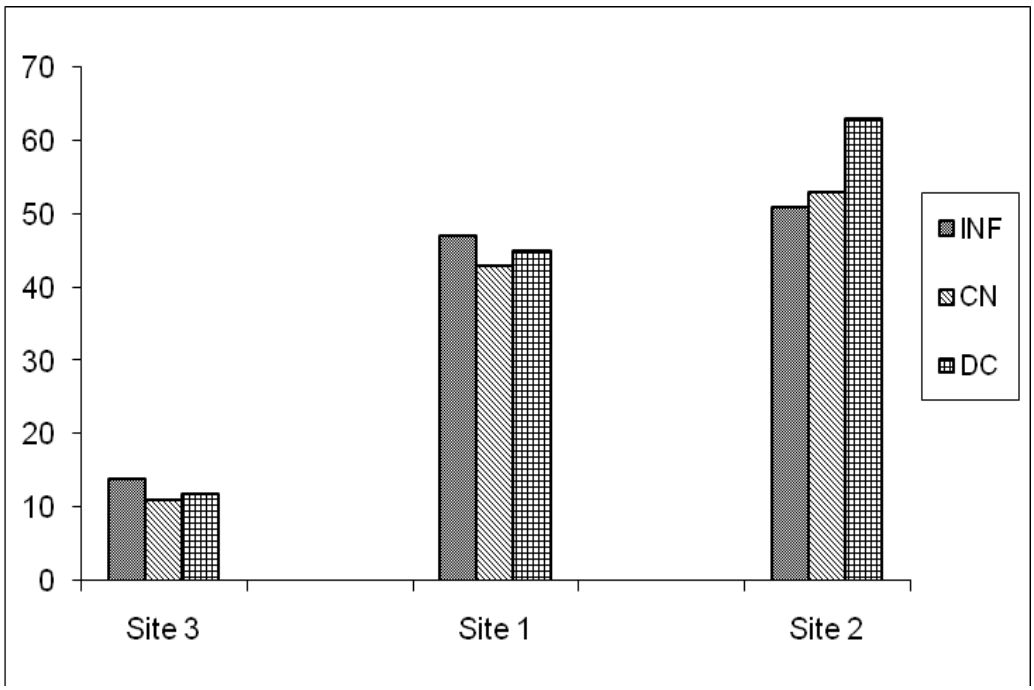


Cyprinus carpio

CDC: Complete degeneration of cillia. INF: Inflammation

CN: Coagulative necrosis DC: Degenerative cillia

Fig. 6.2: Histopathological lesions (%) in intestine of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

INF: Inflammation CN: Coagulative necrosis DC : Degenerative cillia

Fig.6.3: Histopathological lesions (%) in intestine of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

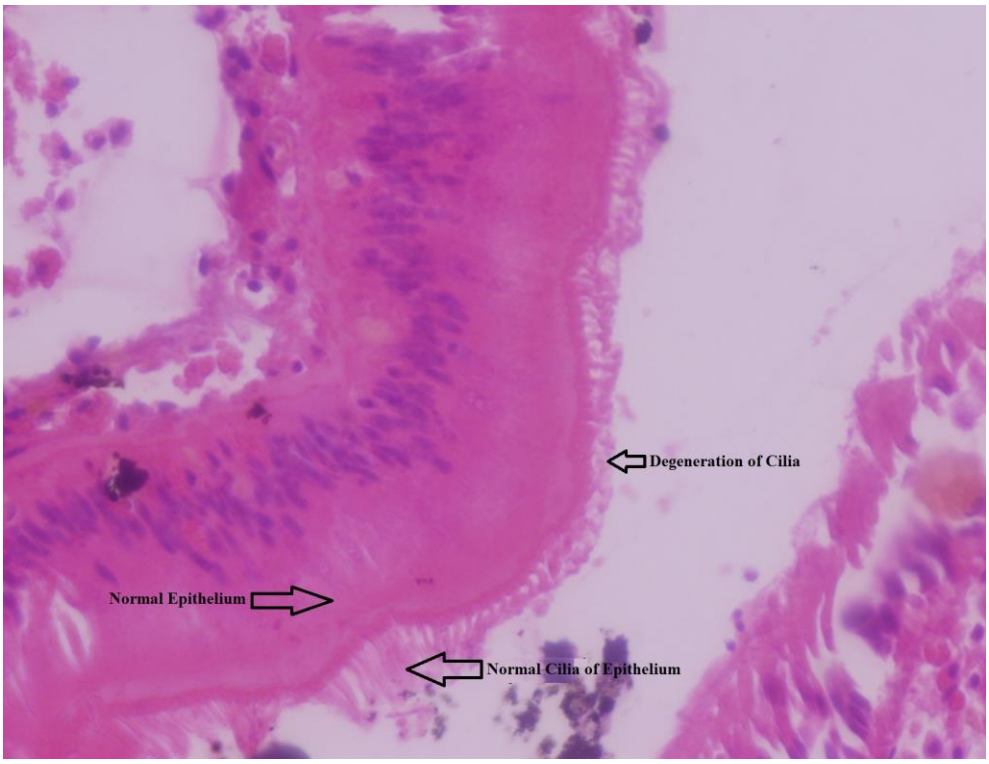


Fig. 6.4: **Image showing normal epithelium, normal cillia of epithelium and degeneration of cillia in intestinal epithelium.**

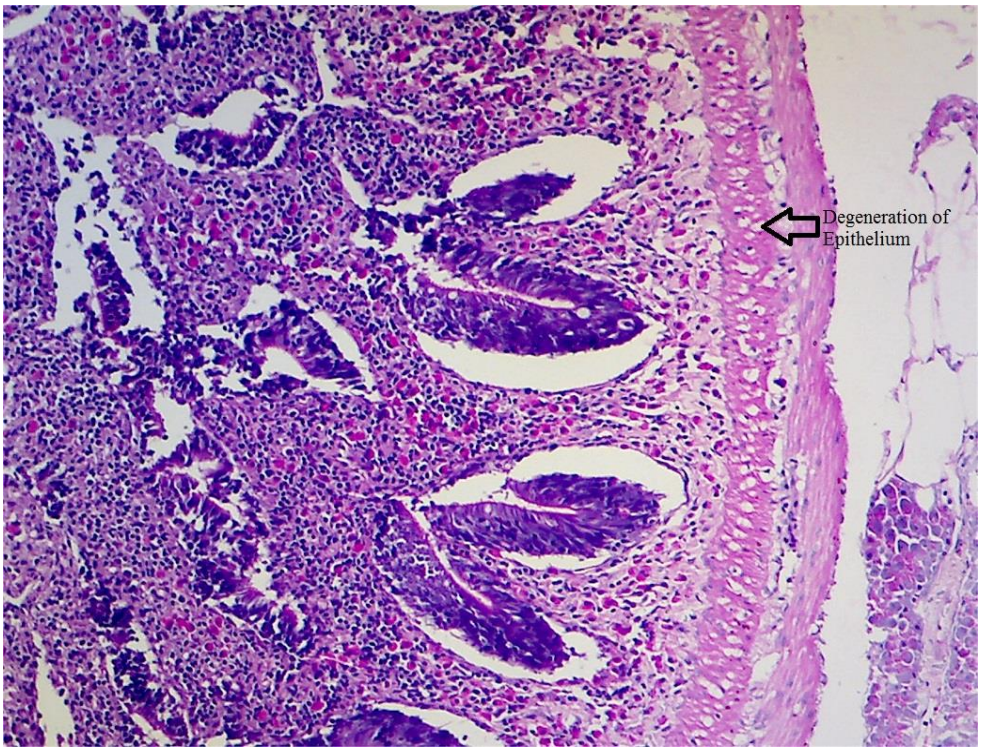


Fig.6.5: **Image showing degeneration of intestinal epithelium.**

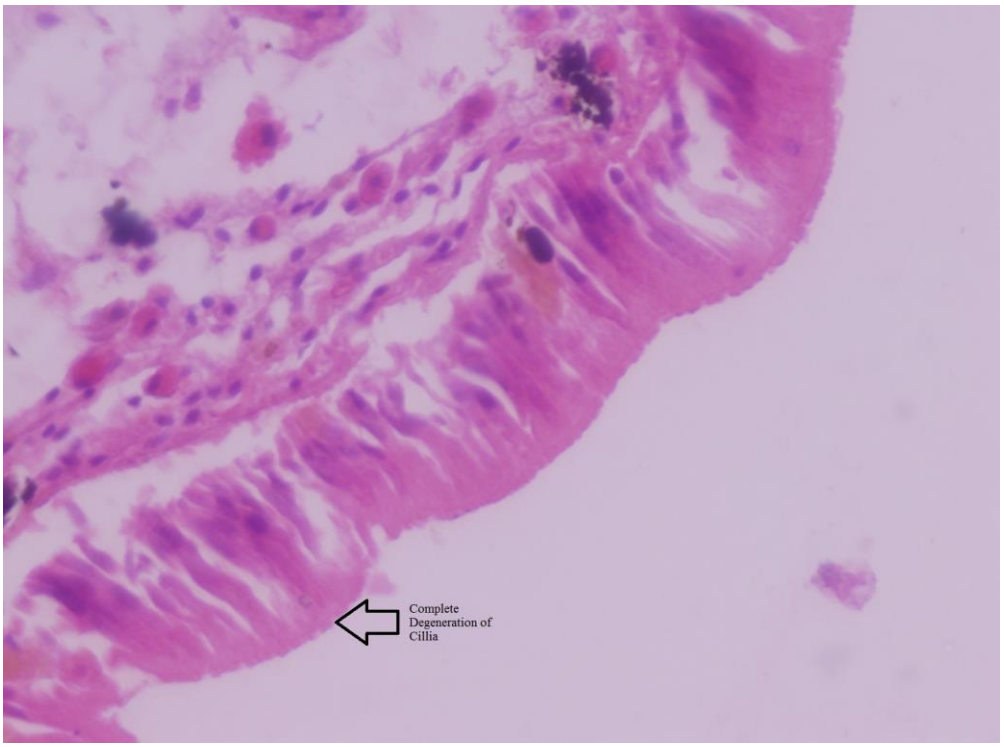


Fig.6.6: Image showing complete degeneration of cillia of epithelium.

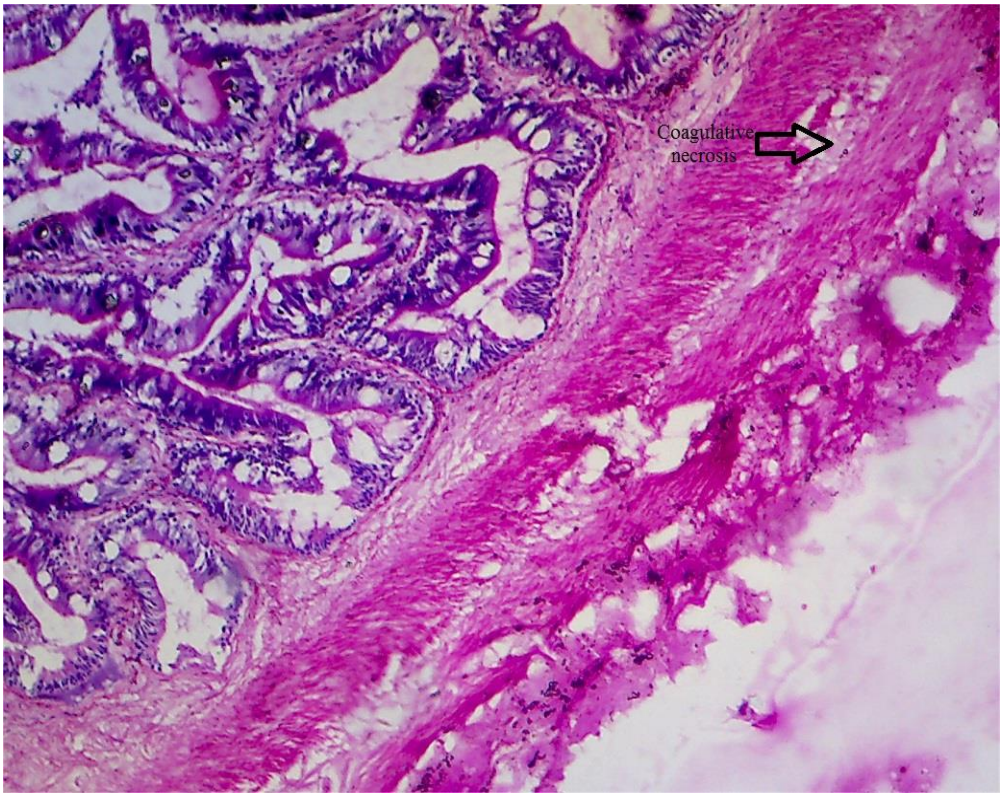


Fig. 6.7: Image showing coagulative necrosis in intestinal epithelium.

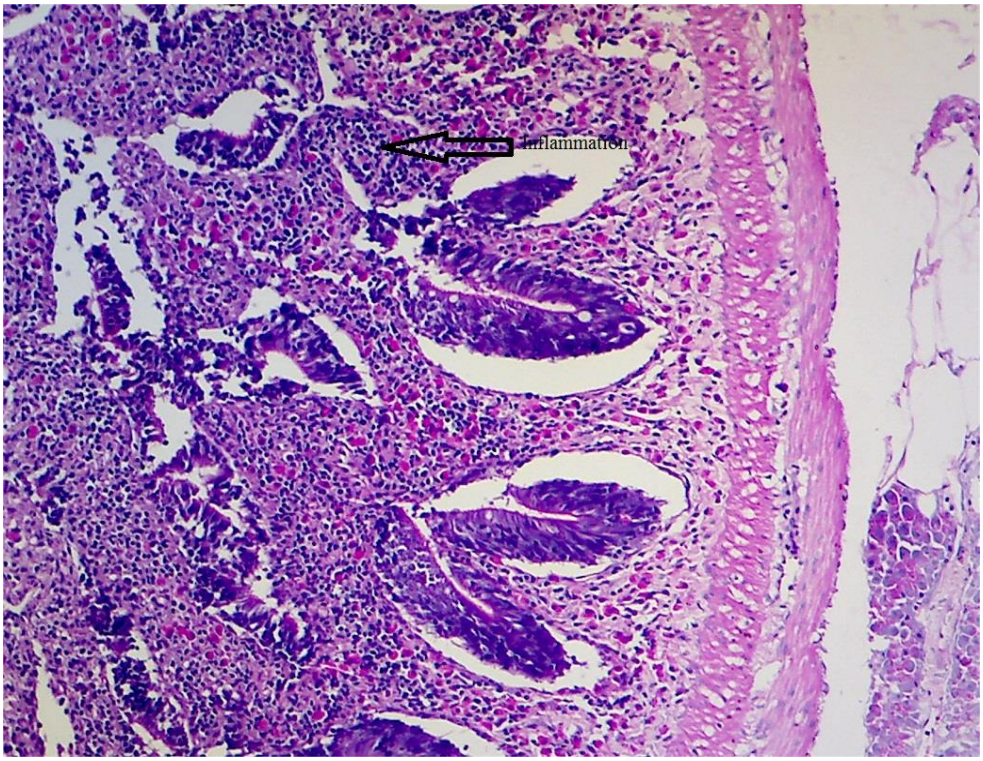


Fig. 6.8: Image showing inflammation in intestine.

6.3.2 Histopathological Lesions in Liver

In the present investigation liver of five different fish species from polluted water showed maximum percentage of histopathological conditions as compare to the fish species from control water, where the minimum percentage of pathological abnormalities was found. The lesions, which were seen in liver of selected examined fish species including inflammatiom, hydropic degeneration, coagulative necrosis, non specific inflammation, liquefactive necrosis and spongiosis hepatis (Table 6.2 and Figs 6.9-6.16).

