Effect of Metal Toxicity in *Cyprinus carpio* Linnaeus and *Schizothorax niger* Heckel with emphasis on Biochemical and Histopathological Parameters

Thesis

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Certificate

This is to certify that the Ph.D thesis entitled, **"Effect of Metal Toxicity in *Cyprinus carpio* Linnaeus and *Schizothorax niger* Heckel with Emphasis on Biochemical and Histopathological Parameters"** is the original and bonafide work of **Miss Ruqaya Yousuf** and is being submitted for the first time. It is further certified that this thesis is fit for submission for the degree of Doctor of Philosophy in Zoology and the candidate has fulfilled all the statutory requirements for the completion of the doctoral programme.

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ABBREVATIONS

AAS	Atomic Absorption Spectrophotometer
H&E	Harris Haematoxylin and Eosin
LDH	Lactate Dehydrogenase
nM	Nanomole
RER	Rough Endoplasmic Reticulum
SDH	Succinate Dehydrogenase
μM	Micromole

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

Water, the most precious natural resource is essential to the livelihood of both aquatic and terrestrial animals. More than two third of the earth's surface is covered with water. Most of it is the ocean, while the total freshwater in lakes and rivers make up less than one percent (Hutchinson, 1967). The State of Jammu and Kashmir is famous for its natural freshwater lakes and rivers which due to their rich ichthyofauna invited the attention of J. Heckel in 1838, who later on described sixteen species of fish from the collection of Von Hugel which were new to science. The icthyological study of Heckel proved to be the milestone for new workers and researchers. The valley of Kashmir abounds in numerous water bodies, both lentic and lotic. The lentic habitats are represented by tarns, lakes, ponds, wetlands and roadside ditches and similar other aquatic systems. The lotic habitats on the other hand consist of river Jhelum and numerous cold water hill streams which directly or indirectly join the river Jhelum.

1.1. Dal Lake

The Dal Lake is one of the beautiful lakes in the world situated at about 10 km in the north east of Srinagar city with a mean latitude of $34^0 7'$ north and longitude of $74^0 52'$. It is surrounded on the east by Mahadev mountain range and the south by the Kohisulaiman. On the northern and

western banks (Map I) lies the Srinagar City. A total water spread of the lake at the turn of 20th century was 25 km^2 but due to encroachment and siltation, the lake has been reduced to 12.4 km^2. It is a shallow lake with a depth of 1.5-2.0 meters, but at certain places like Sonalank and Rupa Lank the depth exceeds 3.5 meters. The depth of the lake varies seasonally due to amount of rainfall and stream flow. On its north the lake receives water from Dagwan nallah, which joins it at Tailbal that brings water from Mansar Lake and from the agricultural fields. On the southwestern side the lake discharges water into the river Jhelum through a channel called Tsutikol which leaves from Gagribal basin at Dalgate.

1.2. River Jhelum

Jhelum- the Hydaspes of the ancient Greeks and Romans, the vetesta of the Hindus and 'Veth' in Kashmir arises from a beautiful spring called Verinag (Map-II). The river Jhelum orginates from Pir Panjal about one kilometer ahead of Verinag. The Jhelum river is navigable from Khanabal to Baramulla, a distance of about 170 Kms. It flows in loops through the valley till it enters the Wular. Emerging from the Wular it takes a southwestern direction which it pursues up to Baramulla. It finally passes into Pakistan through the Baramulla- Uri gorge. On the right bank above Khanabal, the river Jhelum is joined by the Sandran, Bringi, Arapatkol, Lidder, Arapal, Harwan, Sindh, Erin, Madhumati, Pohru and Viji- Dakil. The other important tributaries of the river include: Vishav, Ramsiora, Sarsara, Romushi, Doodganga, Sukhnag, Ferozepur Nirgal, Uroosa, Nambla (Haji-pir nallah), Rambiara, Pohru and Boniyaar etc.

The oldest economic activity lined with the river Jhelum and Dal Lake is fishing and as the home of all indigenous fish fauna, the Jhelum and Dal Lake are of great economic importance. After Heckel (1838, 1844) who listed

16 species of fishes in Kashmir Valley and surrounding areas, Silas (1960), Das and Subla (1963, 1964), Saxena and Koul (1966), and Nath (1986) summarized most of the collection and provided new checklists which contain increasing number of species. Both the water bodies serves as an important source of indigenous (*Schizothorax* spp.) as well as exotic fishes (*Cyprinus carpio* spp.). The fish catch for previous some years from Dal lake and river Jhelum is shown in Table 1.1 (Department of Fisheries, Kashmir).

Table I: Fish catch in kgs for the year 2004-05; 2005-06.

Water body	**Fish**	**Year**	**No. of Fishermen**	**Total fish captured**
Dal lake	*Schizothorax niger*	2004-05	783	4071qtls
		2005-06	786	
	Cyprinus carpio spp.	2004-05	783	4526 qtls
		2005-06	786	
River Jhelum	*Schizothorax niger*	2004-05	303	3169 qtls
		2005-06	317	
	Cyprinus carpio spp.	2004-05	303	3010 qtls
		2005-06	317	

Source: Mr.G. Q. Chilloo, Assistant Director (Planning)
Department of Fisheries.Kashmir

1.3. Economic importance

Freshwater fish form one of the important food sources in both the developed as well as under-developed countries. Fish remain the major source of protein, whereas processed fishmeal plays a prominent though indirect role as an important component in the production of meat in human nutrition (Braunbeck *et al.,* 1993). The recreational value of angling is also important in most developed countries (Lloyd, 1992). The natives of Kashmir valley divide all types of fishes broadly into two categories of local (Kashmiri) and non-local (Punjabi) fish, zoologically known as endemic and exotic fish spp. Important ichthyofauna of two water bodies under study comprises of the family Cyprinidae.

Family Cyprinidae

Fish Species	**Local Name**
Shizothorax niger (Heckel)	Ale-gaad
Cyprinus carpio communis (Linn.)	Scale carp
Cyprinus carpio specularis (Linn.)	Mirror carp

Concern

As a cultural symbol of Kashmir, river Jhelum is as healthy as ever, but as a river it is fast losing its significance. Its water is today repugnant. The source of pollution in the river is sewage and effluents. The problem assumes greater dimension due to dense human settlement along the banks dispensing and dumping the whole municipal garbage into the river. The Jhelum flows besides the capital city of Srinagar through three major cities, Anantnag, Sopore and Baramulla. It is through these stretches that the river receives maximum of its pollutants.

1.4. Threat to the aquatic life - Pollutants

The fish population, especially the local fish *Schizothorax* has been experiencing a continuous and considerable reduction both in Dal lake and river Jhelum over the last decade (Department of Fisheries, Kashmir, Report 2004-2005). The species being sensitive can not withstand unclear waters. Since the water quality in the river and lake has deteriorated over the years, the *Schizothorax* finds it difficult to thrive in water with depleted oxygen levels (Hussain, 2003).

A fish's condition reflects its ability in finding and storing energy under prevailing environmental condition. Condition not only reflect the health, growth and reproductive state of a fish, but also reflects environmental characteristics such as habitat quality and prey availability (Busacker *et al*., 1990). Condition indices have been widely used as indicators of relative health (Brown and Murphy, 1991; Childress, 1991), since these provide relatively simple and rapid indicators of how well the fish cope with their environment (Goede and Barton, 1990).

Aquatic organisms are often exposed to high levels of pollutants through bioconcentration and/or bioaccumulation. Currently, it is difficult to find any source of water that does not carry fingerprints of human activity (United Nations Environment Programme, 2004). Acid precipitation causing leaching of metals from surrounding soils (Norton, 1982) and increasing numbers of synthetic organic compounds and metabolized pharmaceuticals finding their way into surface waters in unlikely places (Huang and Xia, 2001), and makes their identification by untargeted chemical analysis prohibitively expensive. As a result, fish have become an indispensable model system for the evaluation and/or measurement of the extent of aquatic pollution.

Public concern regarding pesticides, fertilizers, agricultural products and metals in recent years have escalated, particularly following major fish kills (Heath and Claassen, 1999).

Modern agricultural practices significantly contribute towards polluting the aquatic habitat. The rapidly increasing use of pesticides, chemicals and fertilizers poses a serious threat to the fisheries, especially to the *Schizothorax* species. Increasing agro-chemical pollution of the Dal lake and River Jhelum has become a matter of great concern. Pesticides are used in agricultural fields and enter the river through land drainage or with surface run-off during floods and excessive rains. These include herbicides, rodenticides, fungicides, wormicides, insecticides, etc (like chlorinated hydrocarbons, organophosphates, carbamates and phenols etc). These are lethal to fish if they exceed the tolerable limits.

Siltation in the river Jhelum is another threat to fish life. The run-off from agricultural fields, denuded forests and spent mine areas results in siltation of the riverbed. At various sites the River Jhelum is gradually becoming narrower. Siltation of the river, besides diminishing the flow of water, results in destruction of breeding grounds of fishes and the benthic fauna, migration of fishes and decline in overall productivity of the river. Siltation has affected the *Schizothorax* species in the Dal lake near Telbal area where the shoals of fish used to migrate. Metals enter the aquatic food chain through direct consumption of water or biota and through non-dietary routes such as uptake through gills in the case of fish.

On the other hand trace metals are introduced into the environment by a wide spectrum of natural and anthropogenic sources. There has been a general global increase in industrial activity over the past few decades,

resulting in significant application of metals in various processes, in turn causing a great escalation of metals in the environment. Industrial activities as well as agriculture and mining make up potential source of heavy metal pollution in aquatic environment (Gumgum *et al*., 1994; Unlu *et al*., 1996; Leland *et al*., 1978; Corbett, 1977; Mance, 1987; Langston, 1990; Kouadio and Trefry, 1987; Ajmal *et al*., 1987). Contamination of a river with heavy metals may have devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organisms becomes limited with the extent of contamination (Suziki *et al*., 1988). It is well known that heavy metals accumulate in tissues of aquatic animals and their measurement in tissues of aquatic animals can reflect past exposures (Canli and Atli, 2003; Kalay *et al*., 1999; Yilmaz, 2003, 2005). Sub lethal effects of heavy metals are of concern as they accumulate and are transferred through food chain to humans. The impact of pollutants on aquatic ecosystems is either acute (due to exposure to immediate lethal dose) or insidious/chronic (due to gradual accumulation of lethal concentrations in body tissues) (Heath and Claassen, 1999). Metals like lead and cadmium may present a health risk even at extremely low concentration, since they may influence enzymatic activity in living systems (Brock and Madigan, 1991). A disease known as plumbism has been known to be caused by acute lead poisoning (Stofen, 1974). Cadmium has also been regarded toxic at very low concentrations (Bryan, 1971) and with hazardous effects on humans (Hagino and Yoshioka, 1961). Biomonitoring of trace metal pollutants has been gaining attention since different organisms can accumulate these substances and transfer them in large concentration to animals or human beings, when consumed (Fadrus *et al*., 1979).

Due to scanty information regarding the metal toxicity in water bodies of Kashmir valley and their effects on the aquatic fauna, the present study was designed with the following objectives:

1) To study the toxicity of some metals *viz*. copper, zinc, iron, and manganese in Dal lake and River Jhelum.

2) To study the concentration of these metals through Atomic absorption spectrophotometer in various organs/tissues viz. gills, liver, kidneys, and muscles of *Schizothorax niger* and *Cyprinus carpio* spp.

3) To study the subsequent effects of metals on biochemical parameters *viz*. total protein, albumin, globulin, blood glucose, urea, serum creatinine and cholesterol in both the fishes.

4) To study the subsequent effects of these metals, on the histomorphology of gills, liver, kidney and muscles of both the fishes.

The perusal of literature indicates a widespread contamination of water bodies leading to the harmful effects on the aquatic fauna. The heavy metal research in the agricultural, environmental and life sciences has emerged only over the last three decades, also urged by the growing social concern with excess concentrations of a number of elements in the environment as a consequence of anthropogenic emission (Davies, 1992). Environmental conditions are certainly not static, and human influence has greatly accelerated the rate of deleterious changes, for example by continuously loading water systems with chemical xenobiotics. Particularly in the 20th century, thousands of organic trace pollutants have been produced, and in part released into the environment (Van der Oost *et al*., 2003). In the mid 20th century, the utilization of huge quantities of heavy metals has resulted from the ever expanding technological development (Nriagu, 1988). Heavy metals can enter the soil by a number of pathways and their behavior and fate in soil differ according to their source and nature. The most important agricultural sources of heavy metals include commercial fertilizers, sewage sludges, urban wastes, waste waters, liming materials and agrochemicals and other wastes used as soil amendments (Rao, 1998).

2.1. Source of metals

According to Odum (1986), environmental pollution is becoming one of the most important limiting ecological factors. Heavy metals are the main group of pollutants, with deleterious effects on organisms. Linnik (1986) noted that, unlike all other toxicants of organic nature that more or less

dissolve in natural waters, heavy metal ions should be considered persistent components in the aquatic environments. An important ecological specificity of these pollutants is also the fact that these are practically no self-cleaning mechanisms (Linnik, 1986).

Acid precipitation causes leaching of metals from surrounding soils (Norton, 1982; Spry and Wiener, 1991) and such wastes containing heavy metals are directly discharged into the water bodies. Despite the focus on the sources and impacts of metals originating from anthropogenic activities (Norberg-King *et al*., 1991; Sarakinos *et al*., 1999; Bailey *et al*., 2000; Van Sprang and Janssen, 2001), recent studies have demonstrated that natural geochemical materials, containing potentially toxic metals (Conveney and Glassock, 1989; Kim and Thornton 1993; Chon *et al*., 1996; Lee *et al*., 1998; Petsch, 2000; Ogendi *et al*., 2004b).

2.2. Source of fertilizers

Heavy use of fertilizers has been a key factor in achieving green revolution but at the same time posed longterm ecological problems. Fertilizers have been found to be a potential source of heavy metals, which they carry as impurities. Rock phosphate or its products have been found to carry a significant amount of lead and cadmium forming a potential source of soil contaminants. Combination of low analysis and straight fertilizers add more lead and cadmium to soils than high analysis and mixed fertilizers. Heavy metals applied to the soil through different derivatives of rock phosphate accumulate almost completely in the surface layer of the soil are easily available to plants. Regular use of phosphate fertilizers over many years has been incriminated for increase in the cadmium content of most cultivated soils (Rao, 1998). In contaminated soils heavy metals have been found to be easily available to plants. This has been found to be in soils with coarse textures and acidic reaction when compared with those containing

large amount of clay and with alkaline reaction. Besides the effect on soil ecology and cultivation these fertilizers along with heavy metal contaminants leach into the water bodies leading to aquatic pollution and disturbed aqua system (Sharma, 2000). Though in India relatively low level of fertilizers about 16-20 kg/ha compared to the world average of 54 kg/ha are used. Yet their accumulation over the years has lead to an alarming situation warranting the immediate monitoring system for contamination levels as well as the effects on the various biotic forms.

2.3. Source of sewage sludge (Biosolids)

Application of sewage sludge in agricultural land for enhancing productivity has been a common practice for many years. However, it has been found to be a potential source of the toxic heavy metal cadmium, chromium, silver, nickel, lead, selenium and mercury (Rao, 1998).

2.4. Source of poultry litter

Poultry farming is an important agricultural industry in many countries. It is estimated that 1 kg of litter is generated for each kilogram of broiler produced. The value of poultry manure and litter as a fertilizer is well recognized due to its nitrogen, phosphorus and potassium content (Rao, 1998). Mitchel *et al*. (1992) showed that, copper and zinc accumulated to toxic levels in fields with a long history of broiler litter applications. Copper concentrations of more than 500 mg/kg have been reported (Mitchel and Browne, 1992).

Fish are particularly sensitive to water-borne environmental contamination, and are recognized as a useful model for indicating water quality (Mathis and Kevern, 1975; Hinton *et al*., 1987, 1992; Whitfield and Elliott, 2002).

Golubev (1973) showed that heavy metals penetrate through skin and are distributed irregularly in organisms, forming depots. According to Tinisli (1982), metals penetrate the organisms by two ways *viz.* direct water adsorption or fish feed. According to Forstner and Prosi (1979) metals are non-biodegradable, and once they enter the environment, bio-concentration may occur in fish tissue by means of metabolic and bio-absorption processes (Hodson, 1988; Carpene *et al*., 1990; Wicklund-Glyun, 1991). Toxicants rarely occur singly; hence the natural water systems are more likely to contain a number of different pollutants at one time (Hellawell, 1986). These toxic metals are accumulated by fish and other organisms (Burton, 1990) resulting in acute and chronic toxicity in sub flora and fauna.

They may cause lethal and sublethal effects upon the resident biota which in turn may lead to declines in taxa richness and abundance, and shifts of community compositon due to elimination of metal-sensitive taxa within the affected acquatic ecosystem (Clements, 1994; David, 2003; Van Greithuysen *et al*., 2004).

According to Ramade (1987), the toxic effect of salts of heavy metals on fish is multidirectional and is manifested by numerous physiological and biochemical changes. Cytoplasmic inclusions seem to be a fairly common occurrence with fish that have been exposed to either metals or organic xenobiotics (Patton and Couch, 1984; Hinton *et al*., 1987). Adverse physiological or physio- toxicological changes induced by metals include poisoning of the nervous system and the damage is usually irreversible (Ramade, 1987). The seriousness and persistence of heavy metals in water are compounded by the fact that they are generally water soluble, non-degradable and have strong affinity for polypeptides and proteins (Jhingren, 1991).

Carnivores at the top of the food-chain obtain most of their heavy metal burden by way of their food, especially where fish are present, and so

there exists the potential for considerable biomagnifications (Mance, 1987; Langston, 1990; Cumbie, 1975). Similar studies conducted by Badsha and Sainsbura (1977), Komarovskii *et al*. (1988), Wachs (1998), Velcheva (1998), Storelli and Marcotrigiano (2001), Fent (2003), and considering fish species as heavy-metal bioindicators. Since the toxic effects of metals have been recognized, heavy metal levels in the tissues of aquatic animals are occasionally monitored. Because the heavy metal concentration in tissues reflects past exposure via water and/or food, it can demonstrate the current situation of the animals before toxicity affects the ecological balance of populations in the aquatic environment (Canli and Kalay, 1998).

2.5. Toxicity of copper

Copper acts as a cofactor for a number of key proteins (i.e., superoxide dismutase, ceruloplasmin) and enzymes including cytochrome C oxidase and thus plays a vital role in cellular respiration. Higher levels of copper have been recognized as one of the most important heavy metal toxins affecting fish (Mance, 1987). Accumulation of copper in organs of different fish species have been reported (Griffin *et al*., 1977; El Deek and Ahmed 1996; McGeer *et al*., 2000; Anderson *et al*., 2001). The elevated ambient copper concentrations can lead to excess copper accumualtion in several tissues (Lauren and McDonald, 1985, 1987a, 1987b; De Boeck *et al*., 1995; Grosell *et al*., 1997, 1998a, 2001b, 2003b; De Boeck *et al*., 2001, Grosell and Wood, 2002; Kamunde *et al*., 2002). Following absorption from gills and intestine, copper is transported as metallothionein into the blood circulation and some of it accumulates in different internal organs especially liver and kidney. It has also been shown to affect various blood parameters (McKim *et al*., 1970; Christensen *et al*., 1972) and enzyme activities in blood (Christensen and Tucker, 1976) and liver (Jackim *et al*., 1970). Singh and Reddy (1990) reported a rise in plasma sodium and potassium from exposure to copper in a fresh water fish. Copper has subtle effects on aspects of digestive physiology

such as enzyme activity, nutrient uptake and neural control of digestion (Clearwater *et al*., 2002). Osomoregulatory organ, of aquatic organisms, which are in direct contact with the envornment are targets for these effects (Wood, 2001; Grosell and Wood, 2002). It has been found to cause the inhibition of Na^+ transport across the gills of freshwater fish which co-incide with inhibition of the Na^+ /K^+ -ATPase enzyme (Lauren and McDonald, 1985; 1987a, 1987b) and inhibition of branchial carbonic anhydrase in crustaceans (Vitale *et al*., 1999). Farag *et al*. (1994) found increased lipid peroxidation in the kidney of Rainbow trout fed with metal-contaminated invertebrates; lipid peroxidation was also increased in the large intestine, liver and pyloric caecae of free-ranging brown trout collected from the Clark Fork River (Farag *et al*., 1995).

The characteristic clinical symptoms of intoxication with copper and copper compounds are laboured breathing and in cyprinids, gasping for air on the water surface. The typical patho-anatomic finding includes a large amount of mucus on body surface, under the gill covers and in the gills. Acute copper intoxication can be diagnosed on the basis of a chemical analysis of the gills in which the concentration of copper is much greater than in other parts of the body of the fish (Svobodova *et al*., 2006). Conversely, high concentrations of waterborne copper affect branchial function, the main toxic action being a perturbation of sodium homeostasis (Lauren and McDonald, 1985). Copper affects swimming performance (Waiwood and Beamish, 1978b), growth, (Lett *et al*., 1976; Waiwood and Beamish, 1978a) and reproductive success (Horning and Nieheisel, 1979) in a variety of teleosts.

2.6. Toxicity of zinc

Zinc is essential for structural and/or catalytic importance in more than 300 proteins that play important roles in piscine growth, reproduction, development, vision and function (Watanabe *et al*., 1997). In fish, zinc is second in quantitative importance only, to iron (Watanabe *et al*., 1997). Although an essential micronutrient for all organisms, it has been found to be toxic at higher concentrations (Vallee and Falchuk, 1993; Hogstrand and Wood, 1996).

Zinc is easily bioaccumulated in stream invertebrates- an important food source for juvenile salmonids while rearing in freshwater systems. Fish fed on diets contaminated with zinc have been observed to exhibit reduced survival, growth, and increased incidence of disease (Farag *et al*., 1994; Balasubramanian *et al*., 1995). At acutely toxic concentrations zinc appears to exert its lethal effects in fish at surface epithelia such as the gills (Evans, 1987) but there may also be chronic effects at tissue/biochemical level (Alabaster and Lloyd, 1982). Since zinc is an essential trace element, (Alabaster and Lloyd, 1982) there may be specific pathways for the metabolism of metals; such trace metals in fish tissue may be subjected to homeostatic control (Goodyear and Boyal, 1972; Weiner and Geisy, 1979).

Shukla (1978) found the relative susceptibility to zinc sulphate to be in the order of *Labeo rohita*, *Cirrhina mrigala*, *Puntius sophore* and *Channa punctatus*. Wide range of physiological disturbances due to zinc intoxication in salmonids under varying conditions of pH and water hardness, have been reported which encompases e.g., respiratory impairment (Skidmore and Tovell, 1972; Hughes and Adeney, 1977), impaired branchial ion regulation (Spry and Wood, 1985; Everall, 1987), inhibition of branchial ATPases (Shephard and Simkiss, 1978; Watson and Beamish, 1981), erythrocyte haemolysis (Kodama *et al*., 1982), tissue inflammatory responses (Hughes

and Gray, 1972), impaired antibody production (O'Neill, 1981) and tissue melano-macrophage induction (Everall, 1987).

The ubiquitous nature of zinc is governed by its ability to form a wide range of coordination complexes, allowing it to interact with a wide range of cellular entities (Vallee and Falchuk, 1993; McCall *et al*., 2000). Furthermore, zinc is redox inert, enabling the formation of relatively stable associations within the cellular environment (Vallee and Falchuk, 1993). In contrast to copper and iron, zinc does not form the free radical ions, and infact has antioxidant properties (Powell, 2000). However, zinc toxicity in fish has been incriminated to its interference with calcium homeostasis (Spry and Wood, 1985; Hogstrand and Wood, 1996).

2.7. Toxicity of iron

Iron is a vital micronutrient for teleost fish, being an integral component of proteins. It is one of the most abundant metals on the earth and is essential to almost all orgamisms. It has a number of fundamental roles in cellular biochemistry and metabolism. These include oxygen binding to heme proteins and the formation of active centres in enzymes involved in the mitochondrial electron transport chain (De Silva *et al*., 1996; Aisen *et al*., 2001). Iron positioning in the haem moiety of hemoglobin increases oxygen binding and carrying capacity. One of iron's key cellular functions is to confer redox activity to the cytochromes involved in respiration, due to its ability to exchange electrons in aerobic conditions.

Excess waterborne iron may be toxic to fish, due to the formation of iron 'flocs' on the gills, resulting in gill clogging and respiratory perturbations (Peuranen *et al*., 1994; Dalzell and MacFarlane, 1999). The iron content of fish is, in general, considerably lower than that of other vertebrates (Van Dijk *et al*., 1975), but the precise daily iron requirements for fish are at present

unknown. Aside from the generally lower levels of iron, it is widely assumed that iron metabolism and function in teleost fish is similar to that in other vertebrates (Lall, 1989). Animals lose iron through defecation and epithelial sloughing, and this loss is compensated for by absorption from the diet. In fact, the regulation of iron homeostasis is governed by intestinal absorption, as a regulated excretory mechanism is not known for iron in higher vertebrates (Andrews, 2000). In small intestine, non-haem bound iron is reduced *via* a membrance-bound ferric reductase (Mckie *et al*., 2001) and ferrous iron enters the cell via a proton/Fe^{2+} symporter (Gunshin *et al*., 1997). This latter protein belongs to the family of proteins termed natural resistance associated macrophage proteins (Nramp), or solute carrier 11 type 2a (Sla 11 2a). However, the protein is more commonly known as the divalent metal transporter (DMT), because it has been shown to transport other divalent cations (Gunshin *et al*., 1997; Talkvist *et al*., 2006; Bannon *et al*., 2002). Physiological evidence indicates that iron preferentially crosses the apical membrane of both the gills and intestine in the ferrous (Fe^{2+}) state (Bury and Grosell, 2003). Consequently, based on molecular evidence, the genes encoding proteins involved in epithelial iron uptake appear to be present in teleost freshwater fish (Bury and Gorsell, 2003).

Iron forms insoluble ferric (hydro) oxides at neutral pH (Aisen *et al*., 2001) and molecular evidence suggests that the small fraction of Fe^{3+} presumably present in the gut lumen will be reduced to Fe^{2+} prior to import into the gut enterocytes of fish (Bury *et al*., 2003). In mamals, ferrireductase activity in the brush border of the intestinal mucosa facilitates the reduction of Fe^{3+} to Fe^{2+} (Riedel *et al*., 1995; Mckie *et al*., 2001). Fe^{2+} is absorbed three times faster than Fe^{3+}. Intracellular Fe is stored as Fe^{3+} by ferritin, a 450 KDa protein with a spherical cavity capable of carrying 4500 iron atoms (Aisen *et al*., 2001). Ferritins are an ancient group of proteins conserved in bacteria,

plants and man (Aisen *et al*., 2001), and have also been found in fish (Andersen, 1996).

Many freshwater organisms have evolved mechanisms for mobilising and sequestering iron. A number of freshwater bacteria, algae and cyanobacteria produce organic compounds, siderophores, which are released to the environment (Wilhelm and Trick, 1994). These siderophores have exceptionally high binding affinity (~log K = 19) for iron (Witter *et al*., 2000), thus maintaining iron in solution, and the iron siderophore complexes are taken up (Wilhelm and Trick, 1994, Cowart, 2002). Some algal species adopt a different mechanism, whereby a plasma membrane ferric chelate reductase utilizes intracellular reducing power to liberate iron from its organic ligand and reduces Fe (III) to Fe (II) after which Fe (II) is subsequently transported into the cell (Robinson *et al*., 1999; Weger *et al*., 2002). In addition, photolysis of siderophore bound iron results in the formation of lower affinity Fe (III), ligands and the reduction of Fe (III), increasing the bioavailable Fe (II) fraction to organisms in the eutrophic zone (Barbeau *et al*., 2001).

2.8. Toxicity of Manganese

Manganese is a naturally occuring substance that is present in surface waters and biota. It occurs in soil, sediments and water both naturally and as a result of enviromental contamination. Manganese is a component of enzymes such as pyruvate carboxylase, mitochondrial superoxide dismutase and arginase, glycosyl transferases, kinases, prolinase and phosphotranferases (COMA, 1991).

An increase in manganese can cause the enrichment of manganese in the bony tissue and scales of the exposed fish (Lockhart and Lutz, 1977; Fraser and Harvey, 1982; Moreau *et al*., 1983). Manganese forms weak

organic complexes, which may be unavailable to fish (Stumm and Bilinski, 1973). Redox conditions within a lake could alter the bioavailability of manganese to the fish (Danvison and Tipping, 1984). The tissue metal levels may be the result of indirect uptake from food or ingested sediments, rather than that obtained directly through concentrations of dissolvd metals (Patrick and Loutit, 1978; Ney and Van Hassel, 1983; Wren *et al*., 1983).

Manganese is known to cause neurotoxicity by increasing oxidative stress and also disturbing neurotransmitter metabolism (Erikson *et al*., 2004).

Agarwal and Srivastava, 1980 reported blood dyserasia in fish exposed to experimental manganese poisoning. *Colisa fasciatus*, a fresh water teleost, showed significant decrease in the total erythrocyte count, number of erythrocytes/1000 cells of all types, and in the hepatosomatic index (relative liver wt.) after exposure for 90 hours to 2500 mg/l manganese sulphate; the 97 h LC 50 value was 2850 mg/L The exposure also evoked leucocytosis due to increase in the number of small lymphocytes

Gonzalez *et al*., 1990, reported a primary toxic action by manganese to brook charr, *Salvelinus fontinalis*, at concentrations near or above the 96 h LC_{50} cause the disruption of sodium regulation. Body and plasma sodium concentration was declined by 52 and 40% during exposure to 109m M manganese (In 250 μM $CaCl_2$) and all fishes died within 36 hours.

The gill epithelium is the main site of toxic activity for manganese, at least at high ambient concentrations (Gonzalez *et al*., 1990). Reader and Morris (1988) demonstrated that calcium uptake was inhibited by manganese and *in vitro* studies by Bansal *et al*., (1985) indicated that manganese inhibited branchial Ca-ATPase activity of the teleost *Saccobranchus fossilis*. Lewis (1976) showed that exposure to a manganese concentration of 180 μM cause 30% mortality of rainbow trout eggs. Beisinger and Christensen (1972) found that a manganese concentration of 74 μM caused a 16% reduction in

reproductive out put of *Daphnia magna*, and a concentration of 95µ M caused a 50% reduction.

2.9. Metal-induced histopathology of gills

Gills are multifunctional organs with a complex internal organization that is similar in most teleosts (Hughes, 1984; Laurent *et al*., 1995). The gill of teleost fish is covered by a complex epithelium whose function is controlled by perfusion through a rather intricate vascular system (Evans, 1987). The gill epithelium is the dominant site of gas exchange, ionic-regulation, acid-base balance, and nitrogenous waste excretion for fish (Houlihan *et al*., 1982; Hoar and Randall, 1984). In 1920s and 1930s there were reports that fish exposed to heavy metals (e.g., zinc) in water suffocated due to damage to the gills and/or clogging of the lamellae with mucus (Jones, 1964). Gills have an extensive surface area and minimal diffusion distance between dissolved oxygen and blood capillary for efficient gaseous exchange. This organ system remains protected by a thin layer of mucous coating (Hughes *et al*., 1979; Powell *et al*., 1992). Electron microscopic investigations have shown that the surface of the gill epithelia is provided with numerous micro-ridges which anchor the mucus film (Hughes and Wright, 1970). The number and pattern of the micro ridges are disturbed following exposure to stress conditions, heavy metals, which may diminish the capacity of gas exchange by reducing both the lamellar area and micro-turbulence (Karlsson-Norrgren *et al*., 1986a; 1986b). Epithelial lifting in the lamellae is a typical inflammatory reaction, and can be interpreted as an initial reaction of the gills to stress to a variety of pollutants (Pawert *et al*., 1998). Morgan and Tovell (1998) observed that epithelial lifting resulted in an enlargement of the water-blood diffusion barrier. The highly branched structural organization of the gill, increased surface area with the large volume of water passing through it and the highly vascular physiological state with small biomass when

compared to their surface area make the gill a prime site for metal accumulation (Mayer *et al*., 1991). Further gills are directly exposed to metals occurring in the external environment and the increased concentrations of these substances cause serious damage (Mallatt, 1985; Dutta *et al*., 1996; Wendalaar Bonga, 1997). Lloyd (1961) proposed that acute toxicity of several poisons, including heavy metals, lead to hyperventilation. The morphological anomalies commonly include "hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia (marked dilation of terminal blood vessels), edema with epithelial separation from basement membranes, general necrosis, and/or epithelial desquamation" (Meyers and Hendricks, 1985). The hypertrophy and hyperplasia of the gill epithelium and the separation of the basal membrane has been observed in the nitrite-exposed fish such as juvenile carp, *Cyprinus carpio* L, (Korwin-Kossakowski and Ostaszewska, 2003), *Oncorhynchus mykiss* (Wedemeyer and Yasulake 1978), *Clarias lazera* (Michael *et al*., 1987) and *Bidyanus bidyanus* (Frances *et al*., 1998)

Experimental exposure of fishes to copper sulphate has been reported to cause the degenerative changes in gills such as hypertrophy of mucus cells, necrosis and disquamentaion of gill epithelium resulting in exposure of supporting pilastar cells and blood capillaries and subsequently affecting the respiratory and osmoregulatory ability of the fish (Sultan and Khan, 1982). In general, the alterations in gill include periodic deformation of lamellar elements, haemorrhages, necrosis and sloughing off of the respiratory epithelium (Zheng *et al*., 1997), hyperplasia, intercellular vacuolization and occasional fusion of secondary lamellae, resulting in increased thickness of primary and secondary lamellae. (Zhang *et al*., 2005).

Skidmore and Tovell (1972) demonstrated that exposure of rainbow trout (*Samlo gairdneri*) to 40 ppm Zn^{2+} for approximately 3hours resulted in severe curling and edema of the secondary lamellae, with the epithelium lifted

away from the basement membrane. In fish with gills damaged by zinc, accumulation of lactic acid and carbon dioxide in the blood produced a combined metabolic and respiratory acidosis (Spry and Wood, 1984). Simkiss (1984) also detected a proliferation of chloride cells in the gills of fish exposed to heavy metals. Olson *et al*. (1973) described some morphological changes such as decreased height of lamellar cell ridges, appearance of vacuolated epithelial cells, and chloride cell degeneration) after exposure of rainbow trout to either mercuric chloride or methyl mercury (approximately 50 ppb for 1 week or 0.25 ppb for 6-8 weeks). Matthiessen and Brafield (1973) examined the effect of exposing sticklebacks (*Gasterosteus aculeatus*) to 0.5 to 1.0 ppm Zn^{2+} for 1 to 3 days in distilled water (usually fatal) or 2 to 6 ppm Zn^{2+} for up to 29 days in hard water (not fatal). Baker (1969) observed gross changes in gill of *Pseudopleuronectus americanus* due to copper poisoning. Gill hyperplasia was also observed in fish cultivated in water contaminated with ammonia (Larmouex and Piper, 1973). Thurston *et al*. (1978) confirmed hypertrophy in the plates of the gill epithelium, necrosis in the epithelial cells and the separation of the epithelium from the gill arch in trout fry reared for 29 days with elevated ammonia levels. The first reaction of the gill to the presence of toxic compounds in water is increased mucus production in gill epithelium. This phenomenon was observed in fish inhabited in acidic (Ultsh and Gros, 1979) and alkaline waters (Daye and Garside, 1976; Jezierska 1988; Ostazewska *et al*., 1999). Further studies revealed the diminished respiratory ability of fish when exposed to nitrite in alkaline waters (Jensen *et al*., 1987; Alcaraz and Espina, 1997; Murthy *et al*., 1981) or acidic waters (Korwin-Kossakowski and Jezierska, 1985). Zhang *et al*. (2005) while studying the heavy metal (Cadmium, Lead and Zinc) accumulation and tissue damage in Goldfish *Carassius auratus*, found that gill epithelium exhibited hypertrophy, pyknosis and vague boarders between the epithelial cells. The thickened respiratory epithelium due to the

hyperplasia of epithelial cells increased the diffusion distance between the ambient and vascular components. Vasodilation in the secondary lamellae of gills and periodic fluctuations in the mucous cell density were also observed at various stages of $ZnCl_2$ exposure (Hemalatha *et al*., 1997). Ferguson (1989) stated that cellular proliferation in respiratory lamellar epithelium may lead to a great disturbance of gas exchange.

A significant interaction was observed between cadmium treatment and exposure time, indicating that cadmium exerted a significant effect at 60 days (75% prevalence in starved fish), though not at 30 days (Randi *et al*., 1996). Lamellar telangiectasis and hyperplasia of chloride cells were specific results of exposure to cadmium (Randi *et al*., 1996). According to Roberts (1981), gill lamellar telangiectasis is caused by the disruption of pillar cells, which results in capillary distension and blood accumulation may lead to further fibrosis. Randi *et al*. (1996) observed only distal telangiectasis, in terms of erythrocyte accumulation, at the tip of secondary lamellae. The same pathology was reported as an effect of ammonia on fish (Smith and Piper, 1975) and in samples of fish from pesticide-contaminated waters (Ramano and Cueva, 1988). McCraren *et al*. (1969) found the same lesion after 21 days of exposure to the pesticide diuron, while Richmonds and Dutta (1989) detected hyperplasia of the secondary lamellae in blue gills after 96 hr of exposure to malathion.

2.10. Metal-induced histopathology of liver

The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some situations, materials may accumulate in the liver to toxic levels and cause pathological alterations (Meyers and Hendricks, 1985; Ferguson, 1989; Braunbeck *et al*., 1990). The liver not only represents a central organ concerning basic metabolism (Gingerich, 1982), but

is also a major site of the accumulation, biotransformation and excretion of xenobiotic compounds (Meyers and Hendricks, 1985). It is the first organ to be exposed by the portal circulation to toxicants ingested by the body (Hibiya, 1982). Because of its unique position and proximity to the venous drainage of the digestive tract, the liver is susceptible to damage from absorbed toxic materials (Leeson and Leeson, 1976). The high degree of metabolic activity of hepatocytes renders them vulnerable and toxins can easily affect them. The harmful effects of ingested toxic substances are primarily exerted within the liver cells (Llyod, 1992). Thus, hepatocytes may be expected to be primary targets of toxicity lesions. Subsequentially, hepatocytes respond to changes in the external and internal environments by alterations in both cellular structure and function (Wheater *et al*., 1985).

There have been many reports of histopathological changes occurring in fish tissues from exposure to pollutants and it is evident that a wide variety of chemicals cause lesions in the livers of fish (Gingerich, 1982; Patton and Couch, 1984; Hinton *et al*., 1987, 1992). These chemicals include the metals arsenic, cadmium, copper and mercury; assorted industrial wastes such as ammonia, Aroclor, chlorine and phenol, both organophosphate and organochlorine pesticides such as endrin, dieldrin and diazinon; the carbonate insecticide carbofuran and petroleum hydrocarbons (Heath, 1995). However, species differences do occur with regards to nature and extent/degree of exposures. There are many studies of liver ultrastructural alterations induced by heavy metals in aquatic environments (Koyama *et al*., 1979; Khangarot, 1992). The type of liver injury is often dependent upon not only the particular agent and its mechanism of action, but also on the length of exposure (Jacobson-Kram and Keller, 2001). The histological changes observed in various studies on livers taken from fish exposed to pollutants include cytoplasmic vacuolization, enlarged lysosomes, changes in nuclear shapes,

focal necrosis (death of cells in a localized area), ischemia (blockage of capillary circulation), hepatocellular shrinkage, regression of hepatocytic microvilli at the bile canaliculi, fatty degeneration, and loss of glycogen (Heath, 1995).

Total protein is used as an indicator of liver impairment. Increased concentrations can be caused by structural liver alterations reducing aminotransferease activity, with concurrent reduction in the deamination capacity (Burtis and Ashwood, 1996).

2.11. Metal-induced histopathology of kidney

The kidneys are highly metabolically active tissues, which are altered by exposure to chemicals. Heavey metals were contained as renal concentrations within vacuoles in the kidney epithelial cells and were referred to as nephrolithes (Carmichael *et al*., 1979).

According to Kent (1998), the liver and Kidneys are involved in the detoxification and removal of toxic substance circulating in the blood stream. Moreover, liver and kidneys, being the major organs of metabolic activities including detoxification (Klavercamp *et al*., 1984) metals might also be transported into these organs from other tissues, including gills and muscle, for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs.

Javed, (2005) while studying the growth responses of *Catla catla*, *Labeo rohita* and *Cirrhina mrigals* for bio- accumulation of zinc during chronic exposure found fish liver, as a major organ for metabolism and kidney accumulated significantly higher concentrations of zinc than that of gills, skin, muscle and scale. In Atlantic Salmo it has been shown that iron concentration in liver (Bjornevik and Maage, 1993; Andersen *et al*., 1996)

and kidney (Bjornevik and Maage, 1993) are sensitive to dietary iron level and can be used to assess iron status. Kock *et al*. (1989) investigated the fish kidney from five regions of Styrian water for their content of cadmium, lead, copper and zinc. The copper content in the kidneys of fish was significantly higher.

Schreck and Lorz (1978) observed that kidneys of copper-exposed fish had glomerular atrophy. Mazon, (1997) and Mazon *et al*. (2002) reported high amount accumulation of copper by kidneys during acute exposure and preliminary morphological examination showed pathological changes, even at low concentration in water.