Heavy metals like chromium, lead, mercury, zinc, copper and nickel are among the most toxic metallic pollutants. Bioaccumulation of these metals is known to adversely affect liver and other tissues of fish, disturbs metabolism and hampers development and growth of fish (Sephar, 1976). In the present study liver of *Wallago attu* from polluted water had 43% and 49% inflammatiom, 51% and 53% hydropic degeneration and 41% and 45% coagulative necrosis and had 9% inflammatiom, 8% hydropic degeneration and 10% coagulative necrosis from reference water (Warsak dam) respectively. Liver of this fish had high percentage of hydropic degeneration and low percentage of coagulative necrosis. These results are supported by the findings of Sastry and Gupta (1978), who had observed shrinkage in liver cells, degenerated nuclei and focal necrosis in liver of *Channa punctatus* fish after exposure to lead intoxication. Comparing this study with the investigations of other researchers highlights that heavy metals are associated with pathological disorders in aquatic and other animals. This could be the result of heavy metals toxicity.

The histopathological conditions like non specific inflammation, liquefactive necrosis and spongiosis hepatis were observed in liver of *Aorichthys seenghala* from polluted and non polluted water of River Kabul. Liver of *Aorichthys seenghala* from polluted sites contained 45% and 56% non specific inflammation, 47% and 55%

liquefactive necrosis and 51% and 59% spongiosis hepatis and contained 13% non specific inflammation, 11% liquefactive necrosis and 10% spongiosis hepatis from Warsak dam (control) respectively. Liver of *Aorichthys seenghala* contained highest percentage of spongiosis hepatis and lowest percentage of non specific inflammation. In a past finding Benedetti *et al* (1981) had observed pathological disorder like cytoplasmic vacuolation in liver of *Ictaburus nebulous* fish after exposure to copper. Comparing the present result with the findings of previous workers showed that heavy metals are toxic in nature and induce pathological alterations in the fish and other animals.

Liver of *Labeo dyocheilus* from polluted site 1 and site 2 showed 46% and 50% non specific inflammation and 12% from control site 3, *Labeo dyocheilus* from polluted site 1 and site 2 showed 49% and 53% liquefactive necrosis and showed 9% from site 3 respectively. In liver of this fish maximum percentage was observed for liquefactive necrosis and lowest percentage for non specific inflammation. These results are in agreement with the findings of Kumar and pant (1981), who have also observed the same pathological abnormalities in liver of *Puntius conchonius* after exposure to heavy metals like copper and zinc. Comparing our data with the findings of above mentioned studies and other workers indicates that heavy metals are toxic in nature and can induce pathological abnormalities in aquatic and other organisms. The result also showed heavy metals pollution in the River Kabul.

Liver of *Cyprinus carpio* from polluted water showed more percentage of pathological abnormalities as compare to control water, where the percentage of histopathological conditions was low. Liver of *Cyprinus carpio* from both polluted sites 1 and 2 had 48% and 54% coagulative necrosis and had 16 % from control site 3, had 49% and 58% non specific inflammation from polluted water and had 14% from reference site and had 48% and 57% liquefactive necrosis from polluted sites and had 13% from control site respectively. In this organ the pathological condition like

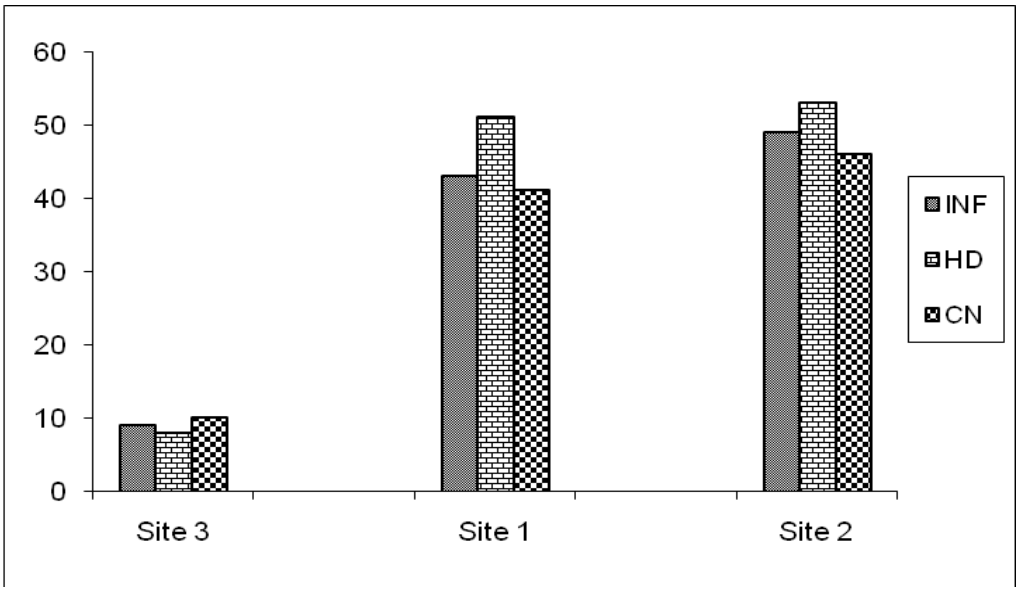
non specific inflammation showed greater percentage and coagulative necrosis showed lowest percentage. Several histopathological lesions in the fish liver after exposure to heavy metals were reported and supported by many other studies and deals with monitoring fish health and environmental pollution in natural water bodies (Stent ford *et al*., 2003). Comparing the present result with the findings of previous workers reveales that heavy metals accumulated in different tissues of the fish, which can induce various histopathological disorders in fish.

The lesions, which were seen in liver of *Ompok bimaculatus* from polluted and control sites including inflammatiom, hydropic degeneration, liquefactive necrosis and non specific inflammation. Liver of *Ompok bimaculatus* from polluted sites 1 and 2 showed 51% and 53% inflammatiom, 53% and 55% hydropic degeneration, 44% and 48% liquefactive necrosis, 41% and 50% non specific inflammation and showed 11% inflammatiom, 10% hydropic degeneration, 11% liquefactive necrosis and showed 9% non specific inflammation from Warsak dam respectively. Liver of *Ompok bimaculatus* had maximum percentage of hydropic degeneration and minimum percentage of liquefactive necrosis. For overall pathological changes liver came first followed by intestine, gills and muscle. In previous finding Singh (1983) has observed various pathological abnormalities like vacuolation and necrosis in liver of *Colisa fasciatus* fish after exposure to copper sulphate. In another finding Dalela *et al* (1984) have reported various pathological disorders like necrosis, hypertrophy and atrophy, loss of polygonal shape of cells, splitting of the cells and formation of spaces in the liver tissues after exposure of *Cyprinus carpio* to lethal and sublethal concentration of copper and cadmium. The present study found the liver to be contained maximum percentage of pathological conditions as compare to intestine, gills and muscle as already mentioned in the previous studies (Peters *et al*., 1987; Ramlingam, 1988; Roncero *et al*., 1992).The percentage of examined lesions in liver was in the sequence of non specific inflammation > liquefactive necrosis >hydropic degeneration > inflammation >

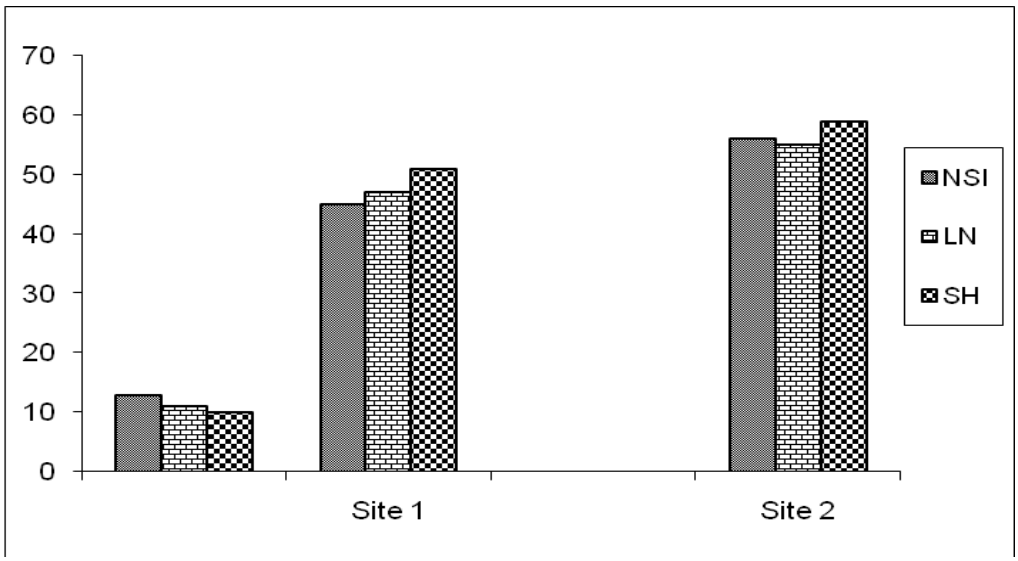
coagulative necrosis > spongiosis hepatis and in different fish species was *Ompok bimaculatus* > *Aorichthys seenghala* > *Cyprinnus carpio* > *Wallago attu* > *Labeo dyocheilus.*The greater lesions in *Ompok bimaculatus* could be attributed to greater level of heavy metals in this organ. This may be due to omnivorous nature of this fish. Being omnivorous nature this fish is more exposed to metal bioaccumulation by many food chains. Comparing the present result with above mentioned studies indicates that heavy metals are toxic in nature and can induce pathological disorders in both animals from both aquatic and terrestrial environments. The present study found more pathological disorders in liver from polluted water as compare to those from control water. This may be attributed to more accumulation of metals, low metabolic rate and low detoxification mechanism of this tissue. Liver came first followed by intestine, gills and muscle for histopathological abnormalities, when overall comparison is made.

Table 6.2: Histopathological lesions (%) in liver of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Lesions	Site 3 (%)	Site 1 (%)	Site 2 (%)
Wallago attu	Inflammation	9	43	49
	Hydropic degeneration	8	51	53
	Coagulative necrosis	10	41	46
Aorichthys seenghala	Non specific inflammation	13	45	56
	Liquefactive necrosis	11	47	55
	Spongiosis hepatis	10	51	59
Labeo dyocheilus	Non specific inflammation	12	46	50
	Liquefactive necrosis	9	49	53
Cyprinus carpio	Coagulative necrosis	16	48	53
	Non specific inflammation	14	49	58
	Liquefactive necrosis	13	48	57
Ompok bimaculatus	Inflammatiom	11	53	51
	Hydropic degeneration	10	53	55
	Liquefactive necrosis	11	44	48
	Non specific inflammation	9	41	50



Wallago attu

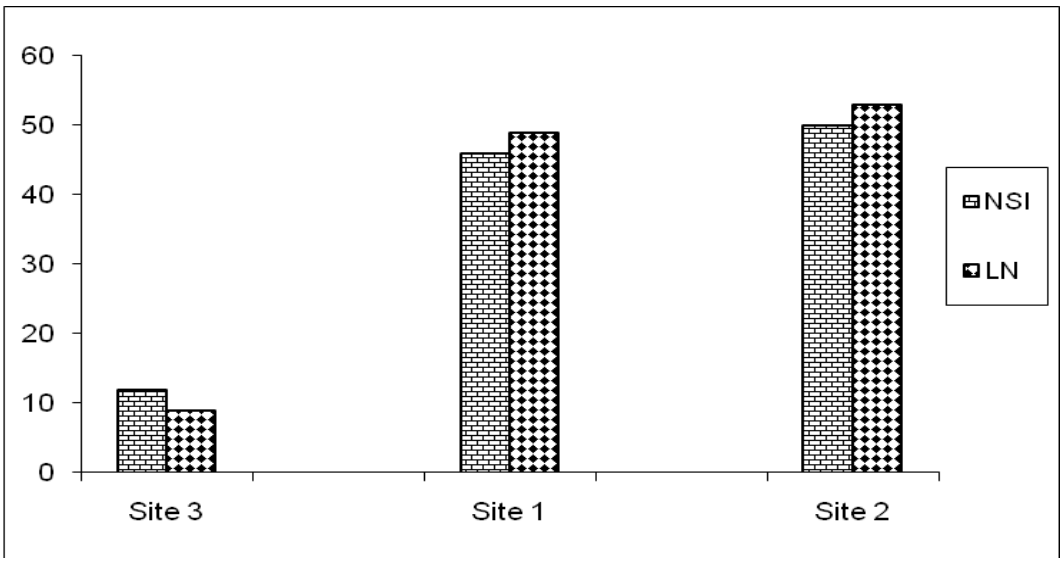


Aorichthys seenghala

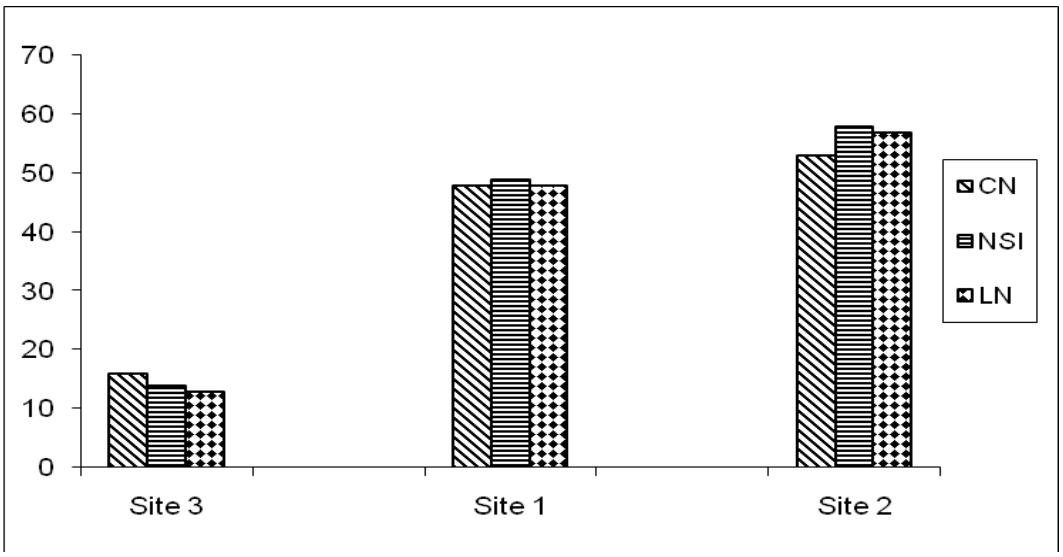
INF: Inflammation HD: Hydropic degeneration CN: Coagulative necrosis

NSI: Non specific inflammation LN: Liquefactive necrosis SH: Spongiosis hepatis

Fig. 6.9: Histopathological lesions (%) in liver of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Labeo dyocheilus