2.12. Metal-induced histopathology of muscle

Asztalos and Nemcsok (1985) reported the damage to sketelal muscle in carp after copper sulphate treatment. Medina *et al*. (1986) measured the concentrations of heavey metals in the muscle of *Mullus barbatus* and *Mullus Surmetalus* collected from Spanish coasts. They found that cadmium, lead and copper levels ranged within 0.02 – 0.19, 0.03 – 0.8 and 0.02 – 0.64 μg/g dry weight, respectively. Windom *et al*. (1987) reported low concentrations of cadmium, coppeu and lead in the muscle of the fish *Coryphaenoides armatus* from the Atlantic and Pacific oceans. Concentration of Cd, Cu and Pb ranged within 0.025 – 0.027, 0.03 – 0.09 and 0.01 – 0.02mg/kg day weight, respectively. Peyghan *et al*. (2006) studied the bioaccumulation of copper in liver and muscle of common carp *Cyprinus carpio* after copper sulfate bath. Radhakrishnaiah *et al*., 1993, while studying the effect of sublethal concentration of mercury and zinc on the energetics of a fresh water fish *Cyprinus carpio* (Linneaus) observed the distinct changes in the energy metabolism of gill, liver and muscle. The changes were: a) The rate of oxygen consumption and succinate dehydrogenase (SDH) activity decreased in the organs of mercury-exposed fish at all the three exposure periods in the order

1>15<30 days, whereas an increase was observed in these parameters in the organs of zinc-exposed fish in the order 1>15> 30 days, b) The activity of lactate dehydrogenase (LDH) and the levels of pyruvate and lactate increased in all the three organs of the fish at the three exposure periods studied in both the metal media. Andreji *et al.* (2005) studied the concentration of selected metals (Fe, Mn, Zn, Cu, Ni, Cr) in muscle of four common Slovak fish species (Chub - *Leuciscus cephalus*, barbel – *Barbus berbus,* perch - *Perca fluviatilis*). The concentration of metals (mg/kg wet weight basis) ranged as follows: Fe 3.4, 3.51-15.64; Cu 0.25 –0.78.

Yamashita *et al*. (2006) reported distinct regional profiles of trace element contnt in muscle of Japnese eel *Anguilla japonica* from Japan, Taiwan, and China. The levels of six trace elements, selenium, mercuy copper, manganese, zinc and arsenic, in muscle tissue varied among eels of different origins.

In the muscle of *Engraulis encrasicholus* and *Mullus barbatus,* Capelli *et al.* (1981) found values ranging from 2.40 μgg^{-1} to 26.40 μgg^{-1} for zinc and from 0.34 μgg^{-1} to 1.50 g μgg^{-1} for copper, whereas in the muscle of black marlin, *Makaira indica*, copper varies between 0.2 and 1.2 g μgg^{-1} and zinc between 5.7 and 14.6 μgg^{-1} (Mackay *et al.*, 1975).

2.13. Metal-induced biochemical alterations

Previous studies have shown that certain metals can either increase or decrease hemoglobin, hematocrit, plasma protein, plasma osmolarity, cortisol, glucose and blood enzymes depending on the metal type, species of fish, water quality, and length of exposure (Mckim *et al*., 1970; Christensen *et al*., 1972; O' Neill 1981; Cyriac *et al*., 1989; Munoz *et al*., 1991). Many of these parameters respond rapidly following exposure to sublethal concentrations of metals as part of a nonspecific stress response. The response is transient if the

animal can compensate for the stressor or if the stressor is removed (Thomas, 1990).

The plasma or serum proteins are known to play a vital role in the maintenance of osmolarity, buffering capacity and pH of blood, besides acting as carriers of various nutrients, metabolites and metal ions. Brett and Groves (1979) reported that protein is the major source of energy. Plasma proteins also have great importance in the defence mechanism of the body. The composition of plasma proteins and its variations, therefore, reflect changes in different physiological and biochemical processes in the organism. Several detailed reviews on fish plasma proteins have appeared in recent past (Booke, 1964; Hunn, 1967, Feeney and Brown, 1974).

El-Naga *et al*. (2005) reported decline in plasma proteins due to exposure of cadmium and copper. Monteiro *et al*. (2005) alo reported decrease in protein content due to copper toxicity. Field *et al*. (1943) reported total protein concentration of 3.6 and 3.0 gm % in carp and trout respectively. Phillips (1958) found the albumin levels in Brooke and brown trout to be 1.4 and 0.9 gm % respectively and globulin values of 0.6 and 1.2 gm %. The total plasma proteins of fish have been found to vary from species to species (Deutsch and Mcshan, 1949; Tsuyuki *et al*., 1966). Dwivedi and Menezes (1978) reported 9.1 gm% of total serum protein in *Mugil cephalus* and 8.2 gm% in *Mugil parsia* and also demonstrated 7 major components in serum proteins.

Many workers have studied subfractionation of fish plasma proteins. They found six to twelve different fractions of proteins depending upon the techniques used. Fine *et al*. (1964) working with fresh water eel reported 5 fractions in plasma proteins. Similar results have been found in carp by Goloner *et al*. (1984), whereas Creyssel *et al*. (1964), Silberzahn *et al*. (1967)

and Komarov *et al.* (1975) have shown 3 or 4 fractions only. Sulya *et al*. (1960) concluded that albumin is lacking in primitive fishes, and that there is generally an increase in the complexity of plasma proteins with progression form lower to higher forms.

Protein synthesis in fish has been found to vary with temperature of environment. Carp hepatocytes from summer acclimatized fish have been shown to exhibit high rate of protein synthesis as compared to those of cold acclimatized fish (Saez *et al*., 1982) whereas Das and Prosser (1967) showed 100-500% higher rate of protein synthesis in tissues from cold acclimatized goldfish than the warm acclimatised fish. Helmy *et al*. (1974) reported variations in plasma proteins between summer and winter attribute the difference to temperature variations in the environment. Pamparathi Rao (1965) observed that in cold acclimatized animals the free amino acids in body fluids decrease, while bound amino acids and total proteins within the cells and tissues increase. Haschemeyer (1969) found that low environmental temperatures during winter induce the synthesis of plasma protein due to increased metabolic activity of liver.

Large numbers of studies have been made to study the utilization of glucose and maintenance of its plasma levels, in different species of fishes. Glucose concentration in the blood of fish is considered to be a highly sensitive indicator of processes like hormonal balance, quality of food, activity of enzymes in metabolic pathways, rate of metabolism, spawning and maturity and the activity of fish in general. The mechanism of blood glucose regulation in fish has not been thoroughly worked out. However, it is known that insulin reduces its concentration in blood, while adrenaline is known to raise blood glucose levels, (Benttey and Follet, 1965). Glucose concentrations in the blood have been reported to be greatly affected by temperature changes

in the environment and are also know to be increased during high carbohydrate diet (Love, 1970; Connors *et al*., 1978).

Nakono and Tomlinson (1967); Lorz *et al*. (1978) reported that heavy metals, including copper, may produce general stress effects in fishes increasing blood sugar, catecholamine and cortisol concentrations in blood plasma. Scarfe *et al*. (1982) and Nemcsok and Hughes (1988) reported an increase in blood glucose levels due to copper sulphate ($CuSO_4$) exposure. Further, the author suggested that $CuSO_4$ could produce stress in fishes, which is reflected by their elevated locomotor activity. El-Naga *et al*. (2005) also reported an increase in glucose due to cadmium and copper toxicity.

Urea is the major end-product of protein and purine metabolism in freshwater fishes in addition to ammonia (Goldstein and Forster, 1965, Cvancare, 1969 a, b; Watts and Watts, 1974). It is excreted mainly through kidneys and to a limited extent through gills. Marine fishes show high blood urea concentration where it plays an important role in maintaining osmolarity (Price and Creaser, 1967; Urist and Van de Pulte, 1967; Holmes and Donaldson, 1969).

Freshwater fishes on the other hand show low blood urea levels mainly because it does not play any major osmoregulatory role. In starvation, fishes have been shown to exhibit significant fall in blood urea (Vellas and Sarfaty, 1967; Tandon and Masih, 1983). Seasonal variations in blood urea concentration in fishes, therefore, reflect varying food sources and osmotic fluctuations in the environment.

The urea levels ranging between 4 – 6.7 mg% have been reported in gold fish and various species of trout (Field *et al*., 1943; Phillips and Brockway, 1958; Sano, 1962). Seasonal variations in urea content were found in *Salmo giardneri* with the lowest values in April – July (Sano, 1962).

Pora and Precoop (1960) found that *Cyprinus carpio* starved for 90 days excreted less organic nitrogen per day than fed ones. Sano (1962) who measured the urea output of *Anguilla japonica* during starvation found that it dropped sharply during first five days and then examined steadily upto 90th day when the experiment was concluded. The nitrogen excretion in the same species of fish starved for 60 days showed a decrease. The levels in summer caught fish were higher presumably because of the higher temperature and metabolic rate (Inui and Ohshima, 1966).

Creatinine is also an excretory product derived from muscle creatine and phosphoscreatine. It is not metabolised in fishes and is known to be completely excreted through the renal tubules. Very limited work is reported in literature on blood creatinine levels. The creatinine clearance is however; known to reflect tubular secretion in freshwater fishes (Hickman and Trump, 1969).

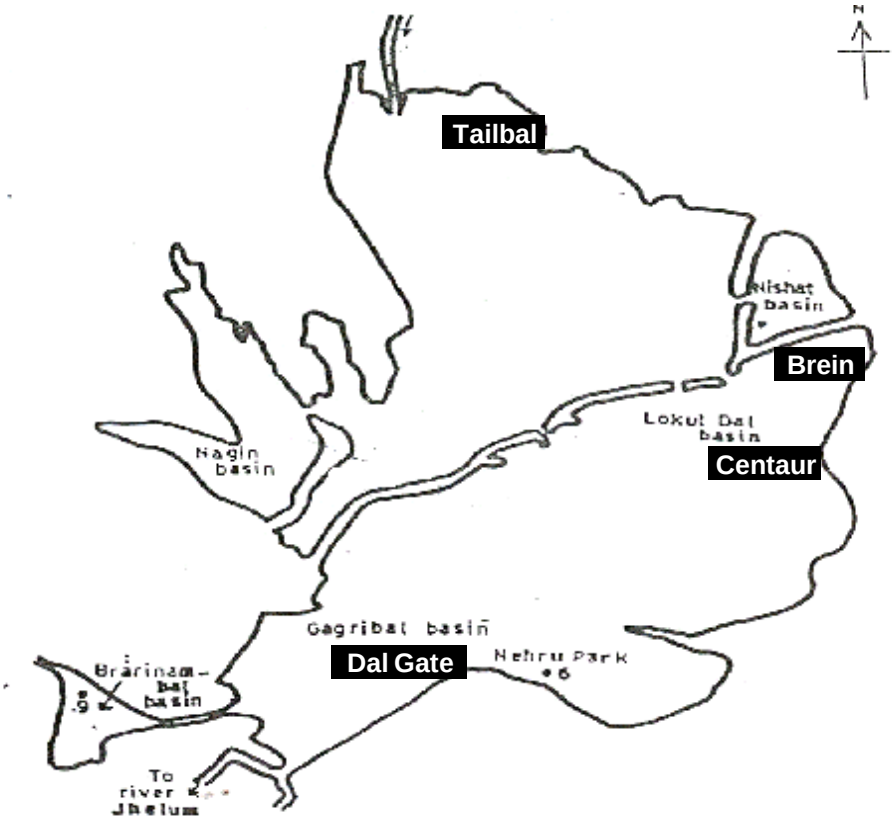
Chang and Idler (1960) reported a high level of creatine (7mg %) in the normal sockaye salmon male as against 2mg % in the female. In the migratory forms, the male gonad creatine level increases to 100mg % while in the female it increases to 12mg % only. The greater increase in the male gonad indicates the formation of the phosphagen store of the spermatozoa required for efficient motile gametes.

Fish blood possesses creatine but the presence of creatine synthesising system has not yet been unequivocal shown (Alekseeva and Arkhangel'skaia, 1964). This is presently due to plentiful supply of creatine in the environment. Blood creatine and creatine levels were found usually in the range of 0.5 – 2.0 mg % (Smith, 1930 a; Field *et al*., 1943; Sano, 1962).

The present study was conducted with the seasonal collection of two fishes viz. *Schizothorax niger* Heckel and *Cyprinus carpio* Linnaeus from different sites of Dal Lake and River Jhelum and estimation of heavy metals in water and fish tissues by atomic absorption spectrophotometry; and examination of their deleterious effects by histopathological and histochemical methods. Consequently biochemical estimations were carried out for selected parameters, from fish serum to examine the alterations subsequent to these toxic exposures.

3.1. Collection sites

In the present study, two water bodies (Figure 1 and 2) were selected viz. Dal Lake and River Jhelum in and around Srinagar area. Representative fish specimens were collected from these two sources at selected sites and pooled together as a sample size (Table II).



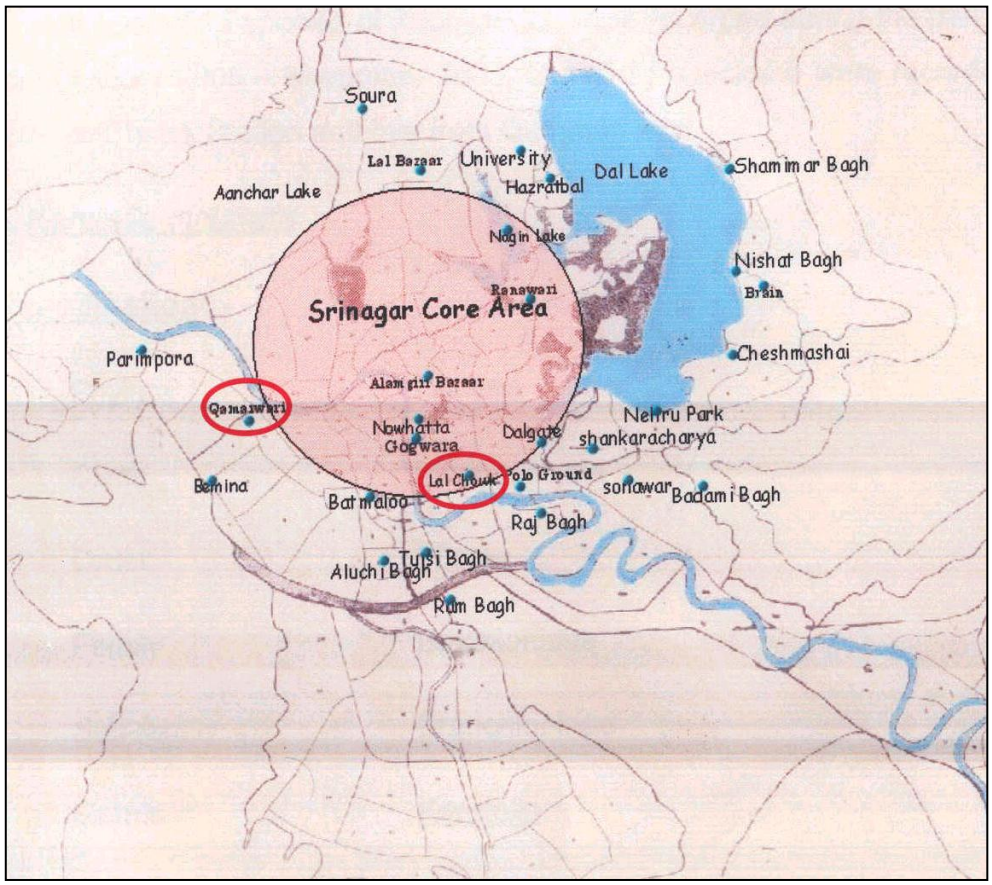
Source: LAWDA

Figure 1: Map showing different collection sites of Dal Lake

Table II: Sources of water bodies and collection sites in and around Srinagar city

Dal Lake

Collection sites	
Site I	Dal gate
Site II	Centaur
Site III	Brein
Site IV	Nishat



Source: LAWDA

Figure 2: Map showing different collection sites of River Jhelum

River Jhelum

Collection sites	
Site I	Chattabal
Site II	Qamarwari
Site III	Gadhanji Pora (Jawahar Nagar)
Site IV	Habba Kadal

Fishes were collected from both the water bodies with the help of local fishermen and were brought alive in plastic buckets to the laboratory for investigating the different parameters.

3.2. Species and number of fish used

The study was conducted using two representative fish species- *Cyprinus carpio* Linnaeus and *Schizothorax niger* Heckel, naturally and readily found in both the water bodies. Pooled specimens from any of the collection sites of both the water bodies to make a sample size of 25 fish of each species (of either sex) with an average length of 30-40 cms were collected. The study was repeated for each season for the year-I (2005-2006) and again during Year-II (2006-2007). A total of 800 fishes were used for the present study.

3.3. Seasonal classification

The study was conducted in four seasons, each with duration of 3 months, during the year 2005-2006 and 2006-2007. The four seasons were categorized as:

Season	Month	Duration
Spring	March-May	3 months
Summer	June-August	3 months
Autumn	September-November	3 months
Winter	December-February	3 months

After collection of the fishes from the Dal lake and the River Jhelum, the fishes were identified on the basis of key provided by Kullander *et al*. (1999).

The fishes were carried to the laboratory in plastic bucket. Every effort was made to keep the fish alive. The blood was collected in different labeled collection vials. The fishes were dissected midventrally and all the visceral organs were taken out in a big tray. Then various visceral organs viz. gills, liver, kidney and muscle were placed in separate petridishes containing normal saline and were later processed for the various selected parameters.

The methodology adopted in the present study was divided into four section viz. Section I, Section II, Section III and Section IV dealing with biochemistry, metal detection, histochemistry and histopathology respectively.

3.4. Biochemical estimation of fish serum

In order to study the effects of heavy metals on fish biochemical parameters, serum was collected from live fish specimens. The following serum parameters were studied- total protein, albumin, globulin, blood glucose, urea, creatinine and cholesterol.

3.4.1. Collection of blood

Blood samples for biochemical estimation were collected from live fishes by either severing/puncturing the caudal vessel or puncturing the heart/dorsal aorta, which ever method was convenient at the time of collection.

Handling of blood: - Blood samples for harvesting of serum were collected in different labeled vials containing no anticoagulants. Blood samples before processing for biochemical estimation were centrifuged at 3000 rpm for 10-15 minutes and the liquid fraction in the form of serum was obtained.

3.4.2. Estimation of total protein

Total protein content of the serum was estimated using commercial estimation kit- Liquichem Total Protein (Recorders and Medicare Systems (P) Ltd., India), by the Biuret method (Tietz, 1976). The method is based on the principle that proteins and peptides containing at least two adjacent peptide bonds react with cupric ions in alkaline solution forming a violet coloured complex having absorption maximum at 550 nm using an Auto Analyzer.

Procedure

1. 1.0 ml of total protein reagent was dispensed into tubes labeled reagent, blank, standard and test.

	Blank	**Standard**	**Test**
Reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

2. 10 µl specimen was placed into appropriately labeled tubes and were mixed well. Distilled water was used as sample for reagent blank.
3. The test samples were permitted to stand at room temperature (15-30^0C) for 15 minutes.
4. The instrument was set to zero at 540 nm using the reagent blank.
5. The absorbance values were recorded for standard, control and test within 60 minutes after colour development.

Calculation of results

$$[\text{Total protein}](\text{mg/dl}) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 4(\text{g/dl})$$

3.4.3. Albumin estimation

The serum albumin was estimated using commercial estimation kit-Liquichem Albumin (Recorders and Medicare Systems (P) Ltd., India), by the BCG method (Lynch, 1969). This method is based on the principle that serum albumin can bind with certain dyes such as bromocresol green, forming coloured complexes. The blue green complexes formed had maximum absorption at 630 nm.The concentration of albumin in serum is obtained by comparing the intensity of coloured solution of an unknown to a known albumin concentration when read at 630 nm.

Procedure

1. 1.0ml albumin reagent was dispensed into tubes: reagent, blank, standard and test.

	Blank	**Standard**	**Test**
Reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

2. 0.01ml specimen was placed into appropriately labeled tubes and were mixed well. Deionized water was used as specimen for reagent blank.
3. Test samples were permitted to stand at room temperature (15–30^{0}C) for 3 minutes.
4. The instrument was adjusted to zero absorbance at 630 nm using reagent blank.
5. Absorbance values were recorded.

The final colour was stable for at least 60 minutes.

Calculation

$$\text{Albumin}(\text{g/dl}) = \frac{\text{Absorbance of test}}{\text{Absorbance of Std.}} \times 4$$

3.4.4. Globulin

The amount of total globulin was calculated by deducting the calculated value of albumin from calculated value of total protein.

Thus total Globulin

Globulin g/dl = Total protein g/dl – Albumin g/dl

3.4.5. Blood sugar estimation

For the analysis of blood sugar enzymatic kit method as described by Tietz (1976) was employed using commercial estimation kit.

Glucose → Gluconic acid + Hydrogenperoxide→Hydroxibenzoate + 4-aminophenazene.

Preparation of working glucose reagent

6 × 100 ml pack working glucose reagent was prepared by transferring the contents of one vial of glucose reagent-1 to black plastic bottle, after reconstituting it to 100 ml with distilled water, 5 ml of phenol reagent (reagent 3) was added and these were mixed well and stored at cool dry place at 2 to 8^{o}C. This working solution remains stable for 45 days at 2 to 8^{o}C.

Protocol for spectrophotometry

Reagent	**Blank (B)**	**Standard (S)**	**Test (T)**
Serum/Plasma	-	-	0.2 ml
Reagent 2: Glucose standard 100 mg%	-	0.2 ml	-
Working glucose reagent	1.5 ml	1.5 ml	1.5 ml
Distilled water	1.5 ml	1.5 ml	1.5 ml

The contents of tubes were mixed well and incubated at 37°C for 30 minutes. The colour intensity was measured in a spectrophotometer at 510 nm against distilled water.

Calculations

$$\text{Serum/plasma glucose mg/100ml} = \frac{\text{Optical Density of Test} - \text{Optical Density of Blank}}{\text{Optical Density of Standard} - \text{Optical Density of Blank}} \times 100$$

3.4.6. Estimation of blood urea

Berthelot method (Trinder, 1969).

Urea + Urease $\longrightarrow$ Ammonia + CO_2

Ammonia + Hypochlorite + Salicylate $\longrightarrow$ Sodium Nitroprusside

Green colour

Reagent preparation: - 25ml of distilled water was added to 1 vial of R_1 and the contents were dissolved slowly without frothing.

Assay procedure

Dispose	Blank	Standard	Sample
R_1	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl
Mix, incubate for 5 minutes at 37^0C, then add			
R_2	1000µl	1000µl	1000µl

Mix, incubate for 5 minutes at 37^0C.

Calculation: - Optical density was measured at 578 nm against reagent blank. Concentration of urea was measured in sample in mg/dl.

$$\frac{\text{Optical Density of sample}}{\text{Optical Density of standard}} \times \text{conc. of standard (i.e., 40)}$$

3.4.7. Estimation of serum creatinine

"Alkaline picrate method" of Baum (1975)

Working standard solution was prepared by diluting 0.1 ml of stock standard to 10 ml, with diluted water. Firstly, the serum was deproteinized as under:

Serum	:	1 ml
Distilled water	:	1 ml
Creatinine reagent A	:	6.0 ml

These were mixed in a test tube and kept in boiling water for one minute. After cooling the tube was centrifuged till the supernatant became clear.

Test procedure

Three borosil test tubes were selected and marked as blank, standard and test, respectively.

Reagent	**Blank (B)**	**Standard (S)**	**Test (T)**
Supernatant step I	-	-	4 ml
Working standard	-	1 ml	-
Distilled water	1 ml	-	-
Creatinine reagent A	3 ml	3 ml	-
Alkaline reagent	1 ml	1 ml	1 ml

The tubes were mixed well at room temperature for 20 minutes and the optical density measured against distilled water at 520 nm in a spectro-photometer.

Calculation

$$\text{Serum creatinine in mg/dl} = \frac{\text{Optical Density of Test} - \text{Optical Density of Blank}}{\text{Optical Density of Standard} - \text{Optical Density of Blank}} \times 30$$

3.4.8. Cholesterol estimation

The serum cholesterol was estimated by Cholesterol oxidase method (Trinder, 1969). It is based on the principle that cholesterol assay involves sequential enzymatic reactions. Cholesterol oxidase then oxidizes cholesterol to form cholest – 4 – en – 3 – one and hydrogen peroxide. Peroxidase catalyzes the hydrogen peroxide oxidation of 4-aminophenazone with subsequent coupling to p-hydroxybenzensulfonate. The end product is a quinoneimine dye which absorbs at 520nm. The order intensity at 520nm is directly proportional to serum cholesterol concentration.

Cholesterol esters $\xrightarrow{CHE}$ Cholesterol - fatty acid

Cholesterol + O_2 $\xrightarrow{CO}$ Cholest-4-en-3-one + H_2O

H_2O_2 + 4-AAP + Phenol $\xrightarrow{POD}$ Red quinoneimine + H_2O

Procedure

1. Kit was prewarmed for five minutes at 37^0C or until reached at room temperature.

	B	S	U
Reagent	1000µl	1000µl	1000µl
Distilled Water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

2. 1.0 ml cholesterol reagent was dispensed into tube labeled.
   reagent, blank, standard and test.
3. 0.01ml specimen was placed into appropriately labeled tubes and were mixed well. Distilled water was used as sample for reagent blank.
4. All tubes were incubated at 37^0C for 10 minutes or at room temperature for 20min.
5. The instrument was set to zero at 510 nm using reagent blank.

6. Absorbances were recorded for standard, control and test within 30 minutes after colour development.

Calculation

$$\text{Total cholesterol}(\text{mg/dl}) = \frac{\text{Absorbance of test}}{\text{Absorbance of Std.}} \times 200\,(\text{Std. Conc. mg/dl})$$

3.5. Metal analysis of water and fish tissue

For detection of metals in water, the samples were collected in conical flasks, filtered through Whatman's filter paper and processed in atomic absorption spectrophotometer (AAS) for estimation of various metal concentrations.

Since gill, liver, kidney and muscle are the sensitive targets to metals, the accumulation and histological lesions in these organs are reported to be predominant. These organs were processed for detection of metals viz. Zinc, Copper, Manganese and Iron. The following methodology was undertaken:

3.5.1. Estimation of Zinc, Copper, Iron and Manganese

Principle

Zn, Cu, Mn, Fe are generally determined by Atomic Absorption Spectrophotometer (AAS). Metallic elements which normally remain at ground state, when subjected to radiations of specific wave length under flame they absorb energy. The absorption of radiations is proportional to the concentration of atoms of the element in a solution. Therefore, concentration of Zn, Cu, Mn and Fe was measured with the help of AAS having greater sensitivity and accuracy.

Apparatus

Atomic absorption spectrophotometer (Electronic Corporation of India, ElementAS AAS 4141), digestion assembly (Hot plate), conical flask, pipette volumetric flask, sample extract vials, etc.

Reagents

Nitric acid (HNO_3).

Procedure: - For the analysis of metal concentration, Lindsay and Norwell (1978) method was employed. The tissue samples viz. gills, liver, kidney and muscles were dried at 120^0C for 48 hours weighed and incinerated at 550^0C for 8-10 hours. 1gm of oven dried fish sample was taken and transferred into 100 ml volumetric flask and 20 ml of nitric acid was added to it and kept overnight. The contents of the flask were heated on hot plate till the fumes of NO_2 stop or 3 to 5ml of digested material remained. The digestion was deemed to be completed when contents of flask became colourless.

1. After cooling, double distilled water was added to the contents of the flask so as to make the volume up to 100 ml.
2. The contents of the flask were filtered through Whatman's No. 1 filter paper and this extract was used for the determination of Zn, Cu, Mn and Fe.
3. The final standard of solutions from stock solutions of Zn, Cu, Mn and Fe were prepared and the readings on a graph paper were noted i.e., concentration on x-axis and AAS readings on y-axis and standard curve was prepared.

Method

The different standard stock solutions were made from the stock solution (1000 ppm) for estimation of metals in the fish tissue.

i) For iron: Standard stock solution = 1000 ppm.

The different standard stock solution such as 2 ppm, 4 ppm, 8 ppm and 16 ppm were made

i) For Cu make 0.1, 0.2, 0.3, 0.4, 0.5, 1.0 ppm

ii) For Zn make 0.5, 1.0, 1.5, 2.0 ppm

iii) For Mn make 1, 2, 3, 5, 7 ppm.

5. After calibration the samples were fed one by one and the readings were taken.

Observation and calculation

1. weight of sample taken = 1 gm
2. volume of extract prepared = 100 ml
3. ppm of Zn or Cu, or Mn or Fe in fish = Rx100.

Where R = ppm of respective element

For example, If reading for Iron = 7.0

It was calculated by multiplying it with dilution factor: 1 gm x 100 ml (0.7) i.e., 7.0 x 100 = 700 ppm i.e., sample contains 700 ppm (Fe). Water samples were simply filtered through Whatman's filter paper No. 42.

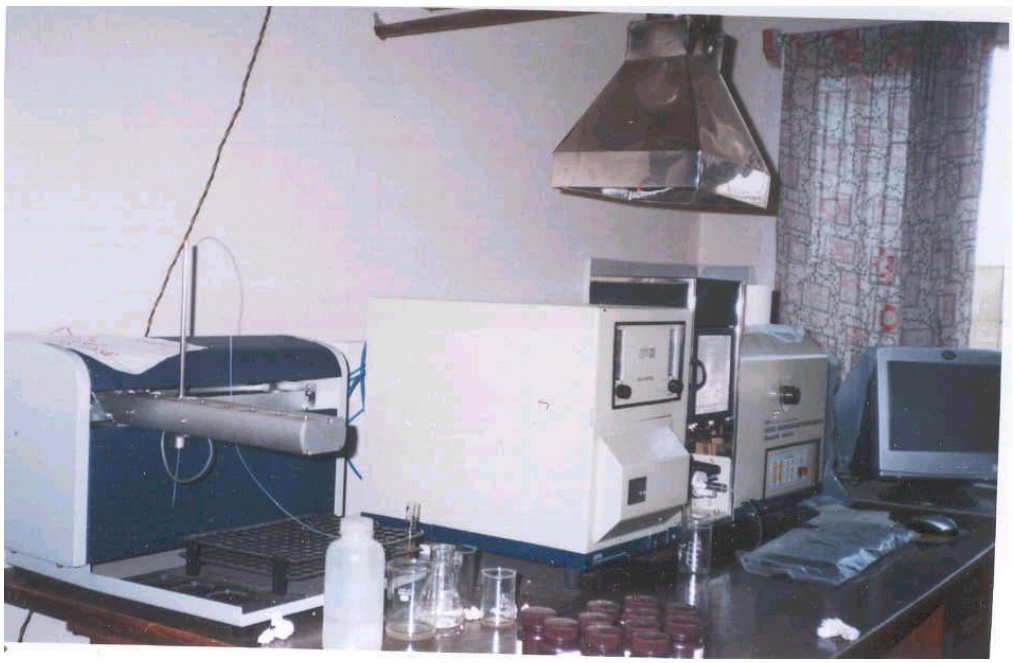


Fig. 3.5.1. Electronic Corporation of India, Atomic absorption spectrophotometer, Element AS AAS 4141

3.6. Histochemical demonstration of metal residues in fish tissues

For detection of metals viz copper, iron and zinc in fish gills, liver, kidney and muscles following hitochemical methods were used.

Perl's method for Iron (Luna, 1988)

Solutions required

10 % Potasium ferrocyanide solution (stock)

Potassium ferrocyanide	-	10.0 gm
Distilled water	-	100.0 ml.

10 % Hydrochloric acid solution (stock)

Hydrochloric acid, concentrated	-	10.0 ml
Distilled water	-	90.0 ml.

Potassium ferrocyanide – Hydrochloric acid solution (Working)

Potassium ferrocyanide solution (stock)	-	70.0 ml
Hydrochloric acid solution (stock)	-	30.0 ml

Mixed just before use.

Nuclear fast red (Kernechtrot) Solution

0.1 gm nuclear fast red was dissolved in 100 ml of 5% solution of aluminium sulfate with aid of heat, filtered and grains of thymol were added as preservative.

Staining Procedure: -

1. The slides were deparaffinized and hydrated to distilled water.
2. Potassium ferrocyanide solution was stocked for 5 minutes.

3. The slides were placed in working potassium ferrocyanide-hydrochloric acid solution for 20 minutes.
4. The slides were rinsed well in distilled water.
5. Nuclear fast red solution was used as counter stain in for 5 minutes.
6. Then slides were washed well in running water.
7. Dehydration was done in 95% alcohol, absolute alcohol and then clearing in Xylene, two changes each.
8. Mount with Permount or DPX.

Result: - Sites of iron (hemosiderin)- blue.

Dithiooxamide method for copper

Solution required

Dithiooxamide (rubeanic acid)	0.2g
70% aqueous ethanol	200ml
Sodium acetate (CH_3 COONa)	0.4g

Procedure

1. Frozen or paraffin sections were taken to water.
2. Then immersed in the dithiooxamide reagent (prepared by combining 0.2g of dithiooxamide and 0.4g of sodium acetate) for 30 min.
3. Rinsed in two changes of 70% ethanol.
4. Dehydrated, cleared and mounted in a resinous medium.

Result: - Sites of copper dark green to black.

Notes: - According to pearse (1985), protein-bound copper can be released by placing the slides (after de-waxing) face downwards over a beaker of concentrated hydrochloric acid for 15 minutes followed by placing for 15 minutes in absolute ethanol.

Dithizone method for zinc

Solution required

A. Dithizone stock solution

Dithizone	100 mg
Absolute acetone	100 ml

B. Complexing solution

Sodium thiosulphate ($Na_2S_2O_3.5H_2O$)	55 g
Sodium acetate (anhydrous)	5.4 g
Potassium cyanide (KCN) (Caution: Poisonous)	1.0 g
Water	100 ml.

Salts were dissolved in water. Then little dithizone was dissolved in 200 ml of carbon tetrachloride. The aqueous solution was shaken in a separatory funnel with successive 50 ml aliquots of the solution of dithizone in CCl_4 until the CCl_4 layer was clear green colour. This manipulation extracts traces of zinc from the reagents.

C) 1.0 M acetic acid

Glacial acetic acid.	60 ml
Water	to 1000 ml

D) Sodium potassium tartarate solution

$NaKC_4H_4O_6.4H_2O$	2 g
Water	to 100 ml

E) Working dithizone solution.

This was mixed when needed and used immediately.

Solution A	24 ml

Water 18 ml

1.0 M acetic acid (Solution C) was placed in 0.1 ml aliquots until the pH was 3.7 (about 2ml required)

Solution B 5.8ml

Solution D 0.2ml

F) Chloroform.

Required for rinsing.

Procedure

1) Cryostat sections were allowed to dry on coverslips or slides.
2) Paraffin section were de-waxed in xylene (three changes) and allowed to dry by evaporation.
3) The slides or coverslips bearing the sections were immersed in freshly mixed working dithizone solution (E) for 10 min.
4) The slides or coverslips were rinsed in two changes of chloroform (F), with agitation, for 30seconds.
5) The chloroform was poured off and the slides or coverslips were allowed to drain but the solvent was not let to evaporate completely (to avoid cracking of the sections).
6) Rinsed in water, allowed most of the water to drain off into filter paper and a drop of an aqueous mounting medium was put onto each section. Slides or coverslips were applied according to the type of preparation.

Results: - A red to purple color is formed where zinc is present in the tissues.

Notes

1) The complexing solution was mixed with the dithizone to prevent the formation of coloured chelates with metals other than zinc; 24 ml of solution A may be diluted with 16ml of water instead of with solution B, C and D as described above. This simple dithizone solution will form

colored complexes with Ag, Au, Bi, Cd, Co, Cu, Fe, Hg, In, Mn, Ni, Pb, Pt, Sn and Tl, as well as with zinc.

2) Zinc was removed by treating control sections for 5 minutes with 1% aqueous acetic acid before staining.

3.7. Histopathological methods

In order to check the deleterious effects of metals on different organs of the fish, the gills, liver, kidneys and muscles were analyzed histologically. The procedure is given as:

3.7.1. Fixation

The tissues were immediately placed in Bouin's fluid, keeping the volume of fixatives 30 to 50 times the volume of the tissue. The procedure for preparation of the fixative is as follows:

Bouin's Fluid

Composition:

Picric acid, saturated aqueous solution	750.0 ml
37–40 % formalin	250.0 ml
Glacial acetic acid	50.0 ml

Depending upon the size of tissue, fixation was done between 4-12 hours. Preferably fixation was done for 12 hours to several days.

After fixing in Bouin's solution, it was washed in several changes of 50% alcohol for 4-6 hours, agitating constantly, to ensure proper removal of the picric acid, and then stored in 70% alcohol. The removal of acid from tissues was most essential in order to ensure proper staining of the tissue sections.

10% Neutral buffered formalin

Composition:

Sodium dihydrogen phosphate, monohydrate ($Na_2H_2PO_4$-H_2O) 4.0 g

Sodium monohydrogen phosphate anhydrous (Na_2HPO_4) 6.0 g

After fixing the material in bouin's fluid, the tissues were washed in 70% alcohol for several hours.

3.7.2. Post fixation treatment

Since fixed tissue was not firm enough to section on the microtome, the tissue was infiltrated and embedded with some supporting substance such as paraffin. The supporting substance furnished stability and held the tissue components in proper relationship to each other. As paraffin and water are not miscible, the tissues were first dehydrated and cleared, then infiltrated and embedded in paraffin.

3.7.3. Dehydration of the tissues

It was the removal of all extractable water by a dehydrant such as ethanol, isopropyl alcohol, dioxin or acetone. Here the tissues were passed through different types of graded alcohol series (80%, 90%, 100%) keeping the volume of the alcohol 10 times the size of the tissue. The time duration in each alcoholic concentration is given below in the flow chart:

Flow Chart for Dehydration

Step	Timing	Solution
1	Holding point	80% alcohol
2.	Two hours (1st change)	95% alcohol
3.	One hour (2nd change)	95% alcohol
4.	One hour (1st change)	100% alcohol
5.	One hour (2nd change)	100% alcohol
6.	One hour (3rd change)	100% alcohol

3.7.4. Clearing/ Dealcoholization

Since alcohol and paraffin are immiscible, therefore the immediate step between dehydration and paraffin infiltration was clearing. Clearing reagent removed the dehydrant and made tissue clear and translucent signifying the completion of the process. The most widely used clearing agent was xylene, but it hardened the tissue unless the clearing time was controlled.

The tissues after dehydration were cleared in xylene keeping the volume of the clearing agents 10 times the size of the tissues. The process of clearing with duration was as follows:

Step	Timing	Solution
7.	One hour (1st change)	Xylene
8.	One hour (2nd change)	Xylene

3.7.5. Infiltration or Impregnation

After the tissue was thoroughly permeated with the clearing agent, the tissue was infiltrated with a supporting medium such as paraffin. The tissues placed in the paraffin beaker in the paraffin for a period of 2 hours. The temperature of the oven was maintained at 60^0C and the volume of the paraffin wax was kept 15 to 20 times the volume of the tissue. The tissues were then transferred to the second and third paraffin beakers for two hours and 1½ hours respectively.

Step	Timing	Solution
9.	2 hours (1^{st} change)	Paraffin
10.	2 hours (2^{nd} change)	Paraffin
11.	1½ hours (3^{rd} change)	Paraffin

3.7.6. Embedding or Casting or Blocking

Embedding was the orientation of tissue in melted paraffin, which when solidified, provides a firm medium for keeping intact all parts of the tissue when sections were cut. In suitable embedding molten paraffin, the tissue was placed so that it is sectioned in the proper plane. The paraffin was cooled and hardened within and around the tissue, enclosing the tissue in a solid mass. The classic paper boat or L-shaped iron rods were used as mold for embedding.

Embedding paper boats were prepared with a depth of at least twice the thickness of tissue. These boats were filled with molten paraffin. With the help of warm forceps, the tissues were picked up gently and placed into mold keeping the tissue towards the bottom of the mold and centered, leaving a

margin of several millimeters around the tissue. Manipulation of the tissue in the mold with proper orientation was quick, so that paraffin did not begin to harden. The paraffin block was allowed to harden and then immersed into shallow, cool (10^0C) water bath for 10 to 15 minutes to hasten solidification of the paraffin. When the paraffin was completely hardened, it was then removed from mold and labeled with a code number.

3.7.7. Trimming of the block

The paraffin block was carefully trimmed attached to the microtome peg with the help of a good scalpel with a clean even edge; the excess paraffin was removed so as to maintain proper size of the block. This made the blocks transparent. The paraffin tissue block was attached to a base of peg for clamping into the microtome. With the help of heated spatula, a central cavity was melted into the surface of the block holder and the trimmed tissue block was immediately placed into the cavity so that the front of the block (the face along which sections were to be cut) was uppermost, the tissue block was to be gently but firmly pressed down against the peg. The tissue block was kept exactly perpendicular to the surface of the block holder. While setting the base, the block holder containing the tissue block was immersed in cold water in a beaker for complete setting.

A label with code number was attached to the block holder/peg by encircling it with a piece of cellophane tape.

3.7.8. Microtomy or Sectioning

The solid paraffin block containing the tissue was sliced into thin desired sections of 3 to 7 microns on a rotary microtome (WESWOX).

After the desired numbers of sections were taken, all blocks were sealed with paraffin so as to prevent drying and other damage of the exposed tissue/material.