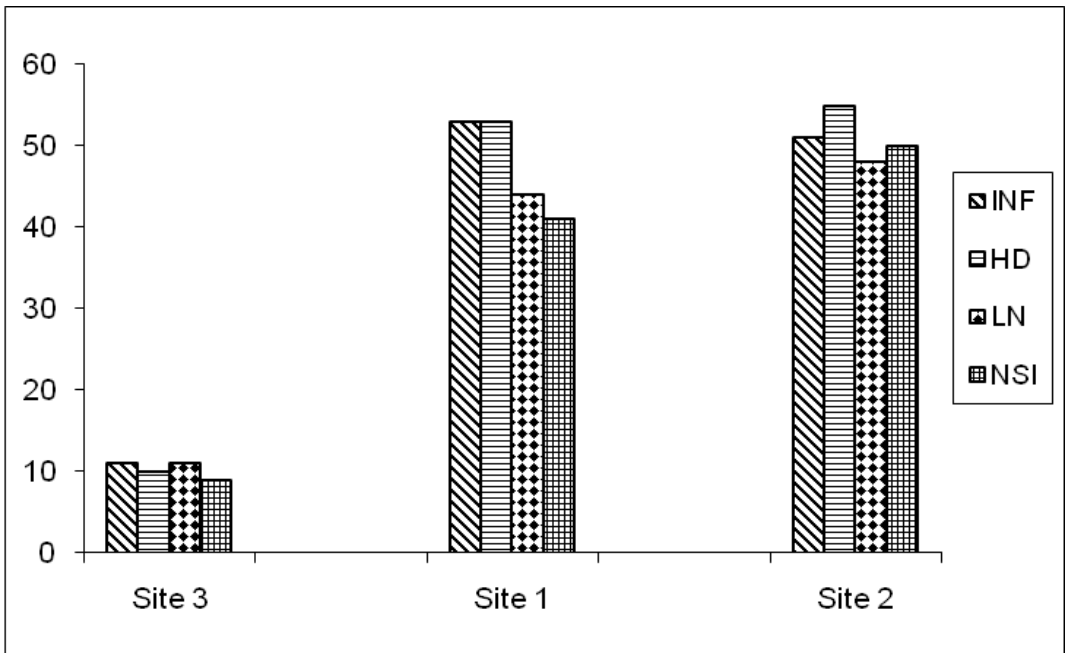


Cyprinus carpio

NSI: Non specific inflammation LN: Liquefactive necrosis

CN: Coagulative necrosis

Fig. 6.10: Histopathological lesions (%) in liver of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

INF: Inflammation HD: Hydropic degeneration

LN: Liquefactive necrosis NSI: Non specific inflammation

Fig. 6.11: Histopathological lesions (%) in liver of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

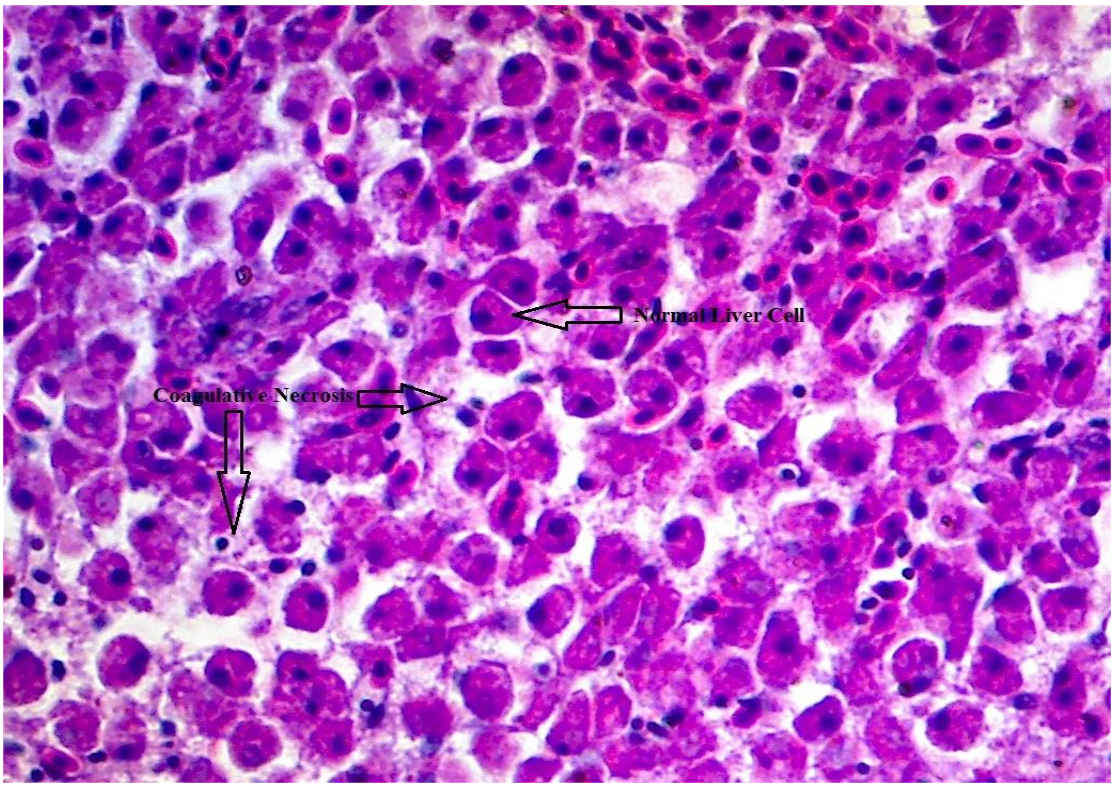


Fig. 6.12: Image showing normal liver cells and coagulative necrosis in liver.

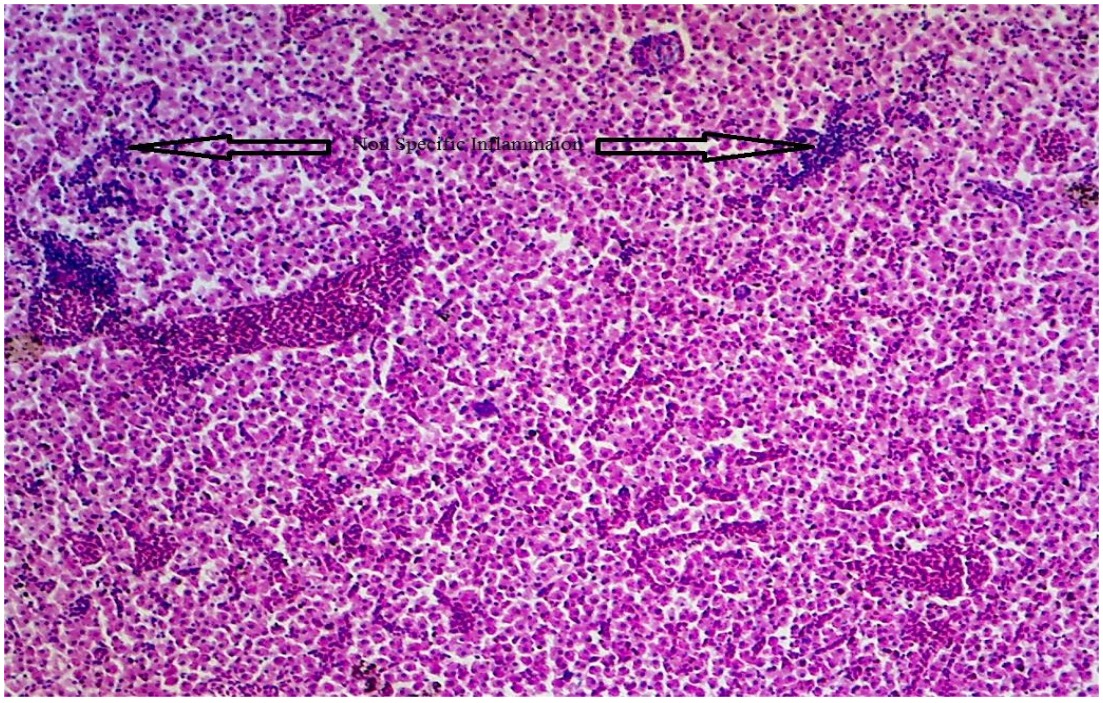


Fig. 6.13: Image showing non-specific inflammation in liver cells.

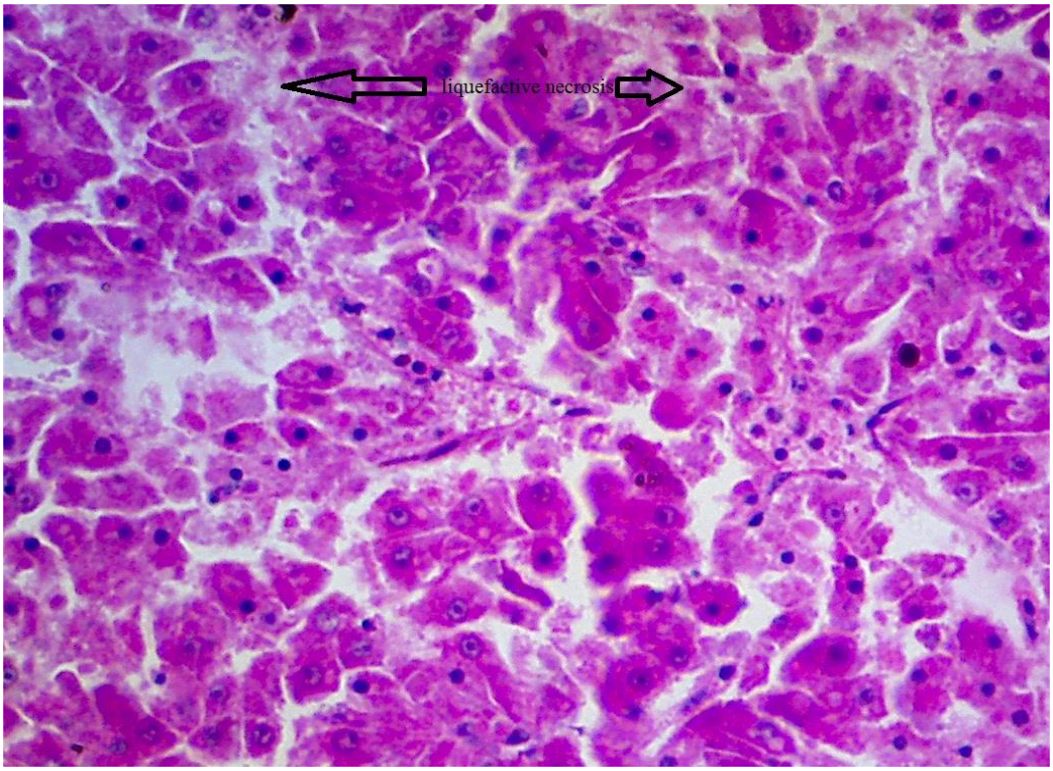


Fig. 6.14: Image showing liquefactive necrosis in liver cells.

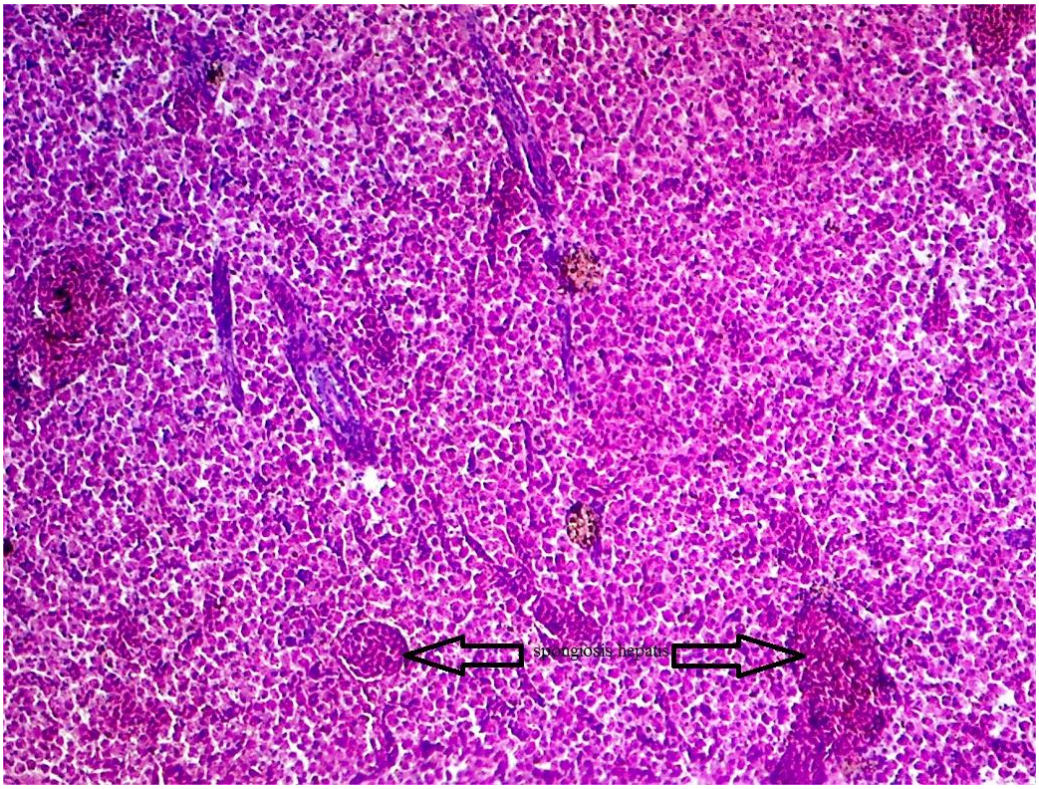


Fig. 6.15: Image showing spongiosis necrosis in liver cells.

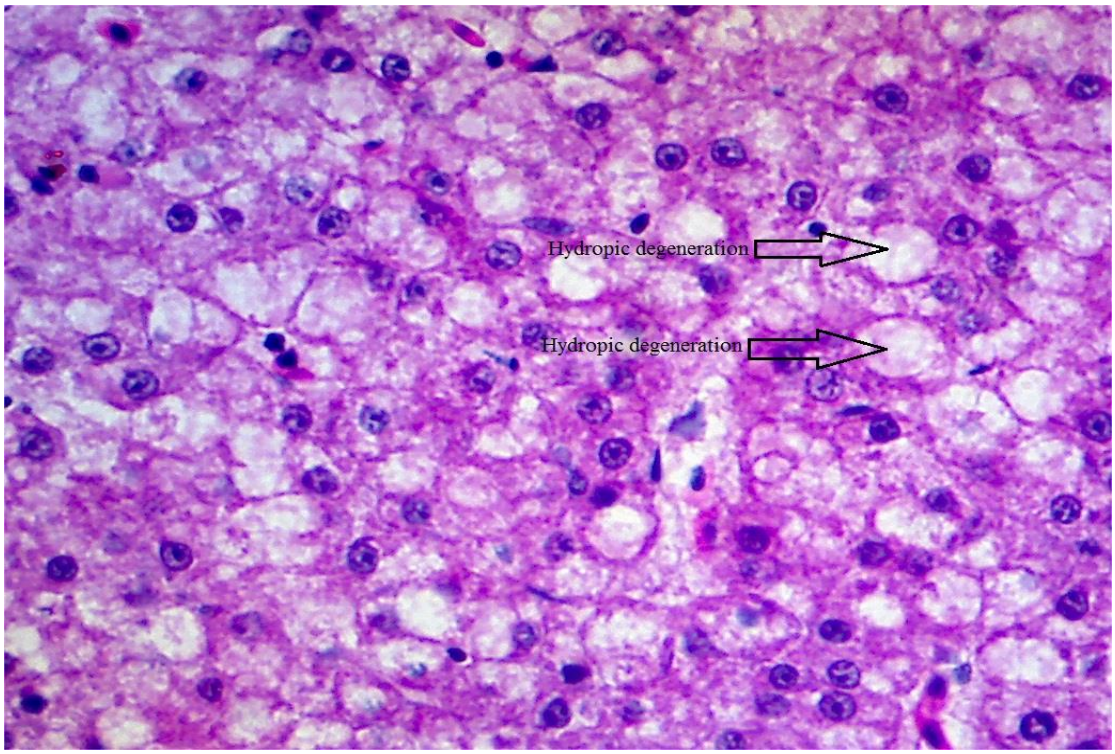


Fig. 6.16: Image showing hyropic degeneration in liver cells.

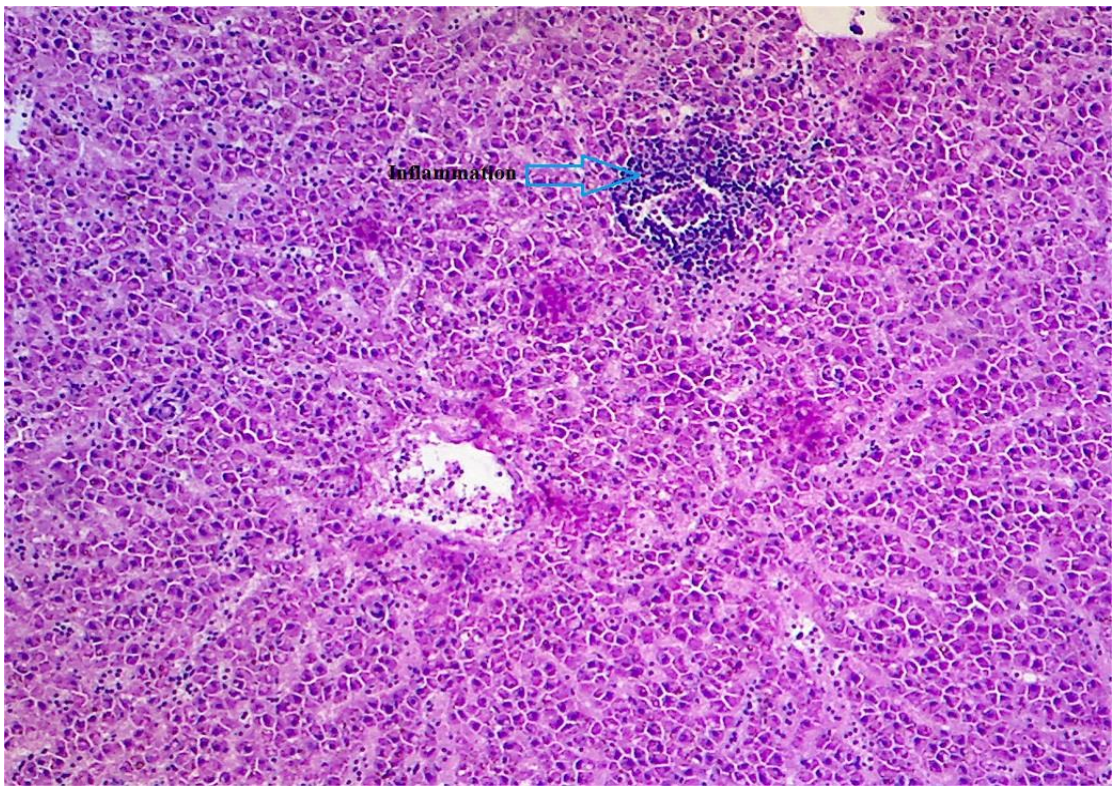


Fig. 6.17: Image showing inflammation in liver cells.

6.3.3 Histopathological Lesions (%) in Gills

The gills from selected fish species was removed and processed for different histopathological abnormalities. Gills of different studied fish from polluted water showed maximum pecentage of histopathological lesions as compare to the fish species from control water. The lesions which were observed in gills of the selected fish species including disquamation and distortion of secondary lamellae with epithelial cell exudate, clumping of gills lamellae, necrotic area, epithelial disquamation, non specific inflammation, vacuolation and oedema (Table 6.3 and Figs 6.17-6.26).

In the present finding gills of *Wallago attu* from polluted sites showed 33% and 41% disquamation and distortion of secondary lamellae with epithelial cell exudate and showed 6% from control site 3, showed 42% and 48% clumping of gills lamellae from both sites 1 and 2 and showed 5% clumping of gills lamellae from control site respectively. Gills of this fish was found to be contained greater percentage of clumping of gills lamellae and lower percentage of disquamation and distortion of secondary lamellae with epithelial cell exudate. The present results of higher percentage for various pathological disorders in this organ of different examined fish species agree with the findings of Swee *et al* (1997), who has also investigated the same histopathological alterations in gills of feral fish after exposure to heavy metals.The present result found more pathological disorders in gills from polluted area as compare to those from reference site, where the percentage of disorders was less. This could be attributed to higher concentration of heavy metals in this tissue and the result also confirmed heavy metals pollution in River Kabul.

The histopathological abnormalities such as necrotic area and epithelial disquamation were observed in gills of *Aorichthys seenghala* from polluted and control sites. The observed percentage values in gills of this fish from polluted sites were 35% and 40% for necrotic area, 38% and 44% for epithelial disquamation and

was 9% for necrotic area and was 7% for epithelial disquamation from control site 3 respectively. In this organ greater percentage was found for epithelial desquamation and lower for necrotic area. The present study found greater percentage of pathological disorders as compare to the findings of Mohammad *et al* (2013), who have reported less percentage for various histopathological alterations in gills of *C. gariepinus.* The present investigation found more percentage for pathological disorders in gills from polluted water than control water. This is because of greater concentration and toxicity of heavy metals in this tissue. This result also indicates heavy metals pollution in water of River Kabul in study area of River Kabul. Gills is highly susceptible to toxic chemicals of environmental pollutants, because of direct contact and constantly exposition to environmental pollutants.The absorption of toxic chemicals through gills is enhanced by increasing the permeability to water and ions of gill epithelium.

The observed pathological alterations in gills of *Labeo dyocheilus* from polluted and control sites are including necrotic area, epithelial disquamation, non specific inflammation and vacuolation and oedema. Gills of this fish from polluted sites 1 and 2 had 44% and 48% necrotic area, 46% and 50% epithelial disquamation, 43% and 53% non specific inflammation and had 45% and 51% vacuolation and oedema and had 15% necrotic area, 11% epithelial disquamation, 12% non specific inflammation and 9% vacuolation and oedem from control site respectively. The gills of this examined fish showed more percentage of pathological lesions like non specific inflammation and lower percentage of necrotic area. Histopathological alterations in gills of *Labeo dyocheilus* from River Kabul being observed in this study are in agreement with many studies that examined the effects of different pollutants on fish gills (Jon, 2011; Mirjana *et al.,* 2011).The marked histopathological alterations in fish gills of this study may be due to the cumulative effect of increased metal concentration in the gills. This study found more histopathological disorders in gills

from polluted sites than those from reference site. This could be related to heavy metals toxicity in the gills from polluted water.

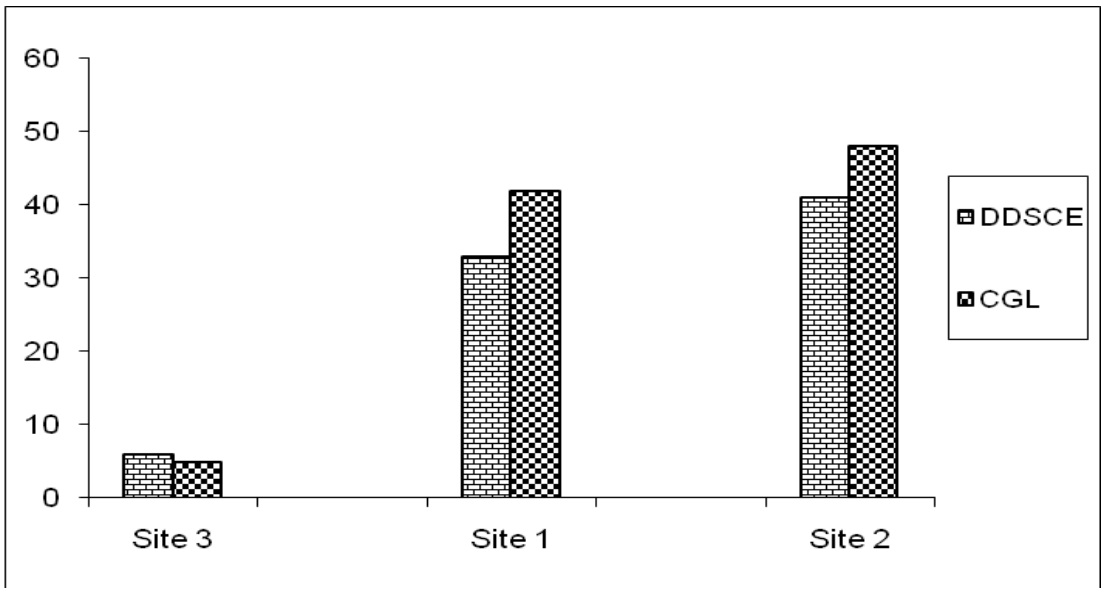
Gills of *Cyprinus carpio* from polluted and control sites showed three pathological abnormalities like epithelial disquamation, non specific inflammation and necrotic area. The gills of this fish from polluted sites showed 39% and 46% epithelial disquamation, 36% and 48% non specific inflammation and 37% and 43% necrotic area and showed 8% epithelial disquamation, 9% non specific inflammation and 11% necrotic area from control water (Warsak dam) respectively. In this fish pathological abnormalities like non specific inflammation showed high percentage and necrotic area showed low percentage. Significantly higher Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe and Hg content in gills could be linked to the occurrence of above mentioned alterations in gills of *Cyprinus carpio* in response to metal exposure of this fish to polluted water of River Kabul. Histopathological changes associated with heavy metals in fish have been studied by many authors (Mohammad *et al*., 2013; Rask *et al*., 1990). The present study found more pathological alterations in gills than muscle and less disorder than liver and intestine. This could be because of gerater concentration of heavy metals and directly and constantly exposur of this tissue to heavy metals in water. Gills came third after liver and intestine followed by muscle for pathological abnormalities.

Two histopathological lesions such as clumping of gills lamellae and non specific inflammation were found in gills of *Ompok bimaculatus* from polluted and control sites of River Kabul respectively. Gills of *Ompok bimaculatus* from polluted water had 51% and 57% clumping of gills lamellae and had 41% and 50% non specific inflammation and had 9% clumping of gills lamellae and 11% non specific inflammation from control water respectively.This fish showed greater percentage of clumping of gills lamellae and lower percentage of non specific inflammation. High zinc intake leads to enfeeblement, retardation of growth and may bring about

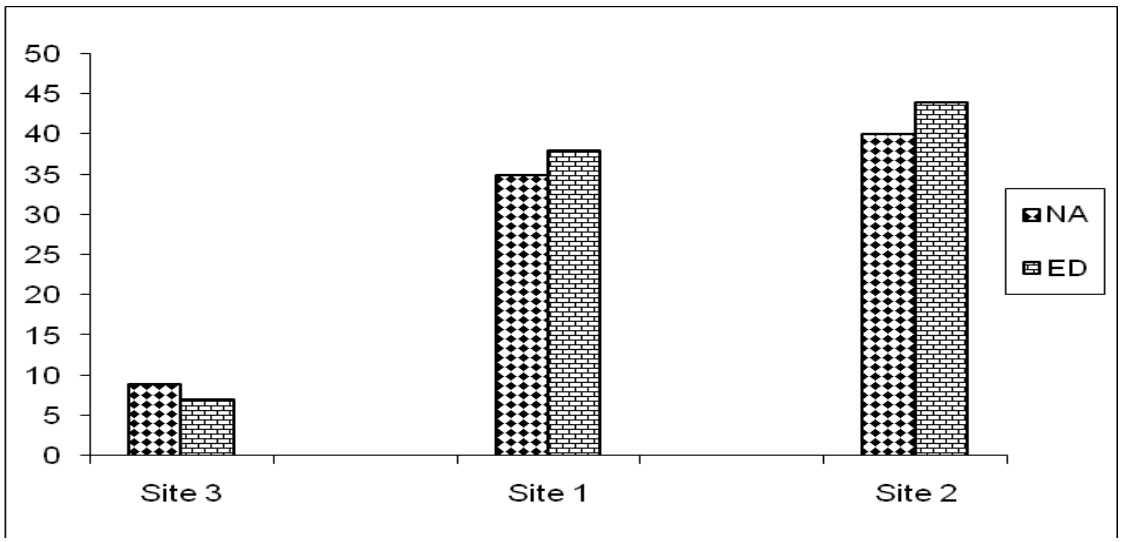
metabolic and pathological changes in various organs in fish (Ambrose *et al*., 1994).The overall order of percentage of different abnormalities in gills was non specific inflammation > epithelial disquamation > necrotic area > clumping of gills lamellae > disquamation and distortion of secondary lamellae with epithelial cell exuudate and in different studied fish species was *Labeo dyocheilus* > *Cyprinnus carpio* > *Ompok bimaculatus* > *Wallago attu*> *Aorichthys seenghala.* This reveales that non specific inflammation showed more percentage and disquamation and distortion of secondary lamellae with epithelial cell exudate showed less percentage. Similarly *Labeo dyocheilus* showed maximum pathological lesions and *Aorichthys seenghala* showed lowest alterations. Greater pathological conditions in *Labeo dyocheilus* could be attributed to omnivorous nature of this fish and being an omnivorous it is more exposed to toxic heavy metals accumulation. Due to heavy metals toxicity, the gills of this fish showed maximum frequency of patholgical lesions.

Table 6.3: Histopathological lesions (%) in gills of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Lesions	Site 3 (%)	Site 1 (%)	Site 2 (%)
Wallago attu	Disquamation and distortion of secondary lamellae with epithelial cell exudates	6	33	41
	Clumping of gills lamellae	5	42	48
Aorichthys seenghala	Necrotic area	9	35	40
	Epithelial desquamation	7	38	44
Labeo dyocheilus	Necrotic area	15	44	48
	Epithelial desquamation	11	46	50
	Non specific inflammation	12	43	53
	Vacuolation and oedema	9	45	51
Cyprinus carpio	Epithelial desquamation	8	39	46
	Non specific inflammation	9	36	48
	Necrotic area	11	37	43
Ompok bimaculatus	Clumping of gills lamellae	9	51	57
	Non specific inflammation	11	41	50



Wallago attu

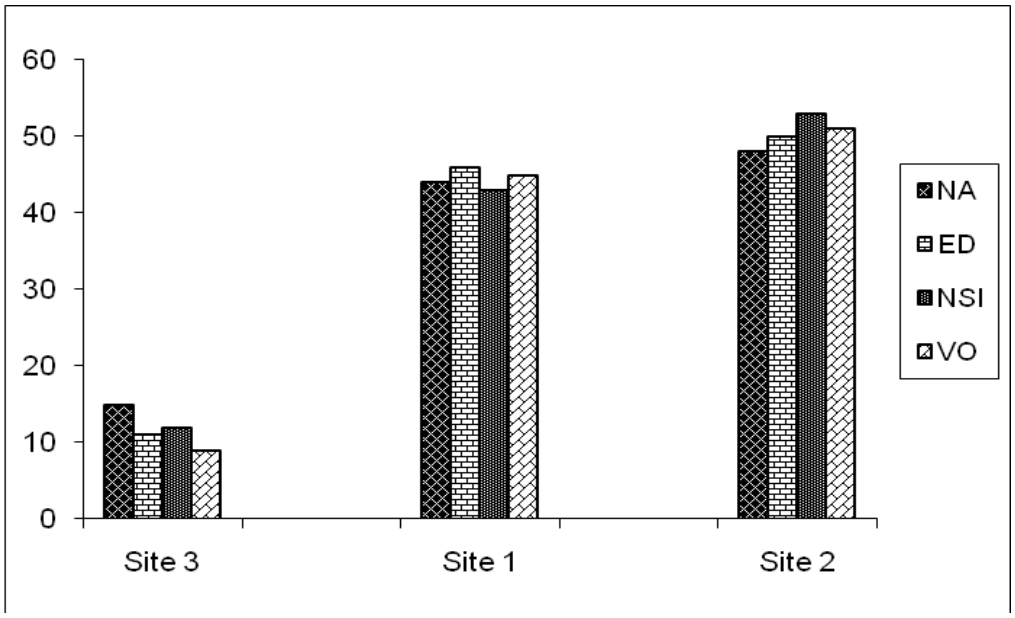


Aorichthys seenghala

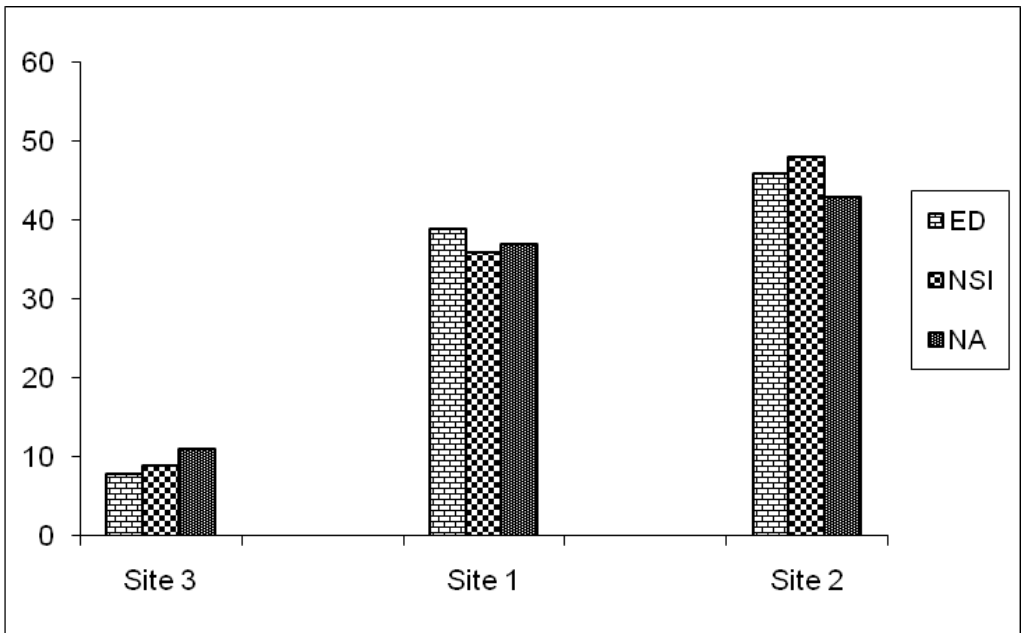
DSCE: Disquamation and distortion of secondary lamellae with epithelial cell exudate