3.7.9. Affixing

Before affixation, the slides were cleaned scrupulously. The sections of tissues were attached to these slides using Mayer's affixative with the following composition:

Mayer's affixative

Egg albumin	:	50.0 ml
Glycerin	:	50.0 ml
Sodium salicylate	:	1 gm

A small drop of Meyer's egg albumin was smeared over the surface of the slide with the finger and rubbed. Sections were put on the albuminized slides. The slides were then placed on a flat surface and a few drops of floating medium (preboiled distilled water) were put to the center of each slide with the aid of a brush. The sections were drained approximately one minute before final drying at 45-50^{0}C in oven for 30 minutes or on a slide-warming plate. Using a diamond pencil each slide was clearly labeled.

3.7.10. Staining

The routine Haematoxylin and Eosin stain (Weesner, 1968) was used for histological / histopathological study.

3-7 microns thick paraffin sections of the material were stained in the said stains.

Reagents required

1) Harris Haematoxylin

Haematoxylin crystals	- 5.0 gm
Ethanol	- 50.0 ml
Ammonium or Potash alum	- 100.0 gm

Distilled water - 1000.0 ml

Mercuric oxide (red) - 2.5 gm

Haematoxylin was dissolved in the alcohol, the alum in the water with the aid of heat. Both the solutions were mixed and brought to boil as rapidly as possible. After removing from heat, mercuric oxide was added slowly and was reheated till dark purple color developed. The solution was allowed to cool in a basin of cold water.

2) Eosin solution

1% stock alcoholic eosin

Eosin y, water soluble - 1.0 gm

Distilled water - 20.0 ml

Dissolve and add:

Alcohol (95%) - 80.0 ml

Working Eosin Solution

Eosin stock Solution - 1 part

Alcohol (80%) - 3 parts

Just before use, 0.5ml of glacial acetic acid was added to each 100 ml of stain and stirred.

3) 1% Acid Alcohol

HCl - 1ml

90% alcohol - 100 ml

4) 0.1% Ammonium Water

Ammonia - 0.1ml

Distilled water - 100 ml

Routine Harri's Haematoxylin and Eosin staining method

The progressive method of staining of Harri's Haematoxylin and Eosin (H&E) was employed. The timing/ duration of staining in H&E was as given below:

1. Deparaffinization

Xylene I	- 15 min.
Xylene II	- 15 min.
Xylene III	- 15 min.

2. Hydration

Absolute Alcohol I	- 5 min.
Absolute Alcohol II	- 5 min.
90% Alcohol	- 5 min.
80% Alcohol	- 5 min.
70% Alcohol	- 5 min.
50% Alcohol	- 5 min.
Distilled water	- 15 min

3. Staining

Harris Haematoxylin	- 7 min.
Distilled water	- Rinse
Acid Alcohol (1%)	- one dip
Distilled water	- Rinse
Ammonia water (0.1%)	- Till optimum blue
Tap water	- Wash
Distilled water	- Rinse

Eosin stain (Alcoholic)	- 4 minutes

4. Dehydration

50% alcohol	- 30 seconds
70% alcohol	- 30 sec
80% alcohol	- 30 sec.
95% alcohol	- 30 sec.
Absolute Alcohol I	- 30 sec.
Absolute Alcohol II	- 30 sec.
Absolute Alcohol III	- 30 sec.

5. Clearing

Xylene I	- 30 min.
Xylene II	- 30 min.
Xylene III	- 60 min.

6. Mounting

Mount in paramount / DPX mountant.

After staining, the stained tissue sections were labelled clearly and stored until the mountant hardened.

3.7.11. Photomicrography

The microscpic study of the stained tissue sections for histopathological study was done with the help of trinocular microscope (Labomed, India) using different lens combinations. Photomicrographs were taken using OLYMPUS (PM-6).

The present study on metal induced biochemical and histopathological changes of fish revealed the following results:

4.1. Biochemical Estimations

The biochemical parameters viz. total protein, albumin, globulin, blood glucose, blood urea, serum creatinine and blood cholesterol were estimated seasonally in *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum and Dal Lake.

4.1.1. Protein Estimation

Dal lake: The estimation of total proteins in the blood of fishes collected from Dal lake varied seasonally from a low concentration of 1.05 ± 0.05 g/dl to a high concentration of 4.32 ± 0.13 g/dl during the entire period of study (Table XXI-XXIV).

Protein concentration in *Schizothorax niger* varied from1.11 ± 0.02 g/dl to 4.32 ± 0.13 g/dl (Fig. 69). A minimum value of total proteins was observed in winter season of year-II and maximum value in summer season of 2006-07. Further, the total protein estimation during rest of the seasons

showed different concentrations. A concentration of 2.10 ± 0.44 g/dl and 2.16 + 0.07 g/dl in respective spring seasons; 3.95 ± 0.17 g/dl and 4.32 ± 0.13 g/dl in summer season; 2.85 ± 0.29 g/dl and 2.15 ± 0.08 g/dl in autumn seasons and 1.82 ± 0.10 g/dl and 1.11 ± 0.02 g/dl in winter seasons during the entire study period was observed.

In contrast to *Schizothorax niger*, the *Cyprinus carpio* spp. showed a total protein concentration of 1.05 ± 0.05 g/dl to 3.79 ± 0.30 mg/dl during the entire study period (Fig. 70). The total protein values varied from 1.69 ± 0.22 g/dl to 1.82 ± 0.10 g/dl in respective spring seasons of two years of study, 3.79 ± 0.30 g/dl and 3.68 + 0.33 g/dl in respective summer seasons, 2.88 ± 0.42 g/dl and 3.13 ± 0.12 g/dl in respective autumn seasons and 1.05 ± 0.05 g/dl and 1.12 ± 0.02 g/dl in respective winter seasons of two years of study. However, lowest concentration of protein in *Cyprinus carpio* spp. were observed in winter season (year-I) and highest concentration in summer season (year-I).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of total proteins in different seasons during the entire study period (Table XXI-XXIV).

In spring seasons the *Schizothorax niger* showed total protein values of 2.18 ± 0.09 g/dl and 2.33 ± 0.15 g/dl. In summer seasons the values recorded were 3.91 + 0.22 g/dl and 4.23 + 0.43 g/dl, in autumn seasons the values were 3.66 ± 0.15 g/dl and 2.71 ± 0.08 g/dl and in winter seasons 1.55 ± 0.18 g/dl and 1.38 ± 0.12 g/dl values were recorded. The minimum concentration of total proteins were observed in winter season (year-II) and maximum values were recorded during summer season (year-II) (Fig. 71).

Cyprinus carpio spp. collected from river Jhelum showed varied total protein concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 72). Total protein concentrations of 1.92 ± 0.13 g/dl and 1.88 ± 0.18 g/dl in respective spring seasons, 2.35 ± 0.12 g/dl and 3.13 ± 0.40 g/dl in respective summer seasons, 2.26 ± 0.07 g/dl and 2.39 ± 0.22 g/dl in respective autumn seasons and 1.61 ± 0.05 g/dl and 1.86 ± 0.09 g/dl in respective winter seasons were observed.

4.1.2. Albumin Estimation

Dal lake: The estimation of albumin in the blood of fishes collected from Dal lake varied seasonally from a low concentration of 0.99 ± 0.04 g/dl to a high concentration of 3.72 ± 0.38 g/dl during the entire period of study (Table XXI-XXIV)

Albumin concentration in *Schizothorax niger* ranged from 1.02 ± 0.00 g/dl to 2.00 ± 0.10 g/dl (Fig. 73). Minimum value of albumin was found in winter season of year-II and maximum value in summer season of year-I. Further, the albumin estimation during rest of the seasons showed different concentrations. A concentration of 1.41 ± 0.02 g/dl and 2.00 + 0.03 g/dl in respective spring seasons; 2.00 ± 0.10 g/dl and 1.95 ± 0.28 g/dl in summer season; 1.85 ± 0.11 g/dl and 1.12 ± 0.03 g/dl in autumn seasons and 1.09 ± 0.01 g/dl and 1.02 ± 0.00 g/dl in winter seasons during the entire study period was observed.

In contrast to *Schizothorax niger*, the *Cyprinus carpio* spp. showed an albumin concentration of 0.92 ± 0.16 g/dl to 2.66 ± 0.13 mg/dl during the entire study period (Fig. 74). The albumin values varied with 0.09 ± 0.16 g/dl and 1.11 ± 0.02 g/dl in respective spring seasons, 1.66 ± 0.13 g/dl and 1.50 + 0.14 g/dl in respective summer seasons, 1.02 ± 0.00 g/dl and 2.01 ± 0.10 g/dl

in respective autumn seasons and 1.00 ± 0.00 g/dl and 0.99 ± 0.04 g/dl in respective winter seasons. However, lowest concentration of albumin in *Cyprinus carpio* spp. were observed in winter season (year-II) and highest concentration in summer season (year-I).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of total proteins in different seasons during the entire study period (Table XXI-XXIV).

In spring seasons in *Schizothorax niger* albumin values varied from 1.96 ± 0.18 g/dl and 2.18 ± 0.09 g/dl. In summer seasons the values recorded were 3.60 + 0.15 g/dl and 3.72 + 0.38 g/dl, in autumn seasons the values were 3.60 ± 0.28 g/dl and 2.51 ± 0.15 g/dl and in winter the values 1.01 ± 0.02 g/dl and 1.02 ± 0.03 g/dl. The minimum concentration of albumin were observed in winter season (year-I) and maximum values were recorded during summer season (year-II) (Fig. 75).

Cyprinus carpio spp. collected from river Jhelum showed varied albumin concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 76). Albumin concentrations of 1.92 ± 0.13 g/dl and 1.88 ± 0.18 g/dl in respective spring seasons, 2.23 ± 0.21 g/dl and 2.22 ± 0.11 g/dl in respective summer seasons, 2.16 ± 0.07 g/dl and 2.33 ± 0.16 g/dl in respective autumn seasons and 1.01 ± 0.02 g/dl and 1.21 ± 0.07 g/dl in respective winter seasons (2005-2007) were observed.

4.1.3. Globulin Estimation

Dal lake: Estimation of blood globulin fishes of Dal lake varied seasonally from a low concentration of 0.16 ± 0.05 g/dl to a high concentration of 2.37 ± 0.15 g/dl during the entire period of study (Table XXI-XXIV)

Schizothorax niger showed a concentration of 0.09 ± 0.02 g/dl to 2.37 ± 0.15 g/dl (Fig. 77). A minimum value of globulin was observed in winter season of year-II and maximum value in summer season of year-II. Further, the globulin estimation during rest of the seasons showed different concentrations. A concentration of 0.59 ± 0.02 g/dl and 0.16 + 0.04 g/dl in respective spring seasons; 1.95 ± 0.07 g/dl and 2.37 ± 0.15 g/dl in summer season; 1.00 ± 0.18 g/dl and 1.03 ± 0.05 g/dl in autumn seasons and 0.73 ± 0.09 g/dl and 0.09 ± 0.02 g/dl in winter seasons during the entire study period was observed.

In contrast to *Schizothorax niger*, the *Cyprinus carpio* spp. showed a globulin concentration of 0.06 ± 0.05 g/dl to 2.18 ± 0.19 mg/dl during the entire study period (Fig. 78). The globulin values varied with 0.77 ± 0.06 g/dl and 0.71 ± 0.08 g/dl in respective spring seasons, 2.13 ± 0.23 g/dl and 2.18 + 0.19 g/dl in respective summer seasons, 1.86 ± 0.42 g/dl and 1.12 ± 0.02 g/dl in respective autumn seasons and 0.06 ± 0.05 g/dl and 0.13 ± 0.02 g/dl in respective winter seasons. However, lowest concentration of globulin in *Cyprinus carpio* spp. were observed in winter season (year-I) and highest concentration in summer season (year-II).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of globulin in different seasons during the entire study period (Table XXI-XXIV).

In spring seasons the *Schizothorax niger* showed globulin values of 0.22 ± 0.09 g/dl and 0.15 ± 0.06 g/dl respectively. In summer seasons the values recorded were 0.31 + 0.07 g/dl and 0.51 + 0.05 g/dl, in autumn seasons the values were 0.06 ± 0.13 g/dl and 0.20 ± 0.07 g/dl and in winter seasons the values 0.54 ± 0.16 g/dl and 0.36 ± 0.09 were recorded. The minimum

concentration of globulin were observed in winter season (year-II) and maximum values were recorded during summer season (year-II) (Fig. 79).

Cyprinus carpio spp. collected from river Jhelum showed varied globulin concentrations with lowest values during winter season (year-I) and highest values during spring season (year-II) (Fig. 80). Globulin concentrations of 0.89 ± 0.12 g/dl and 0.10 ± 0.11 g/dl in respective spring seasons, 0.02 ± 0.09 g/dl and 0.91 ± 0.29 g/dl in respective summer seasons, 0.10 ± 0.00 g/dl and 0.06 ± 0.01 g/dl in respective autumn seasons and 0.60 ± 0.03 g/dl and 0.65 ±0.02 g/dl in respective winter seasons were observed.

4.1.4. Glucose Estimation

Dal lake: The estimation of glucose in the blood of fishes collected from Dal lake varied seasonally from a low concentration of 137.8 ± 17.00 g/dl to a high concentration of 352.5 ± 24.59 g/dl during the entire period of study (Table XIX-XXII).

Schizothorax niger showed a glucose concentration of 137.8 ± 17.00 g/dl to 340.1 ± 17.00 g/dl (Fig. 81). A minimum value of glucose was observed in winter season of year-I and maximum value in summer season of year-I. Further, the glucose estimation during rest of the seasons showed different concentrations. A concentration of 216.1 ± 17.63 g/dl and 222.3 + 30.97 g/dl in respective spring seasons, 340.1 ± 17.00 g/dl and 336.8 ± 13.84 g/dl in summer season; 178.1 ± 10.27 g/dl and 202.3 ± 14.69 g/dl in autumn seasons and 137.8 ± 17.00 g/dl and 138.0 ± 13.84 g/dl in winter seasons during the entire study period was observed.

In contrast to *Schizothorax niger*, the *Cyprinus carpio* spp. showed a glucose concentration of 196.1 ± 24.59 g/dl to 352.5 ± 24.59 mg/dl during the

entire study period (Fig. 82). The glucose values varied with 237.8 ± 31.32 g/dl and 241.8 ± 11.62 g/dl in respective spring seasons, 352.5 ± 24.59 g/dl and 348.8 + 28.01 g/dl in respective summer seasons, 218.8 ± 27.80 g/dl and 284.8 ± 25.72 g/dl in respective autumn seasons and 196.1 ± 24.59 g/dl and 199.2 ± 32.06 g/dl in respective winter seasons. However, lowest concentration of glucose in *Cyprinus carpio* spp. were observed in winter season (2005-06) and highest concentration in summer season (year-I).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of glucose in different seasons during the entire study period (Table XXI-XXIV).

In spring seasons the *Schizothorax niger* showed glucose values of 192.3 ± 18.02 g/dl and 202.8 ± 29.38 g/dl respectively (Fig. 83). In summer seasons the values recorded were 332.1 + 17.14 g/dl and 337.6 + 12.12 g/dl, in autumn seasons the values were 176.3 ± 19.92 g/dl and 181.8 ± 13.22 g/dl and in winter seasons the values 125.5 ± 17.11 g/dl and 133.1 ± 13.01 g/dl were recorded. The minimum concentrations of glucose were observed in winter season (year-I) and maximum values were recorded during summer season (year-II).

Cyprinus carpio spp. collected from river Jhelum showed varied glucose concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 84). Glucose concentrations of 218.8 ± 39.20 g/dl and 222.6 ± 66.52 g/dl in respective spring seasons, 341.6 ± 25.01 g/dl and 342.9 ± 28.77 g/dl in respective summer seasons, 220.3 ± 28.88 g/dl and 272.0 ± 27.72 g/dl in respective autumn seasons and 136.1 ± 25.01 g/dl and 143.2 ± 23.00 g/dl in respective winter seasons (2005-2007) were observed.

4.1.5. Urea and Creatinine Estimation

Dal lake: The estimation of urea and creatinine in the blood of fishes collected from Dal lake varied seasonally from a low concentration of 16.1 ± 1.41 g/dl and 0.15 ± 0.05 g/dl to a high concentration of 23.3 ± 2.99 g/dl and 1.19 ± 0.07g/dl during the entire period of study (Table XXI-XXIV)

Schizothorax niger showed a urea concentration of 16.1 ± 1.41 g/dl to 22.8 ± 3.13 g/dl. A minimum value of urea was observed in winter season of year-I and maximum value in summer season of year-II (Fig. 85). Further, the urea estimation during rest of the seasons showed different concentrations. A concentration of 18.0 ± 0.37 g/dl and 19.1 + 1.12 g/dl in respective spring seasons; 22.4 ± 1.08 g/dl and 22.8 ± 3.13 g/dl in summer season; 18.6 ± 1.66 g/dl and 18.9 ± 1.46 g/dl in autumn seasons and 16.1 ± 1.41 g/dl and 17.8 ± 2.23 g/dl in winter seasons during the entire study period was observed.

The *Cyprinus carpio* spp. showed a urea concentration of 16.2 ± 0.43 g/dl to 23.3 ± 2.99 mg/dl during the entire study period (Fig. 86). The urea values varied with 22.0 ± 1.43 g/dl and 22.8 ± 1.59 g/dl in respective spring seasons, 23.1 ± 1.01 g/dl and 23.3 + 2.99 g/dl in respective summer seasons, 20.0 ± 1.38 g/dl and 20.9 ± 3.03 g/dl in respective autumn seasons and 16.2 ± 1.43 g/dl and 15.9 ± 1.45 g/dl in respective winter seasons. However, lowest concentration of urea in *Cyprinus carpio* spp. were observed in winter season (year-I) and highest concentration in summer season (year-II).

Further, *Schizothorax niger* showed a creatinine concentration of 0.15 ± 0.05 g/dl to 1.19 ± 0.07 g/dl. A minimum value of creatinine was observed in winter season of year-I and maximum value in summer season of year-II (Fig. 87). Further, the creatinine estimation during rest of the seasons showed different concentrations. A concentration of 0.86 ± 0.12 g/dl and 0.87 + 0.04

g/dl in respective spring seasons; 1.14 ± 0.05 g/dl and 1.16 ± 0.05 g/dl in summer season; 0.91 ± 0.04 g/dl and 0.92 ± 0.30 g/dl in autumn seasons and 0.15 ± 0.05 g/dl and 0.17 ± 0.02 g/dl in winter seasons during the entire study period was observed.

The *Cyprinus carpio* spp. showed a creatinine concentration of 0.19 ± 0.09 g/dl to 1.19 ± 0.07 mg/dl during the entire study period (Fig. 88). The creatinine values varied with 0.88 ± 0.03 g/dl and 0.89 ± 0.14 g/dl in respective spring seasons, 1.18 ± 0.09 g/dl and 1.19 ± 0.07 g/dl in respective summer seasons, 0.93 ± 0.04 g/dl and 0.94 ± 0.08 g/dl in respective autumn seasons and 0.19 ± 0.09 g/dl and 0.22 ± 0.08 g/dl in respective winter seasons. However, lowest concentration of creatinine in *Cyprinus carpio* spp. were observed in winter season (year-I) and highest concentration in summer season (year-II).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of urea and creatinine in different seasons during the entire study period (Table XXI-XXIV).

In spring seasons, the *Schizothorax niger* showed urea values of 17.2 ± 0.88 g/dl and 17.8 ± 1.52 g/dl respectively (Fig. 89). In summer seasons the values recorded were 21.6 + 1.72 g/dl and 21.7 + 3.59 g/dl, in autumn seasons the values were 18.1 ± 2.11 g/dl and 18.3 ± 1.36 g/dl and in winter the values were 17.0 ± 0.59 g/dl and 17.1 ± 2.33 g/dl recorded. The minimum concentrations of urea were observed in winter season (year-I) and maximum values were recorded during summer season (year-II). *Cyprinus carpio* spp. collected from river Jhelum showed varied urea concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 90). Urea concentrations of 20.9 ± 1.60 g/dl and 22.2 ± 1.44

g/dl in respective spring seasons, 23.8 ± 1.99 g/dl and 24.0 ± 2.12 g/dl in respective summer seasons, 19.2 ± 1.33 g/dl and 19.6 ± 3.08 g/dl in respective autumn seasons and 16.1 ± 1.50 g/dl and 15.2 ± 1.44 g/dl in respective winter seasons were observed.

However, the concentration of creatinine in *Schizothorax niger* varied from 0.09 ± 0.22 to 1.11 ± 0.06 g/dl respectively (Fig. 91). A minimum value of creatinine was observed in winter season of year-I and maximum value in summer season of year-I. Further, the creatinine estimation during rest of the seasons showed different concentrations. A concentration of 0.72 ± 0.11 g/dl and 0.81 ± 0.06 g/dl in respective spring seasons; 1.09 ± 1.20 g/dl and 1.11 ± 0.06 g/dl in summer season; 0.84 ± 0.04 g/dl and 0.86 ± 0.22 g/dl in autumn seasons and 0.09 ± 0.22 g/dl and 0.11 ± 0.04 g/dl in winter seasons during the entire study period was observed.

Cyprinus carpio spp. collected from river Jhelum showed varied creatinine concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 92). Creatinine concentrations of 0.74 ± 0.02 g/dl and 0.77 ± 0.13 g/dl in respective spring seasons, 1.12 ± 0.12 g/dl and 1.14 ± 0.09 g/dl in respective summer seasons, 0.88 ± 0.50 g/dl and 0.91 ± 0.09 g/dl in respective autumn seasons and 0.14 ± 0.10 g/dl and 0.16 ± 0.09 g/dl in respective winter seasons were observed.

4.1.6. Cholesterol estimation

Dal lake: The estimation of cholesterol in the blood of fishes from Dal lake varied seasonally from a low concentration of 37.21 ± 2.60 g/dl to a high concentration of 98.22 ± 2.98 g/dl during the entire period of study (Table XXI-XXIV).

Schizothorax niger showed a concentration of 37.21 ± 2.60 g/dl to 95.31 ± 5.30 g/dl (Fig. 93). A minimum value of cholesterol was observed in winter season of year-I and maximum value in summer season of year-II. Further, the cholesterol estimation during rest of the seasons showed different concentrations. A concentration of 52.71 ± 8.66 g/dl and 63.71 ± 2.22 g/dl in respective spring seasons; 89.34 ± 4.12 g/dl and 95.31 ± 5.30 g/dl in summer season; 58.27 ± 2.17 g/dl and 60.33 ± 2.81 g/dl in autumn seasons and 37.21 ± 2.60 g/dl and 42.82 ± 4.33 g/dl in winter seasons during the entire study period was observed.

In contrast to *Schizothorax niger*, the *Cyprinus carpio* spp. showed a cholesterol concentration of 42.66 ± 3.82 g/dl to 98.22 ± 2.98 mg/dl during the entire study period (Fig. 94). The cholesterol values varied with 54.46 ± 2.99 g/dl and 69.12 ± 1.87 g/dl in respective spring seasons, 95.47 ± 3.89 g/dl and 98.22 ± 2.98 g/dl in respective summer seasons, 66.75 ± 3.54 g/dl and 69.28 + 2.92 g/dl in respective autumn seasons and 42.66 ± 3.82 g/dl and 49.12 ± 3.12 g/dl in respective winter seasons. However, lowest concentration of cholesterol in *Cyprinus carpio* spp. were observed in winter season (year-I) and highest concentration in summer season (year-II).

River Jhelum: *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the river Jhelum showed a varied concentrations of cholesterol in different seasons during the entire study period (Table XXi-XXIV).

In spring seasons the *Schizothorax niger* showed cholesterol values of 51.66 ± 9.01 g/dl and 52.44 ± 3.12 g/dl respectively. In summer seasons the values were 99.34 ± 4.55 g/dl and 100.01 ± 5.88 g/dl, in autumn seasons the values were 59.77 ± 2.22 g/dl and 65.42 ± 2.99 g/dl and in winter seasons the values were 36.42 ± 2.71 g/dl and 51.42 ± 4.20 g/dl. The minimum

concentrations of cholesterol were observed in winter season (year-I) and maximum values were recorded during summer season (year-II) (Fig. 95).

Cyprinus carpio spp. from river Jhelum showed cholesterol concentrations with lowest values during winter season (year-I) and highest values during summer season (year-II) (Fig. 96). Cholesterol concentrations of 63.20 ± 3.01 g/dl and 63.20 ± 1.55 g/dl in respective spring seasons, 82.77 ± 3.52 g/dl and 92.03 ± 3.01 g/dl in respective summer seasons, 60.11 ± 3.55 g/dl and 67.30 ± 3.01 g/dl in respective autumn seasons and 40.11 ± 3.22 g/dl and 48.76 ± 3.14 g/dl in respective winter seasons were observed.

4.2. Metal Concentrations in Water and Fish Tissues

4.2.1. Metal concentrations in water

Results of metal concentration in two different water bodies are represented in the Table III & IV. The seasonal variations in the concentration of copper, zinc, iron and manganese in water samples from Dal lake and River Jhelum during the period of investigation (Year-I, 2005-2006 and Year-II, 2006-2007) are shown in Fig 3 & 4 respectively. Table III & IV shows the concentration of the metals in water correlated with W.H.O. standards.

In Dal lake the concentration of copper was in the range of 1.020 to 1.070 ppm, with maximum concentration found in summer season (year-II) and the minimum in winter season (year-I). The iron concentration ranged between 0.110 to 0.191ppm. The highest value was observed during summer season (year-II) and the minimum in winter season (year-I). The zinc concentration ranged between 0.150 to 0.542 ppm, with maximum value observed in summer season (year-II) and the lowest values in winter season (year-I) and the manganese concentration ranged between 0.021 to 0.083 ppm

with maximum value observed in summer season year-II and the lowest values in winter season (year-I).

In River Jhelum concentration of copper ranged between 1.002 to 1.006 ppm with maximum concentration found in summer season year-II and the minimum in winter season (year-I). The iron concentration ranged between 0.129 to 0.168 ppm. The highest value was observed during summer season in (year-II) and the minimum in autumn season (year-I). The zinc concentration ranged between 0.100 to 0.483 ppm with maximum value observed in summer (year-II) and the lowest values in winter season (year-I) and the manganese concentration ranged between 0.0056 to 0.053 ppm with maximum value observed in summer (year-II) and the lowest values in winter season (year-I).

4.2.2. Metal concentrations in fish tissues

The concentration/accumulation of metals viz. copper, zinc, iron and manganese in different organs/tissues of the selected fishes viz. an indigenous fish (*Schizothorax niger*) and the exotic fish (*Cyprinus carpio* spp.) were investigated seasonally from March 2005 to February 2007. Results of metal concentration observed seasonally in *Schizothorax niger* and *Cyprinus carpio* spp. are represented as mean values (Tables III to XVI). All results are expressed on dry weight basis.

4.2.2.1. Copper accumulation in fish tissues

Dal Lake: In Dal lake, the concentration of copper in the gills of *Schizothorax niger* varied from 13.52 ± 1.12 to 21.84 ± 2.49 ppm (Table V-VIII) during the entire study period. The highest concentration with a mean value of 21.84 ± 2.49 ppm was observed in the summer season, (year-II) and the lowest concentration with a mean value of 13.52 ± 1.12 ppm was observed

during the spring season, 2005-2006 respectively (Fig. 5). Further the concentration of copper in gills during rest of the seasons differ when compared with each other. A concentration of 15.71 ± 1.21 ppm and 16.26 ± 1.67 ppm in respective autumn seasons (2005-2007), 14.92 ± 1.21 ppm and 16.01 ± 1.72 ppm in respective winter seasons was observed. However, a concentration of 15.52 ± 1.24 ppm in spring season (year-II) and 20.66 ± 2.59 ppm in summer (year-I) was observed.

The liver contained the highest concentration of copper in comparison to other organs among the selected tissues/organs. The mean concentration ranged from 66.77 ± 3.12 to 81.68 ± 3.51 ppm (Table V-VIII). The maximum value of 81.68 ± 3.51 ppm was observed in summer (year-II) and the lowest value of 66.77 ± 3.12 was observed in winter season (year-I). The concentration of copper in liver during other seasons showed mean values of 70.01 ± 2.12 and 74.54 ± 3.24 ppm in spring seasons of year-I and year-II, 68.52 ± 2.12 and 72.82 ± 3.24 ppm in autumn seasons of year 2005-2007. However, a concentration of 76.52 ± 2.81 ppm in summer season (year-I) and 70.54 ± 3.12 ppm in winter season (year-II) respectively was observed (Fig. 9).

In kidney, the concentration varied in the range of 64.61 ± 3.10 to 78.90 ± 3.42 ppm, with the highest concentration observed during the summer season, (year-II) and the lowest in the winter season (year-I) (Table V-VIII). The concentration of copper in kidneys during other seasons showed mean values of 69.11 ± 3.71 and 72.18 ± 3.84 ppm in spring seasons of year-I and year-II, 66.12 ± 3.52 and 70.62 ± 3.72 ppm in autumn seasons of year-I and 2006-07. However, a concentration of 73.31 ± 3.24 ppm in summer season (year-I) and 68.79 ± 3.18 ppm in winter season (year-II) respectively was observed (Fig. 13).

Among the selected tissues, muscle showed the lowest concentration. The concentration varied between 07.81 ± 0.54 to 12.72 ± 1.10 ppm (Table V-VIII). Here also the maximum concentration was observed during the summer season, (year-I) and the minimum during the winter season (year-I). When compared the concentration of copper in muscle during other seasons showed mean values of 08.53 ± 1.20 and 09.85 ± 1.26 ppm in spring seasons of (year-I and year-II), 08.32 ± 0.85 and 09.01 ± 0.95 ppm in autumn seasons of year 2005-2007. However, a concentration of 12.11 ± 1.12 ppm in summer season (year-II) and 08.34 ± 0.71 ppm in winter season (year-II) respectively was observed (Fig. 17).

However, in *Cyprinus carpio* spp. collected from Dal lake, the concentration of copper in gills ranged between 16.83 ± 1.82 to 25.99 ± 2.71 ppm (Table V-VIII). The highest value of 25.99 ± 2.71 ppm was observed during the summer season (year-II) and the lowest value of 16.83 ± 1.82 ppm was observed in the winter season (year-I). Further, the concentration of copper in gills during rest of the seasons was different when compared with each other. A concentration of 16.90 ± 1.52 ppm and 19.13 ± 1.90 ppm in respective spring seasons (2005-2007), 19.97 ± 2.52 ppm and 20.66 ± 1.80 ppm in respective autumn seasons was observed. However, a concentration of 24.98 ± 2.91 ppm in summer season (year-I) and 18.11 ± 1.61 ppm in winter season (year-I) was observed (Fig. 6).

In liver the concentration varied between 99.41 ± 3.01 to 139.22 ± 4.24 ppm (Table V-VIII). The highest concentration was found in the summer season (year-II) and the minimum concentration was observed in the spring season year-I. Further, the concentration of copper in gills during rest of the seasons was different when compared with each other. A concentration of 113.24 ± 2.92 ppm and 114.11 ± 4.01 ppm in respective autumn seasons

(2005-2007), 110.62 ± 3.91 ppm and 112.99 ± 3.92 ppm in respective winter seasons was observed. However, a concentration of 103.66 ± 3.99 ppm in spring season (year-II) and 132.01 ± 3.21 ppm in summer (year-I) was observed (Fig. 10).

In kidney, the concentration ranged between 96.52 ± 3.52 to 132.83 ± 4.54 ppm (Table III-VI). The maximum value of 132.83 + 4.54 ppm was observed in summer (year-II) and the minimum value of 96.52 ± 3.10 ppm was observed in spring (year-I). The concentration of copper in kidneys during other seasons showed mean values of 110.22 ± 3.12 and 112.27 ± 3.24 ppm in autumn seasons of year-I and year-II, 108.07 ± 3.55 and 109.85 ± 3.71 ppm in winter seasons of year 2005-2007. However, a concentration of 125.45 ± 5.01 ppm in summer season (year-I) and 100.21 ± 3.61 ppm in winter season (year-II) respectively was observed (Fig. 14).

The concentration of copper in muscle varied between 09.99 ± 1.24 to 17.58 ± 2.93 ppm with maximum value found in summer year-II and the minimum value observed in winter season year-I (Table III-VI). The concentration of copper in muscle during other seasons showed mean values of 10.55 ± 0.80 and 11.21 ± 0.88 ppm in spring seasons of year-I and year-II, 11.72 ± 1.60 and 12.16 ± 1.71 ppm in autumn seasons of year 2005-2007. However, a concentration of 16.58 ± 2.85 ppm in summer season (year-I) and 10.98 ± 1.76 ppm in winter season (year-II) respectively was observed (Fig. 18).

River Jhelum: In gills of *Schizothorax niger* from the River Jhelum, the copper concentration varied between 10.13 ± 1.06 to 19.54 ± 2.40 ppm (Table V-VIII). The highest value was observed in summer season (year-I) and the minimum in winter season (year-II). Further, the concentration of copper in

gills during rest of the seasons was different when compared with each other. A concentration of 12.81 ± 1.29 ppm and 14.33 ± 1.12 ppm in respective spring seasons (2005-2007), 14.71 ± 1.61 ppm and 12.11 ± 2.12 ppm in respective autumn seasons was observed. However, a concentration of 16.33 ± 2.52 ppm in summer season (year-II) and 13.99 ± 1.70 ppm in winter season (year-I) was observed (Fig. 7).

Liver showed the concentration ranging between 63.69 ± 3.69 to 79.52 ± 3.81 ppm with maximum value in the summer season (year-II) and minimum in winter season, year-I (Table V-VIII). When compared the concentration of copper in liver during other seasons showed mean values of 68.12 ± 2.54 and 73.21 ± 3.24 ppm in spring seasons of year-I and year-II, 66.12 ± 2.66 and 71.23 ± 3.24 ppm in autumn seasons of year 2005-2007. However, a concentration of 74.61 ± 2.84 ppm in summer season (year-I) and 68.55 ± 3.12 ppm in winter season (year-II) respectively was observed (Fig. 11).

In kidney, the concentration varied between 62.54 ± 2.99 to 77.64 ± 3.52 ppm (Table V-VIII). The highest concentration was obtained in the summer season (year-II) and the lowest concentration was observed in the winter season (year-I). The concentration of copper in kidney during other seasons showed mean values of 67.11 ± 3.12 and 71.69 ± 3.12 ppm in spring seasons of year-I and year-II, 64.24 ± 3.42 and 68.12 ± 3.12 ppm in autumn seasons of year 2005-2007. However, a concentration of 71.84 ± 3.12 ppm in summer season (year-I) and 66.92 ± 3.10 ppm in winter season (year-II) respectively was observed (Fig. 15).

The muscle concentration ranged between 06.33 ± 0.50 to 12.72 ± 1.10 ppm, with maximum value of observed in the summer season (year-I) and the minimum value of being observed in the winter season (year-I) (Table III-VI). The concentration of copper in muscle during other seasons showed mean

values of 07.35 ± 0.99 and 08.12 ± 1.17 ppm in spring seasons of year-I and year-II, 07.98 ± 0.77 and 08.72 ± 0.84 ppm in autumn seasons of year 2005-2007. However, a concentration of 11.03 ± 1.04 ppm in summer season (year-II) and 07.26 ± 0.68 ppm in winter season (year-II) respectively was observed (Fig. 19).

In case of *Cyprinus carpio* spp. derived from the River Jhelum, the concentration of copper in gill ranged between 14.82 ± 1.75 to 23.24 ± 2.54 ppm (Table V-VIII). The maximum value of 23.24 ± 2.54 ppm was observed in the summer 2006-07 and the minimum value of 14.82 ± 1.75 ppm was observed in the spring season, year-I. Further, the concentration of copper in gills during rest of the seasons showed different values when compared with each other. A concentration of 16.01 ± 2.31 ppm and 18.79 ± 1.77 ppm in respective autumn seasons (2005-2007), 15.03 ± 1.10 ppm and 17.48 ± 1.80 ppm in respective winter seasons was observed. However, a concentration of 16.11 ± 1.20 ppm in spring season (year-II) and 21.25 ± 2.15 ppm in summer (year-I) was observed (Fig. 8).

The concentration in liver ranged between 97.62 ± 3.99 ppm to 131.99 ± 4.52 ppm being maximum in the summer season of year-II and the minimum in the spring season (year-I) (Table V-VIII). Further, the concentration of copper in liver during rest of the seasons showed different values when compared with each other. A concentration of 109.11 ± 3.24 ppm and 111.12 ± 3.90 ppm in respective autumn seasons (2005-2007), 107.85 ± 3.12 ppm and 110.50 ± 3.54 ppm in respective winter seasons was observed. However, a concentration of 102.62 ± 4.01 ppm in spring season (year-II) and 129.99 ± 3.91 ppm in summer (year-I) was observed (Fig. 12).

In kidney the concentration varied from 94.33 ± 3.25 to 129.62 ± 4.12 ppm (Table V-VIII). The highest value of 129.62 ± 4.12 was observed in summer season of year-II and the lowest value of 94.33 ± 3.25 was observed in spring (year-I). Further, the concentration of copper in kidney during rest of the seasons showed different values when compared with each other. A concentration of 108.66 ± 3.10 ppm and 109.62 ± 3.82 ppm in respective autumn seasons (2005-2007), 106.11 ± 3.14 ppm and 107.65 ± 3.24 ppm in respective winter seasons was observed. However, a concentration of 99.99 ± 3.92 ppm in spring season (year-II) and 123.51 ± 4.97 ppm in summer (year-I) was observed (Fig. 16).

The muscle concentration ranged between 08.24 ± 1.01 to 16.27 ± 2.83 ppm (Table V-VIII). The maximum value being observed in the summer, year-II and the minimum in the winter season (year-I). The concentration of copper in muscle during other seasons showed mean values of 09.88 ± 0.50 and 10.22 ± 0.78 ppm in spring seasons of year-I and year-II, 10.91 ± 1.50 and 11.27 ± 1.54 ppm in autumn seasons of year 2005-2007. However, a concentration of 14.11 ± 1.55 ppm in summer season (year-I) and 09.17 ± 1.59 ppm in winter season (year-II) respectively was observed (Fig. 20).

4.2.2.2. Zinc accumulation in fish tissues

Dal Lake: In *Schizothorax niger* collected from Dal lake, the concentration of zinc in gill, liver, kidney and muscle was 52.11 ± 2.12 to 72.44 ± 3.92 ppm; 73.81 ± 2.52 to 97.84 ± 4.62 ppm; 88.77 ± 3.52 to 101.99 ± 4.03 ppm; and 29.93 ± 2.55 to 39.72 ± 3.81 ppm respectively (Table IX-XII). In all tissues i.e. gills, liver, kidney and muscle, the maximum values i.e. 72.44 ± 3.92 ppm; 97.84 ± 4.62 ppm, 101.99 ± 4.03 ppm and 39.72 ± 3.81 ppm respectively were observed in the summer season of year-II and the minimum

value of 52.11 ± 2.12 ppm in gills was observed in the spring season of year-I, in case of liver and kidney the minimum values of 73.81 ± 2.52 ppm; 88.77 ± 3.52 ppm was observed during the winter seasons of year-I and in case of muscle the minimum value of 29.93 ± 2.55 ppm was observed during the autumn seasons of year-I. Further, the concentration of zinc in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 55.36 ± 2.51 ppm and 64.72 ± 2.99 ppm in respective autumn seasons (2005-2007), 54.81 ± 2.99 ppm and 64.61 ± 3.28 ppm in respective winter seasons was observed. However, a concentration of 62.32 ± 4.52 ppm in spring season (year-II) and 57.21 ± 2.55 ppm in summer (year-I) was observed (Fig. 21). When compared the concentration of zinc in liver during other seasons showed mean values of 74.52 ± 2.24 and 81.06 ± 3.44 ppm in spring seasons of year-I and year-II, 75.12 ± 4.77 and 82.52 ± 3.99 ppm in autumn seasons of year 2005-2007. However, a concentration of 90.61 ± 3.92 ppm in summer season (year-I) and 81.06 ± 3.44 ppm in winter season (year-II) respectively (Fig. 25). When compared the concentration in kidney during other seasons showed mean values of 89.41 ± 4.12 and 96.52 ± 3.01 ppm in spring seasons of year-I and year-II, 91.36 ± 2.81 and 98.05 ± 3.11 ppm in autumn seasons of year 2005-2007. However, a concentration of 94.32 ± 4.15 ppm in summer season (year-I) and 95.24 ± 3.92 ppm in winter season (year-II) respectively (Fig. 29). When compared the concentration in muscle during other seasons showed mean values of 31.89 ± 3.11 and 33.24 ± 3.22 ppm in spring seasons of year-I and year-II, 30.88 ± 2.10 and 32.40 ± 2.16 ppm in winter seasons of year 2005-2007. However, a concentration of 37.41 ± 3.52 ppm in summer season (year-I) and 31.92 ± 2.86 ppm in autumn season (year-II) respectively was observed in muscle of the host (Fig. 33). The maximum concentration of zinc

in case of *Schizothorax niger* obtained from Dal Lake was found in kidney followed by liver, gills and muscles.

In case of *Cyprinus carpio* spp. collected from Dal lake, the concentration of zinc in gills, liver, kidney and muscle tissues varied between 56.92 ± 2.52 to 87.25 ± 3.52 ppm; 111.35 ± 4.24 to 152.61 ± 5.26 ppm; 119.84 ± 4.15 to 159.32 ± 4.52 ppm and 34.46 ± 2.12 to 44.52 ± 3.95 ppm respectively (Table IX-XII). The highest values of 56.92 ± 2.52 ppm in gills, 152.61 ± 5.26 ppm in liver, 159.32 ± 4.52 ppm in kidney and 44.52 ± 3.95 ppm in muscle was observed in the summer season (year-II) and the minimum value of 56.92 ± 2.52 in gill was observed in the spring season of the year-I. In liver the minimum value of 111.35 ± 4.24 ppm was observed in the winter season of year-I, in kidney the minimum value of 119.84 ± 4.15 ppm was observed in the autumn season year-I. However, in muscle the minimum value of 34.36 ± 2.12 was observed in the winter season of the year-I. Further, the concentration of zinc in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 59.31 ± 2.90 ppm and 77.20 ± 3.55 ppm in respective autumn seasons (2005-2007), 58.23 ± 2.51 ppm and 76.47 ± 4.52 ppm in respective winter seasons was observed. However, a concentration of 75.61 ± 3.92 ppm in spring season (year-II) and 61.82 ± 3.12 ppm in summer (year-I) was observed (Fig. 22). When compared the concentration in liver during other seasons showed mean values of 115.16 ± 5.21 and 124.76 ± 5.98 ppm in spring seasons of year-I and year-II, 114.01 ± 5.20 and 124.05 ± 4.82 ppm in autumn seasons of year 2005-2007. However, a concentration of 143.24 ± 5.01 ppm in summer season (year-I) and 121.40 ± 4.86 ppm in winter season (year-II) respectively (Fig. 26). When compared the concentration in kidney during other seasons showed mean values of 126.98 ± 4.91 and 135.52 ± 4.18

ppm in spring seasons of year-I and year-II, 122.45 ± 4.51 and 123.66 ± 4.56 ppm in winter seasons of year 2005-2007. However, a concentration of 149.80 ± 5.20 ppm in summer season (year-I) and 129.71 ± 4.91 ppm in autumn season (year-II) respectively (Fig. 30). When compared the concentration in muscle during other seasons showed mean values of 35.22 ± 3.09 and 37.01 ± 3.19 ppm in spring seasons of year-I and year-II, 35.29 ± 2.75 and 37.11 ± 2.86 ppm in autumn seasons of year 2005-2007. However, a concentration of 42.38 ± 3.59 ppm in summer season (year-I) and 36.71 ± 2.21 ppm in winter season (year-II) respectively in muscle was observed (Fig. 34).

River Jhelum: In *Schizothorax niger*, the zinc concentration in gills, liver, kidney and muscle ranged between 50.60 ± 2.50 to 69.24 ± 3.87 ppm; 71.99 ± 3.92 to 95.43 ± 4.53 ppm, 80.42 ± 4.11 to 99.84 ± 4.01 ppm and 27.18 ± 2.15 to 38.12 ± 3.72 ppm (Table IX-XII). The maximum values of 69.24 ± 3.87 ppm in gill, 95.43 ± 4.53 ppm in liver, 99.84 ± 4.01 ppm in kidney and 38.12 ± 3.72 ppm in muscle were observed in the summer months of the year-II and the minimum values of 50.60 ± 2.50 ppm in gill was observed in the spring season (year-I), 71.99 ± 3.92 ppm in liver was observed in the winter season of the year-I, the minimum value of 80.42 ± 4.11 in kidney was observed in spring season year-I and 27.18 ± 2.15 ppm, the minimum value in muscle was observed in autumn season in year-I. Further, the concentration of zinc in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 53.82 ± 2.15 ppm and 62.91 ± 2.82 ppm in respective autumn seasons (2005-2007), 52.72 ± 3.22 ppm and 62.79 ± 3.12 ppm in respective winter seasons was observed. However, a concentration of 61.12 ± 4.43 ppm in spring season (year-II) and 55.62 ± 2.94 ppm in summer (year-I) was observed (Fig. 23).

When compared the concentration in liver during other seasons showed mean values of 73.81 ± 4.10 and 80.15 ± 3.36 ppm in spring seasons of year-I and year-II, 74.32 ± 4.10 and 80.01 ± 3.84 ppm in autumn seasons of year 2005-2007. However, a concentration of 82.59 ± 4.12 ppm in summer season (year-I) and 78.82 ± 4.10 ppm in winter season (year-II) respectively was observed (Fig. 27). When compared the concentration in kidney during other seasons showed mean values of 82.72 ± 4.12 and 85.92 ± 3.92 ppm in autumn seasons of year year-I and year-II, 81.35 ± 3.24 and 79.61 ± 3.52 ppm in winter seasons of year 2005-2007. However, a concentration of 81.56 ± 3.60 ppm in spring season (year-II) and 86.63 ± 3.52 ppm in summer season (year-I) respectively was observed (Fig. 31). When compared the concentration in muscle during other seasons showed mean values of 29.11 ± 3.00 and 32.25 ± 3.19 ppm in spring seasons of year-I and year-II, 28.65 ± 2.01 and 31.09 ± 2.11 ppm in winter seasons of year 2005-2007. However, a concentration of 35.66 ± 3.15 ppm in summer season (year-I) and 30.71 ± 2.74 ppm in autumn season (year-II) respectively was observed (Fig. 35).

In *Cyprinus carpio* spp. the zinc concentration in gills, liver, kidney and muscle varied from 54.79 ± 2.91 to 86.43 ± 3.41 ppm; 109.98 ± 3.92 to 141.81 ± 5.12 ppm; 116.31 ± 4.05 to 148.92 ± 4.27 ppm and 32.17 ± 2.09 to 43.44 ± 3.84 ppm (Table IX-XII). The highest values in all the tissue i.e. 86.43 ± 3.41 ppm in gill, 141.81 ± 5.12 ppm in liver, 148.92 ± 4.27 ppm in kidney and 43.44 ± 3.84 ppm in muscle was observed in the summer season in year-II. The lowest values of 54.79 ± 2.91 ppm in gill was found in spring season in year-I, in liver the minimum value of 109.98 ± 3.92 ppm was observed in winter season year-I, the minimum value of 116.31 ± 4.05 ppm in kidney was observed in autumn season of year-I and in muscle the minimum value of 32.17 ± 2.09 ppm in winter season of year-I. . Further, the

concentration of zinc in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 57.13 ± 2.55 ppm and 75.11 ± 3.24 ppm in respective autumn seasons (2005-2007), 56.59 ± 2.81 ppm and 74.29 ± 4.24 ppm in respective winter seasons was observed. However, a concentration of 73.81 ± 3.72 ppm in spring season (year-II) and 59.11 ± 3.29 ppm in summer (year-I) was observed (Fig. 24). When compared the concentration in liver during other seasons showed mean values of 112.98 ± 3.92 and 121.75 ± 5.84 ppm in spring seasons of year-I and year-II, 113.63 ± 4.72 and 122.12 ± 4.64 ppm in autumn seasons of year 2005-2007. However, a concentration of 137.99 ± 4.12 ppm in summer season (year-I) and 119.22 ± 4.54 ppm in winter season (year-II) respectively was observed (Fig. 28). When compared the concentration in kidney during other seasons showed mean values of 123.22 ± 4.52 and 127.92 ± 4.02 ppm in spring seasons of year-I and year-II, 119.54 ± 4.15 and 125.22 ± 3.99 ppm in winter seasons of year 2005-2007. However, a concentration of 143.13 ± 5.12 ppm in summer season (year-I) and 118.11 ± 4.92 ppm in autumn season (year-II) respectively was observed (Fig. 32). When compared the concentration in muscle during other seasons showed mean values of 33.16 ± 2.99 and 36.49 ± 3.15 ppm in spring seasons of year-I and year-II, 33.26 ± 2.55 and 36.92 ± 2.77 ppm in autumn seasons of year 2005-2007. However, a concentration of 40.22 ± 3.41 ppm in summer season (year-I) and 35.52 ± 2.18 ppm in winter season (year-II) respectively was observed (Fig. 36).

4.2.2.3. Iron accumulation in fish tissues

Dal Lake: In *Schizothorax niger* collected from Dal lake, the concentration of iron in gill, liver, kidney and muscle varied between 130.10 ± 5.55 to 192.52 ± 6.54 ppm; 204.92 ± 5.21 to 296.51 ± 4.37 ppm; 200.99 ± 5.04 to 292.61 ±

4.25 ppm and 40.88 ± 2.15 to 49.23 ± 3.84 ppm respectively (Table XIII-XVI). The maximum values of 192.52 ± 6.54 ppm in gills; 296.51 ± 4.37 ppm in liver, 292.61 ± 4.25 ppm in kidney and 49.23 ± 3.84 ppm in muscle were observed in the summer (year-II) and the minimum values 130.10 ± 5.55 ppm in gills; 204.92 ± 5.21 ppm in liver, 200.99 5.04 ppm in kidney and 40.88 ± 2.15 ppm in the muscle were observed in the winter season (year-I). Further, the concentration of iron in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 137.66 ± 5.22 ppm and 140.21 ± 4.77 ppm in respective spring seasons (2005-2007), 139.24 ± 3.51 ppm and 142.01 ± 6.20 ppm in respective autumn seasons was observed. However, a concentration of 188.11 ± 5.12 ppm in summer season (year-I) and 138.24 ± 5.22 ppm in winter (year-II) was observed (Fig. 37). When compared the concentration in liver during other seasons showed mean values of 227.91 ± 6.52 and 241.20 ± 6.96 ppm in spring seasons of year-I and year-II, 228.36 ± 6.55 and 242.54 ± 4.85 ppm in autumn seasons of year 2005-2007. However, a concentration of 284.31 ± 4.29 ppm in summer season (year-I) and 234.56 ± 5.95 ppm in winter season (year-II) respectively was observed (Fig. 41). When compared the concentration in kidney during other seasons showed mean values of 224.34 ± 6.05 and 239.44 ± 6.52 ppm in spring seasons of year-I and year-II, 224.90 ± 6.24 and 238.11 ± 4.15 ppm in autumn seasons of year 2005-2007. However, a concentration of 280.41 ± 4.20 ppm in summer season (year-I) and 230.62 ± 5.55 ppm in winter season (year-II) respectively was observed (Fig. 45). When compared the concentration in muscle during other seasons showed mean values of 41.77 ± 3.51 and 44.22 ± 3.82 ppm in spring seasons of year-I and year-II, 42.22 ± 3.15 and 45.44 ± 3.56 ppm in autumn seasons of year 2005-2007. However, a concentration of 46.62 ± 3.24 ppm in summer

season (year-I) and 43.72 ± 2.52 ppm in winter season (year-II) respectively was observed (Fig. 49).

The iron content in *Schizothorax niger* from Dal lake was found to be higher in liver than kidney followed by gills and the minimum in muscle tissue. In all the tissues maximum concentration was observed in the summer (year-II).

In case of *Cyprinus carpio* spp. from the Dal lake, the concentration of iron in gill, liver, kidney and muscle ranged between 153.72 ± 4.25 to 201.61 ± 5.24 ppm; 290.32 ± 6.51 to 392.21 ± 6.15 ppm; 286.75 ± 6.25 to 388.69 ± 6.12 ppm and 51.67 ± 3.12 to 62.71 ± 3.92 ppm respectively (Table IX-XVI). The highest values of 201.61 ± 5.24 ppm in gills, in liver, 392.21 ± 6.15 ppm, 388.69 ± 6.12 ppm in kidney and 62.71 ± 3.92 ppm in muscle were observed in the summer (year-II) and the lowest values 153.72 ± 4.25 ppm in gills was observed in the spring season (year-I); in liver the minimum value of 290.32 ± 6.51 ppm was observed in the winter season (year-I), in kidney the lowest value of 286.75 ± 6.25 ppm and in muscle the minimum value of 51.67 ± 3.12 ppm was observed in the winter season (year-I). Further, the concentration of iron in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 157.88 ± 4.25 ppm and 162.41 ± 4.28 ppm in respective autumn seasons (2005-2007), 156.67 ± 4.52 ppm and 160.12 ± 4.34 ppm in respective winter seasons was observed. However, a concentration of 159.23 ± 4.34 ppm in spring season (year-II) and 199.35 ± 5.99 ppm in summer (year-I) was observed (Fig. 38). When compared the concentration in liver during other seasons showed mean values of 291.54 ± 6.15 and 310.72 ± 6.25 ppm in spring seasons of year-I and year-II, 304.11 ± 5.14 and 314.52 ± 5.54 ppm in autumn seasons of year 2005-2007. However, a concentration of

379.11 ± 6.12 ppm in summer season (year-I) and 299.23 ± 5.92 ppm in winter season (year-II) respectively was observed (Fig. 42). When compared the concentration in kidney during other seasons showed mean values of 287.52 ± 6.15 and 306.52 ± 6.59 ppm in spring seasons of year-I and year-II, 300.71 ± 5.04 and 311.12 ± 5.19 ppm in autumn seasons of year 2005-2007. However, a concentration of 374.66 ± 6.10 ppm in summer season (year-I) and 296.66 ± 5.19 ppm in winter season (year-II) respectively was observed (Fig. 46). When compared the concentration in muscle during other seasons showed mean values of 52.66 ± 4.01 and 55.42 ± 4.65 ppm in spring seasons of year-I and year-II, 53.11 ± 3.52 and 56.41 ± 3.62 ppm in autumn seasons of year 2005-2007. However, a concentration of 59.38 ± 3.51 ppm in summer season (year-I) and 54.41 ± 3.55 ppm in winter season (year-II) respectively was observed (Fig. 50).

Like, *Schizothorax niger*, the *Cyprinus carpio* spp. collected from the Dal lake also contained maximum concentration in summer season, with the liver containing the maximum concentration followed by kidney, gills and muscle.

River Jhelum: In *Schizothorax niger* collected from River Jhelum the iron concentration in gills, liver, kidney and muscle ranged between 127.52 ± 5.21 to 187.46 ± 6.34 ppm; 208.44 ± 5.12 to 294.62 ± 4.32 ppm; 204.32 ± 5.10 to 289.77 ± 4.52 ppm and 31.90 ± 2.51 to 48.16 ± 3.82 ppm respectively (Table iX-XVI). The highest value of 187.46 ± 6.34 ppm in gills, 294.62 ± 4.32 ppm in liver, 289.77 ± 4.52 ppm in kidney and 48.16 ± 3.82 ppm in muscle was observed in the summer (year-II). The lowest values of 127.52 ± 5.21 ppm in gills, 208.44 ± 5.12 ppm in liver and 204.32 ± 5.10 ppm in kidney were observed in winter (year-I) and 31.90 ± 2.51 ppm in muscle was observed in winter (year-II). Further, the concentration of iron in gills, liver, kidney and

muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 135.62 ± 3.21 ppm and 139.92 ± 4.61 ppm in respective spring seasons (2005-2007), 137.11 ± 3.12 ppm and 140.76 ± 6.12 ppm in respective autumn seasons was observed. However, a concentration of 186.17 ± 4.01 ppm in summer season (year-I) and 136.11 ± 5.10 ppm in winter (year-II) was observed (Fig. 39). When compared the concentration in liver during other seasons showed mean values of 216.12 ± 6.12 and 233.11 ± 6.81 ppm in spring seasons of year-I and year-II, 224.52 ± 4.21 and 240.22 ± 4.72 ppm in autumn seasons of year 2005-2007. However, a concentration of 281.59 ± 4.21 ppm in summer season (year-I) and 239.65 ± 5.84 ppm in winter season (year-II) respectively was observed (Fig. 43). When compared the concentration in kidney during other seasons showed mean values of 213.12 ± 5.85 and 230.52 ± 6.15 ppm in spring seasons of year-I and year-II, 220.79 ± 4.20 and 237.20 ± 4.52 ppm in autumn seasons of year 2005-2007. However, a concentration of 277.5 ± 4.53 ppm in summer season (year-I) and 235.68 ± 4.01 ppm in winter season (year-II) respectively was observed (Fig. 47). When compared the concentration in muscle during other seasons showed mean values of 39.12 ± 3.12 and 43.31 ± 3.79 ppm in spring seasons of year-I and year-II, 40.77 ± 3.10 and 44.78 ± 3.55 ppm in autumn seasons of year 2005-2007. However, a concentration of 44.81 ± 3.12 ppm in summer season (year-I) and 38.39 ± 2.12 ppm in winter season (year-I) respectively was observed (Fig. 51).

Therefore, the highest concentration of iron was observed in the summer season and the maximum concentration was found in liver and the minimum in muscle.

In *Cyprinus carpio* spp. obtained from River Jhelum, the iron concentration in gills, liver, kidney, and muscle ranged between 151.92 ± 3.25

to 199.52 ± 5.12 ppm; 281.81 ± 6.12 to 381.43 ± 6.11 ppm; 278.66± 6.10 to 378.66 ± 6.01 ppm and 49.22 ± 3.10 to 61.07 ± 3.74 ppm respectively (Table IX-XVI). The highest value of 199.52 ± 5.12 ppm in gill, 381.43 ± 6.11 ppm in liver, 378.66 ± 6.01 ppm in kidney and 61.07 ± 3.74 ppm in muscle were observed in the summer season of year-II. The lowest value of 151.92 ± 3.25 ppm in gills was observed in the spring season of the year-II. The minimum value of 281.81± 6.12 in liver was observed in the winter season of year-I, the minimum value of 278.66 ± 6.10 ppm in kidney was observed in the autumn season of year-I and in muscle the lowest concentration of 49.22 ± 3.10 ppm was observed in the winter season of the year-I. Further, the concentration of iron in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 155.59 ± 4.21 ppm and 160.23 ± 4.18 ppm in respective autumn seasons (2005-2007), 154.29 ± 4.12 ppm and 158.26 ± 4.32 ppm in respective winter seasons was observed. However, a concentration of 151.92 ± 3.25 ppm in spring season (year-I) and 196.62 ± 5.21 ppm in summer (year-I) was observed (Fig. 40). When compared the concentration in liver during other seasons showed mean values of 285.61 ± 6.21 and 292.34 ± 6.15 ppm in spring seasons of year-I and year-II, 301.12 ± 5.01 and 312.16 ± 5.32 ppm in autumn seasons of year 2005-2007. However, a concentration of 377.31 ± 6.01 ppm in summer season (year-I) and 307.01 ± 5.74 ppm in winter season (year-II) respectively was observed (Fig. 44). When compared the concentration in kidney during other seasons showed mean values of 281.31 ± 6.24 and 287.92 ± 6.50 ppm in spring seasons of year-I and year-II, 278.66 ± 6.10 and 299.34 ± 4.15 ppm in winter seasons of year 2005-2007. However, a concentration of 373.85 ± 6.10 ppm in summer season (year-I) and 307.52 ± 5.12 ppm in autumn season (year-II) respectively was observed (Fig. 48). When compared the concentration in muscle during other seasons showed

mean values of 50.85 ± 3.92 and 54.66 ± 4.54 ppm in spring seasons of year-I and year-II, 51.90 ± 3.41 and 55.11 ± 3.57 ppm in autumn seasons of year 2005-2007. However, a concentration of 57.62 ± 3.42 ppm in summer season (year-I) and 53.21 ± 3.42 ppm in winter season (year-II) respectively was observed (Fig. 52).

Here the maximum concentration was found in liver followed by kidney, gills and lowest being in the muscle tissue. Also the highest concentration in all tissues was observed in the summer season of year-II.

4.2.2.4. Manganese accumulation in fish tissues

Dal Lake: In *Schizothorax niger* collected from Dal lake, the concentration of manganese in gills, liver, kidney and muscle ranged between 02.71 ± 0.52 to 11.55 ± 2.91 ppm; 01.13± 0.02 to 08.30 ± 1.00 ppm; 0.84 ± 0.06 to 06.95 ± 0.93 ppm and 0.09 ± 0.01 to 03.92 ± 0.88 ppm respectively (Table XVII-XX). The highest values of 11.55 ± 2.91 ppm in gills, 08.30 ± 1.00 ppm in liver, 06.95 ± 0.93 ppm in kidney and 03.92 ± 0.88 ppm in muscle were observed in summer season of year-II. The lowest value of 02.71 ± 0.52 ppm in gills and 01.13 ± 0.02 ppm in liver were observed in the spring season of the year-I, the minimum value in kidney 0.84 ± 0.66 ppm and 0.09 ± 0.01 ppm in muscle were observed in the spring season of year-I. Further, the concentration of manganese in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 02.71 ± 0.52 ppm and 04.95 ± 0.27 ppm in respective spring seasons (2005-2007), 05.43 ± 0.85 ppm and 07.49 ± 1.30 ppm in respective autumn seasons was observed. However, a concentration of 09.72 ± 0.99 ppm in summer season (year-II) and 06.50 ± 0.06 ppm in winter (year-II) was observed (Fig. 53). When compared the concentration in liver during other

seasons showed mean values of 01.13 ± 0.02 and 02.74 ± 0.03 ppm in spring seasons of year-I and year-II, 03.16 ± 0.98 and 04.74 ± 0.11 ppm in autumn seasons of year 2005-2007. However, a concentration of 07.13 ± 0.99 ppm in summer season (year-I) and 03.55 ± 0.07 ppm in winter season (year-II) respectively was observed (Fig. 57). When compared the concentration in kidney during other seasons showed mean values of 01.63 ± 0.55 and 02.66 ± 0.03 ppm in autumn seasons of year-I and year-II, 0.99 ± 0.09 and 01.52 ± 0.02 ppm in winter seasons of years 2005-2007. However, a concentration of 0.95 ± 0.08 ppm in spring season (year-II) and 05.74 ± 0.77 ppm in summer season (year-I) respectively was observed (Fig. 61). When compared the concentration in muscle during other seasons showed mean values of 0.62 ± 0.02 and 0.72 ± 0.04 ppm in autumn seasons of year-I and year-II, 0.20 ± 0.02 and 0.55 ± 0.07 ppm in winter seasons of year 2005-2007. However, a concentration of 0.12 ± 0.05 ppm in spring season (year-II) and 01.32 ± 0.19 ppm in summer season (year-I) respectively was observed (Fig. 65).

The maximum manganese content in *Schizothorax niger* was observed in gill and the minimum concentration was observed in muscle. Summer season contained the maximum values of metals in all seasons.

In *Cyprinus carpio* spp. obtained from the Dal Lake, the manganese concentration in gills, liver, kidney and muscle ranged between 05.99 ± 0.87 to 13.21 ± 2.48 ppm; 03.67 ± 0.09 to 11.72 ± 2.11 ppm; 01.32 ± 0.31 to 09.34 ± 1.59 ppm and 0.06 ± 0.11 to 06.82 ± 1.12 ppm respectively (Table XVII-XX). The highest value of 13.21 ± 2.48 ppm in gills, 11.72 ± 2.11 ppm in liver, 09.34 ± 1.59 ppm in kidney and 06.82 ± 1.12 ppm were observed in summer season of year-II and minimum value of 05.99 ± 0.87 ppm in gills, 03.67 ± 0.09 in liver, 01.32 ± 0.31 ppm in kidney and 0.06 ± 0.11 ppm in muscle were observed in the winter season of the year-I. Further, the

concentration of zinc in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 06.54 ± 0.81 ppm and 08.22 ± 0.91 ppm in respective spring seasons (2005-2007), 07.92 ± 0.79 ppm and 09.54 ± 2.01 ppm in respective autumn seasons was observed. However, a concentration of 11.22 ± 1.12 ppm in summer season (year-I) and 07.33 ± 0.12 ppm in winter (year-II) was observed (Fig. 54). When compared the concentration in liver during other seasons showed mean values of 04.22 ± 0.72 and 05.11 ± 0.81 ppm in spring seasons of year-I and year-II, 05.24 ± 1.10 and 06.77 ± 1.12 ppm in autumn seasons of year 2005-2007. However, a concentration of 10.54 ± 2.10 ppm in summer season (year-I) and 04.92 ± 0.11 ppm in winter season (year-II) respectively was observed (Fig. 58). When compared the concentration in kidney during other seasons showed mean values of 02.34 ± 0.03 and 03.12 ± 0.81 ppm in spring seasons of year-I and year-II, 03.22 ± 0.99 and 04.05 ± 1.01 ppm in autumn seasons of year 2005-2007. However, a concentration of 08.36 ± 1.24 ppm in summer season (year-II) and 02.01 ± 0.01 ppm in winter season (year-I) respectively was observed (Fig. 62). When compared the concentration in muscle during other seasons showed mean values of 0.89 ± 0.81 and 02.91 ± 0.77 ppm in spring seasons of year-I and year-II, 01.66 ± 0.90 and 03.72 ± 0.70 ppm in autumn seasons of year 2005-2007. However, a concentration of 04.11 ± 1.01 ppm in summer season (year-I) and 0.32 ± 0.05 ppm in winter season (year-II) respectively was observed (Fig. 64).

In case of *Cyprinus carpio* spp. from the Dal lake, the maximum concentration of manganese was observed in gills followed by liver and kidney and the minimum was observed in muscle tissue. The values of manganese was also found higher in summer season of the year-II.

River Jhelum: In *Schizothorax niger* collected from the River Jhelum, the concentration of manganese in gills, liver, kidney and muscle ranged between 01.54 ± 0.42 to 10.66 ± 2.01 ppm; 0.87 ± 0.02 to 06.73 ± 0.55 ppm; 0.51 ± 0.08 to 03.61 ± 0.30 ppm and 0.05 ± 0.01 to 01.00 ± 0.10 ppm respectively (Table XVII-XX). The maximum value of 10.66 ± 2.01 in gills, 06.73 ± 0.55 ppm in liver, 03.61 ± 0.30 ppm in kidney and 01.00 ± 0.10 ppm were found in the summer season of the year-II and the minimum concentration of 01.54 ± 0.42 ppm in gills, 0.87 ± 0.02 ppm in liver, 0.51 ± 0.08 ppm in kidney and 0.05 ± 0.01 ppm in muscle were observed in the spring season of the year-I. Further, the concentration of manganese in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 04.33 ± 0.80 ppm and 06.52 ± 1.12 ppm in respective autumn seasons (2005-2007), 03.12 ± 0.50 ppm and 05.00 ± 0.11 ppm in respective winter seasons was observed. However, a concentration of 03.61 ± 0.29 ppm in spring season (year-II) and 08.19 ± 0.91 ppm in summer (year-I) was observed (Fig. 55). When compared the concentration in liver during other seasons showed mean values of 01.76 ± 0.02 and 02.84 ± 0.33 ppm in autumn seasons of year-I and year-II, 0.89 ± 0.12 and 0.93 ± 0.09 ppm in winter seasons of year 2005-2007. However, a concentration of 0.92 ± 0.09 ppm in spring season (year-II) and 05.24 ± 0.59 ppm in summer season (year-I) respectively (Fig. 59). When compared the concentration in kidney during other seasons showed mean values of 0.93 ± 0.15 and 0.98 ± 0.09 ppm in autumn seasons of year-I and year-II, 0.55 ± 0.05 and 0.74 ± 0.05 ppm in winter seasons of year 2005-2007. However, a concentration of 0.52 ± 0.04 ppm in spring season (year-II) and 0.32 ± 0.98 ppm in summer season (year-I) respectively was observed (Fig. 63). When compared the concentration in muscle during other seasons showed mean values of 0.56 ± 0.06 and 0.72 ± 0.17 ppm in autumn seasons of year-I and

year-II, 0.14 ± 0.02 and 0.52 ± 0.09 ppm in winter seasons of year 2005-2007. However, a concentration of 0.63 ± 0.55 ppm in spring season (year-II) and 0.97 ± 0.54 ppm in summer season (year-II) respectively was observed (Fig. 67).

The maximum concentration was observed in gills and the minimum concentration was found in muscle. The maximum concentration was observed in the summer season of the year-I.

In case of *Cyprinus carpio* spp. from River Jhleum, the manganese concentration in gills, liver, kidney and muscle varied between 04.76 ± 0.80 to 12.01 ± 2.12 ppm; 0.97 ± 0.07 to 08.95 ± 1.12 ppm; 0.57 ± 0.09 to 06.74 ± 1.12 ppm and 0.24 ± 0.02 to 03.43 ± 0.09 ppm respectively (Table XVII-XX). The maximum value of 12.01 ± 2.12 ppm in gills, 08.95 ± 1.12 ppm in liver, 06. 74 ± 1.12 ppm in kidney and 03.43 ± 0.09 ppm in muscle was observed in the summer season of year-II. The minimum value of 04.76 ± 0.80 ppm in gills was observed in the winter season of the year-I. In liver the minimum value of 0.97 ± 0.07 ppm was observed in the spring season of year-I, in kidney the minimum value of 0.57 ± 0.09 ppm was found in the winter season (year-I) and the lowest value of 0.24 ± 0.02 ppm in muscle was observed in the winter season of the year-I. Further, the concentration of manganese in gills, liver, kidney and muscle during rest of the seasons showed different concentrations when compared with each other. In gills, a concentration of 05.32 ± 0.71 ppm and 07.12 ± 0.99 ppm in respective spring seasons (2005-2007), 06.25 ± 0.75 ppm and 08.79 ± 1.32 ppm in respective autumn seasons was observed. However, a concentration of 10.54 ± 1.11 ppm in summer season (year-II) and 06.99 ± 0.95 ppm in winter (year-I) was observed (Fig. 56). When compared the concentration in liver during other seasons showed mean values of 03.52 ± 0.41 and 04.61 ± 0.45 ppm in autumn seasons of year-

I and year-II, 01.04 ± 0.19 and 01.99 ± 0.09 ppm in winter seasons of year 2005-2007. However, a concentration of 0.97 ± 0.07 ppm in spring season (year-I) and 07.95 ± 1.10 ppm in summer season (year-I) respectively was observed (Fig. 60). When compared the concentration in kidney during other seasons showed mean values of 0.67 ± 0.09 and 01.62 ± 0.33 ppm in spring seasons of year-I and year-II, 01.99 ± 1.00 and 02.59 ± 0.52 ppm in autumn seasons of year 2005-2007. However, a concentration of 05.54 ± 0.55 ppm in summer season (year-I) and 01.22 ± 0.21 ppm in winter season (year-II) respectively was observed (Fig. 64). When compared the concentration in muscle during other seasons showed mean values of 0.84 ± 0.50 and 0.66 ± 0.60 ppm in spring seasons of year 2005-2007, 01.01 ± 0.10 and 0.85 ± 0.51 ppm in autumn seasons of year-I and 2006-2007. However, a concentration of 03.24 ± 0.99 ppm in summer season (year-I) and 0.62 ± 0.51 ppm in winter season (year-II) respectively was observed (Fig. 68).

Like in *Schizothorax niger* inhabiting the same habitat, *Cyprinus carpio* spp also contained the maximum concentration of manganese in the summer season and in the gills tissues followed by liver, kidney and the lowest value was obtained in the muscle tissue.

4.3. Histochemistry

The tissues of the fishes were analysed histochemically so as to localize the deposition of copper, iron and zinc in gills, liver, kidney and muscles. The analysis was done seasonally and the results showed highest concentration of the metals during summer followed by spring, autumn and winter.

4.3.1. Metal accumulation by gills

Histochemical analysis of Cu, Fe and Zn in gills of *Schizothorax niger* and *Cyprinus carpio* spp. demonstrated an enormous amount of metals in summer seasons during the entire study period. However, the concentration of these metals was found to be comparatively more in *Cyprinus carpio* spp. than in *Schizothorax niger* (Fig. 97-101). Further, the concentration of metals in the gills of fish from Dal lake was comparatively higher than the gills of the fish from River Jhelum. In other seasons the concentration of metals in gills was comparatively less.

4.3.2. Metal accumulation by liver

During the entire study period, liver of both the fishes demonstrated highest concentration of the metals during summer season followed by spring, autumn and winter. Liver of *Cyprinus carpio* spp. collected from Dal Lake demonstrated comparatively more concentration than the liver of *Schizothorax niger* (Fig. 102-106).

4.3.3. Metal accumulation by kidney

Comparatively kidneys demonstrated lesser amount of the metals than liver. However, seasonal analysis showed higher concentration of metals in kidneys of *Cyprinus carpio* spp. and *Schizothorax niger* in summer followed by other seasons (Fig. 107-108). Further, in winter a small amount of metals was found to be deposited in the kidneys.

4.3.4. Metal accumulation by muscle

Muscles of *Cyprinus carpio* spp. and *Schizothorax niger* were found to possess neglegible amount of metals during the entire period of study.

4.4. Histopathology

The subsequent effects of metals, demonstrated by wet digestion-based atomic absorption method and histochemcial methods on gills, liver, kidney, muscle of *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake and River Jhelum were analysed for histological changes. The histological study showed gross changes in gills, liver and kidneys of the fish. However, no histological changes were detected in muscles of both the fishes in either of the water bodies.

4.4.1. Histopathology of gills

In gills of both the fishes collected from either of the water bodies, the general changes included oedema, congestion of the secondary lamellae with epithelial hypertrophy and hyperplasia principally at the origin of the primary lamellae or rakers with proliferation of the basal cell were observed (Fig. 109-111). Further, the apex of the secondary lamellae was often club shaped and blunt. Often the lamellae were fused together at the apex, at the hemi-branch, or the proliferating cells of one lamellae would be fused with the opposite lamellar side. Loss of epithelium, mononuclear cells were sometimes found to infiltrate into the lamellae. Changes were often associated with mucus cell hyperplasia with a compressed nucleus towards the base (Fig. 112-116). However, telangietatisis at the margins of the gill filaments and vacuolar degeneration in the epithelial cells with an ecentric nucleus located more at the tips of the secondary lamellae and deposition of the melanin like substance noticed on the gill filaments were observed in *Cyprinus carpio* spp. collected from Dal lake during summer season (Fig. 117-119).

4.4.2. Histopathology of liver

The liver in both fish hosts showed disruption of the hepatic cords and tubules with congestion and degenerative changes in hepatocytes that varied from mild in winter seasons to severe vascular degeneration in summer season. (Fig. 120-121). Further, kupffer cell hyperplasia was noticed in *Cyprinus carpio* spp. collected from Dal lake during summer season (Fig. 122).

4.4.3. Histopathology of kidney

The general changes observed in *Cyprinus carpio* spp. and *Schizothorax niger* collected from both the water bodies included atrophy of the glomerulus with hypercellularity and hyperplasia (Fig. 123-125). The changes in the kidneys included mild congestion in *Schizothorax niger* during winter seasons (Fig. 126) to severe tubular degeneration in *Cyprinus carpio* spp. during summer seasons (Fig. 127).

From the present study it is inferred that the water bodies of the Kashmir valley particularly Dal lake and River Jhelum possess different metal concentration. These metals have accumulated in the different tissues of icthyofauna of the water bodies. Subsequently, the metals have altered the biochemical and histomorphological features of these economically impotant fishes.

Table III: Showing Metal concentration (ppm) in Dal Lake and River Jhelum (2005-2006 Year-I)

Water Resource	Metal Concentration	Spring	Summer	Autumn	Winter	International Standards W.H.O. (µg/L)
Dal Lake	Copper	1.040	1.060	1.030	1.020	5,000
	Iron	0.120	0.189	0.144	0.110	300
	Zinc	0.500	0.530	0.350	0.150	1,000
	Maganese	0.056	0.080	0.060	0.021	100
River Jhelum	Copper	1.003	1.004	1.003	1.002	5,000
	Iron	0.159	0.166	0.129	0.148	300
	Zinc	0.460	0.472	0.340	0.100	1,000
	Maganese	0.011	0.044	0.033	0.0056	100

Table IV: Showing Metal concentration (ppm) in Dal Lake and River Jhelum (2006-2007 Year-II)

Water Resource	Metal Concentration	Spring	Summer	Autumn	Winter	International Standards W.H.O. (μg/L)
Dal Lake	Copper	1.050	1.070	1.040	1.030	5,000
	Iron	0.124	0.191	0.146	0.112	300
	Zinc	0.533	0.542	0.351	0.152	1,000
	Maganese	0.057	0.083	0.061	0.022	100
River Jhelum	Copper	1.004	1.006	1.005	1.003	5,000
	Iron	0.165	0.168	0.133	0.159	300
	Zinc	0.462	0.483	0.356	0.111	1,000
	Maganese	0.013	0.053	0.034	0.0057	100

Table V: Showing Copper concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Spring Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	13.52 ± 1.12	70.01 ± 2.12	69.11 ± 3.71	08.53 ± 1.20
		2006-07	25	15.52 ± 1.24	74.54 ± 3.24	72.18 ± 3.84	09.85 ± 1.26
	Cyprinus carpio	2005-06	25	16.90 ± 1.52	99.41 ± 3.01	96.52 ± 3.52	10.55 ± 0.80
		2006-07	25	19.13 ± 1.90	103.66 ± 3.99	100.21 ± 3.61	11.21 ± 0.88
River Jhelum	*Schizothorax niger*	2005-06	25	12.81 ± 1.29	68.12 ± 2.54	67.11 ± 3.12	07.35 ± 0.99
		2006-07	25	14.33 ± 1.12	73.21 ± 3.24	71.69 ± 3.12	08.12 ± 1.17
	Cyprinus carpio	2005-06	25	14.82 ± 1.75	97.62 ± 3.99	94.33 ± 3.25	09.88 ± 0.50
		2006-07	25	16.11 ± 1.20	102.62 ± 4.01	99.99 ± 3.92	10.22 ± 0.78

Values are expressed as mean ±SEM

Table VI: Showing Copper concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Summer Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	20.66 ± 2.59	76.52 ± 2.81	73.31 ± 3.24	12.72 ± 1.10
		2006-07	25	21.84 ± 2.49	81.68 ± 3.51	78.90 ± 3.42	12.11 ± 1.12
	Cyprinus carpio	2005-06	25	24.98 ± 2.91	132.01 ± 3.21	125.45 ± 5.01	16.58 ± 2.85
		2006-07	25	25.99 ± 2.71	139.22 ± 4.24	132.83 ± 4.54	17.58 ± 2.93
River Jhelum	*Schizothorax niger*	2005-06	25	19.54 ± 2.40	74.61 ± 2.84	71.84 ± 3.12	12.72 ± 1.10
		2006-07	25	16.33 ± 2.52	79.52 ± 3.81	77.64 ± 3.52	11.03 ± 1.04
	Cyprinus carpio	2005-06	25	21.25 ± 2.15	129.99 ± 3.91	123.51 ± 4.97	14.11 ± 1.55
		2006-07	25	23.24 ± 2.54	131.99 ± 4.52	129.62 ± 4.12	16.27 ± 2.83

Values are expressed as mean ±SEM

Table VII: Showing Copper concentration in *Schizothorax niger*. and *Cyprinus carpio* spp. in Autumn Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	15.71 ± 1.21	68.52 ± 2.12	66.12 ± 3.52	08.32 ± 0.85
		2006-07	25	16.26 ± 1.67	72.82 ± 3.24	70.62 ± 3.72	09.01 ± 0.95
	Cyprinus carpio	2005-06	25	19.97 ± 2.52	113.24 ± 2.92	110.22 ± 3.12	11.72 ± 1.60
		2006-07	25	20.66 ± 1.80	114.11 ± 4.01	112.27 ± 3.24	12.16 ± 1.71
River Jhelum	*Schizothorax niger*	2005-06	25	14.71 ± 1.61	66.12 ± 2.66	64.24 ± 3.42	07.98 ± 0.77
		2006-07	25	12.11 ± 2.12	71.23 ± 3.24	68.12 ± 3.12	08.72 ± 0.84
	Cyprinus carpio	2005-06	25	16.01 ± 2.31	109.11 ± 3.24	108.66 ± 3.10	10.91 ± 1.50
		2006-07	25	18.79 ± 1.77	111.12 ± 3.90	109.62 ± 3.82	11.27 ± 1.54

Values are expressed as mean ±SEM

Table VIII: Showing Copper concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Winter Season in Dal lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Copper accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	14.92 ± 1.21	66.77 ± 3.12	64.61 ± 3.10	07.81 ± 0.54
		2006-07	25	16.01 ± 1.72	70.54 ± 3.12	68.79 ± 3.18	08.34 ± 0.71
	Cyprinus carpio	2005-06	25	18.11 ± 1.61	110.62 ± 3.91	108.07 ± 3.55	09.99 ± 1.24
		2006-07	25	16.83 ± 1.82	112.99 ± 3.92	109.85 ± 3.71	10.98 ± 1.76
River Jhelum	*Schizothorax niger*	2005-06	25	13.99 ± 1.70	63.69 ± 3.69	62.54 ± 2.99	06.33 ± 0.50
		2006-07	25	10.13 ± 1.06	68.55 ± 3.12	66.92 ± 3.10	07.26 ± 0.68
	Cyprinus carpio	2005-06	25	15.03 ± 1.10	107.85 ± 3.12	106.11 ± 3.14	08.24 ± 1.01
		2006-07	25	17.48 ± 1.80	110.50 ± 3.54	107.65 ± 3.24	09.17 ± 1.59

Values are expressed as mean ±SEM

Table IX: Showing Zinc concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Spring Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	52.11 ± 2.12	74.52 ± 2.24	89.41± 4.12	31.89 ± 3.11
		2006-07	25	62.32 ± 4.52	81.06 ± 3.44	96.52 ± 3.01	33.24 ± 3.22
	Cyprinus carpio	2005-06	25	56.92 ± 2.52	115.16 ± 5.21	126.98 ± 4.91	35.22 ± 3.09
		2006-07	25	75.61 ± 3.92	124.76 ± 5.98	135.52 ± 4.18	37.01 ± 3.19
River Jhelum	*Schizothorax niger*	2005-06	25	50.60 ± 2.50	73.81 ± 4.10	80.42 ± 4.11	29.11 ± 3.00
		2006-07	25	61.12 ± 4.43	80.15 ± 3.36	81.56 ± 3.60	32.25 ± 3.19
	Cyprinus carpio	2005-06	25	54.79 ± 2.91	112.98 ± 3.92	123.22 ± 4.52	33.16 ± 2.99
		2006-07	25	73.81 ± 3.72	121.75 ± 5.84	127.92 ± 4.02	36.49 ± 3.15