CGL: Clumping of gills lamellae NA: Necrotic area ED: Epithelial desquamation

Fig. 6.18: Histopathological lesions (%) in gills of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



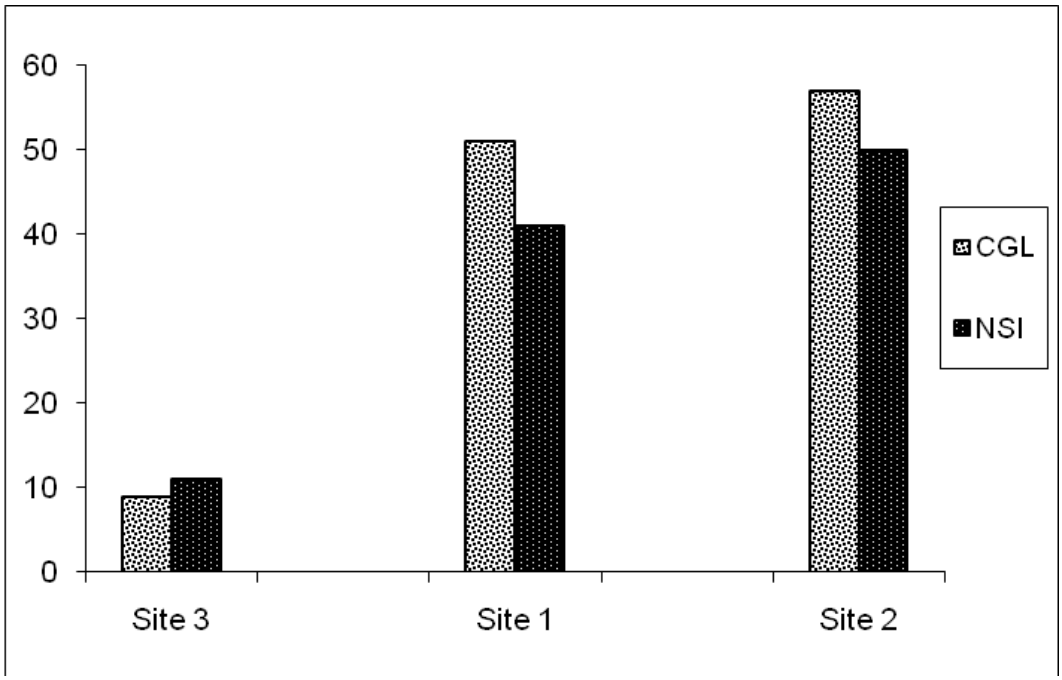
Labeo dyocheilus



Cyprinus carpio

NA: Necrotic area ED: Epithelial disquamation NSI: Non specific inflammation VO: Vacuolation and oedema NA: Necrotic area

Fig. 6.19: Histopathological lesions (%) in gills of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

CGL: Clumping of gills lamellae NSI: Non specific inflammation

Fig. 6.20: Histopathological lesions (%) in gills of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

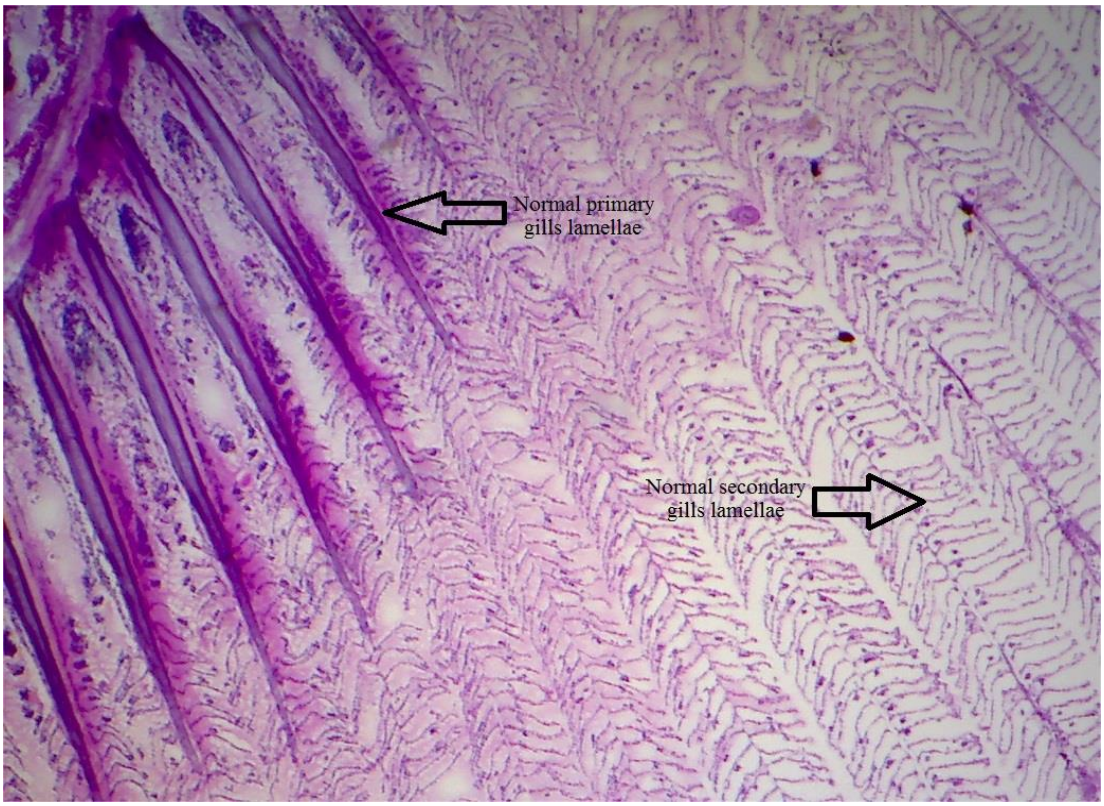


Fig. 6.21: Image of gills showing normal primary and secondary gills lamellae.

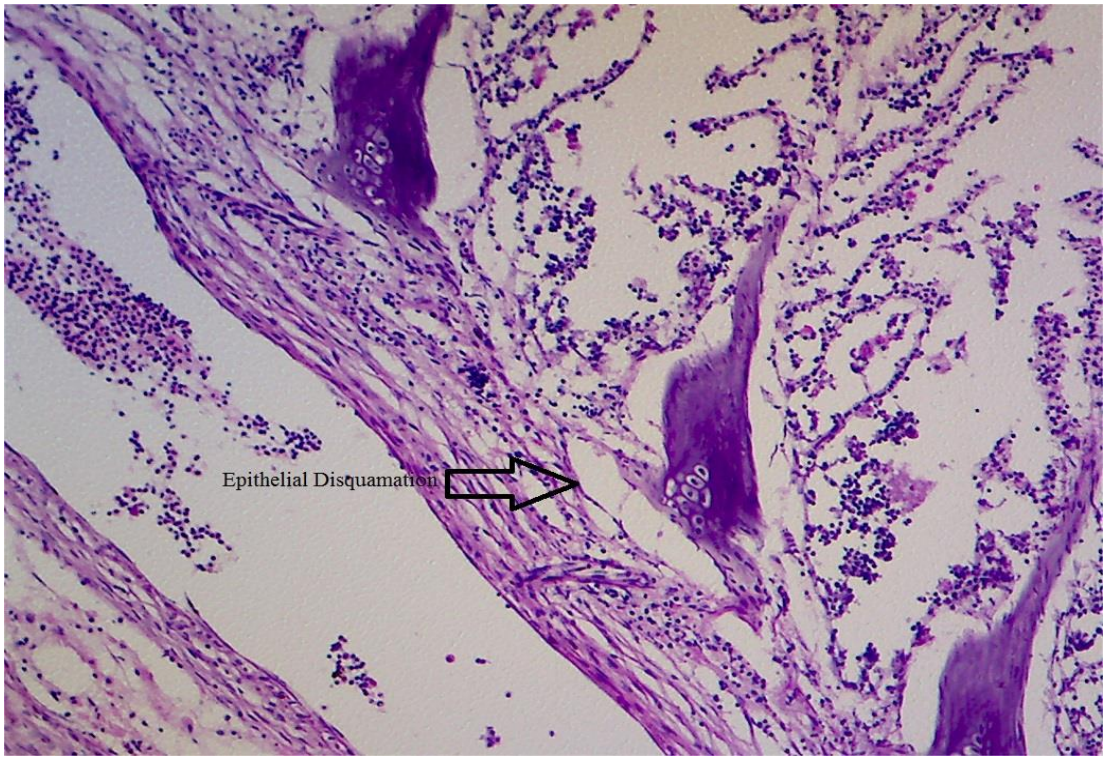


Fig. 6.22: Image showing the disquamation of gills epithelium.

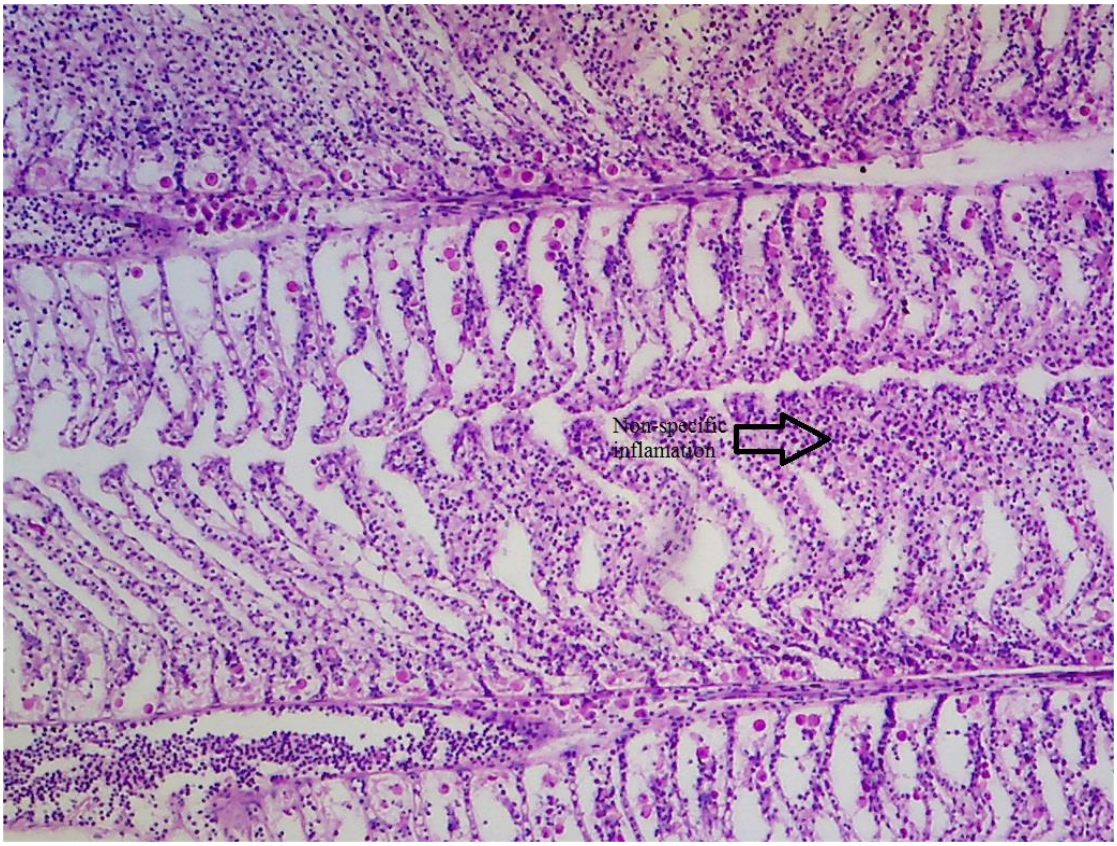


Fig. 6.23: Image showing the non-specific inflammation in gills lamellae.

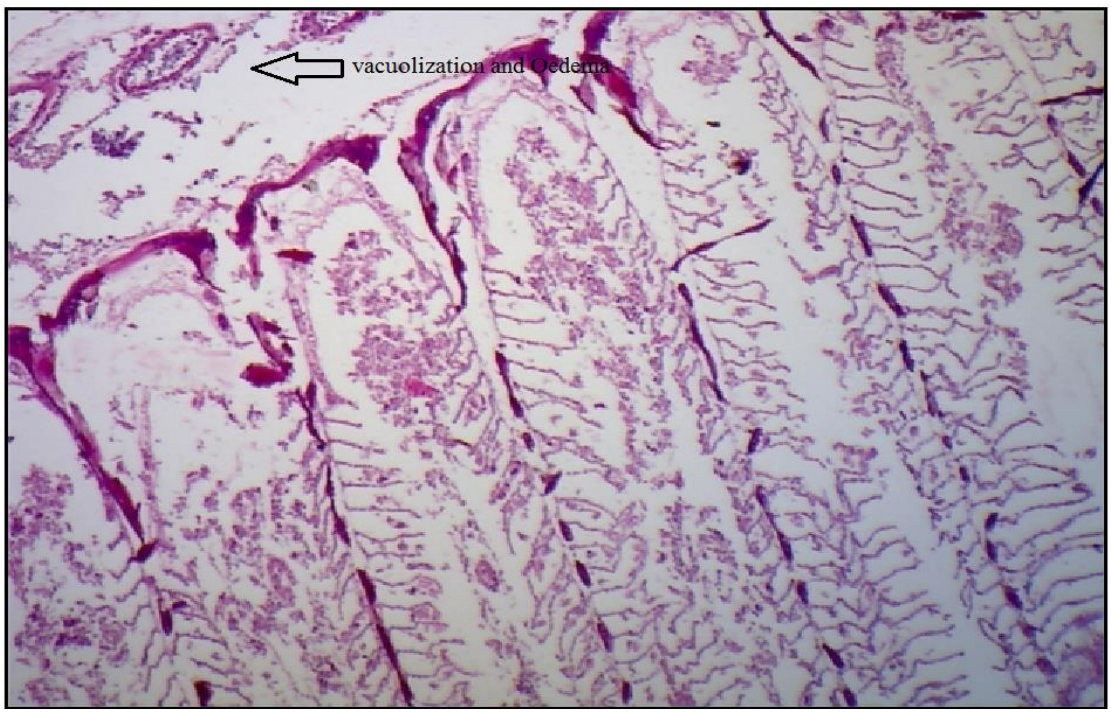


Fig. 6.24: Image showing vacuolization and oedema in gills epithelium.