Values are expressed as mean ±SEM

Table X: Showing Zinc concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Summer Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	57.21 ± 2.55	90.61 ± 3.92	94.32 ± 4.15	37.41 ± 3.52
		2006-07	25	72.44 ± 3.92	97.84 ± 4.62	101.99 ± 4.03	39.72 ± 3.81
	Cyprinus carpio	2005-06	25	61.82 ± 3.12	143.24 ± 5.01	149.80 ± 5.20	42.38 ± 3.59
		2006-07	25	87.25 ± 3.52	152.61 ± 5.26	159.32 ± 4.52	44.52 ± 3.95
River Jhelum	*Schizothorax niger*	2005-06	25	55.62 ± 2.94	82.59 ± 4.12	86.63 ± 3.52	35.66 ± 3.15
		2006-07	25	69.24 ± 3.87	95.43 ± 4.53	99.84 ± 4.01	38.12 ± 3.72
	Cyprinus carpio	2005-06	25	59.11 ± 3.29	137.99 ± 4.12	143.13 ± 5.12	40.22 ± 3.41
		2006-07	25	86.43 ± 3.41	141.81 ± 5.12	148.92 ± 4.27	43.44± 3.84

Values are expressed as mean ±SEM

Table XI: Showing Zinc concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Autumn Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	55.36 ±2.51	75.12 ± 4.77	91.36 ± 2.81	29.93 ± 2.55
		2006-07	25	64.72 ± 2.99	82.52 ± 3.99	98.05 ± 3.11	31.92 ± 2.86
	Cyprinus carpio	2005-06	25	59.31 ± 2.90	114.01 ± 5.20	119.84 ± 4.15	35.29 ± 2.75
		2006-07	25	77.20 ± 3.55	124.05 ± 4.82	129.71 ± 4.91	37.11 ± 2.86
River Jhelum	*Schizothorax niger*	2005-06	25	53.82 ± 2.15	74.32 ± 4.10	82.72 ± 4.12	27.18 ± 2.15
		2006-07	25	62.91 ± 2.82	80.01± 3.84	85.92 ± 3.92	30.71 ± 2.74
	Cyprinus carpio	2005-06	25	57.13 ± 2.55	113.63 ± 4.72	116.31 ± 4.05	33.26 ± 2.55
		2006-07	25	75.11 ± 3.24	122.12 ± 4.64	118.11 ± 4.92	36.92 ± 2.77

Values are expressed as mean ±SEM

Table XII: Showing Zinc concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Winter Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Zinc accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	54.81 ± 2.99	73.81 ± 2.52	88.77 ± 3.52	30.88 ± 2.10
		2006-07	25	64.61 ± 3.28	80.88 ± 4.15	95.24 ± 3.92	32.40 ± 2.16
	Cyprinus carpio	2005-06	25	58.23 ± 2.51	111.35 ± 4.24	122.45 ± 4.51	34.46 ± 2.12
		2006-07	25	76.47 ± 4.52	121.40 ± 4.86	123.66 ± 4.56	36.71 ± 2.21
River Jhelum	*Schizothorax niger*	2005-06	25	52.72 ± 3.22	71.99 ± 3.92	81.35 ± 3.24	28.65 ± 2.01
		2006-07	25	62.79 ± 3.12	78.82 ± 4.10	79.61 ± 3.52	31.09 ± 2.11
	Cyprinus carpio	2005-06	25	56.59 ± 2.81	109.98 ± 3.92	119.54 ± 4.15	32.17 ± 2.09
		2006-07	25	74.29 ±4.24	119.22 ± 4.54	125.22 ± 3.99	35.52 ± 2.18

Values are expressed as mean ±SEM

Table XIII: Showing Iron concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Spring Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Iron accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	137.66 ± 5.22	227.91 ± 6.52	224.34 ± 6.05	41.77 ± 3.51
		2006-07	25	140.21 ± 4.77	241.20 ± 6.96	239.44 ± 6.52	44.22 ± 3.82
	Cyprinus carpio	2005-06	25	153.72 ± 4.25	291.54 ± 6.15	287.52 ± 6.15	52.66 ± 4.01
		2006-07	25	159.23 ± 4.34	310.72 ± 6.25	306.52 ± 6.59	55.42 ± 4.65
River Jhelum	*Schizothorax niger*	2005-06	25	135.62 ± 3.21	216.12 ± 6.12	213.12 ± 5.85	39.12 ± 3.12
		2006-07	25	139.92 ± 4.61	233.11 ± 6.81	230.52 ± 6.15	43.31 ± 3.79
	Cyprinus carpio	2005-06	25	151.92 ± 3.25	285.61 ± 6.21	281.31 ± 6.24	50.85 ± 3.92
		2006-07	25	156.74 ± 4.29	292.34 ± 6.15	287.92 ± 6.50	54.66 ± 4.54

Values are expressed as mean ±SEM

Table XIV: Showing Iron concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Summer Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Iron accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	188.11 ± 5.12	284.31 ± 4.29	280.41 ± 4.20	46.62 ± 3.24
		2006-07	25	192.52 ± 6.54	296.51 ± 4.37	292.61 ± 4.25	49.23 ± 3.84
	Cyprinus carpio	2005-06	25	199.35 ± 5.99	379.11 ± 6.12	374.66 ± 6.10	59.38 ± 3.51
		2006-07	25	201.61 ± 5.24	392.21 ± 6.15	388.69 ± 6.12	62.71 ± 3.92
River Jhelum	*Schizothorax niger*	2005-06	25	186.17 ± 4.01	281.59 ± 4.21	277.55 ± 4.53	44.81 ± 3.12
		2006-07	25	187.46 ± 6.34	294.62 ± 4.32	289.77 ± 4.52	48.16 ± 3.82
	Cyprinus carpio	2005-06	25	196.62 ± 5.21	377.31 ± 6.01	373.85 ± 6.10	57.62 ± 3.42
		2006-07	25	199.52 ± 5.12	381.43 ± 6.11	378.66 ± 6.01	61.07 ± 3.74

Values are expressed as mean ±SEM

Table XV: Showing Iron concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Autumn Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Iron accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	139.24 ± 3.51	228.36 ± 6.55	224.90 ± 6.24	42.22 ± 3.15
		2006-07	25	142.01 ± 6.20	242.54 ± 4.85	238.11 ± 4.15	45.44 ± 3.56
	Cyprinus carpio	2005-06	25	157.88 ± 4.25	304.11 ± 5.14	300.71 ± 5.04	53.11 ± 3.52
		2006-07	25	162.41 ± 4.28	314.52 ± 5.54	311.12 ± 5.19	56.41 ± 3.62
River Jhelum	*Schizothorax niger*	2005-06	25	137.11 ± 3.12	224.52 ± 4.21	220.79 ± 4.20	40.77 ± 3.10
		2006-07	25	140.76 ± 6.12	240.22 ± 4.72	237.20 ± 4.52	44.78 ± 3.55
	Cyprinus carpio	2005-06	25	155.59 ± 4.21	301.12 ± 5.01	297.77 ± 4.89	51.90 ± 3.41
		2006-07	25	160.23 ± 4.18	312.16 ± 5.32	307.52 ± 5.12	55.11 ± 3.57

Values are expressed as mean ±SEM

Table XVI: Showing Iron concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Winter Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Iron accumulation in ppm			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	130.10 ± 5.55	204.92 ± 5.21	200.99 ± 5.04	40.88 ± 2.15
		2006-07	25	138.24 ± 5.22	234.56 ± 5.95	230.62 ± 5.55	43.72 ± 2.52
	Cyprinus carpio	2005-06	25	156.67 ± 4.52	290.32 ± 6.51	286.75 ± 6.25	51.67 ± 3.12
		2006-07	25	160.12 ± 4.34	299.23 ± 5.92	296.66 ± 5.19	54.41 ± 3.55
River Jhelum	*Schizothorax niger*	2005-06	25	127.52 ± 5.21	208.44 ± 5.12	204.32 ± 5.10	38.39 ± 2.12
		2006-07	25	136.11 ± 5.10	239.65 ± 5.84	235.68 ± 4.01	31.90 ± 2.51
	Cyprinus carpio	2005-06	25	154.29 ± 4.12	281.81 ± 6.12	278.66 ± 6.10	49.22 ± 3.10
		2006-07	25	158.26 ± 4.32	307.01 ± 5.74	299.34 ± 4.15	53.21 ± 3.42

Values are expressed as mean ±SEM

Table XVII: Showing Manganese concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Spring Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	02.71 ± 0.52	01.13 ± 0.02	0.84 ± 0.06	0.09 ± 0.01
		2006-07	25	04.95 ± 0.27	02.74 ± 0.03	0.95 ± 0.08	0.12 ± 0.05
	Cyprinus carpio	2005-06	25	06.54 ± 0.81	04.22 ± 0.72	02.34 ± 0.03	0.89 ± 0.81
		2006-07	25	08.22 ± 0.91	05.11 ± 0.81	03.12 ± 0.81	02.91± 0.77
River Jhelum	*Schizothorax niger*	2005-06	25	01.54 ± 0.42	0.87 ± 0.02	0.51 ± 0.08	0.05 ± 0.01
		2006-07	25	03.61 ± 0.29	0.92 ± 0.09	0.52 ± 0.04	0.63 ± 0.55
	Cyprinus carpio	2005-06	25	05.32 ± 0.71	0.97 ± 0.07	0.67 ± 0.09	0.84 ± 0.50
		2006-07	25	07.12 ± 0.99	0.88 ± 0.08	01.62 ± 0.33	0.66 ± 0.60

Values are expressed as mean ±SEM

Table XVIII: Showing Manganese concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Summer Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	09.72 ± 0.99	07.13 ± 0.99	05.74 ± 0.77	01.32 ± 0.19
		2006-07	25	11.55 ± 2.91	08.30 ± 1.00	06.95 ± 0.93	03.92 ± 0.88
	Cyprinus carpio	2005-06	25	11.22 ± 1.12	10.54 ± 2.10	08.36 ± 1.24	04.11 ± 1.01
		2006-07	25	13.21 ± 2.48	11.72 ± 2.11	09.34 ± 1.59	06.82 ± 1.12
River Jhelum	*Schizothorax niger*	2005-06	25	08.19 ± 0.91	05.24 ± 0.59	03.32 ± 0.98	01.00 ± 0.10
		2006-07	25	10.66 ± 2.01	06.73 ± 0.55	03.61 ± 0.30	0.97 ± 0.54
	Cyprinus carpio	2005-06	25	10.54 ± 1.11	07.95 ± 1.10	05.54 ± 0.55	03.24 ± 0.99
		2006-07	25	12.01 ± 2.12	08.95 ± 1.12	06.74 ± 1.12	03.43 ± 0.09

Values are expressed as mean ±SEM

Table XIX: Showing Manganese concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Autumn Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	05.43 ± 0.85	03.16 ± 0.98	01.63 ± 0.55	0.62 ± 0.02
		2006-07	25	07.49 ± 1.30	04.74 ± 0.11	02.66 ± 0.03	0.72 ± 0.04
	Cyprinus carpio	2005-06	25	07.92 ± 0.79	05.24 ± 1.10	03.22 ± 0.99	01.66 ± 0.90
		2006-07	25	09.54 ± 2.01	06.77 ± 1.12	04.05 ± 1.01	03.72 ± 0.70
River Jhelum	*Schizothorax niger*	2005-06	25	04.33 ± 0.80	01.76 ± 0.02	0.93 ± 0.15	0.56 ± 0.06
		2006-07	25	06.52 ± 1.12	02.84 ± 0.33	0.98 ± 0.09	0.72 ± 0.17
	Cyprinus carpio	2005-06	25	06.25 ± 0.75	03.52 ± 0.41	01.99 ± 1.00	01.01 ± 0.10
		2006-07	25	08.79 ± 1.32	04.61 ± 0.45	02.59 ± 0.52	0.85 ± 0.51

Values are expressed as mean ±SEM

Table XX: Showing Manganese concentration in *Schizothorax niger* and *Cyprinus carpio* spp. in Winter Season in Dal Lake and River Jhelum

Water resources	Fish Host	Year	No. Observed	Manganese accumulation (ppm)			
				Gill	Liver	Kidney	Muscle
Dal Lake	*Schizothorax niger*	2005-06	25	04.32 ± 0.52	02.81 ± 0.05	0.99 ± 0.09	0.20 ± 0.02
		2006-07	25	06.50 ± 0.06	03.55 ± 0.07	01.52 ± 0.02	0.55 ± 0.07
	Cyprinus carpio	2005-06	25	05.99 ± 0.87	03.67 ± 0.09	01.32 ± 0.31	0.32 ± 0.05
		2006-07	25	07.33 ± 0.12	04.92 ± 0.11	02.01± 0.01	0.06 ± 0.11
River Jhelum	*Schizothorax niger*	2005-06	25	03.12 ± 0.50	0.89 ± 0.12	0.55 ± 0.05	0.14 ± 0.02
		2006-07	25	05.00 ± 0.11	0.93 ± 0.09	0.74 ± 0.05	0.52 ± 0.09
	Cyprinus carpio	2005-06	25	04.76 ± 0.80	01.04 ± 0.19	0.57 ± 0.09	0.24 ± 0.02
		2006-07	25	06.99 ± 0.95	01.99 ± 0.09	01.22 ± 0.21	0.62 ± 0.51

Values are expressed as mean ±SEM

Table XXI: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Spring Season in Dal Lake and River Jhelum

Water Resources	Fish Host	Year	No. Observed	Tot. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Sr. Urea (mg/dl)	Sr. creatinine (mg/dl)	Tot. Cholesterol (mg/dl)
Dal Lake	*Schizothorax niger*	2005-06	25	2.10 ± 0.44	1.41 ± 0.02	0.59 ± 0.02	216.1 ± 17.63	18.0 ± 0.37	0.86 ± 0.12	52.71 ± 8.66
		2006-07	25	2.16 ± 0.07	2.00 ± 0.03	0.16 ± 0.04	222.3 ± 30.97	19.1 ± 1.12	0.87 ± 0.04	63.71 ± 2.22
	Cyprinus carpio	2005-06	25	1.69 ± 0.22	0.92 ± 0.16	0.77 ± 0.06	237.8 ± 31.32	22.0 ± 1.43	0.88 ± 0.03	54.46 ± 2.99
		2006-07	25	1.82 ± 0.10	1.11 ± 0.02	0.71 ± 0.08	241.8 ± 11.62	22.8 ± 1.59	0.89 ± 0.14	69.12 ± 1.87
River Jhelum	*Schizothorax niger*	2005-06	25	2.18 ± 0.09	1.96 ± 0.18	0.22 ± 0.09	192.3 ± 18.02	17.2 ± 0.88	0.72 ± 0.11	51.66 ± 9.01
		2006-07	25	2.33 ± 0.15	2.18 ± 0.09	0.15 ± 0.06	202.8± 29.38	17.8 ± 1.52	0.81 ± 0.06	52.44 ± 3.12
	Cyprinus carpio	2005-06	25	1.92 ± 0.13	1.03 ± 0.01	0.89 ± 0.12	218.8 ± 39.20	20.9 ± 1.60	0.74 ± 0.02	63.20 ± 3.01
		2006-07	25	1.88 ± 0.18	1.78 ± 0.07	0.10 ± 0.11	222.6 ± 66.52	22.2 ± 1.44	0.77 ± 0.13	63.20 ± 1.55

Table XXII: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Summer Season in Dal Lake and River Jhelum

Water Resources	Fish Host	Year	No. Observed	Tot. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Sr. Urea (mg/dl)	Sr. creatinine (mg/dl)	Tot. Cholesterol (mg/dl)
Dal Lake	*Schizothorax niger*	2005-06	25	3.95 ± 0.17	2.00 ± 0.10	1.95 ± 0.07	340.1 ± 17.00	22.4 ± 1.08	1.14 ± 0.05	89.34 ± 4.12
		2006-07	25	4.32 ± 0.13	1.95 ± 0.28	2.37 ± 0.15	336.8 ± 13.84	22.8 ± 3.13	1.16 ± 0.05	95.31 ± 5.30
	Cyprinus carpio	2005-06	25	3.79 ± 0.30	1.66 ± 0.13	2.13 ± 0.23	352.5 ± 24.59	23.1 ± 1.01	1.18 ± 0.09	95.47 ± 3.89
		2006-07	25	3.68 ± 0.33	1.50 ± 0.14	2.18 ± 0.19	348.8 ± 28.01	23.3 ± 2.99	1.19 ± 0.07	98.22 ± 2.98
River Jhelum	*Schizothorax* niger	2005-06	25	3.91 ± 0.22	3.60 ± 0.15	0.31 ± 0.07	332.1 ± 17.14	21.6 ± 1.72	1.09 ± 1.20	99.34± 4.55
		2006-07	25	4.23 ± 0.43	3.72 ± 0.38	0.51 ± 0.05	337.6 ± 12.12	21.7 ± 3.59	1.11± 0.06	100.01 ± 5.88
	Cyprinus carpio	2005-06	25	2.35 ± 0.12	2.23 ± 0.21	0.02 ± 0.09	341.6 ± 25.01	23.8 ± 1.99	1.12 ± 0.12	82.77 ± 3.52
		2006-07	25	3.13 ± 0.40	2.22 ± 0.11	0.91 ± 0.29	342.9 ± 28.77	24.0 ± 2.12	1.14 ± 0.09	92.03 ± 3.01

Table XXIII: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Autumn Season in Dal Lake and River Jhelum

Water Resources	Fish Host	Year	No. Observed	Tot. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Sr. Urea (mg/dl)	Sr. creatinine (mg/dl)	Tot. Cholesterol (mg/dl)
Dal Lake	*Schizothorax niger*	2005-06	25	2.85 ± 0.29	1.85 ± 0.11	1.00 ± 0.18	178.1 ± 10.27	18.6 ± 1.66	0.91 ± 0.04	58.27 ± 2.17
		2006-07	25	2.15 ± 0.08	1.12 ± 0.03	1.03 ± 0.05	202.3 ± 14.69	18.9 ± 1.46	0.92 ± 0.30	60.33 ± 2.81
	Cyprinus carpio	2005-06	25	2.88 ± 0.42	1.02 ± 0.00	1.86 ± 0.42	218.8 ± 27.80	20.0 ± 1.38	0.93 ± 0.04	66.75 ± 3.54
		2006-07	25	3.13 ± 0.12	2.01 ± 0.10	1.12 ± 0.02	284.8 ± 25.72	20.9 ± 3.03	0.94 ± 0.08	69.28 ± 2.92
River Jhelum	*Schizothorax* niger	2005-06	25	3.66 ± 0.15	3.60 ± 0.28	0.06 ± 0.13	176.3 ± 19.92	18.1 ± 2.11	0.84 ± 0.04	59.77 ± 2.22
		2006-07	25	2.71 ± 0.08	2.51 ± 0.15	0.20 ± 0.07	181.8 ± 13.22	18.3 ± 1.36	0.86 ± 0.22	65.42 ± 2.99
	Cyprinus carpio	2005-06	25	2.26 ± 0.07	2.16 ± 0.07	0.10 ± 0.00	220.3 ± 28.88	19.2 ± 1.33	0.88 ± 0.50	60.11 ± 3.55
		2006-07	25	2.39 ± 0.22	2.33 ± 0.16	0.06 ± 0.01	272.0 ± 27.72	19.6 ± 3.08	0.91 ± 0.09	67.30 ± 3.01

Table XXIV: Showing Biochemical values in *Schizothorax niger* and *Cyprinus carpio* spp. in the Winter Season in Dal Lake and River Jhelum

Water Resources	Fish Host	Year	No. Observed	Tot. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	Sr. Urea (mg/dl)	Sr. creatinine (mg/dl)	Tot. Cholesterol (mg/dl)
Dal Lake	*Schizothorax niger*	2005-06	25	1.82 ± 0.10	1.09 ± 0.01	0.73 ± 0.09	137.8 ± 17.00	16.1 ± 1.41	0.15 ± 0.05	37.21 ± 2.60
		2006-07	25	1.11 ± 0.02	1.02 ± 0.00	0.09 ± 0.02	138.0 ± 13.84	17.8 ± 2.23	0.17 ± 0.02	42.82 ± 4.33
	Cyprinus carpio	2005-06	25	1.05 ± 0.05	1.00 ± 0.00	0.06 ± 0.05	196.1 ± 24.59	16.2 ± 1.43	0.19 ± 0.09	42.66 ± 3.82
		2006-07	25	1.12 ± 0.02	0.99 ± 0.04	0.13 ± 0.02	199.2 ± 32.06	15.9 ± 1.45	0.22 ± 0.08	49.12 ± 13.12
River Jhelum	*Schizothorax* niger	2005-06	25	1.55 ± 0.18	1.01 ± 0.02	0.54 ± 0.16	125.5 ± 17.11	17.0 ± 0.59	0.09 ± 0.22	36.42 ± 2.71
		2006-07	25	1.38 ± 0.12	1.02 ± 0.03	0.36 ± 0.09	133.1 ± 13.01	17.1 ± 2.33	0.11 ± 0.04	51.42 ± 4.20
	Cyprinus carpio	2005-06	25	1.61 ± 0.05	1.01 ± 0.02	0.60 ± 0.03	136.1 ± 25.01	16.1 ± 1.50	0.14 ± 0.10	40.11 ± 3.22
		2006-07	25	1.86 ± 0.09	1.21 ± 0.07	0.65 ± 0.02	143.2 ± 33.00	15.2 ± 1.44	0.16 ± 0.09	48.76 ± 3.14

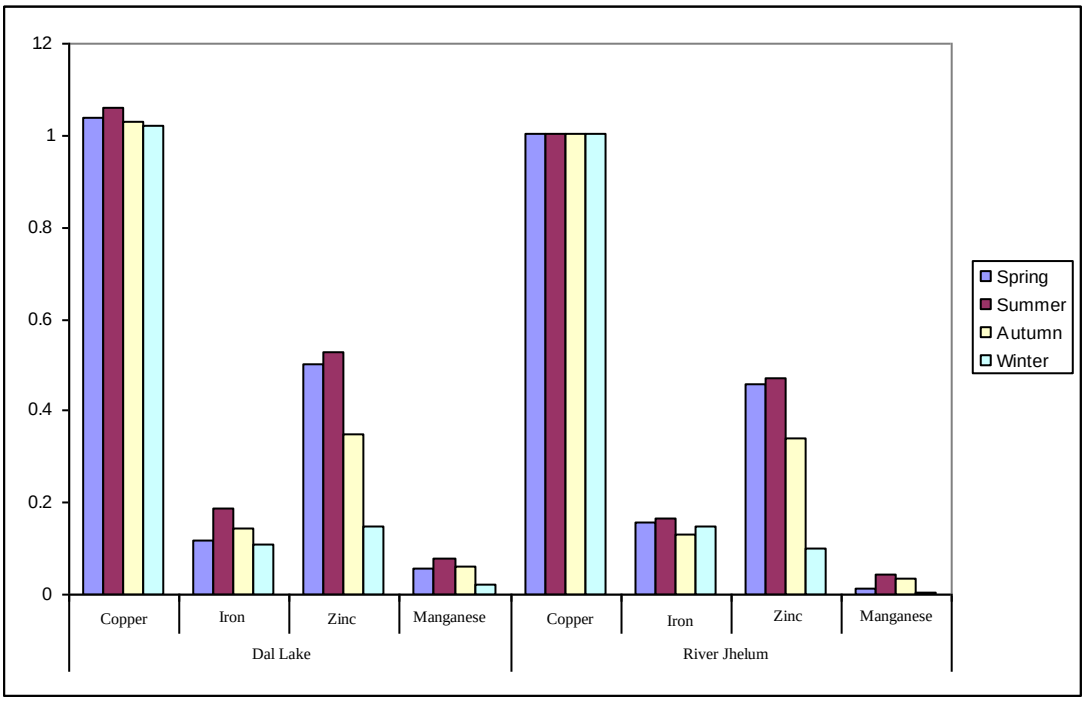


Fig. 3: Showing concentration of Copper, Zinc, Iron and Manganese in Dal and River Jhelum during March 2005 – February 2006.

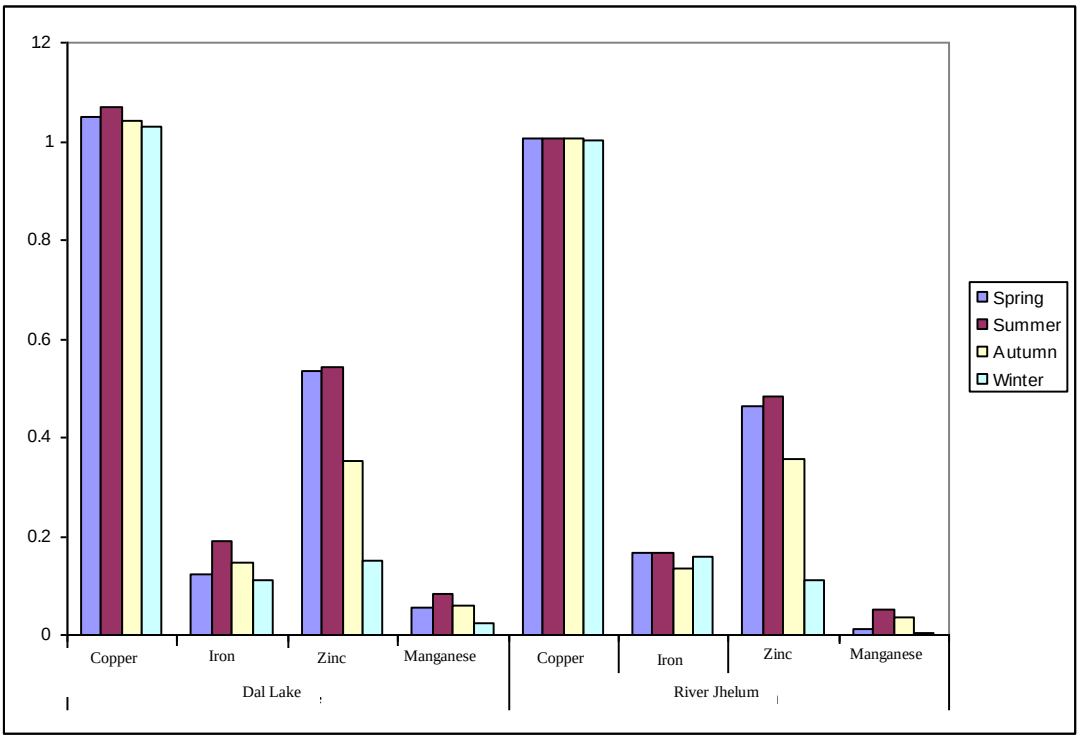


Fig. 4: Showing concentration of Copper, Zinc, Iron and Manganese in Dal and River Jhelum during March 2006 – February 2007.

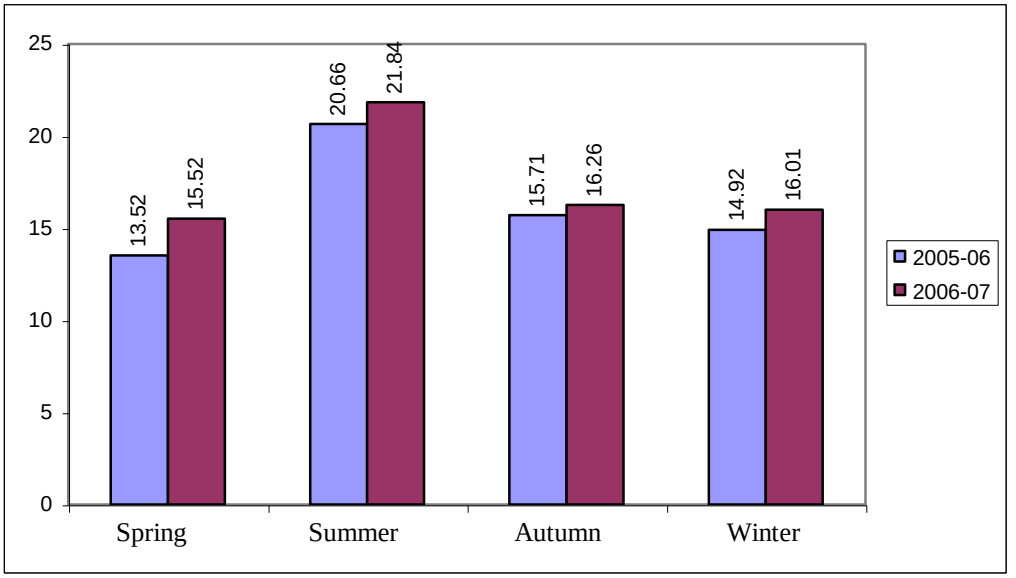


Fig. 5: Showing Copper concentration in gills of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

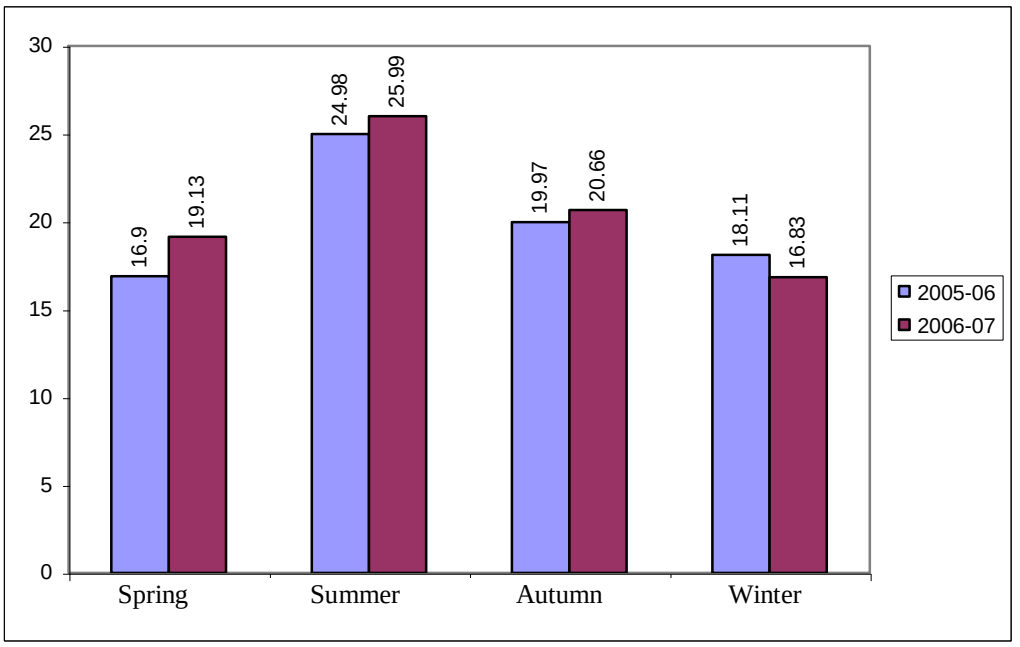


Fig. 6: Showing Copper concentration in gills of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

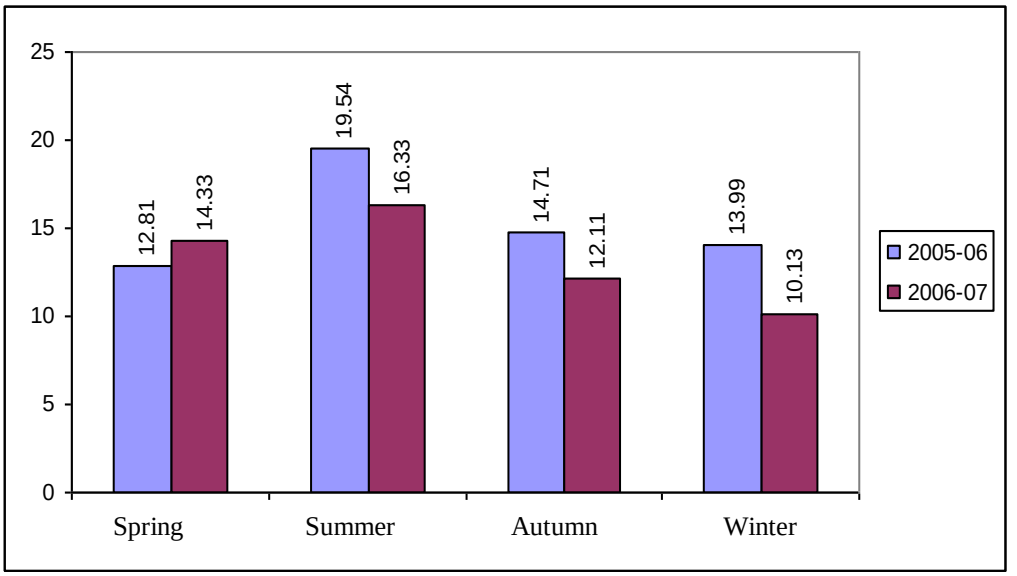


Fig. 7: Showing Copper concentration in gills of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

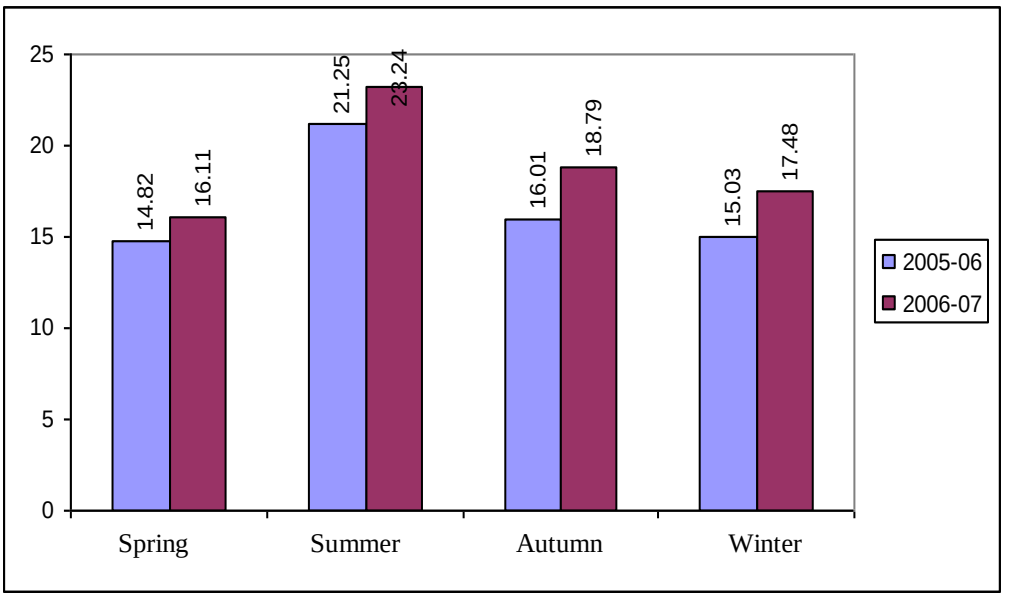


Fig. 8: Showing Copper concentration in gills of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

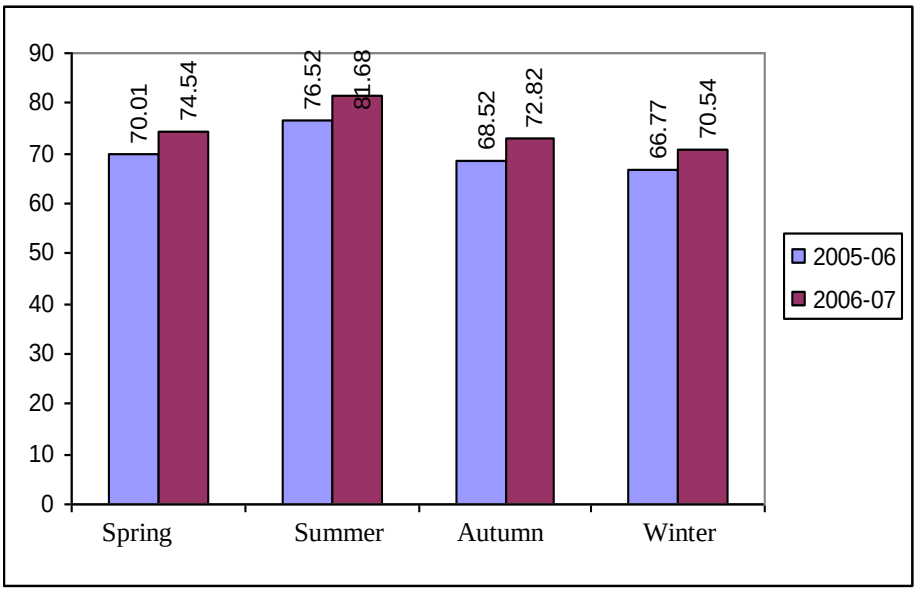


Fig. 9: Showing Copper concentration in liver of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

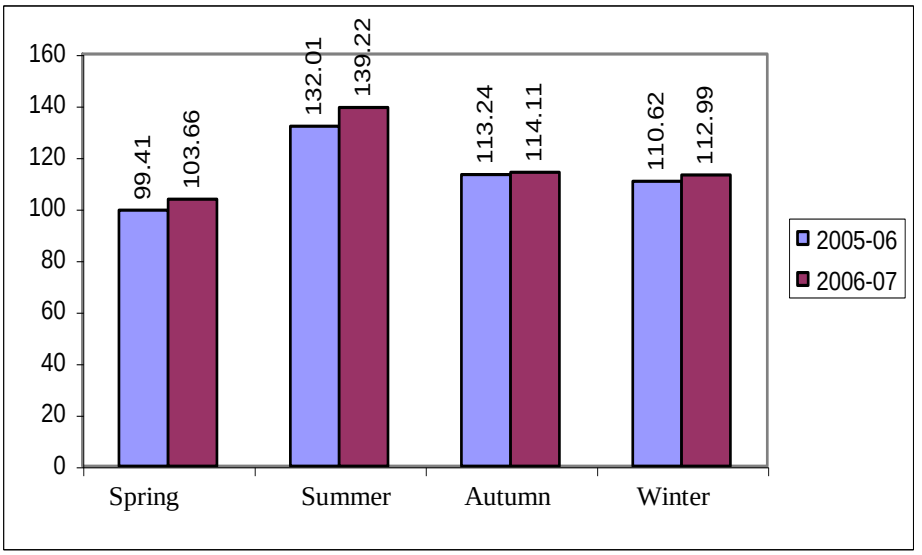


Fig. 10: Showing Copper concentration in liver of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

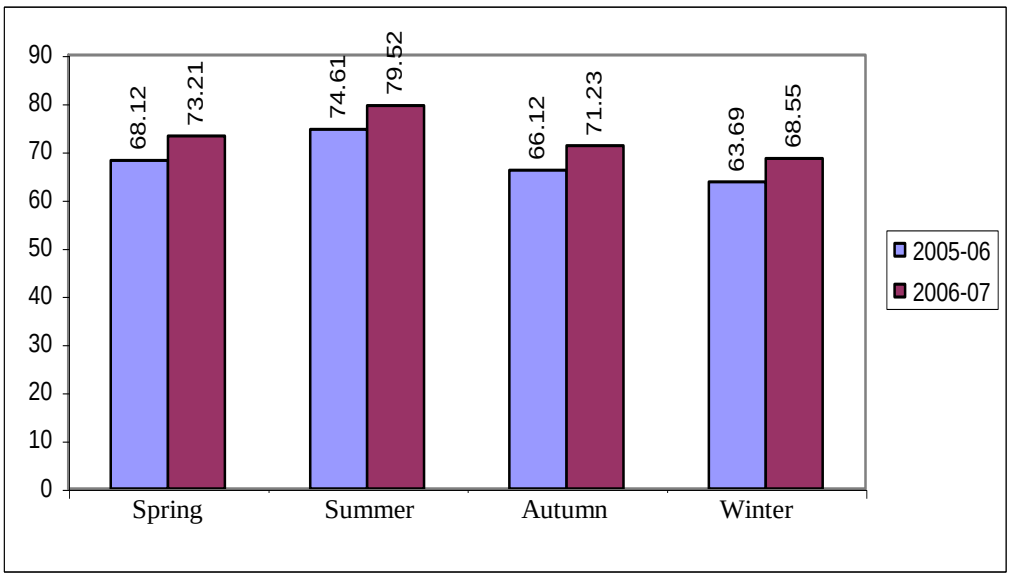


Fig. 11: Showing Copper concentration in liver of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

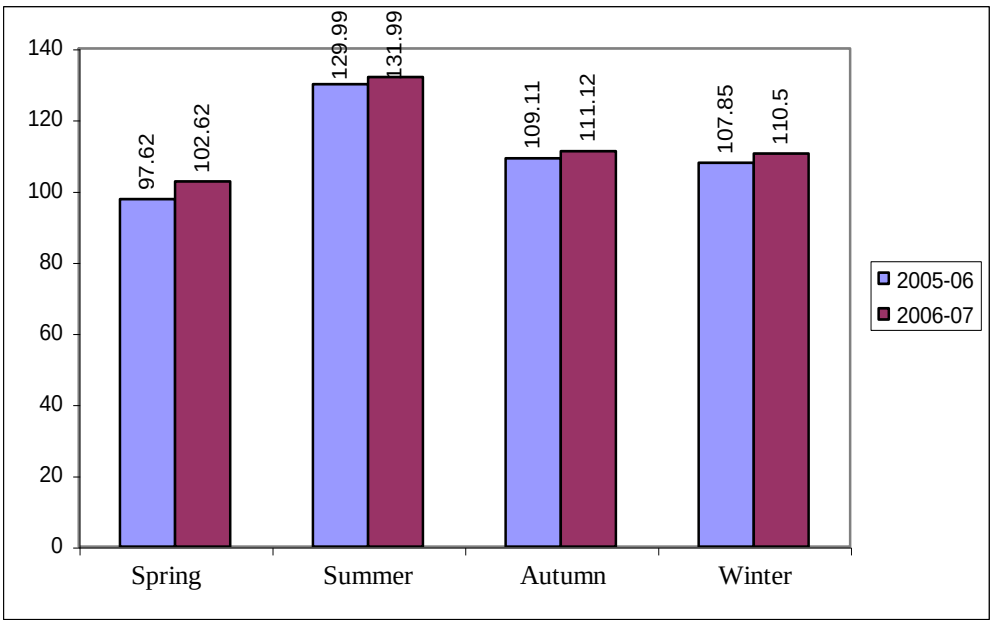


Fig. 12: Showing Copper concentration in liver of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

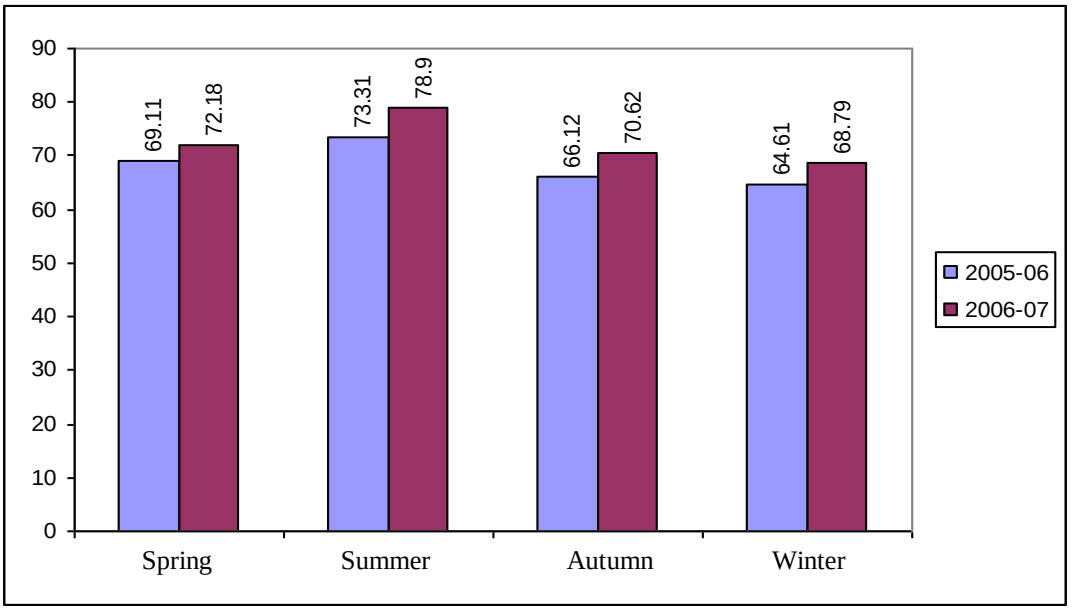


Fig. 13: Showing Copper concentration in kidney of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

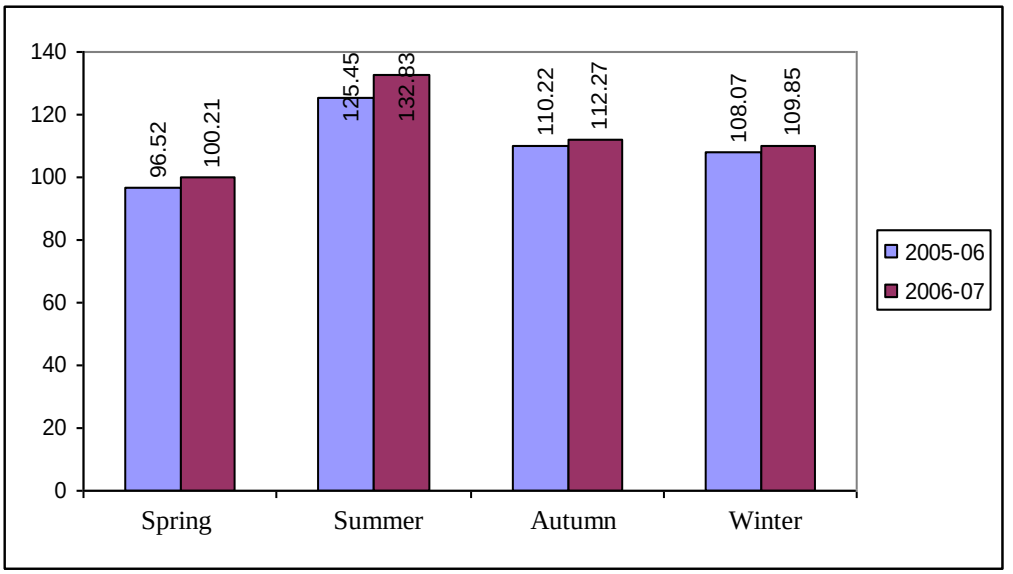


Fig. 14: Showing Copper concentration in kidney of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

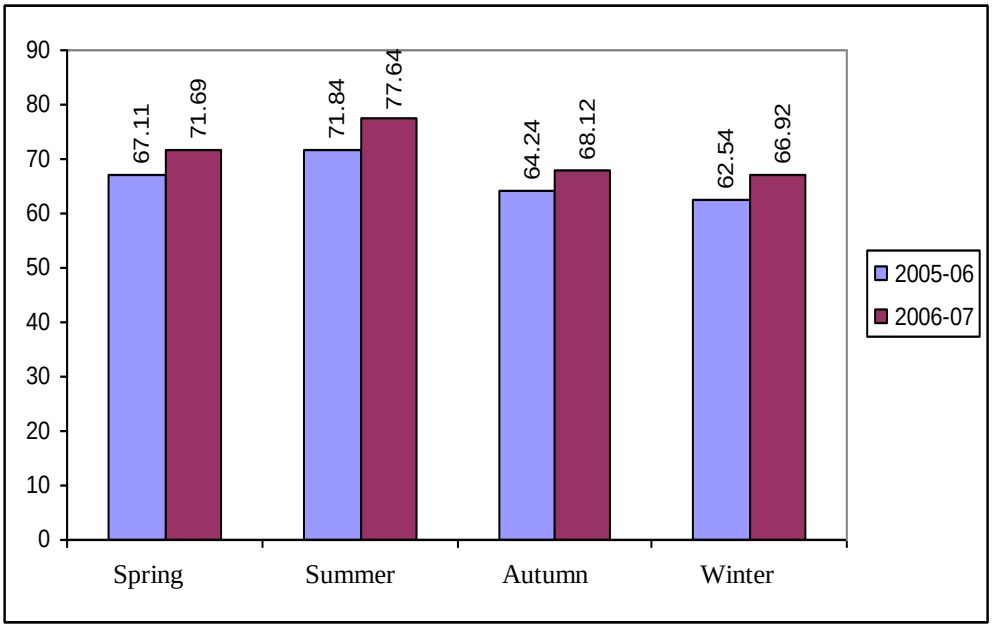


Fig. 15: Showing Copper concentration in kidney of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

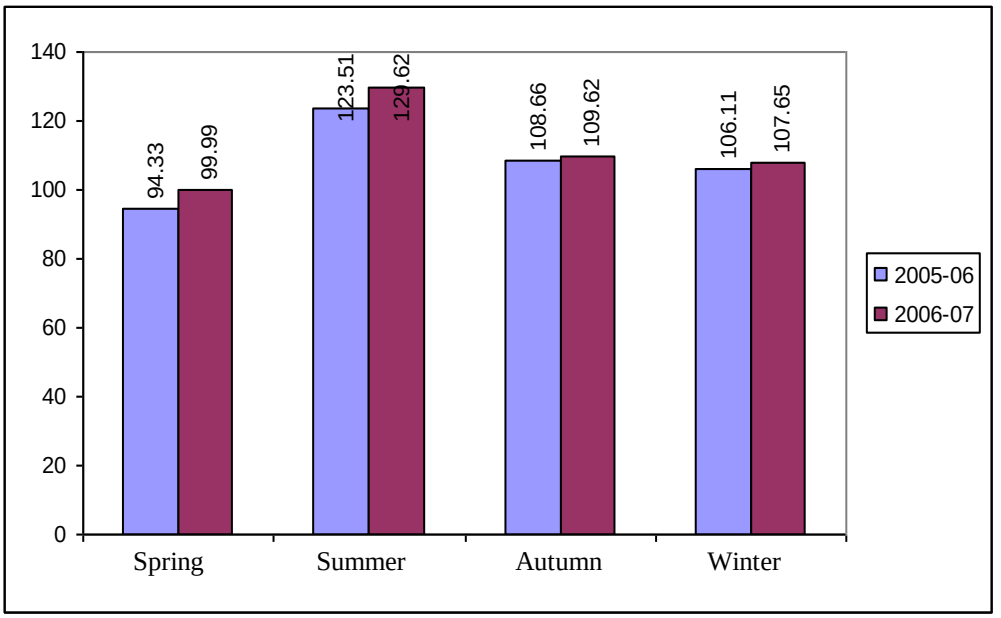


Fig. 16: Showing Copper concentration in kidney of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

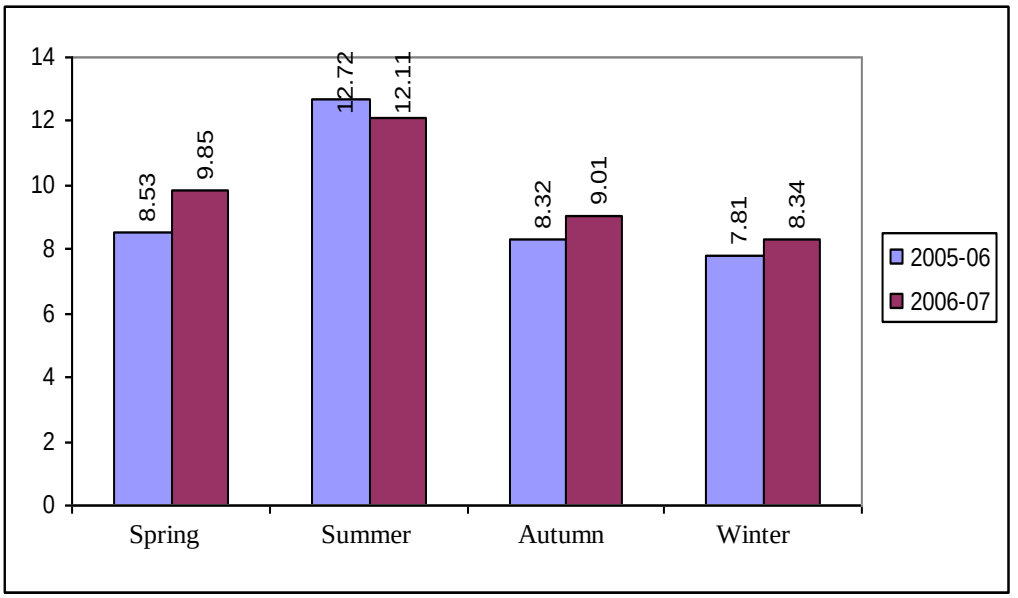


Fig. 17: Showing Copper concentration in muscle of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

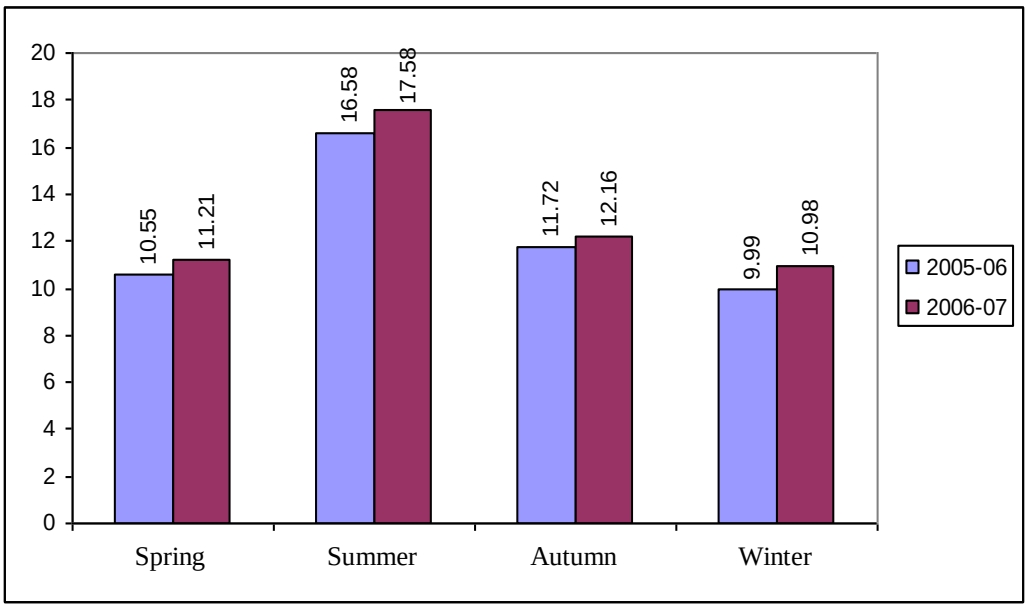


Fig. 18: Showing Copper concentration in muscle of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

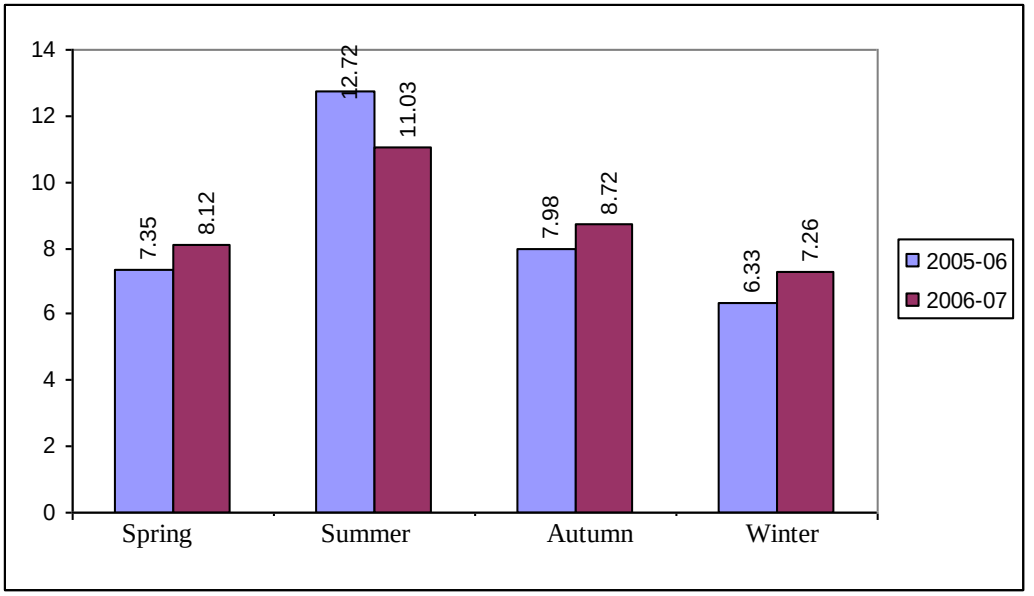


Fig. 19: Showing Copper concentration in muscle of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

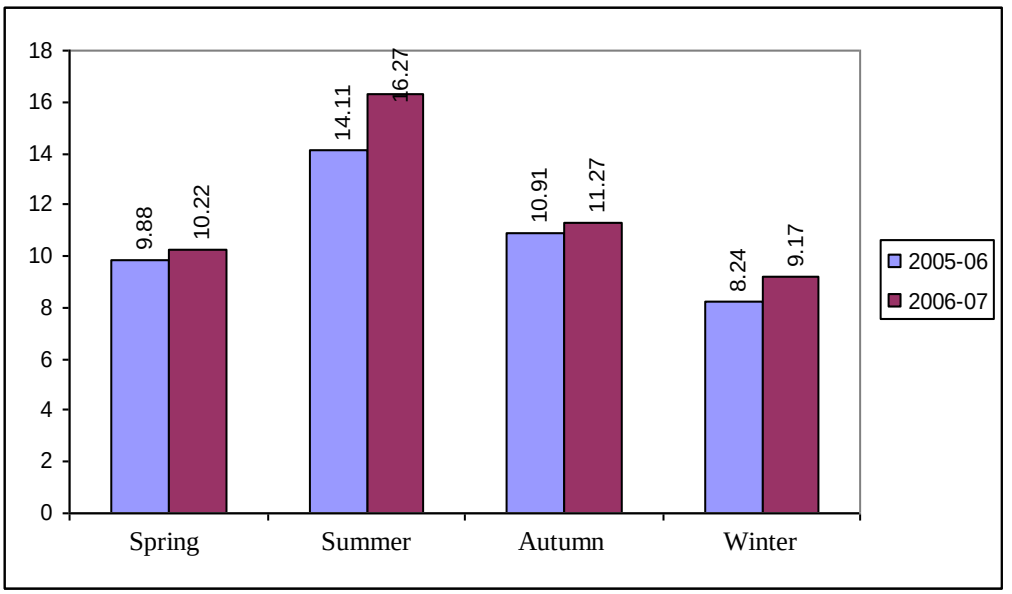


Fig. 20: Showing Copper concentration in muscle of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

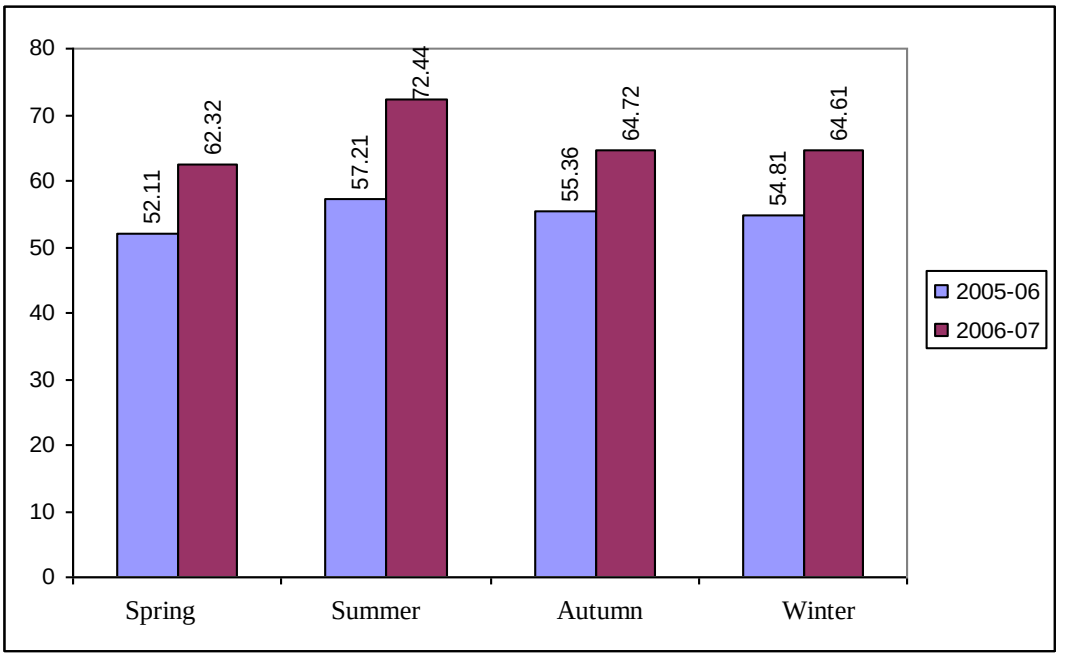


Fig. 21: Showing Zinc concentration in gills of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

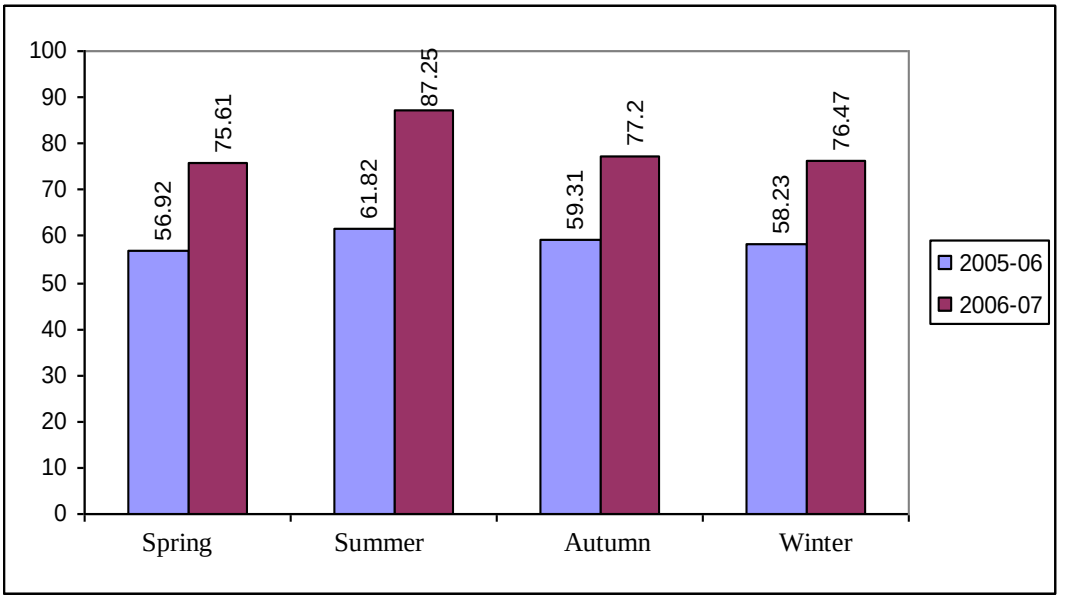


Fig. 22: Showing Zinc concentration in gills of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

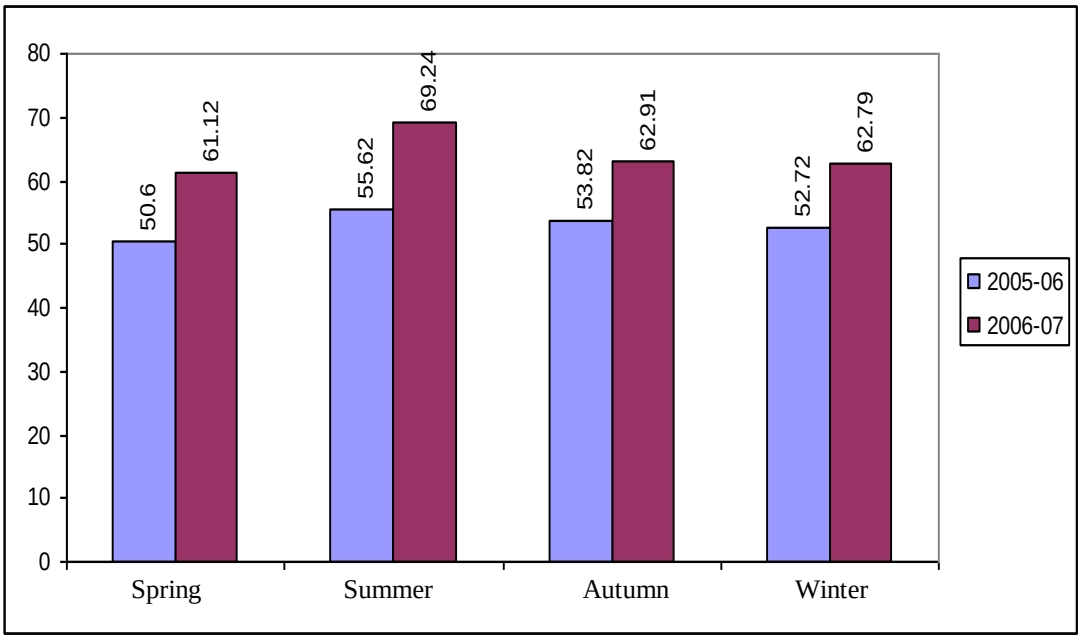


Fig. 23: Showing Zinc concentration in gills of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

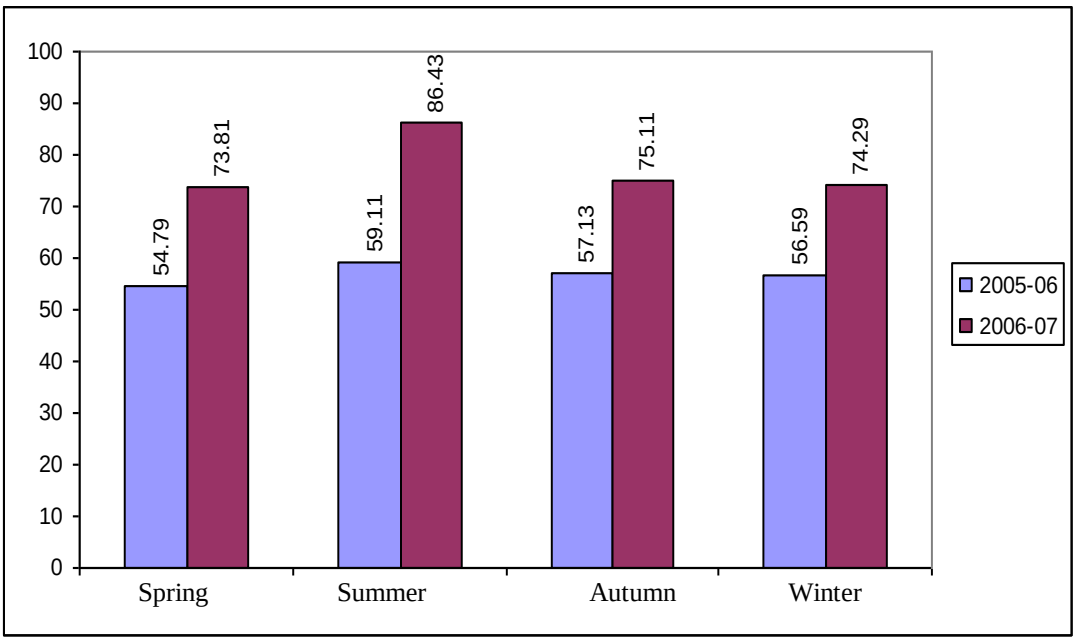


Fig. 24: Showing Zinc concentration in gills of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

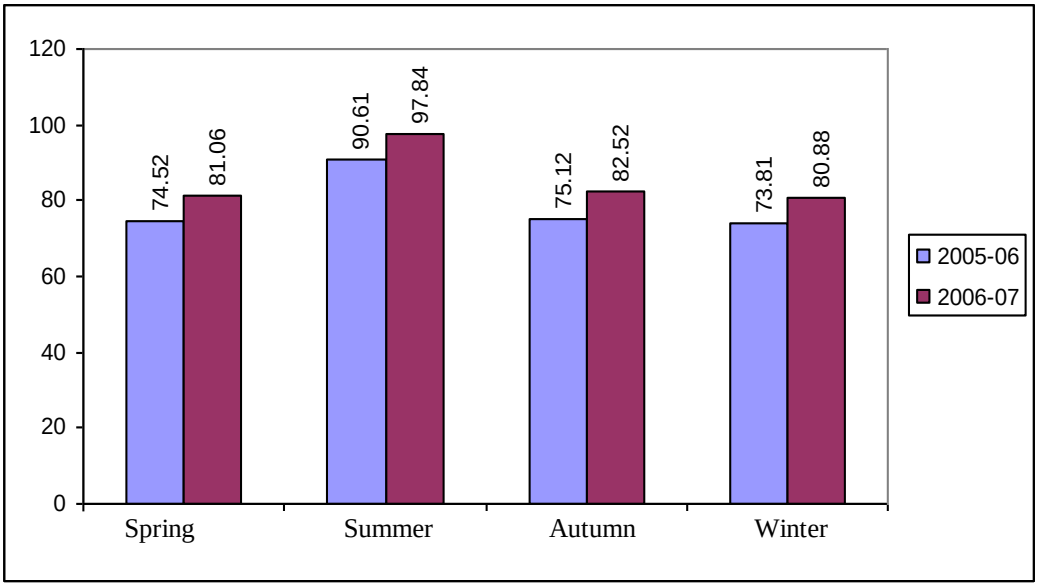


Fig. 25: Showing Zinc concentration in liver of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

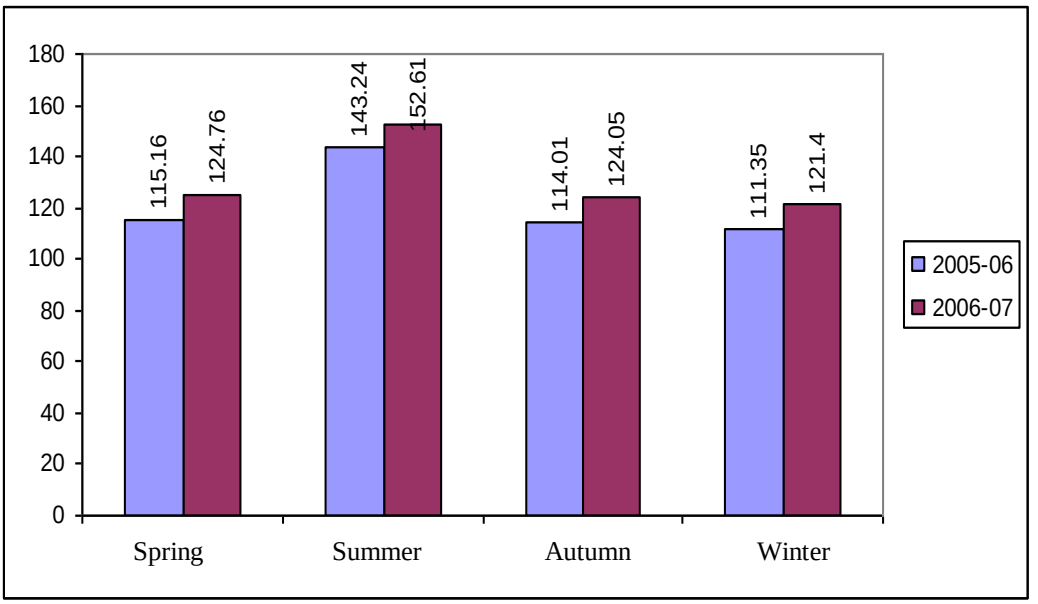


Fig. 26: Showing Zinc concentration in liver of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

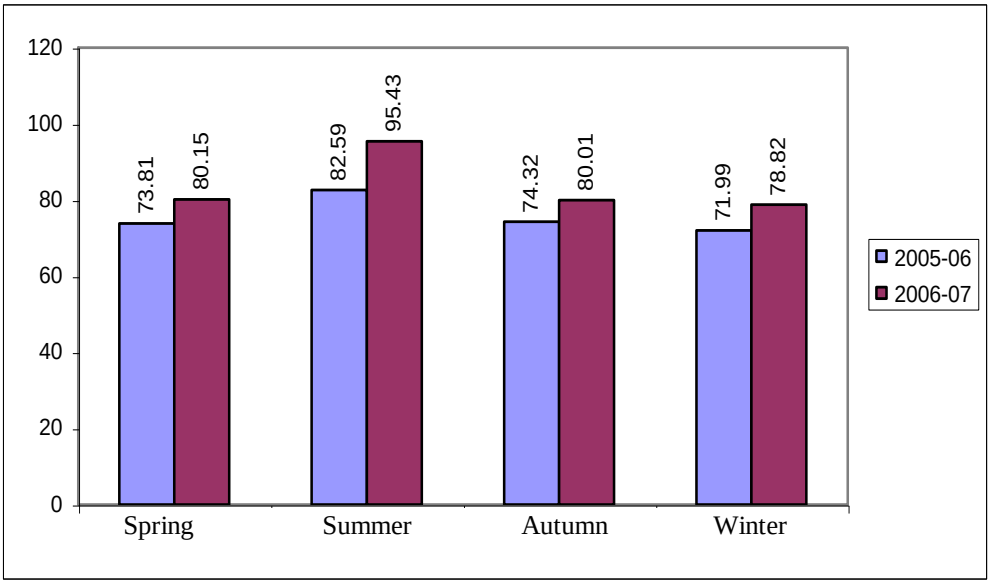


Fig. 27: Showing Zinc concentration in liver of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

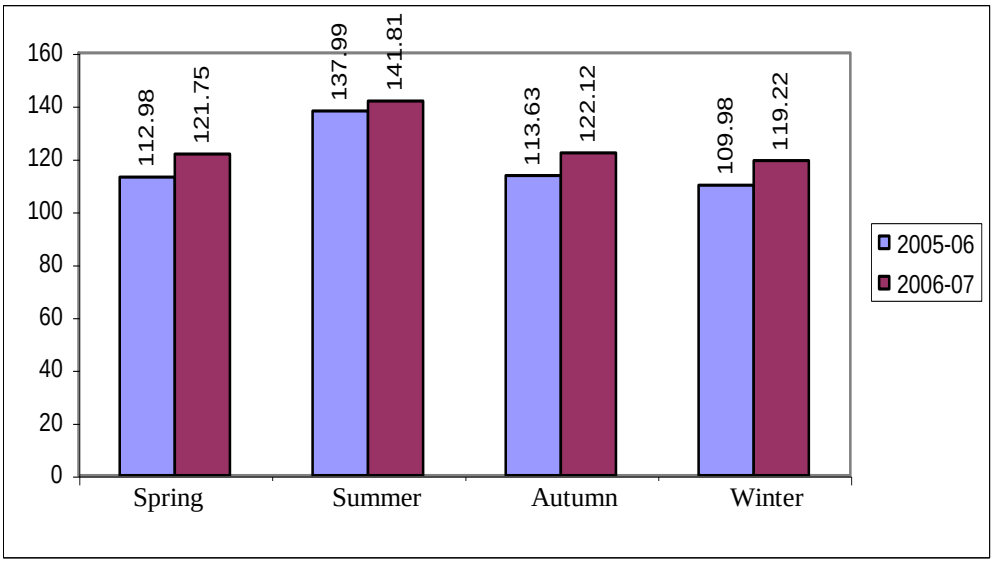


Fig. 28: Showing Zinc concentration in liver of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

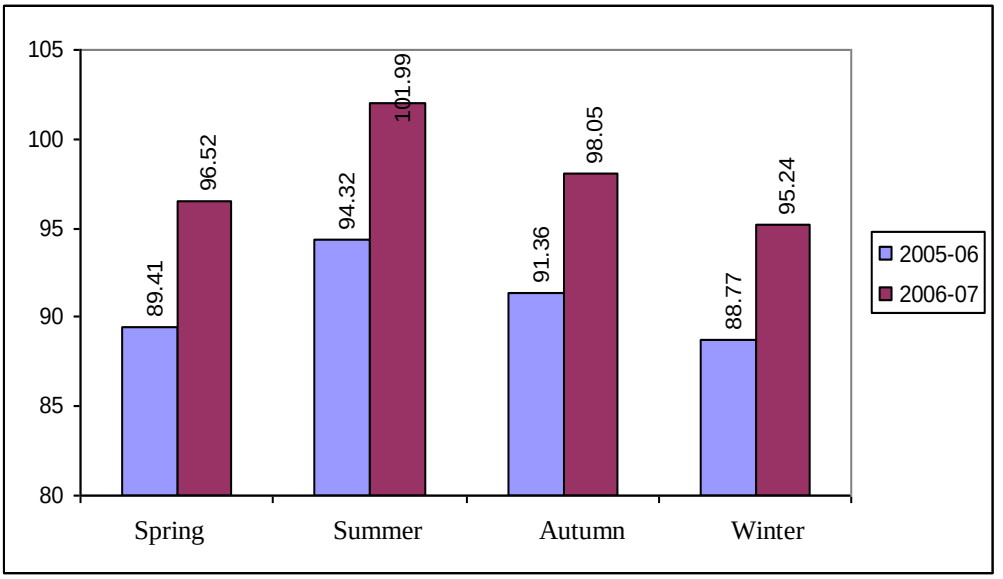


Fig. 29: Showing Zinc concentration in kidney of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

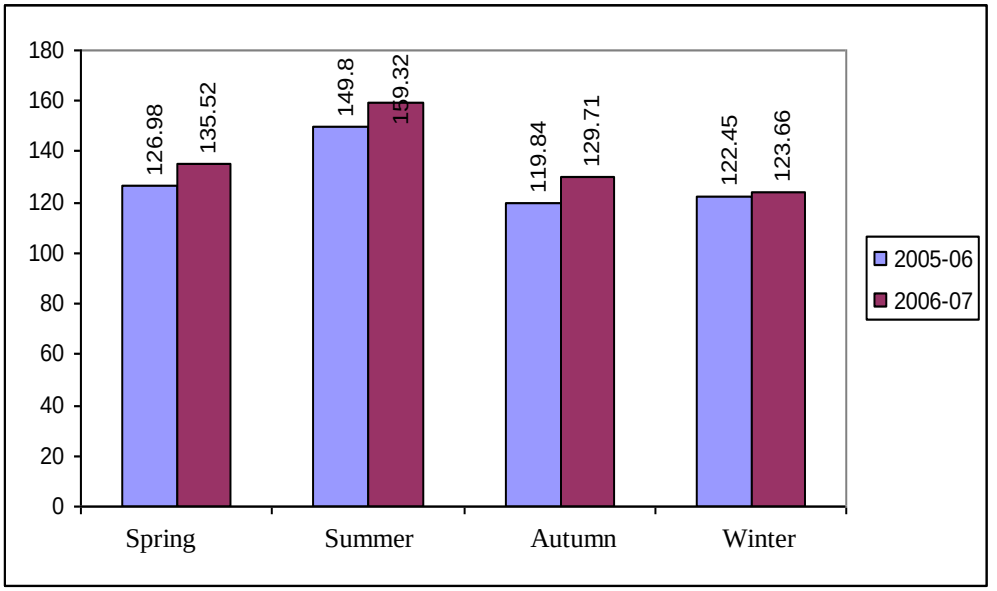


Fig. 30: Showing Zinc concentration in kidney of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

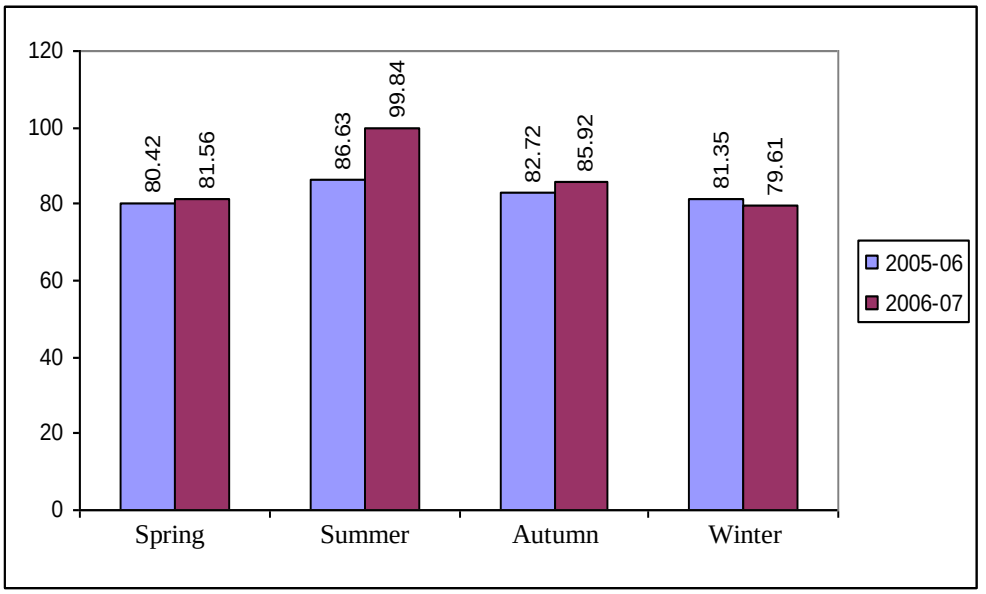


Fig. 31: Showing Zinc concentration in kidney of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

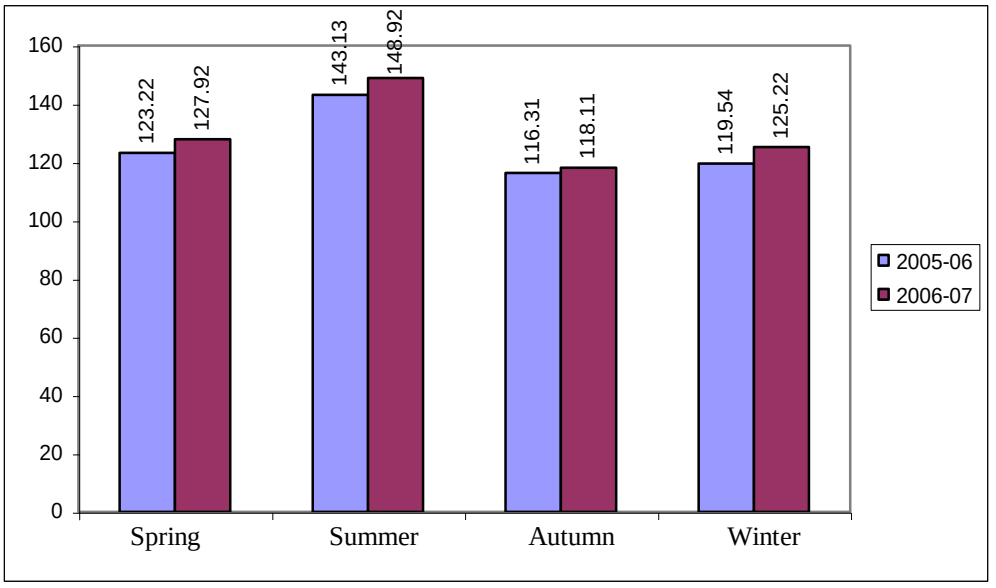


Fig. 32: Showing Zinc concentration in kidney of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