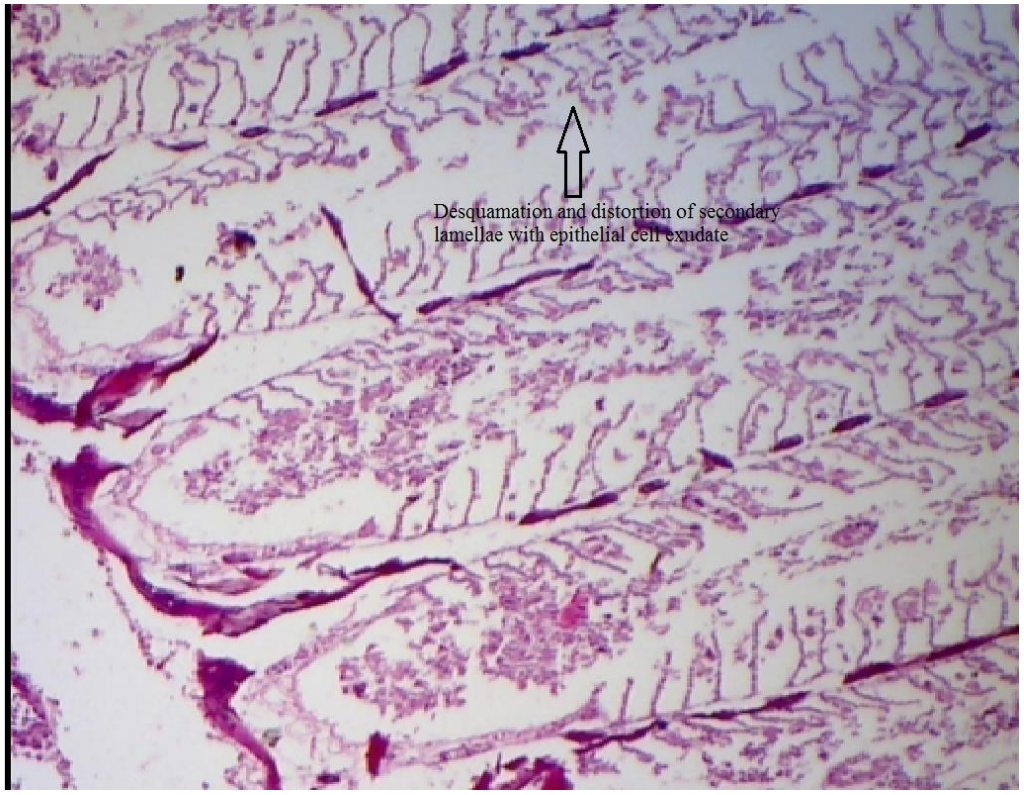


Fig. 6.25: Image showing dessquamation and distortion of secondary gills lamellae with epithelial cells exudate.

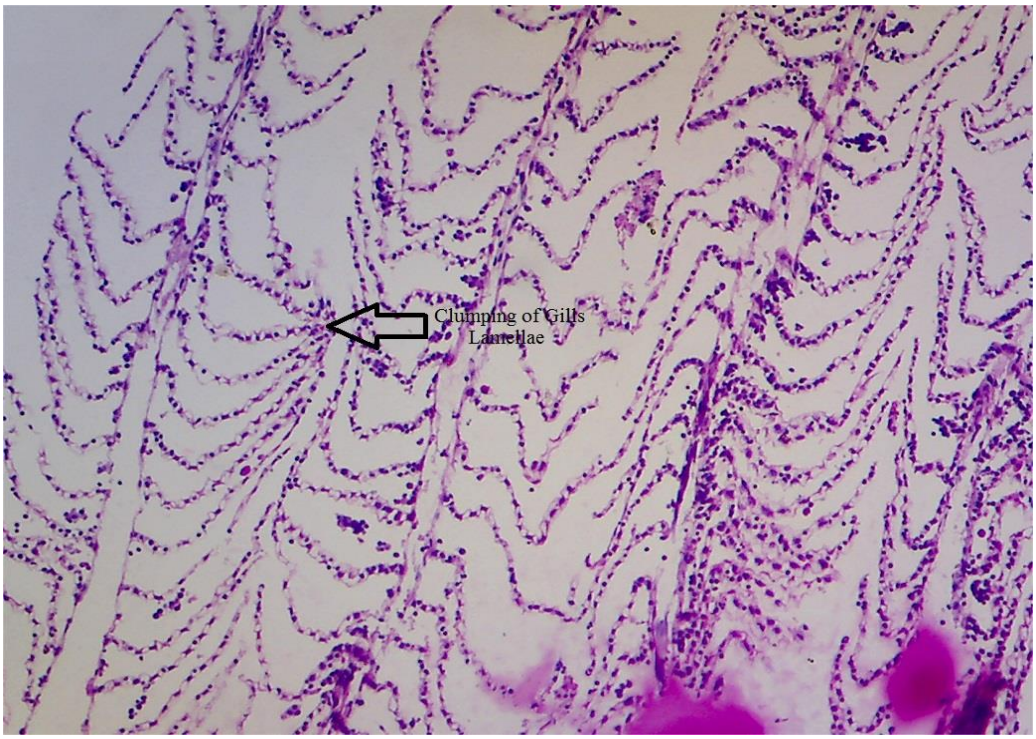


Fig. 6.26: Image showing clumping of secondary gills lamellae.

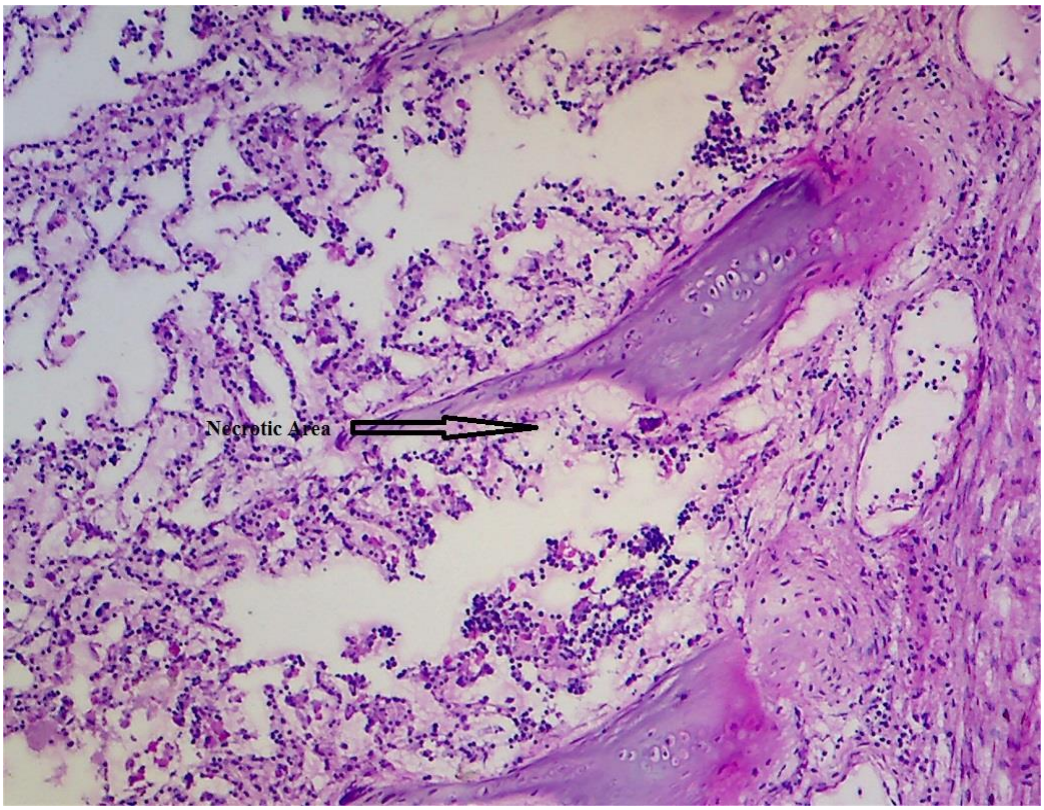


Fig. 6.27: Image showing necrosis in primary gills lamellae (arrow).

6.3.4 Histopathological Lesions (%) in Muscle

The muscle from selected fish species was removed and processed for different pathological conditions. Muscle of different studied fish from polluted water showed greater percentage of histopathological disorders as compare to the fish species from control water, where the smaller percentage of pathological abnormalities was observed. The lesions which were observed in muscle of selected studied fish species including fibrillar degeneration, inflammation, coagulative necrosis and non specific inflammation (Table 6.4 and Figs 6.27-6.33).

Three pathological conditions including degeneration, inflammation and coagulative necrosis were observed in muscle of *Wallago attu* from polluted and control sites. The muscle of *Wallago attu* from polluted water showed 31% and 37% fibrillar degeneration, 34% and 45% inflammation and showed 33% and 36% coagulative necrosis and showed 6% fibrillar degeneration, 5% inflammation and 4% coagulative necrosis from reference site respectively. In this tissue of *Wallago attu,* more percentage was observed for histopathological lesion like inflammation and less for coagulative necrosis. These results were in agreement with the findings of previous investigations by Kaoud and El-Dahshan (2010), who had also observed maximum percentage for various histopathological changes in muscle tissue of *Oreochromis niloticus* fish after exposure to heavy metals. Comparing the present result with above mentioned studies and findings of other workers showed that heavy metals are toxic chemicals and could result into pathological abnormalities in aquatic animals like fish.

Muscle of *Aorichthys seenghala* from both polluted sites showed more percentage of pathological abnormalities than control site. Muscle of this fish from polluted water had 42% and 51% non specific inflammation and had 13% from control water, had 44% and 49% coagulative necrosis from polluted sites and had

11% from reference site 3 respectively. Muscle of this fish showed higher percentage of non specific inflammation and lower percentage of coagulative necrosis respectively. More pathological lesions were found in muscle of examined fish species from polluted water as compare to control water. The lesions in this finding confirmed that exposure of the muscle to heavy metals can induce histological and pathological changes, as already mentioned in other studies (Damek and Sawicka, 2003; Zhang *et al.,* 2005; Martin-Diaz *et al*., 2006; Raldua *et al*., 2007). Comparing the present investigation with previous findings highlights that heavy metals are toxic chemicals and could be correlated to pathological disorders in fish and other animals including human beings. By making overall comparison, muscle came last after liver, intestine and gills for histopathological disorders. This is because that muscle had less heavy metals as compare to other examined tissues like liver, intestine and gills.

Different pathological conditions like inflammation, coagulative necrosis and non specific inflammation were observed in muscle of *Labeo dyocheilus* from polluted and control sites. In muscle of *Labeo dyocheilus* from polluted sites 1 and 2 percentage of inflammation were 43% and 51%, for coagulative necrosis were 36% and 43% and for non specific inflammation were 37% and 42%. From site 3 percentage were 8%, 9% and 7% for inflammation, coagulative necrosis and non specific inflammation respectively. This fish had maximum percentage of inflammation and minimum percentage of coagulative necrosis respectively. Histopathological changes were observed in *Archosargus probatocephalus* exposed to copper (Cardeilhac *et al*,, 1979), in *Tijapia niloiica* exposed to lead acetate, mercuric chloride and cadmium chloride (Balah *et al*., 1993), in *Cyprinion mhaknsis* exposed to copper (Ghazaly *et al*., 1994), in *Macropsobrycon uruguayanae* exposed to cadmium (Randi *et al*., 1996) and in *Salmo trutta* exposed to iron sulphate (Dalzell and Macfarlane, 1999). Comparing the present result with the findings of above mentioned studies indicates that heavy metals are toxic in nature and could result into pathological alterations in fish.

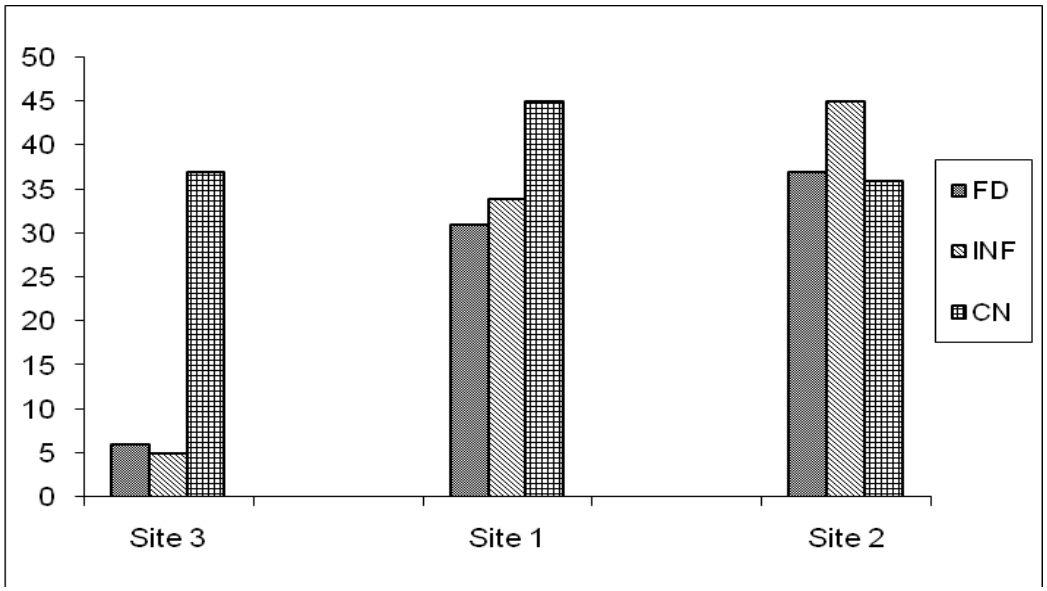
More histopathological abnormalities were observed in muscle of *Cyprinus carpio* from polluted water than control water. Muscle of this fish from polluted sites 1 and 2 contained 32% and 41% coagulative necrosis and 35% and 42% non specific inflammation. From site 3 the muscle contained 7% coagulative necrosis and 6% non specific inflammation respectively. This fish had greater percentage of pathological conditions such as coagulative necrosis and smaller percentage of non specific inflammation. The present result confirmed various pathological disorders in muscle of different fish species after collection from polluted sites of River Kabul. These alterations have also been reported for other fish species exposed to heavy metals particularly lead and cadmium (Deore and Wagh, 2012; Patnaik *et al*., 2011; De Smet and Blust 2001). The present result confirmed different pathological abnormalities in muscle, which are also supported by the findings of other workers. This result proved that heavy metals are toxic in nature and can induce pathological alterations in fish.