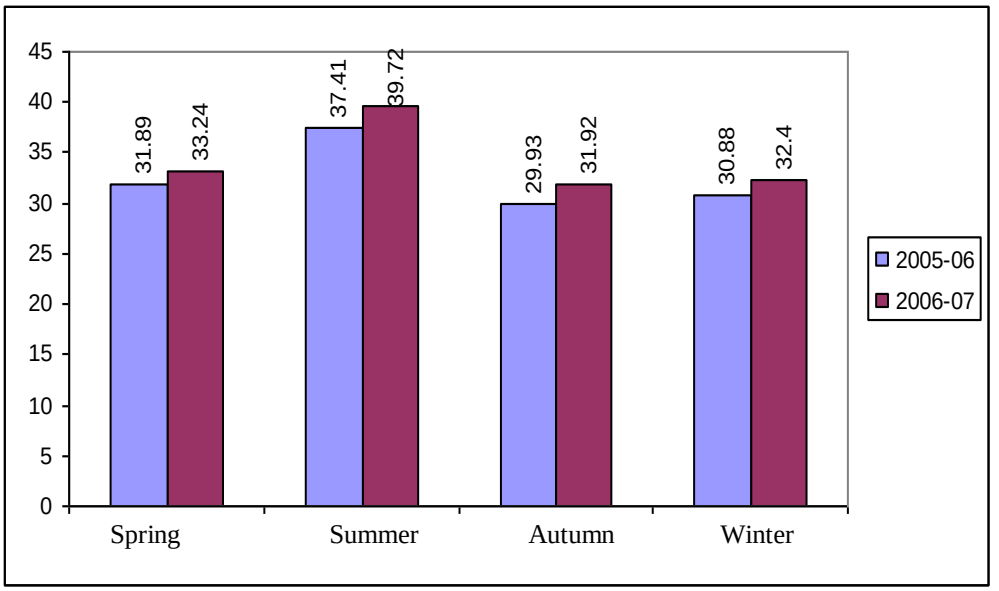


Fig. 33: Showing Zinc concentration in muscle of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

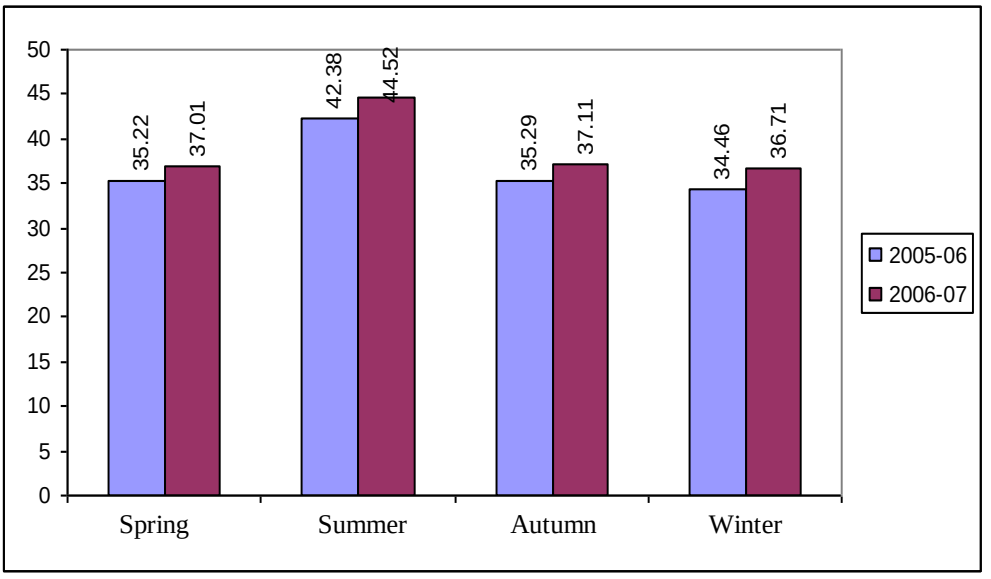


Fig. 34: Showing Zinc concentration in muscle of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

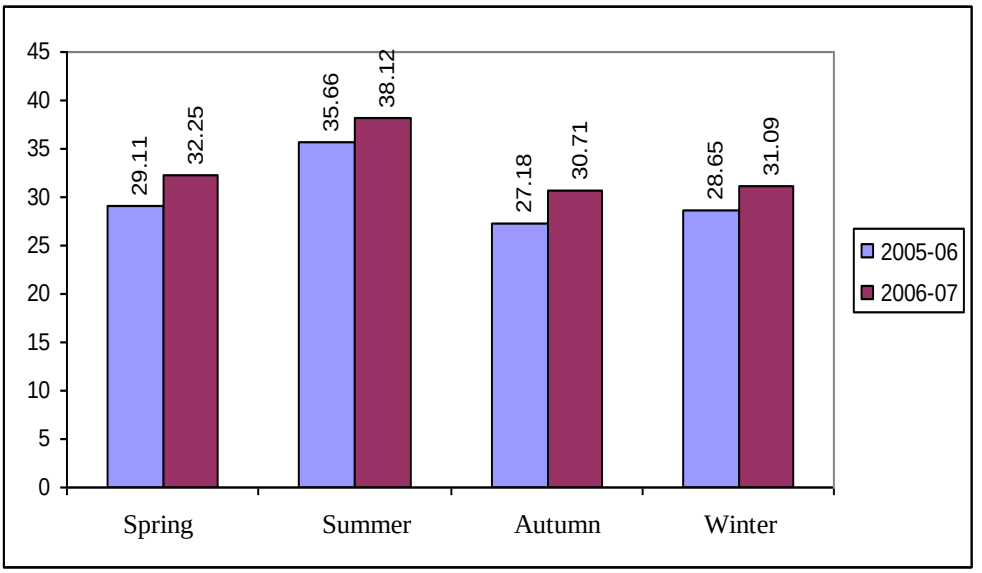


Fig. 35: Showing Zinc concentration in muscle of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

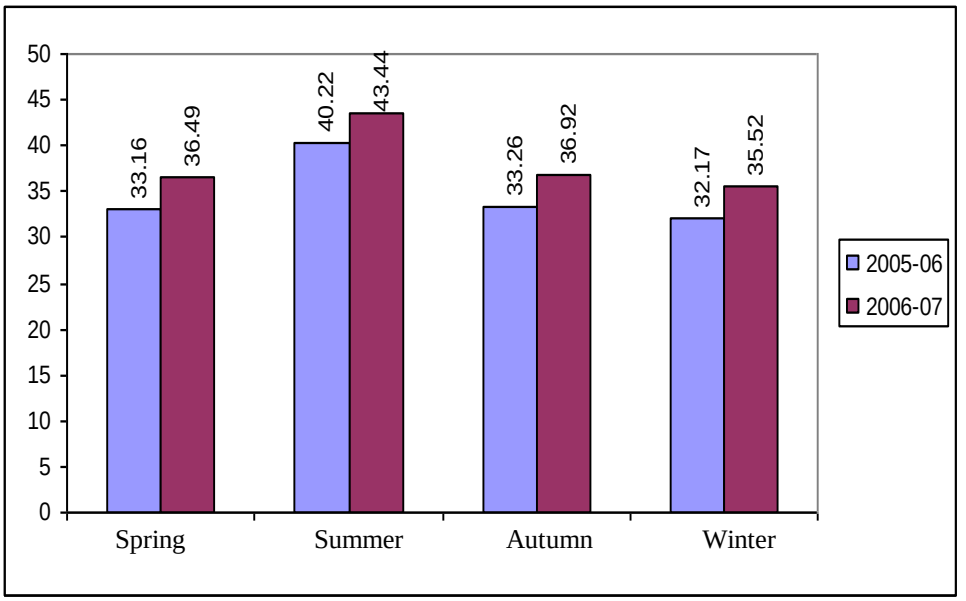


Fig. 36: Showing Zinc concentration in muscle of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

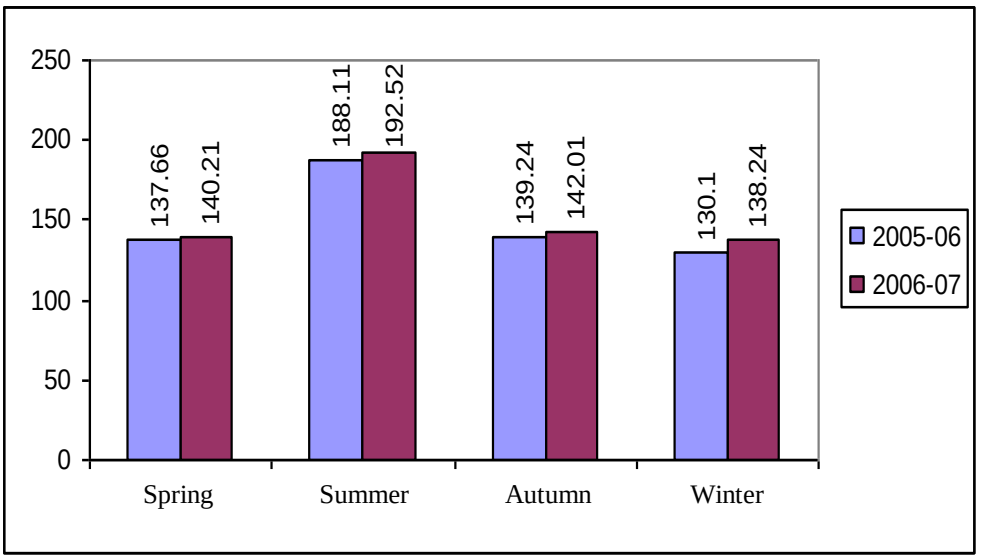


Fig. 37: Showing Iron concentration in gills of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

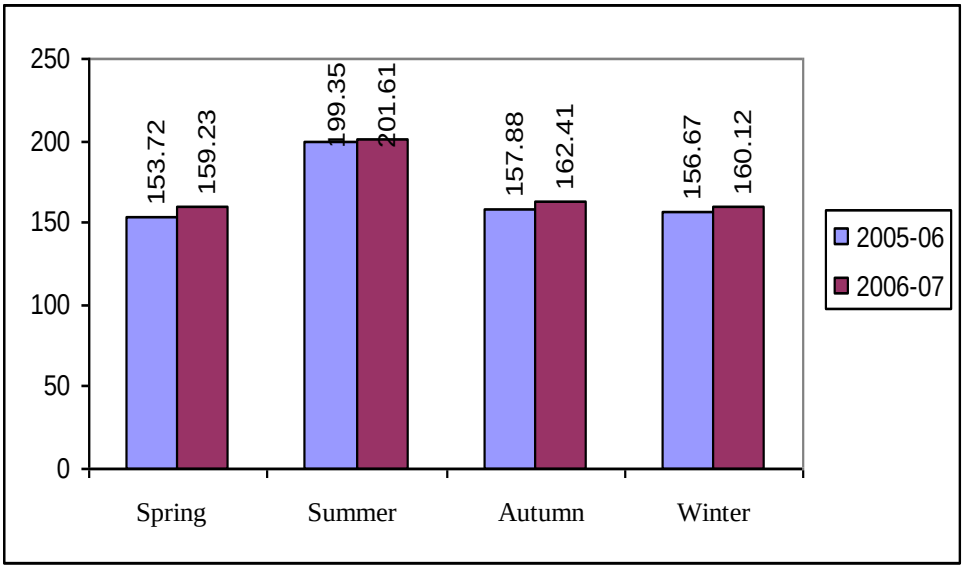


Fig. 38: Showing Iron concentration in gills of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

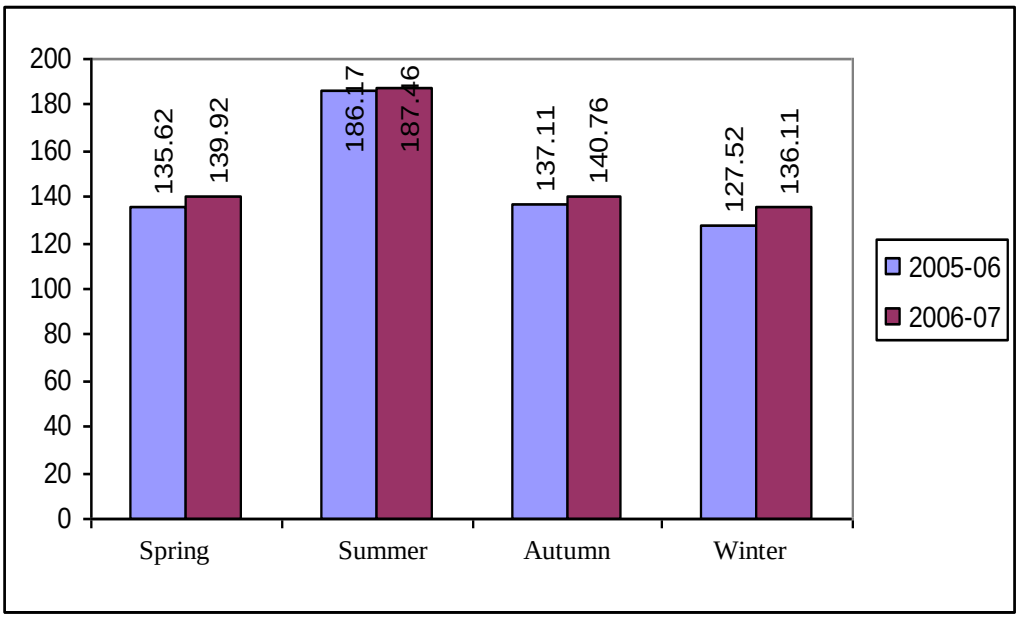


Fig. 39: Showing Iron concentration in gills of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

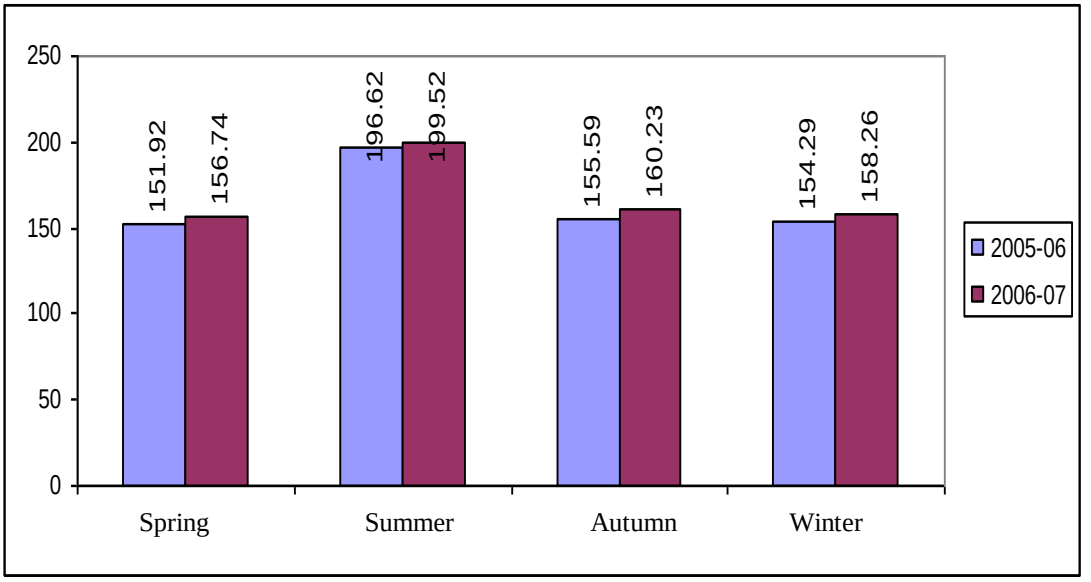


Fig. 40: Showing Iron concentration in gills of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

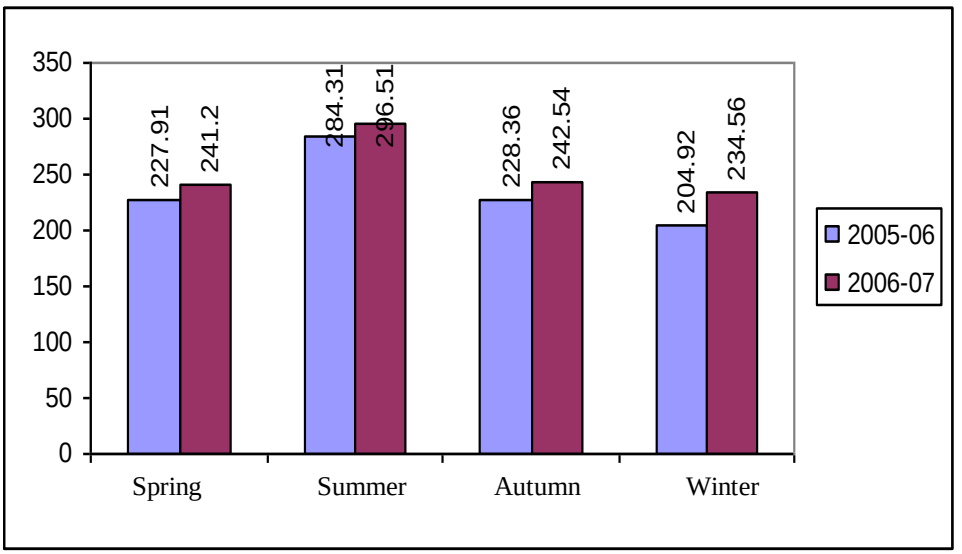


Fig. 41: Showing Iron concentration in liver of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

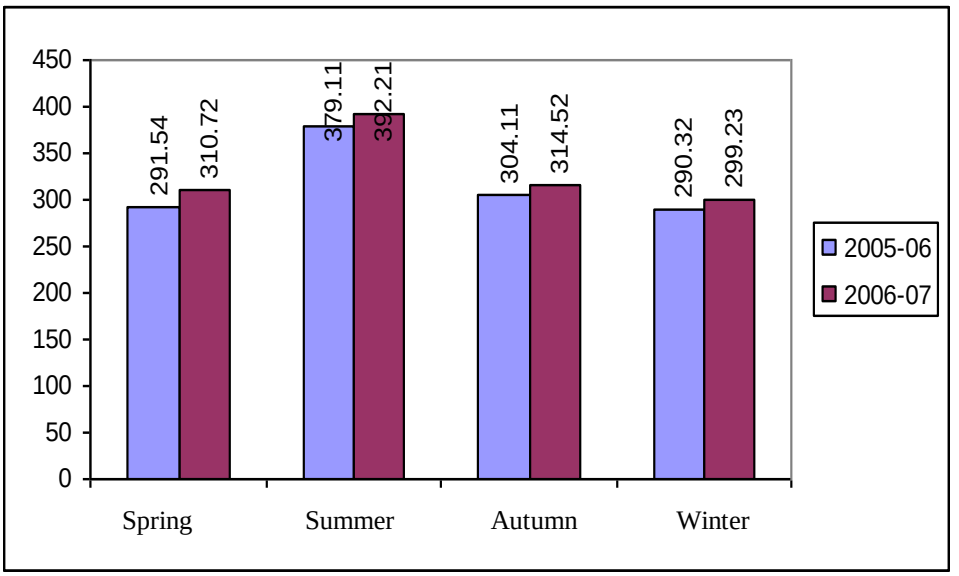


Fig. 42: Showing Iron concentration in liver of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

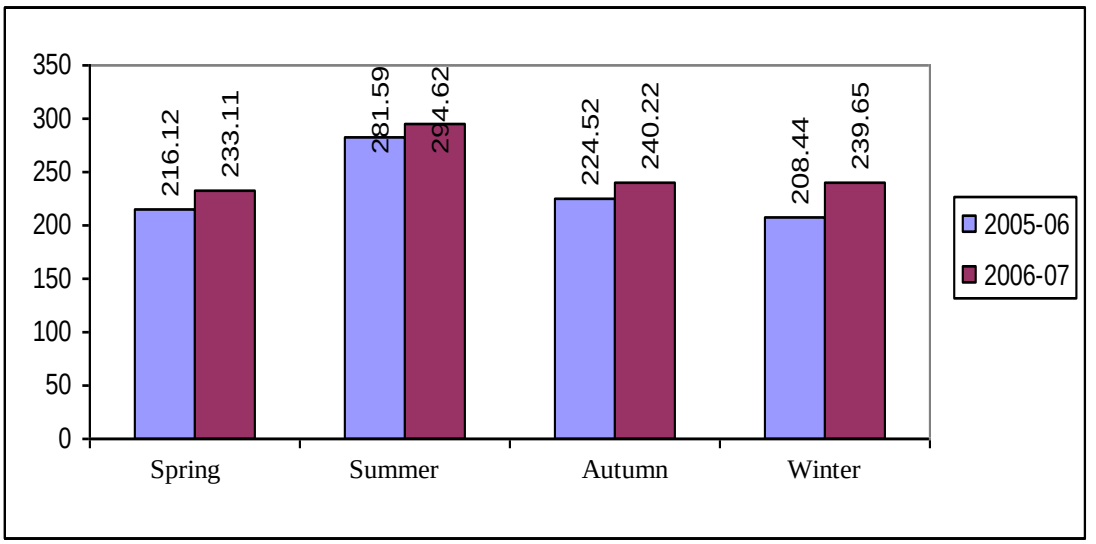


Fig. 43: Showing Iron concentration in liver of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

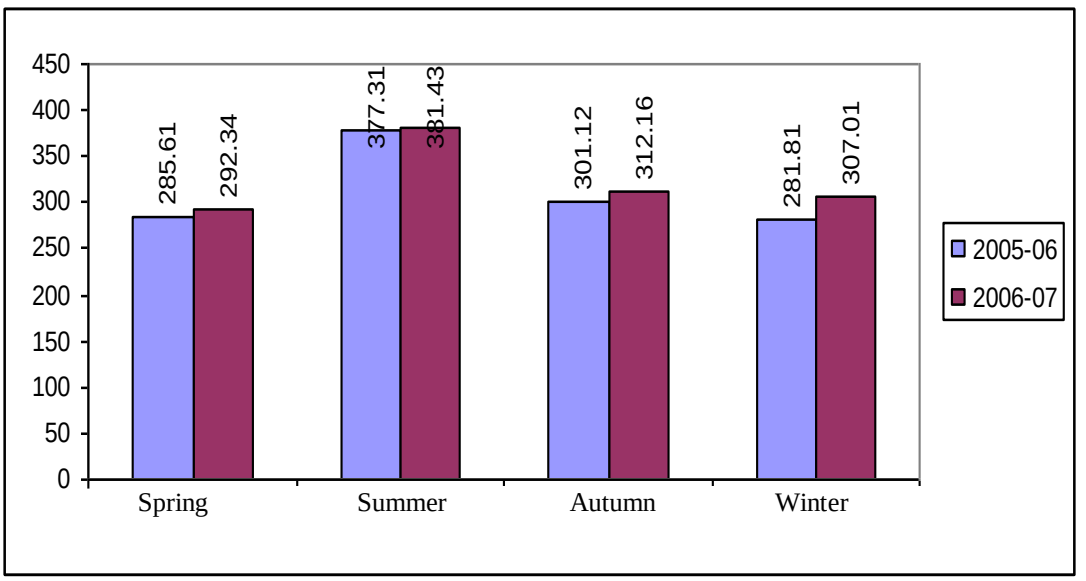


Fig. 44: Showing concentration in liver of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

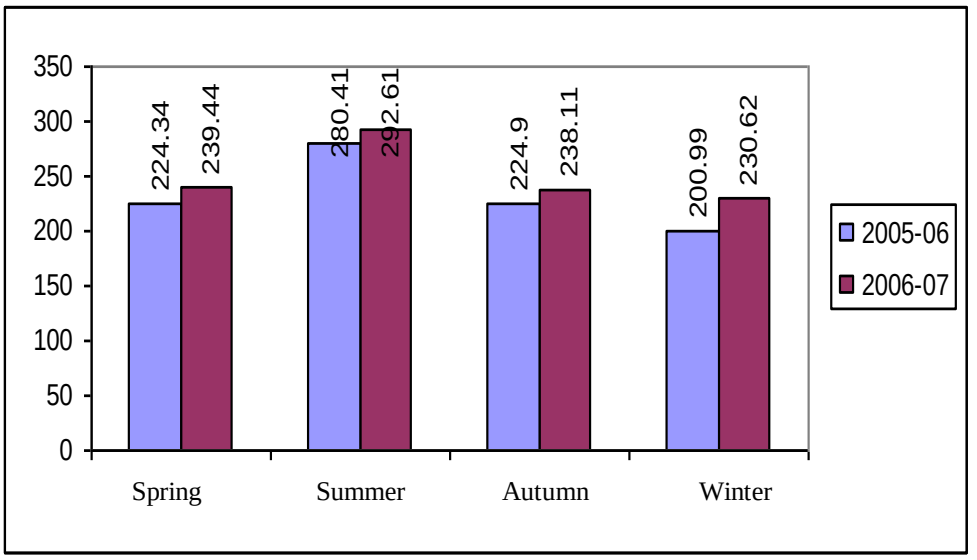


Fig. 45: Showing Iron concentration in kidney of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

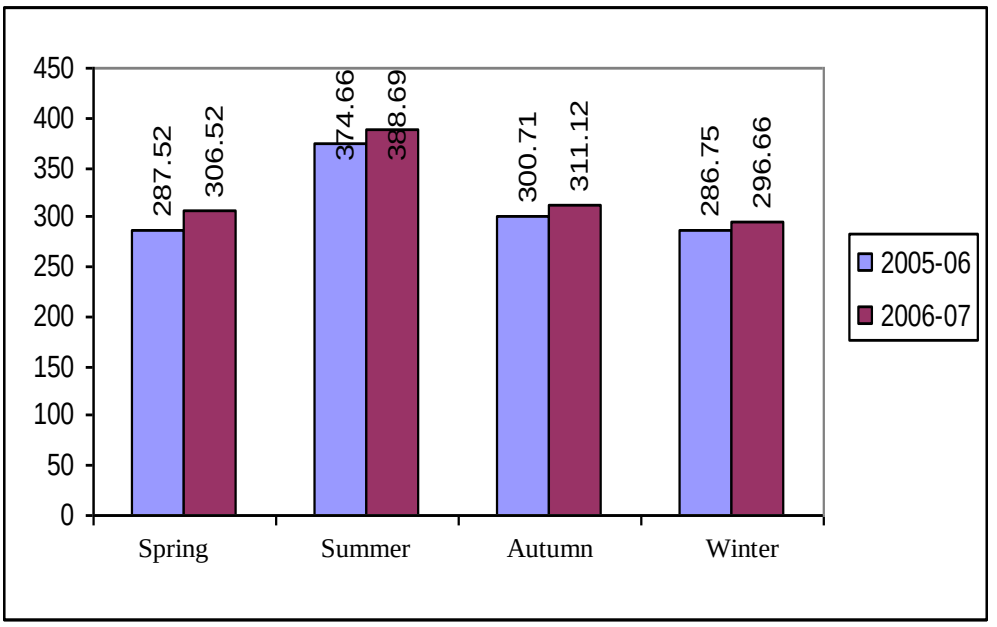


Fig. 46: Showing Iron concentration in kidney of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

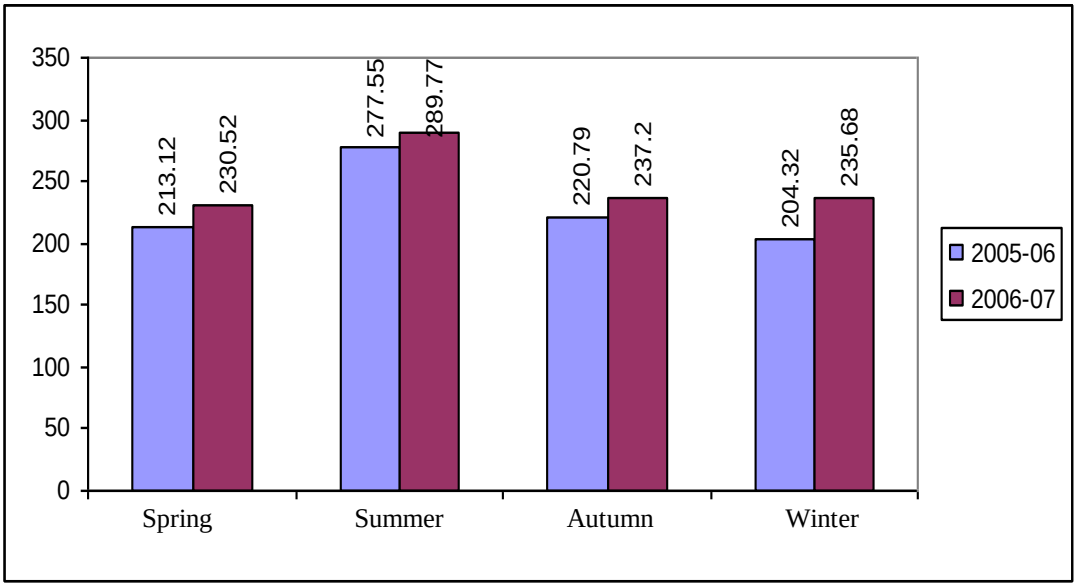


Fig. 47: Showing Copper concentration in kidney of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

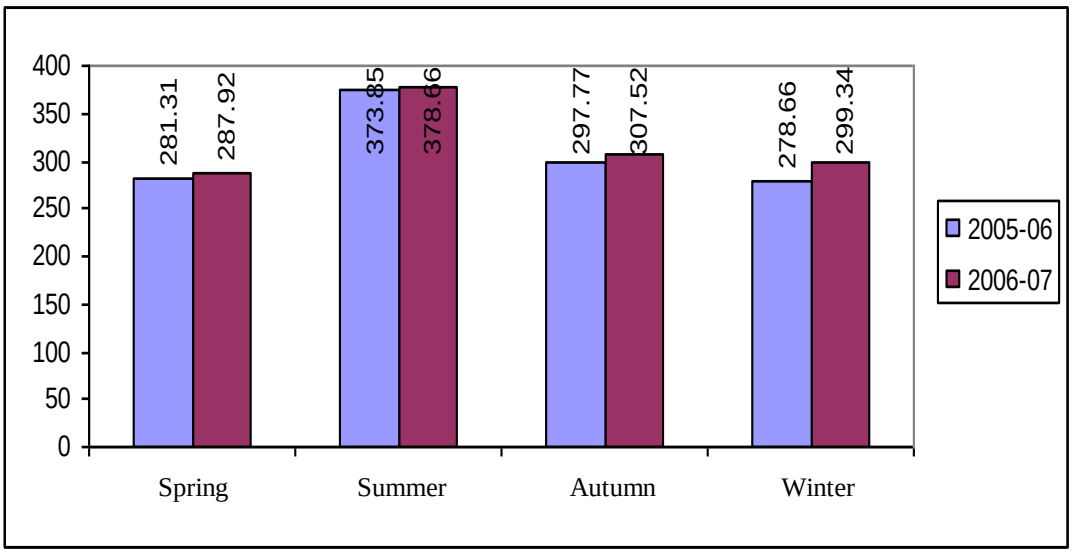


Fig. 48: Showing Iron concentration in kidney of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

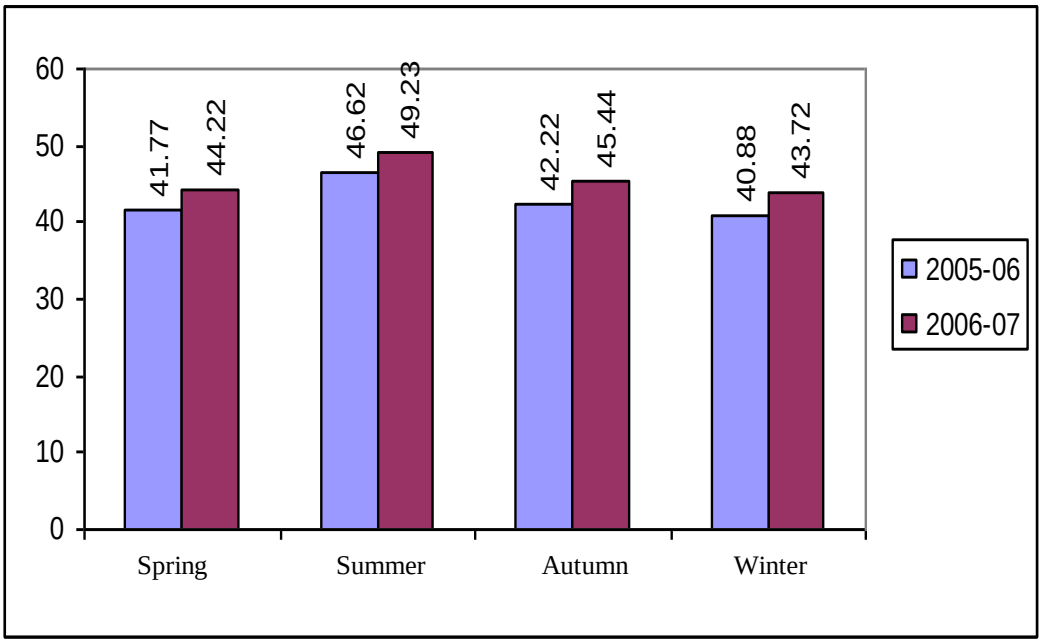


Fig. 49: Showing Iron concentration in muscle of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

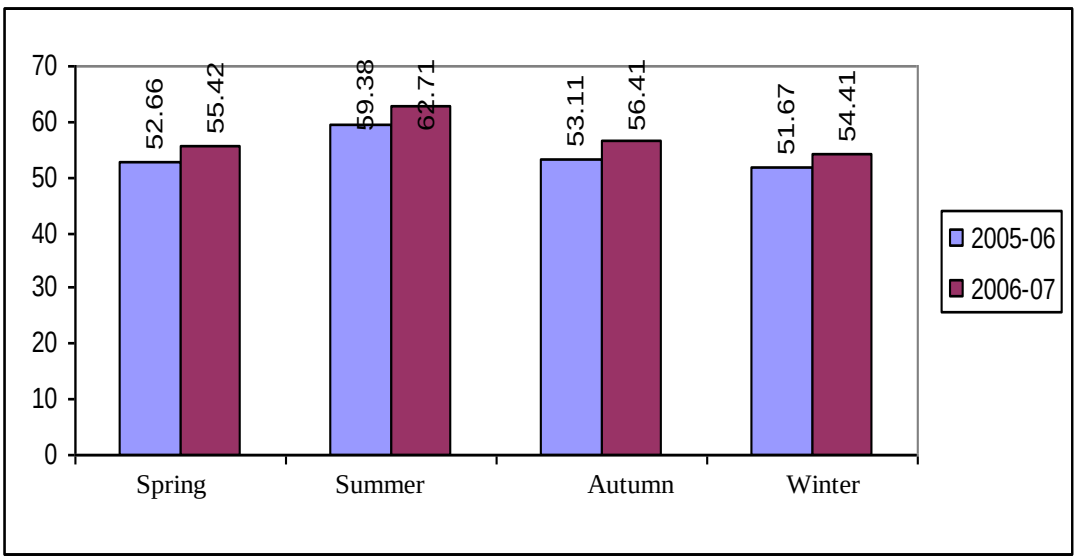


Fig. 50: Showing Iron concentration in muscle of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

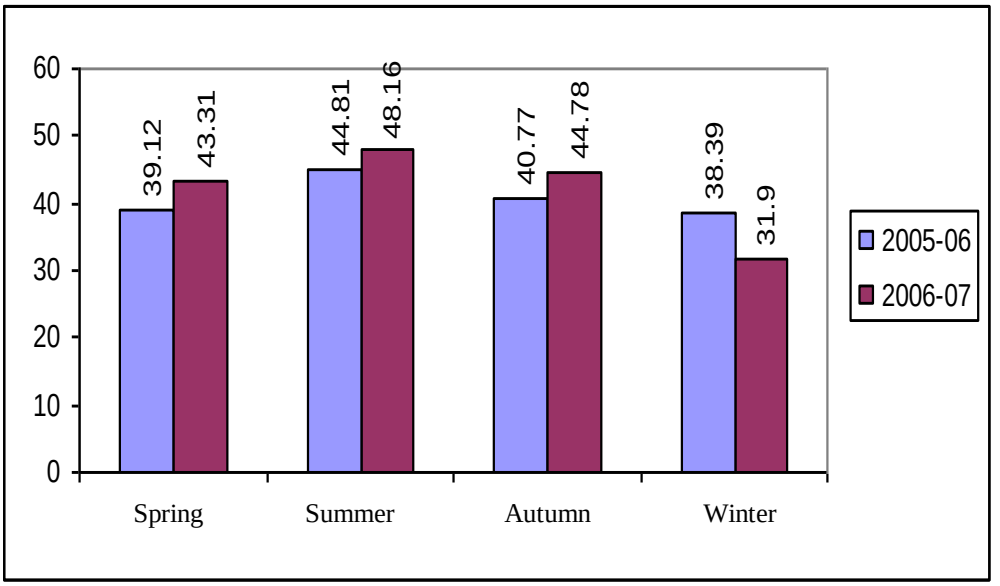


Fig. 51: Showing Iron concentration in muscle of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

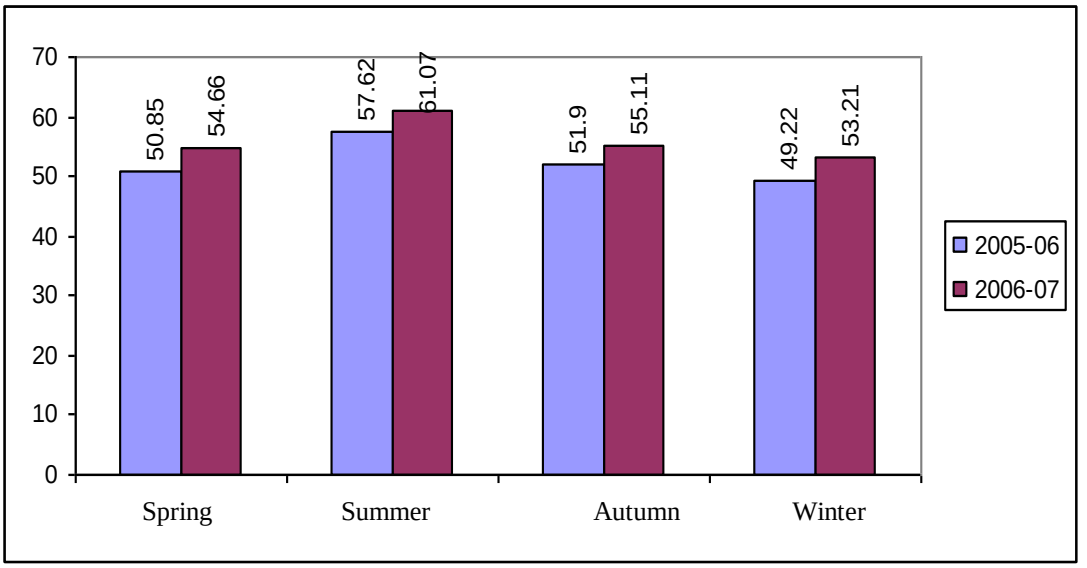


Fig. 52: Showing Iron concentration in muscle of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

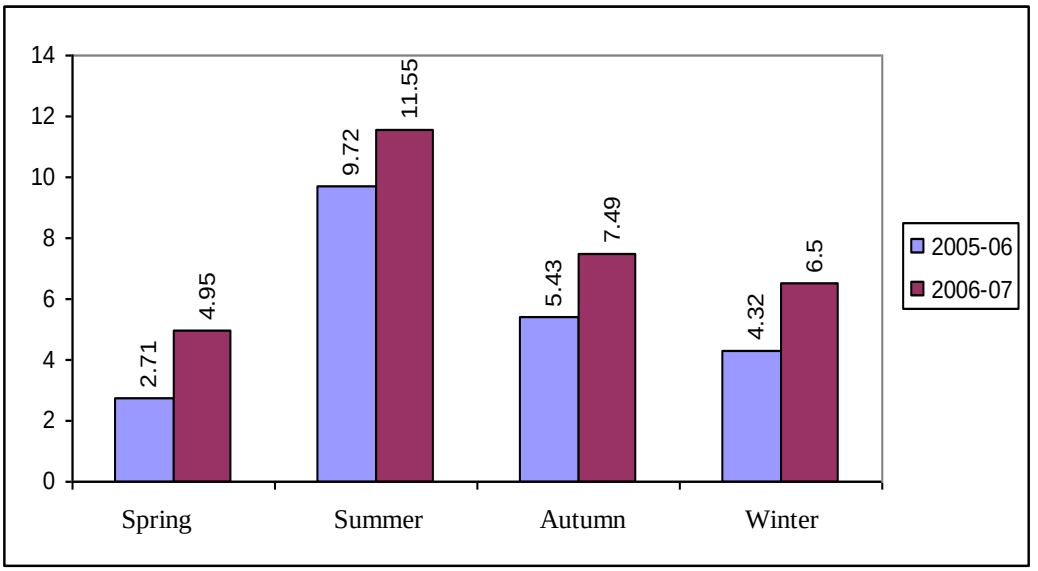


Fig. 53: Showing Manganese concentration in gills of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