Three most prominent histopathological lesions including non specific inflammation, fibrillar degeneration and coagulative necrosis were seen in muscle of *Ompok bimaculatus*. Muscle of this fish from polluted sites showed 38% and 46% non specific inflammation, 41% and 45% fibrillar degeneration and 40% and 47% coagulative necrosis and showed 11% non specific inflammation, 13% fibrillar degeneration and 9% coagulative necrosis from control site 3 respectively. In this organ maximum percentage was observed for coagulative necrosis and minimum percentage for fibrillar degeneration respectively. These results agree with the findings of previous workers (Sabry *et al*., 2005; Deore and Wagh, 2012). The percentage of these lesions in muscle was in the order of coagulative necrosis > non specific inflammation > fibrillar degeneration and in different fish species was *Ompok bimaculatus* > *Labeo dyocheilus*> *Wallago attu*> *Aorichthys seenghala* > *Cyprinus carpio.* This shows that the percentage of coagulative necrosis was highest and that of fibrillar degeneration was the lowest. Similarly more percentage of pathological disorders was seen in *Ompok bimaculatus* and less in *Cyprinus carpio.* Muscle came

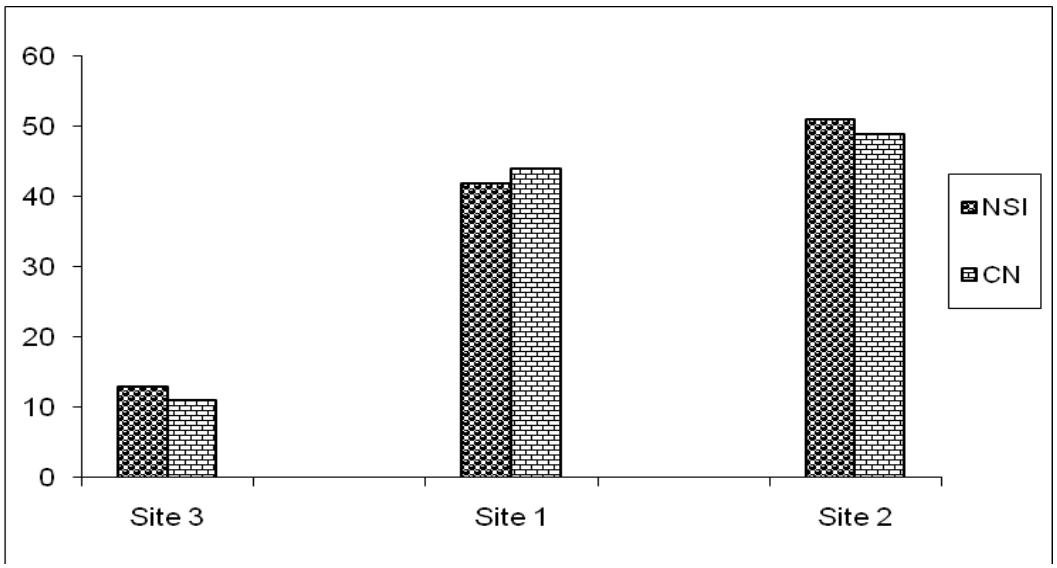
last for pathological conditions as compare to other tissues. This could be attributed to less concentration of metals and high metabolic rate in this tissue. The present result indicates that heavy metal contamination definitely affects the aquatic life of both fresh and marine water. Hence, a scientific method of detoxification is essential to improve the health of these economic fish in any stressed environmental conditions. However, the high concentration of the analyzed metals in the whole body tissues investigated could be due to the storage role played by these tissues. Fish contaminated by heavy metals suffers from various pathological alterations. Our result furtherly showed that heavy metals are toxic in nature and can induce different histopathological abnormalities in aquatic organisms. The result also showed heavy metals pollution in River Kabul.

Table 6.4 Histopathological lesions (%) in muscle of five different fish species netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

Fish	Lesions	Site 3 (%)	Site 1 (%)	Site 2 (%)
Wallago attu	Fibrillar degeneration	6	31	37
	Inflammation	5	34	45
	Coagulative necrosis	4	33	36
Aorichthys seenghala	Non specific inflammation	13	42	51
	Coagulative necrosis	11	44	49
Labeo dyocheilus	Inflammation	8	43	51
	Coagulative necrosis	9	36	43
	Non specific inflammation	7	37	42
Cyprinus carpio	Coagulative necrosis	7	32	41
	Non specific inflammation	6	35	42
Ompok bimaculatus	Non specific inflammation	11	38	46
	Fibrillar degeneration	13	41	45
	Coagulative necrosis	9	40	47



Wallago attu



Aorichthys seenghala

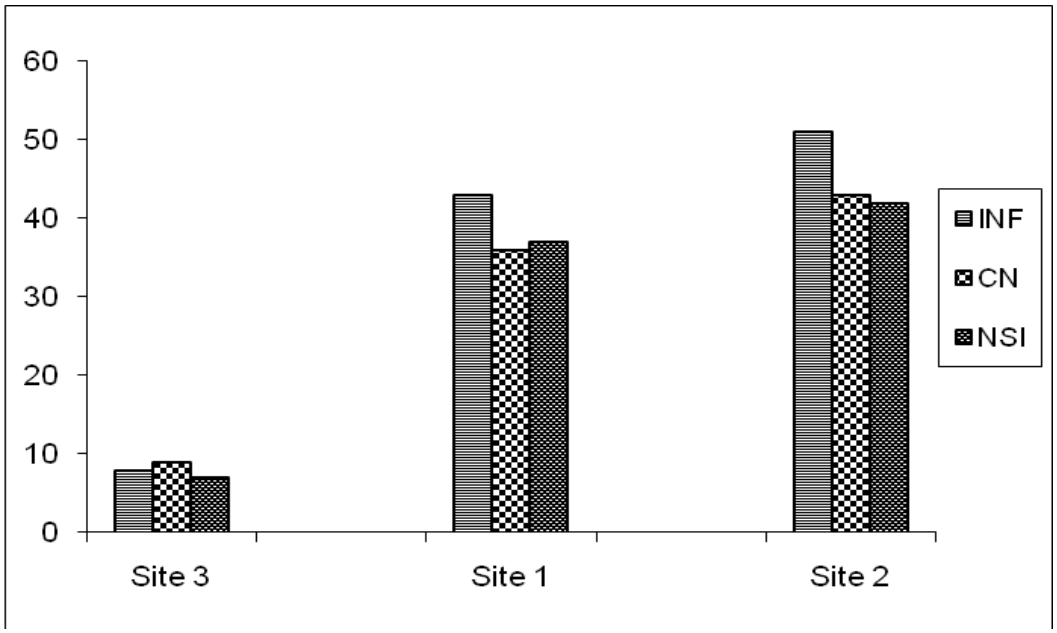
FD: Fibrillar degeneration

INF: Inflammation

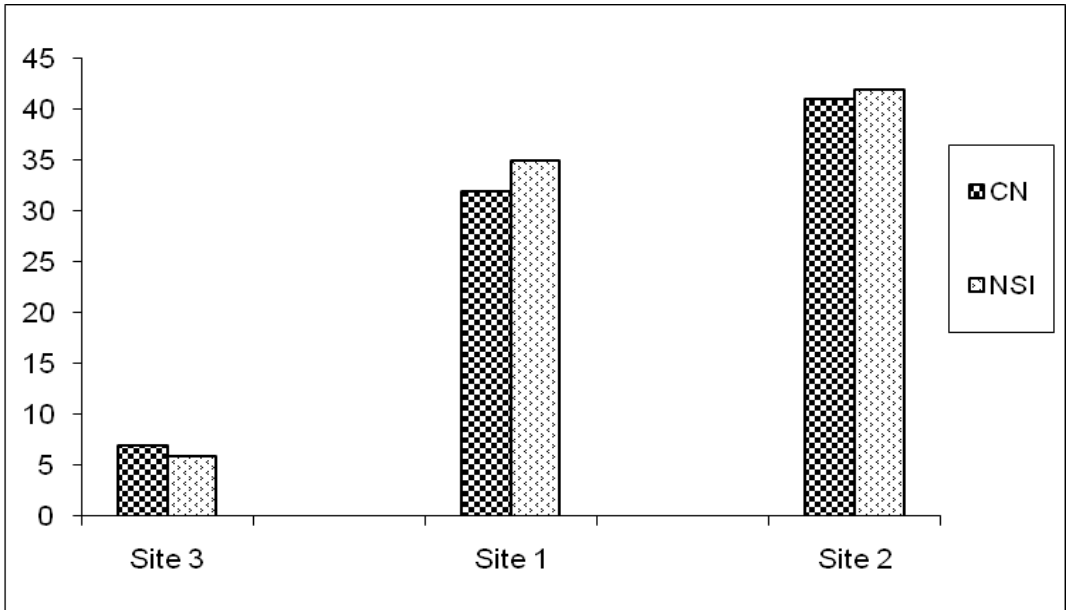
CN: Coagulative necrosis

NSI: Non specific inflammation

Fig. 6.28: Histopathological lesions (%) in muscle of *Wallago attu* and *Aorichthys seenghala* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



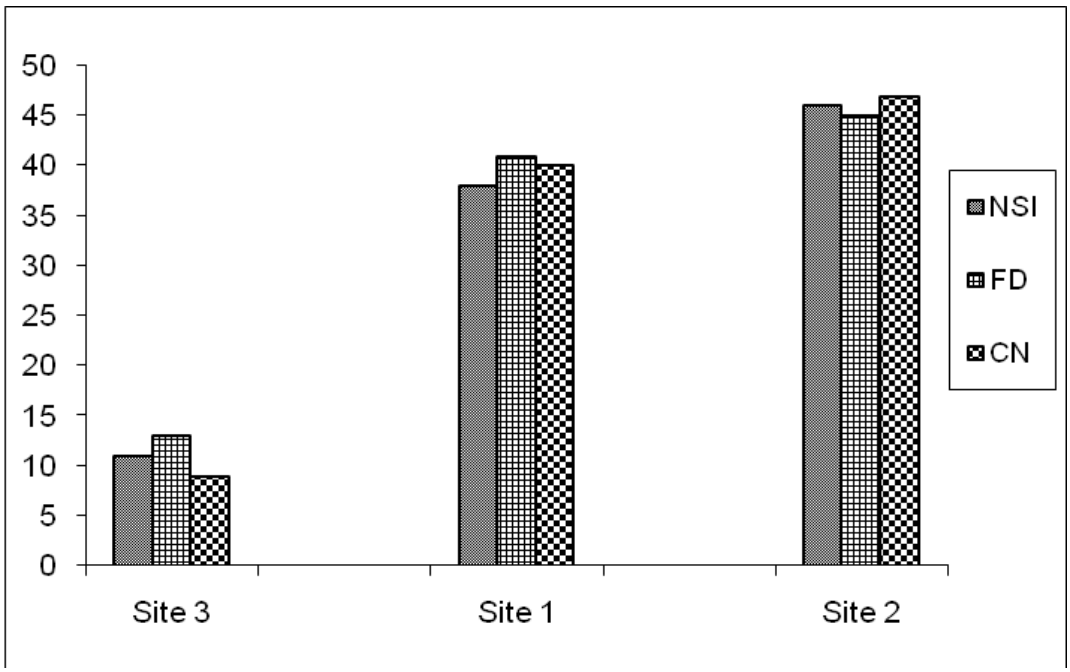
Labeo dyocheilus



Cyprinus carpio

INF: Inflammation CN: Coagulative necrosis NSI: Non specific inflammation

Fig. 6.29: Histopathological lesions (%) in muscle of *Labeo dyocheilus* and *Cyprinus carpio* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.



Ompok bimaculatus

NSI: Non specific inflammation FD: Fibrillar degeneration

CN: Coagulative necrosis

Fig. 6.30: Histopathological lesions (%) in muscle of *Ompok bimaculatus* netted from site 3 (control) and site 1 and site 2 (polluted) of River Kabul receiving city sewages and industrial effluents.

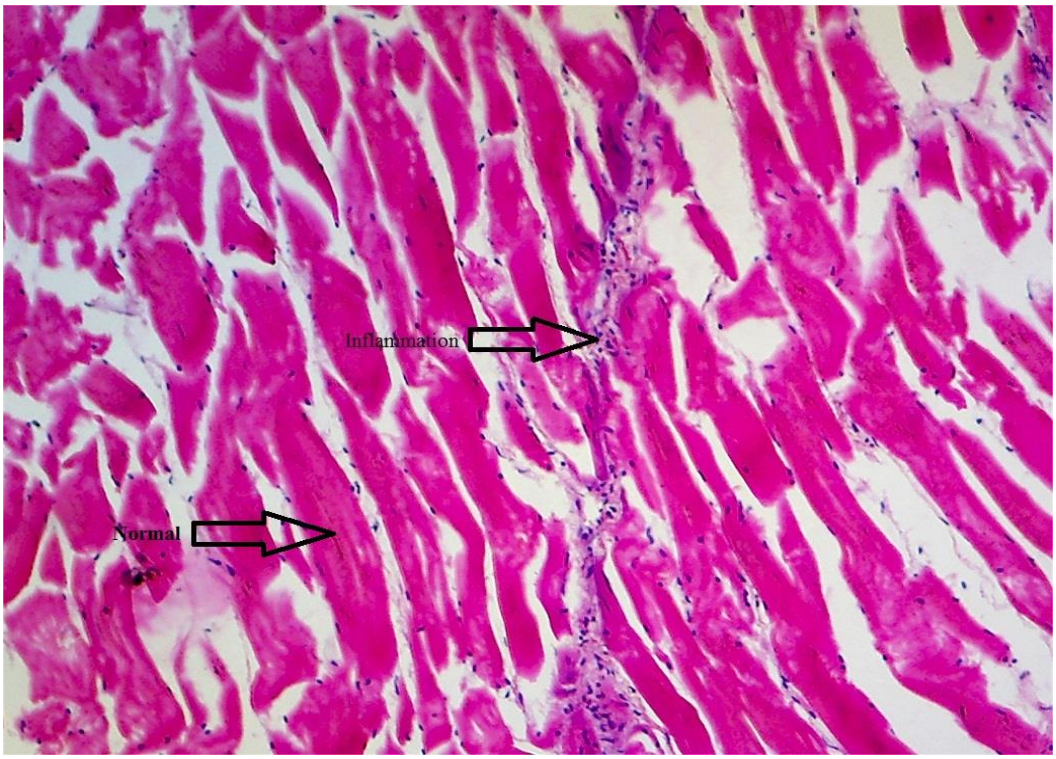


Fig. 6.31: Image showing normal muscle tissue (below) and inflammation in same tissue (above).

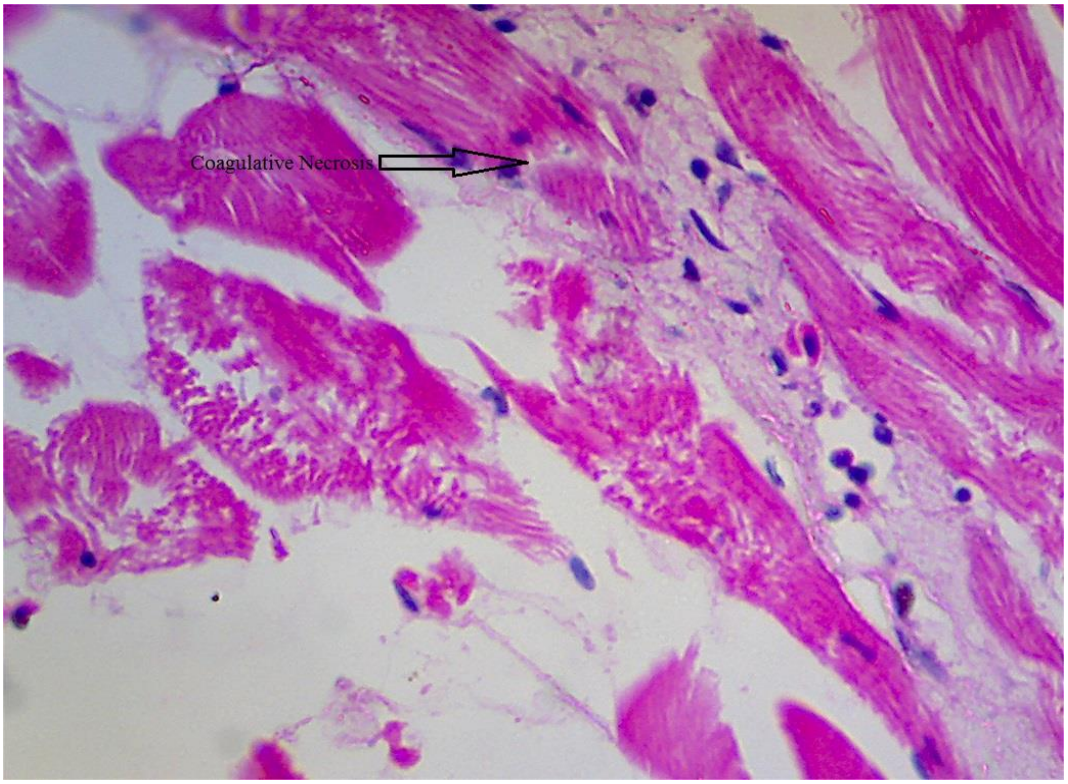


Fig. 6.32: Image showing coagulative necrosis in muscle tissues.

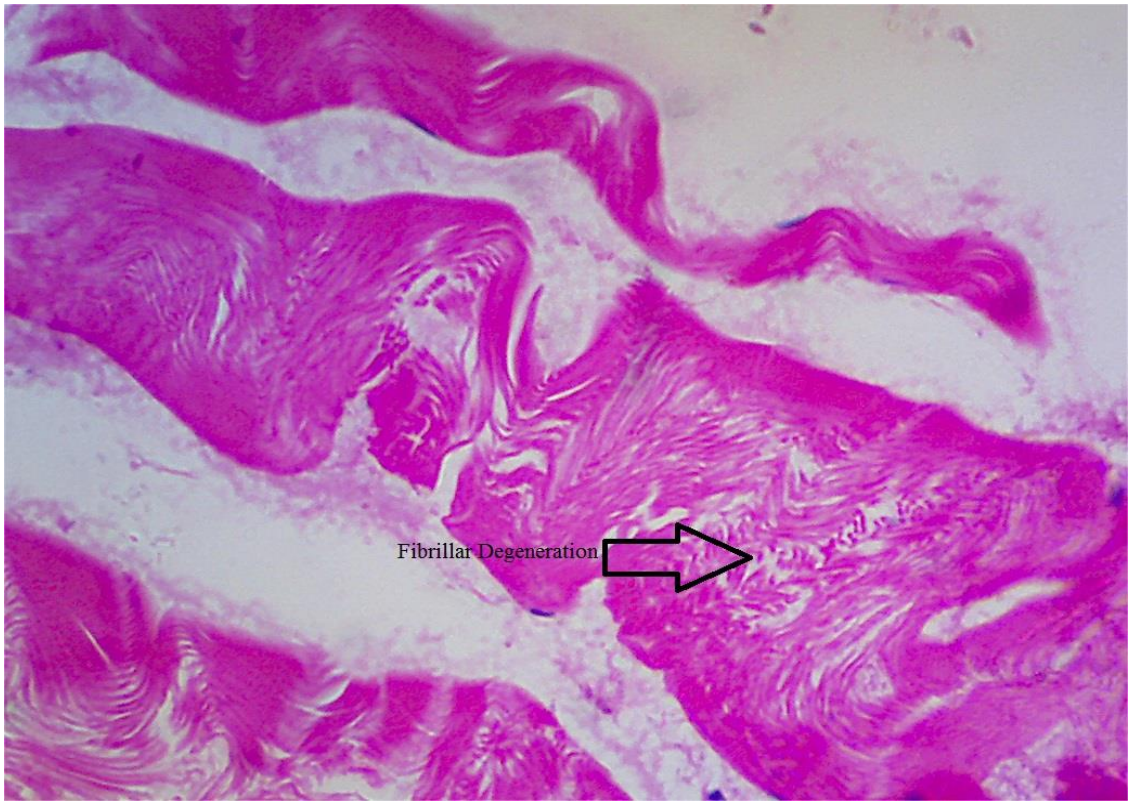


Fig. 6.33: Image showing fibrillar degeneration in muscle tissue.

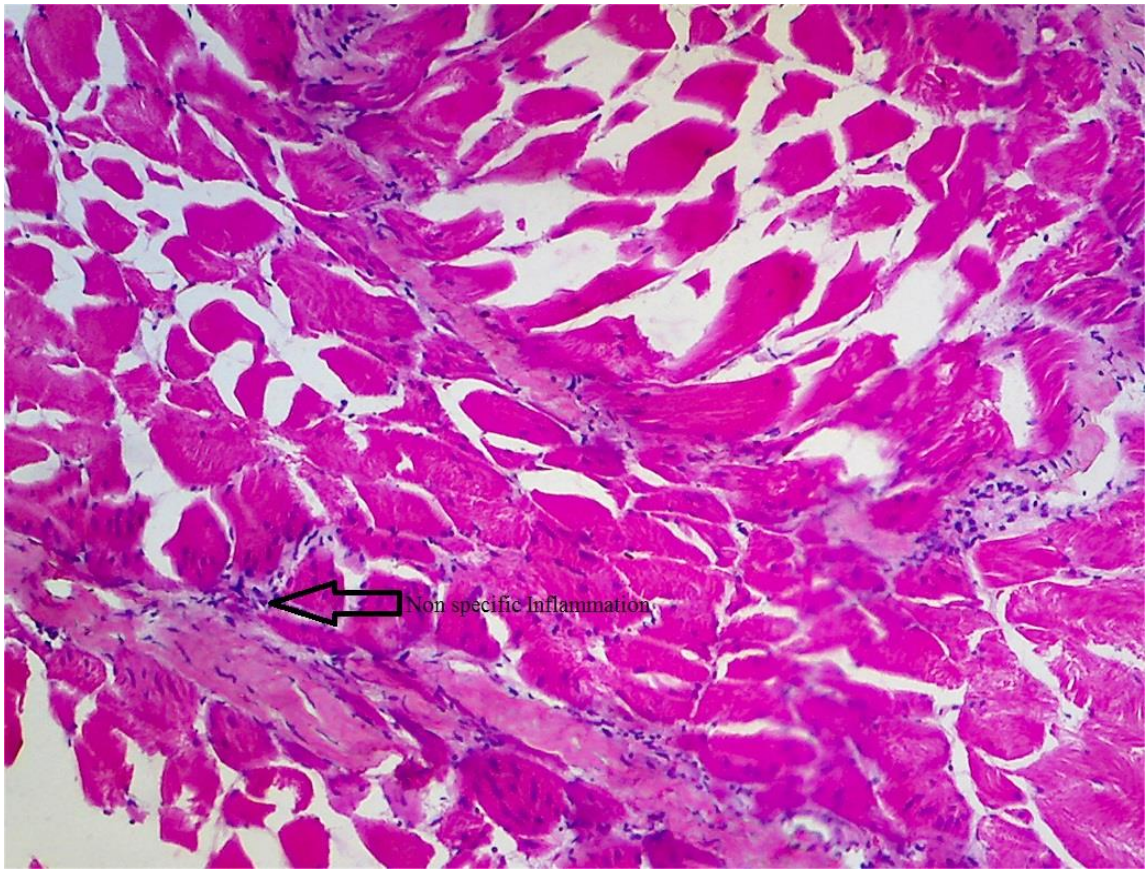


Fig. 6.34: Image showing non-specific inflammation in muscle tissue.

6.3.5 Sequences of Histopathological Conditions (%)

The results highlights that all the examined tissues and fish from polluted water showed greater histopathological abnormalities than those from control water. Liver showed maximum pathological disorders followed by intestine, gills and muscle. Among fish, *Cyprinus carpio* showed more pathological abnormalities followed by *Ompok bimaculatus, Labeo dyocheilus, Aorichthys seenghala* and *Wallago attu.*

The order of different histopathological conditions in intestine of *Wallago attu* was degeneration of epithelium >complete degeneration of cillia, in *Aorichthys seenghala* was degenerative cillia >coagulative necrosis >inflammation, in *Labeo dyocheilus* was complete degeneration of cillia >inflammation, in *Cyprinus carpio* was complete degeneration of cillia >degenerative cillia >coagulative necrosis >inflammation and in *Ompok bimaculatus* was degenerative cillia >coagulative necrosis>inflammation. Overall order of different histopathological abnormalities in intestine of different fish species was inflammation >degenerative cillia >complete degeneration of cillia >coagulative necrosis > degeneration of epithelium and in different fish species was *Cyprinus carpio>Ompok bimaculatus >Aorichthys seenghala > Wallago attu> Labeo dyocheilus.*

The sequence of different pathological lesions in liver of *Wallago attu* was hydropic degeneration >inflammation>coagulative necrosis, in *Aorichthys seenghala* was spongiosis hepatis>liquefactive necrosis>non specific inflammation, in *Labeo dyocheilus* was liquefactive necrosis>non specific inflammation, in *Cyprinus carpio* was non specific inflammation >liquefactive necrosis>coagulative necrosis and in *Ompok bimaculatus* the sequence was hydropic degeneration > inflammatiom>non specific inflammation> liquefactive necrosis. Overall order of different histopathological conditions in intestine of different fish species was non specific inflammation > liquefactive necrosis > hydropic degeneration > inflammation >

coagulative necrosis >spongiosis hepatis and in different fish species was *Ompok bimaculatus > Aorichthys seenghala > Cyprinus carpio > Wallago attu > Labeo dyocheilus.*

The sequence of different pathological lesions in gills of *Wallago attu* was clumping of gills lamellae>disquamation and distortion of secondary lamellae with epithelial cell exudate, in *Aorichthys seenghala* was epithelial disquamation >necrotic area, in *Labeo dyocheilus* was non specific inflammation > vacuolation and oedema>epithelial disquamation >necrotic area, in *Cyprinus carpio* was non specific inflammation>epithelial desquamation >necrotic area and in *Ompok bimaculatus* the sequence was clumping of gills lamellae>non specific inflammation. Overall order of different histopathological conditions in gills of different fish species was non specific inflammation>epithelial disquamation >clumping of gills lamellae>necrotic area > vacuolation and oedema>disquamation and distortion of secondary lamellae with epithelial cell exudate and in different fish species was *Labeo dyocheilus>Cyprinus carpio>Ompok bimaculatus >Wallago attu >Aorichthys seenghala.*

The order of different histopathological abnormalities in muscle of *Wallago attu* was inflammation>fibrillar degeneration>coagulative necrosis, in *Aorichthys seenghala* was non specific inflammation>coagulative necrosis, in *Labeo dyocheilus* was inflammation>non specific inflammation>coagulative necrosis, in *Cyprinus carpio* was coagulative necrosis>non specific inflammation and in *Ompok bimaculatus* was coagulative necrosis > non specific inflammation> fibrillar degeneration respectively. Overall order of different histopathological conditions in different fish species was coagulative necrosis>non specific inflammation> inflammation > fibrillar degeneration and in different fish species was *Ompok bimaculatus > Labeo dyocheilus > Wallago attu > Aorichthys seenghala > Cyprinus carpio.*

6.3.7 Conclusions and Remarks

In the present investigation different histopathological conditions were determined in intestine, gills, liver and muscle of five different fish species including *Wallago attu*,*Ompok bimaculatus*, *Labeo dyocheilus*, *Cyprinus carpio* and *Aorichthys seenghala* netted from both polluted and control sites of River Kabul. The observed lesions in intestine of studied fish species were degeneration of epithelium, complete degeneration of cillia, inflammatiom, coagulative necrosis and degenerative cilia, in gills were disquamation and distortion of secondary lamellae with epithelial cell exudate, clumping of gills lamellae, necrotic area, epithelial disquamation, non specific inflammation, vacuolation and oedem, in liver were inflammatiom, hydropic degeneration, coagulative necrosis, non specific inflammation, liquefactive necrosis and spongiosis hepatis and in muscle were fibrillar degeneration, inflammation, coagulative necrosis and non specific inflammation. The organs level of histopathological alterations in *Wallago attu* was in the sequence of liver >muscle >intestine >gills, in *Aorichthys seenghala* was in the order of liver > intestine >muscle >gills, in *Labeo dyocheilus* was in the order of gills > muscle > liver> intestine, in *Cyprinus carpio* was in the order of intestine >liver > gills > muscle and in *Ompok bimaculatus* was in the order of liver>intestine >muscle>gills. Overall pathological abnormalities in *Wallago attu* were highest in liver and lowest in gills, in *Aorichthys seenghala* were more in liver and less in gills, in *Labeo dyocheilus* were maximum in gills and minimum in intestine, in *Cyprinus carpio* were higher in intestine and lower in muscle and in *Ompok bimaculatus* were greater in liver and smaller in gills. Overall order of pathological lesions in different fish organs was liver >intestine > gills > muscle and in different fish species was *Cyprinus carpio* > *Ompok bimaculatus* > *Labeo dyocheilus* > *Aorichthys seenghala* > *Wallago attu.* This shows that more histopathological abnormalities were found in liver followed by intestine and gills while less lesions were observed in muscle. Similarly maximum histopathological

lesions were observed in *Cyprinus carpio* fish followed by *Ompok bimaculatus*, *Labeo dyocheilus, and Aorichthys seenghala* and minimum in *Wallago attu* fish.

CHAPTER-7
CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

1. The industries in the vicinity of River Kabul discharge their effluents containing high levels of TSS and Hg into the River Kabul. Thus raising the levels of these parameters beyond the recommended levels of National Environmental Quality Standards.

2. Some of the parameters like TSS and Hg still remain higher in downstream water showing sublethal contents of contaminants in the water of River Kabul.

3. The fish accumulated heavy metals like Zn, Ni, Cr, Cu, Cd, Pb, Mn, Fe and Hg in its various organs. The intestine generally accumulated highest metals followed by skin, liver, gills and muscle.

4. The data generated in these studies confirm the presence of sublethal concentrations of pollutants in the River Kabul that fish population is surviving under stressful conditions, which is apparent from the genotoxicological and pathological disorders and heavy metals load in the bodies of inhabitant fish population.

5. The data generated also confirms the presence of pollution plug in the river at Nowshera, which has created a barrier between the fish population of River Indus and River Kabul. The migration of *Cyprinus carpio, Ompok bimaculatus, Aorichthys seenghala, Labeo dyocheilus* and *Wallago attu* from River Indus into spawning areas in River Kabul during breeding season has also stopped, which in another reason for decline of these fish population in River Kabul and also in River Indus.

6. The decline in the fish population, especially *Cyprinus carpio, Aorichthys seenghala, Ompok bimaculatus, Labeo dyocheilus* and *Wallago attu* in River

Kabul can not only attributed to detrimental effect on adult fish health but also on the fish eggs and seeds.

7. The damage to the fish health is indicated by genotoxicological and histopathological abnormalities in the fish samples collected from polluted portions of River Kabul.

7.2 RECOMMENDATIONS

1. The processing of industrial effluents, before their disposal should be regulated by strict vigilance and effective legislation.

2. The industrial effluents and sewages should be detoxified before discharging into River Kabul.

3. Strict environmental laws should be implemented and public awareness should be created.

4. A general biomonitoring programing needed to be established where the hydrological and geomorphological characteristics, the chemical and physical water quality and the river vegetation are taken into consideration as these will affects aquatic system.

5. To avoid harmful accumulation of heavy metals in the human system, the gills, liver, intestine and skin should be discarded while processing fish for consumption. Removal of these organs would drastically reduce the metal intake.

6. The construction of Warsak dam on River Kabul without fish ladder has also limited the *Cyprinus carpio, Ompok bimaculatus, Aorichthys seenghala, Labeo dyocheilus* and *Wallago attu* into zones in Pakistan and Afghanistan. This dam has blocked the migration of these fish from Pakistan to the breeding areas in Afghanistan. Construction of the fish ladder in Warsak dam could be helpful in the rural propagation of the fish population.

7. Illegal and indiscriminate fishing, even during spawning seasons, is another reasons of *Cyprinus carpio, Ompok bimaculatus, Aorichthys seenghala, Labeo dyocheilus* and *Wallago attu* decline. As this practice is not only harming the adult's population but is also destroying the fish seeds. Therefore strict supervision by supervisory staff and social awareness is highly needed.

8. Uses of brutal means for fishing like electric currents, dynamite and addition of chemicals should be avoided in the River Kabul and its tributaries. Because these practices are destroying the fish population and its seeds and are even detrimental to men and consuming chemical baited fish.

9. The general biomonitoring programme is needed to be established to check the level of heavy metals and physico-chemical parameters in the River water on rountine basis.

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