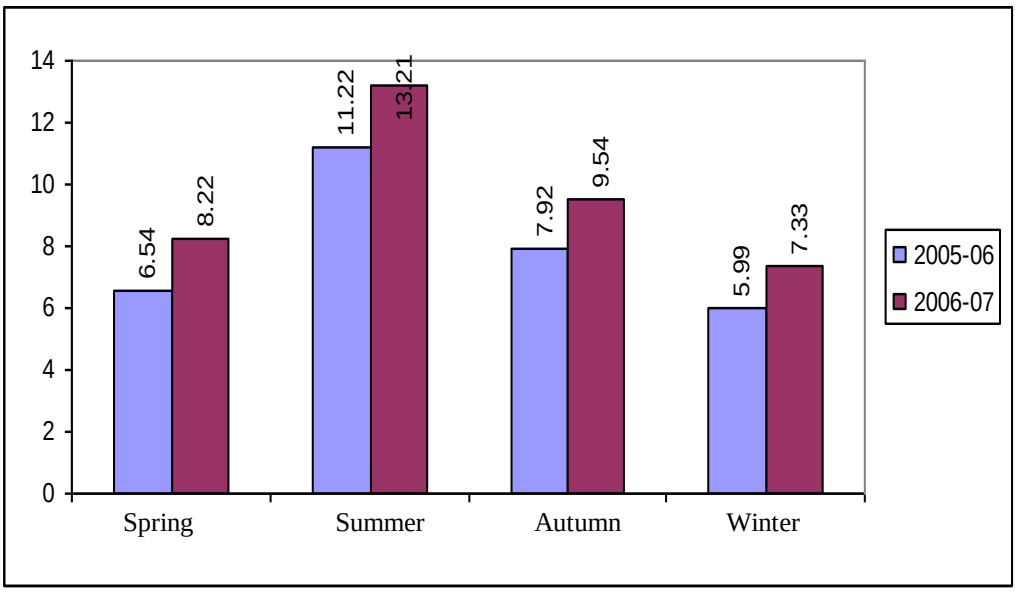


Fig. 54: Showing Manganese concentration in gills of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

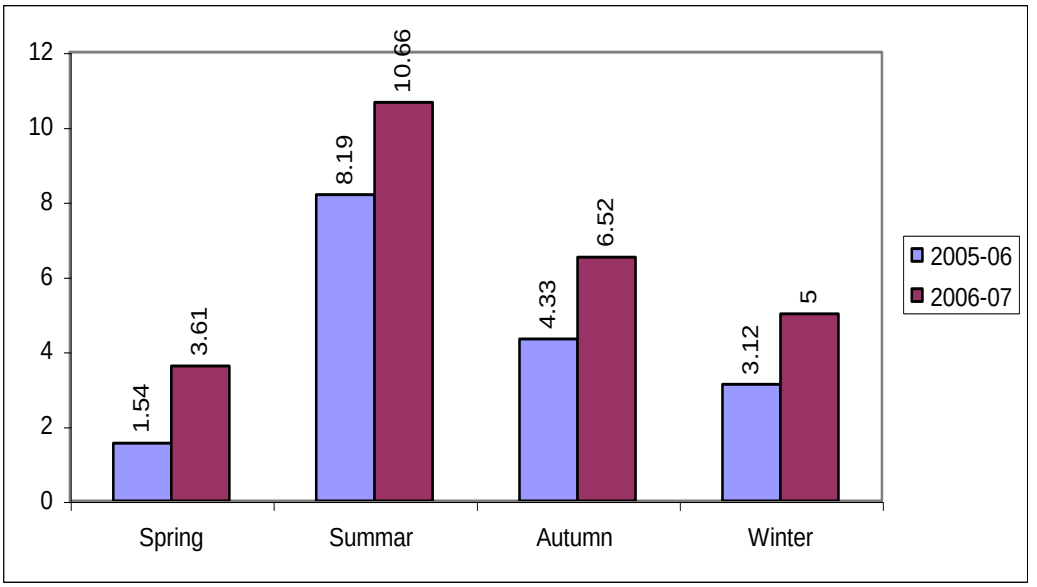


Fig. 55: Showing Manganese concentration in gills of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

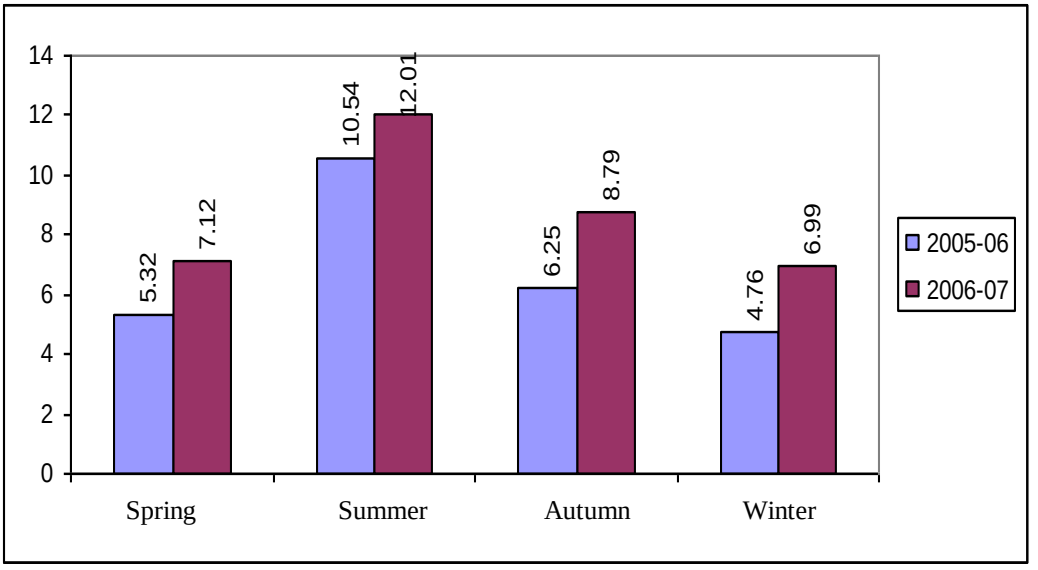


Fig. 56: Showing Manganese concentration in gills of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

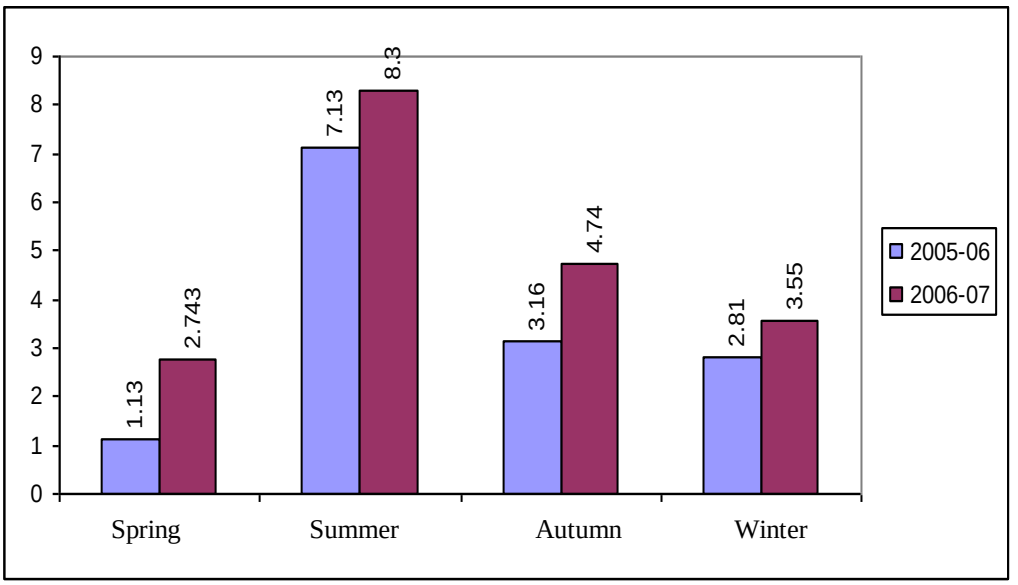


Fig. 57: Showing Manganese concentration in liver of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

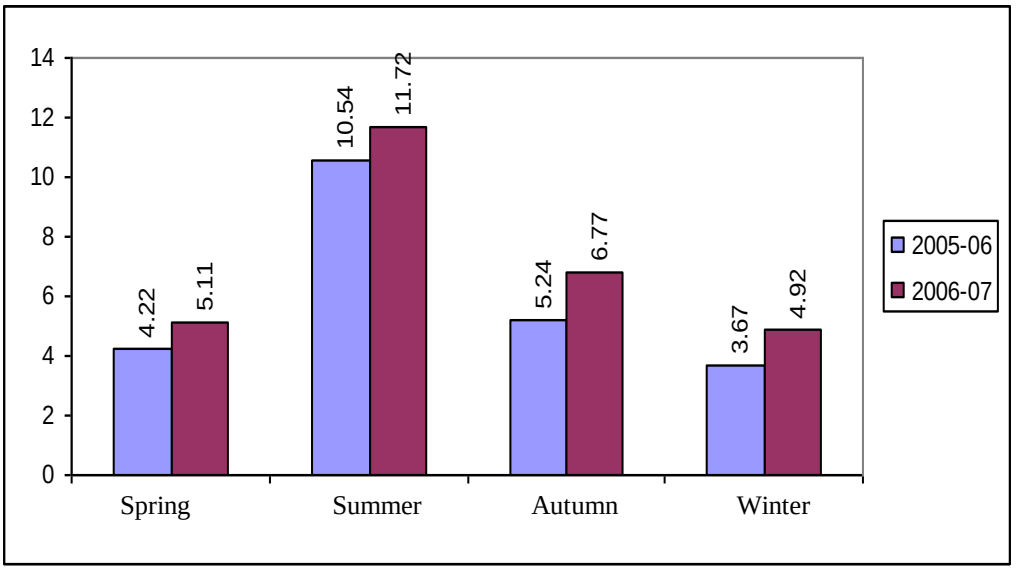


Fig. 58: Showing Manganese concentration in liver of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

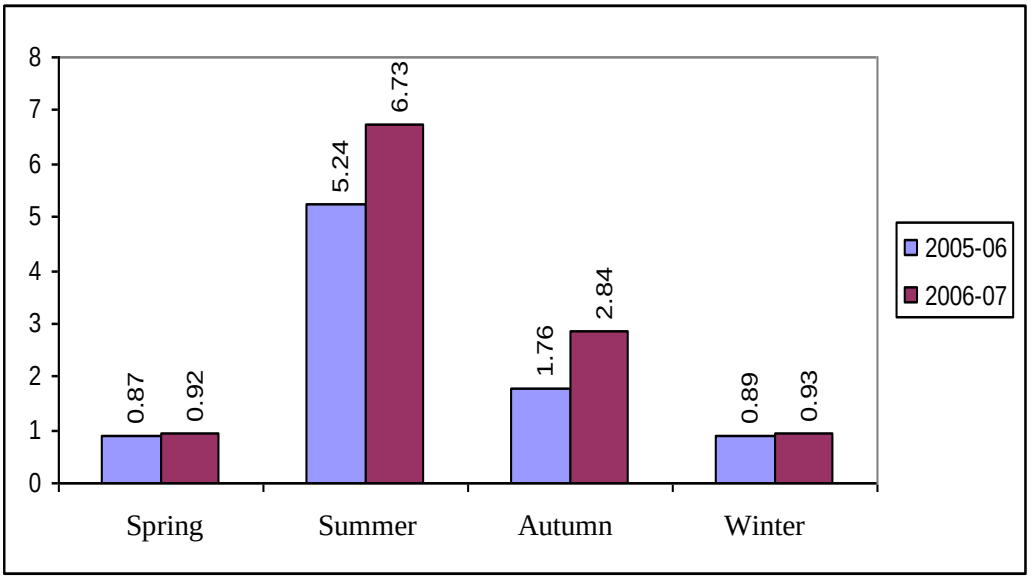


Fig. 59: Showing Manganese concentration in liver of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

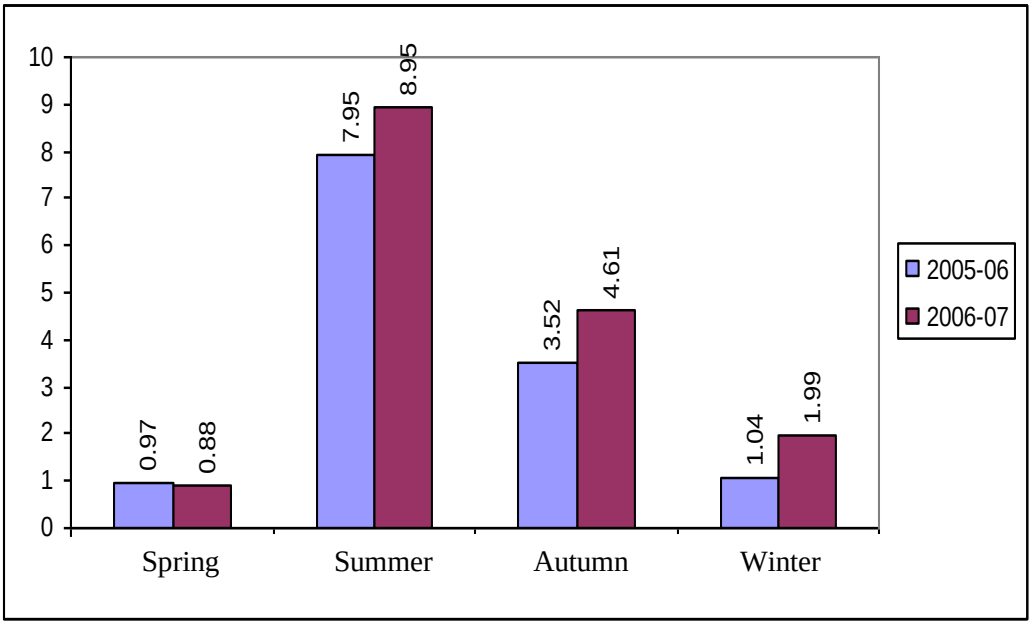


Fig. 60: Showing Manganese concentration in liver of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

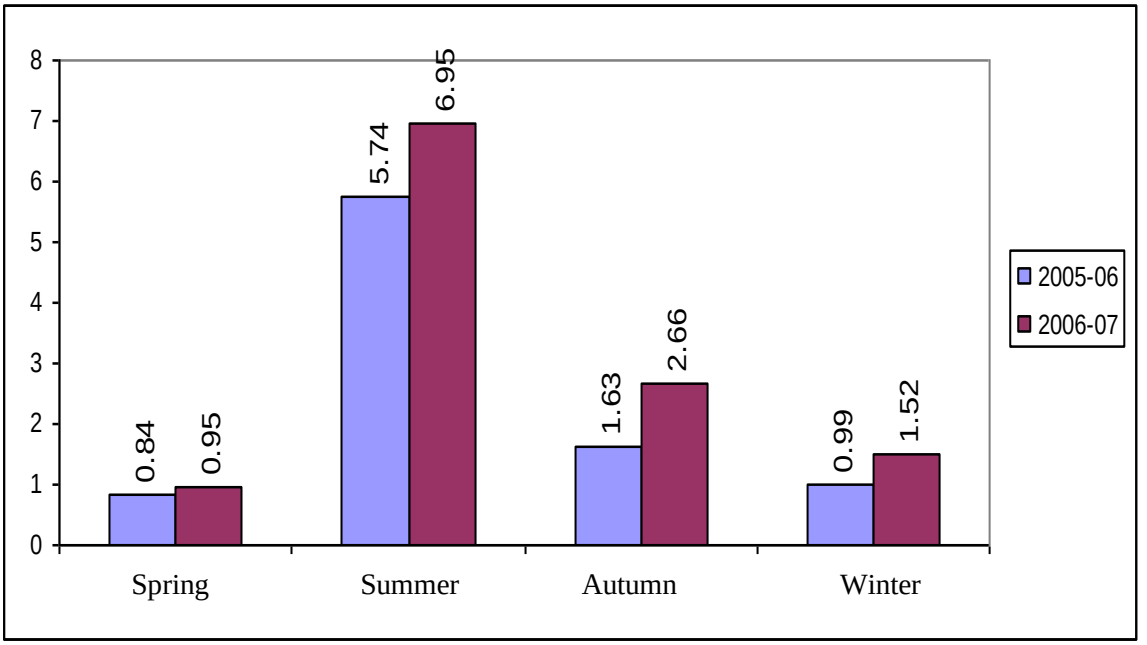


Fig. 61: Showing Manganese concentration in kidney of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

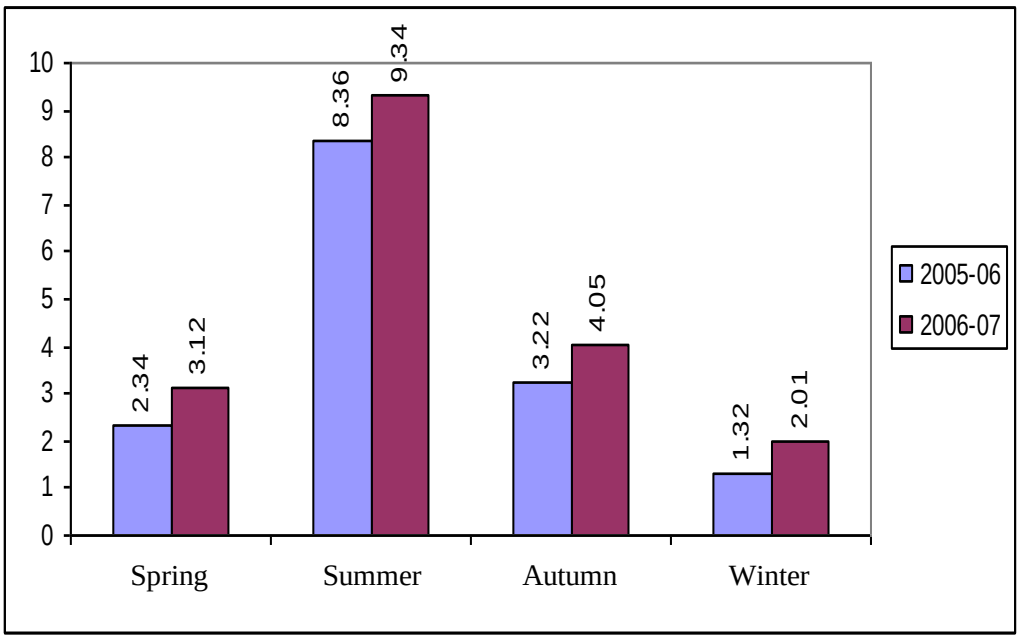


Fig. 62: Showing Manganese concentration in kidney of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

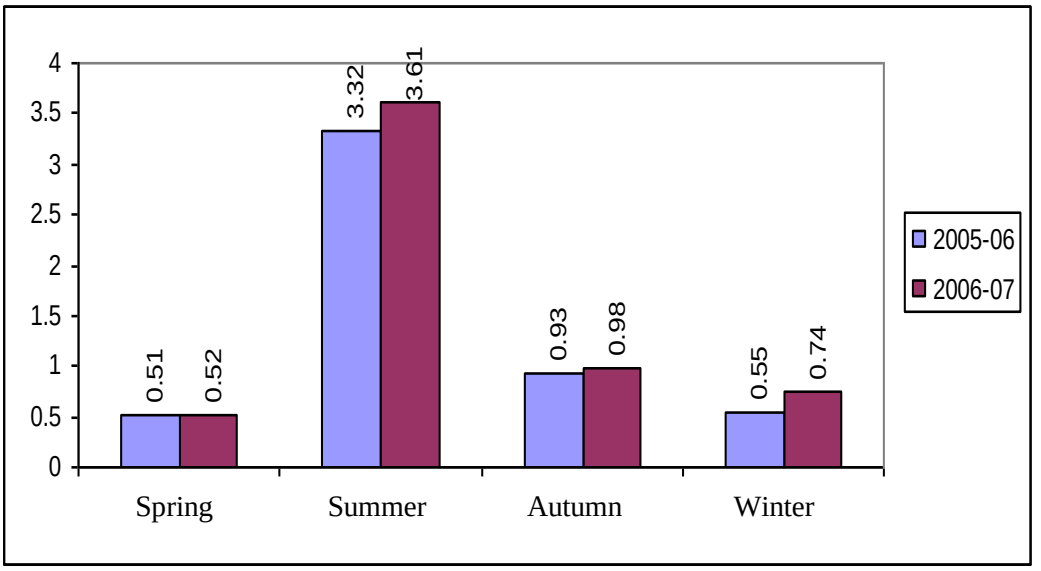


Fig. 63: Showing Manganese concentration in kidney of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

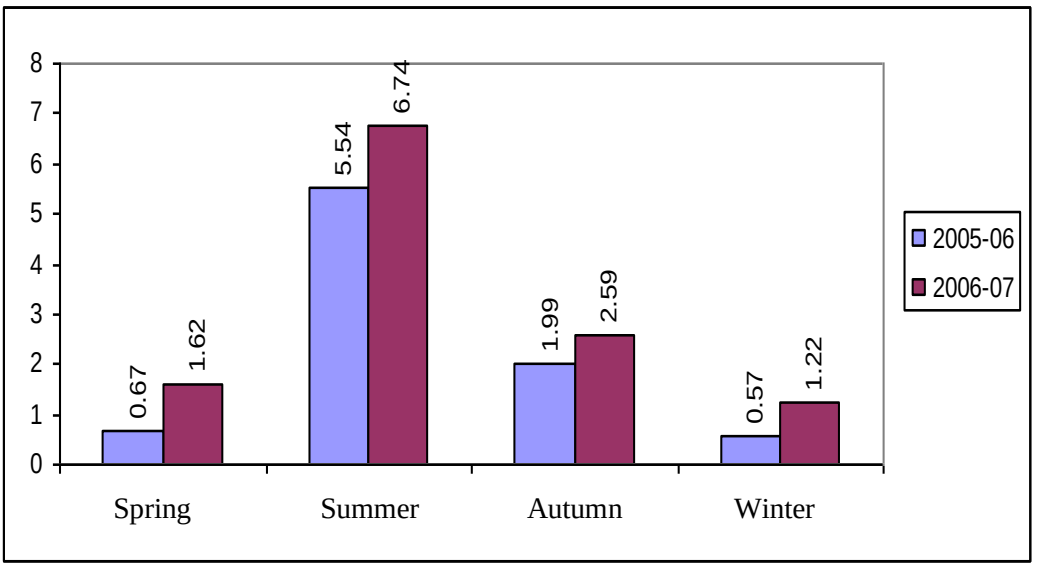


Fig. 64: Showing Manganese concentration in kidney of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

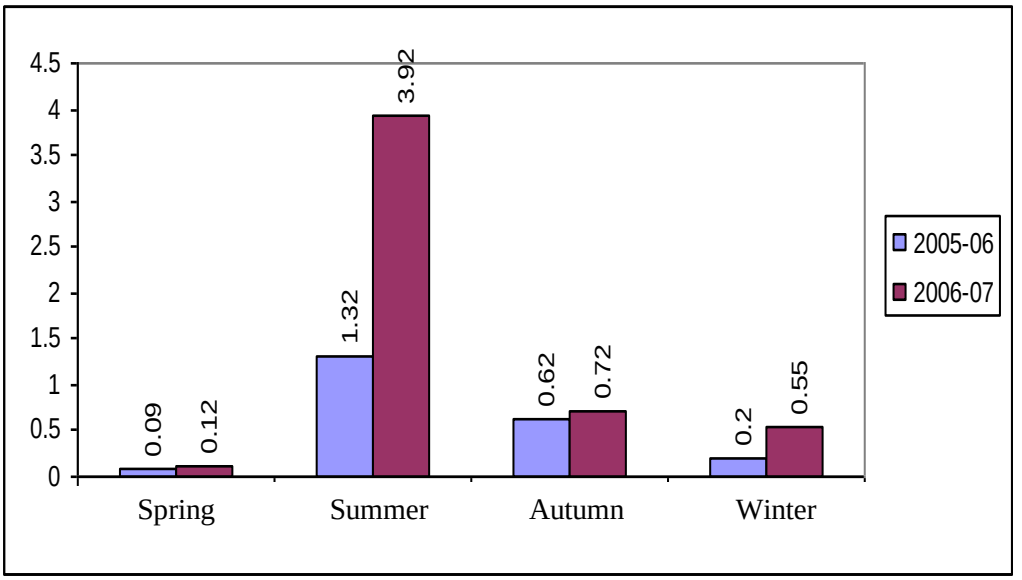


Fig. 65: Showing Manganese concentration in muscle of *Schizothorax niger* from Dal lake during March 2005 to February 2007.

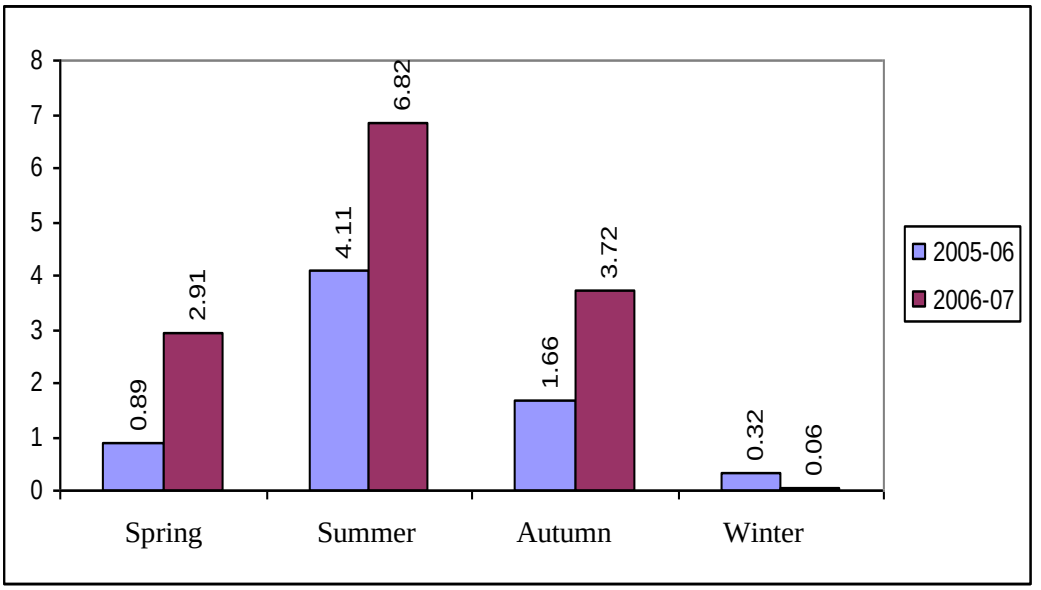


Fig. 66: Showing Manganese concentration in muscle of *Cyprinus carpio* spp. from Dal lake during March 2005 to February 2007.

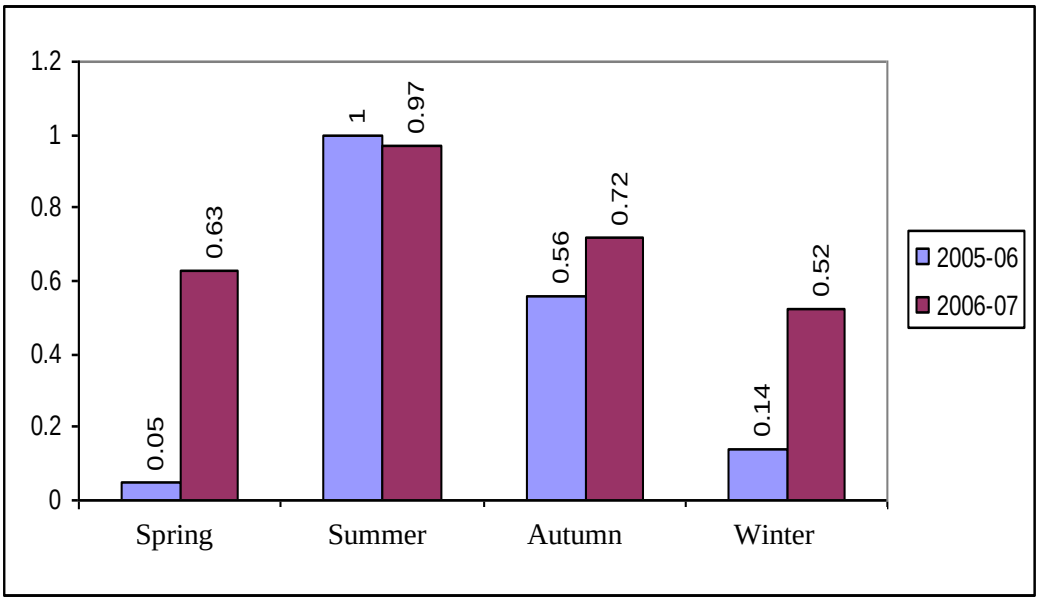


Fig. 67: Showing Manganese concentration in muscle of *Schizothorax niger* from River Jhelum during March 2005 to February 2007.

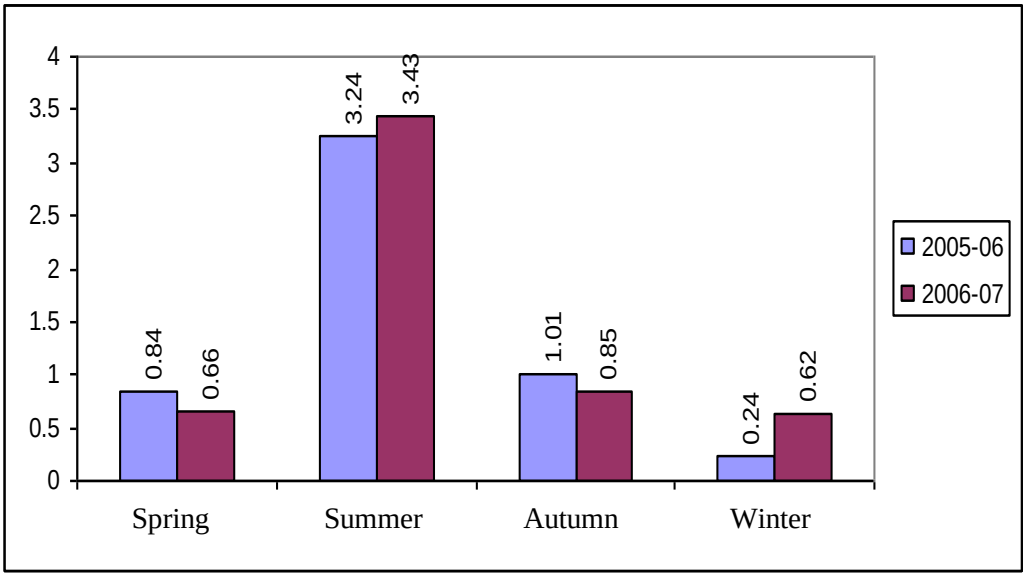


Fig. 68: Showing Manganese concentration in muscle of *Cyprinus carpio* spp. from River Jhelum during March 2005 to February 2007.

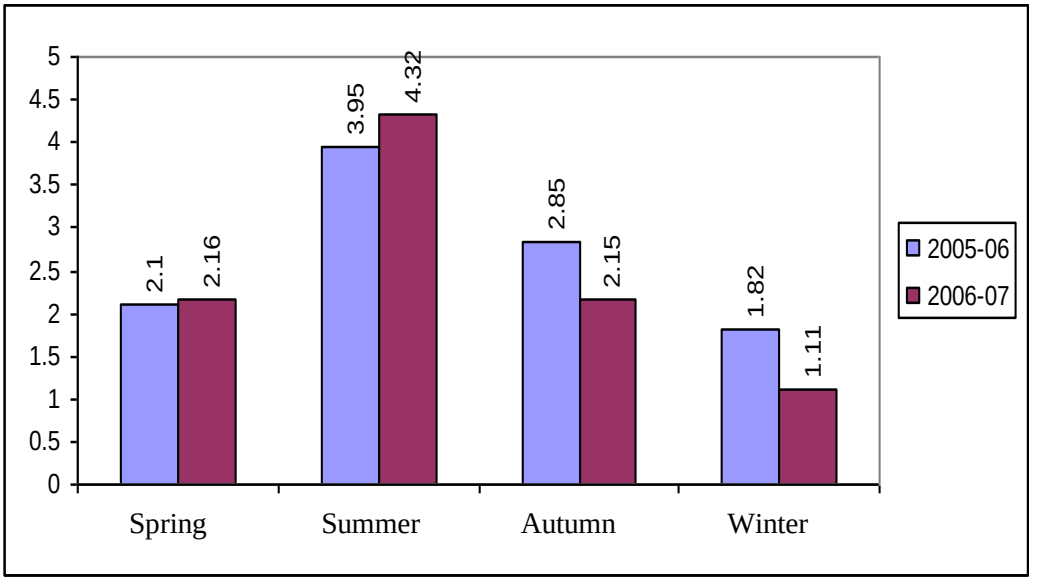


Fig. 69: Showing seasonal estimation of total protein in *Schizothorax niger* collected from Dal lake during March 2005 to February 2007.

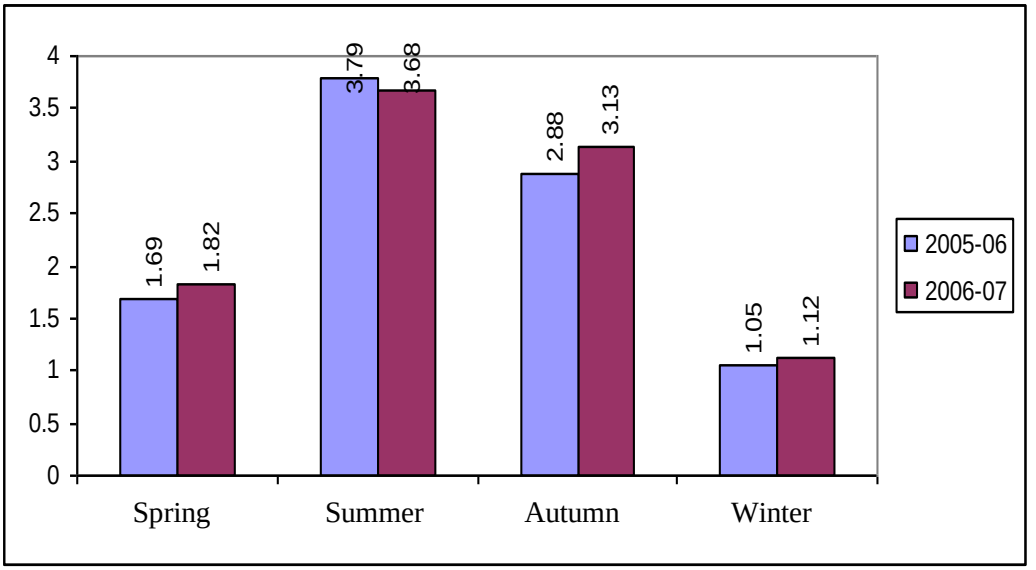


Fig. 70: Showing seasonal estimation of total protein in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

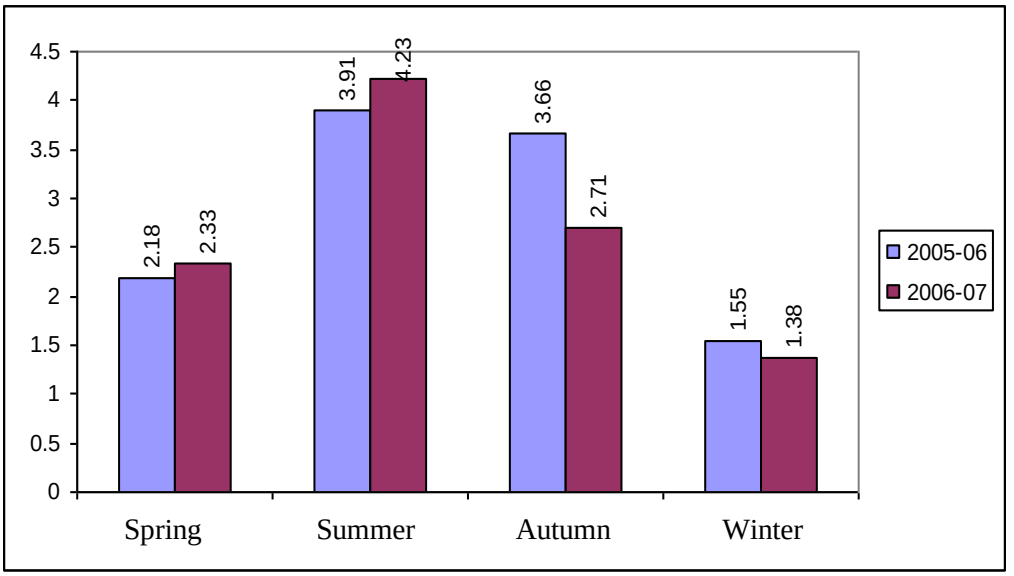


Fig. 71: Showing seasonal estimation of total protein in *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

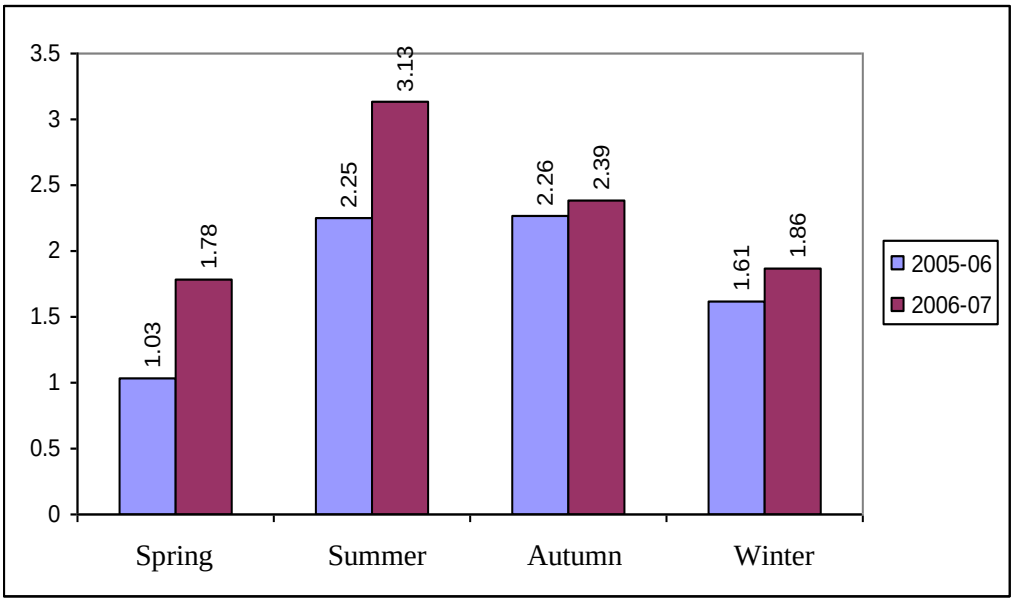


Fig. 72: Showing seasonal estimation of total protein in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007.

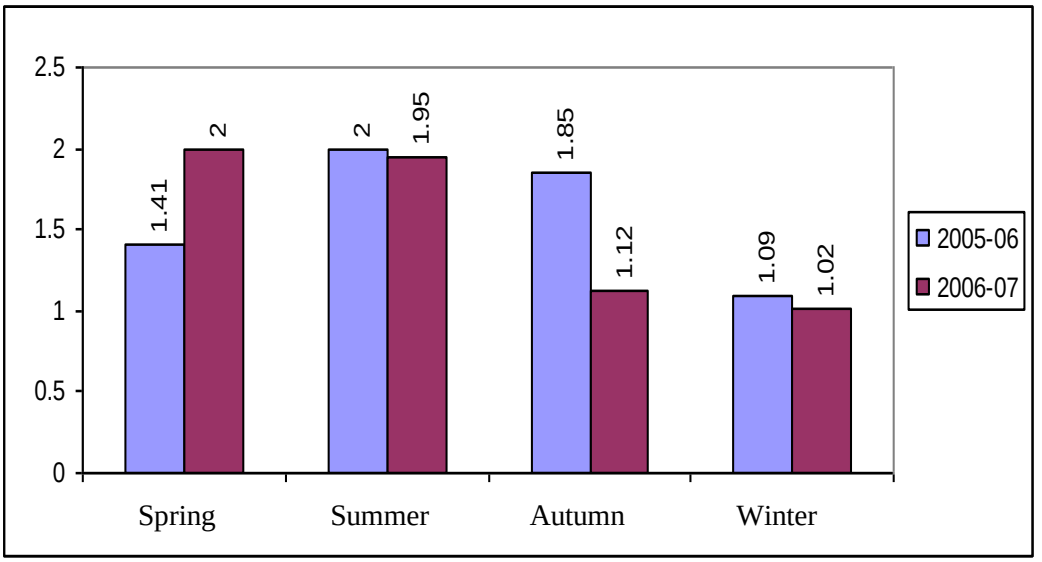


Fig. 73: Showing seasonal estimation of albumin in *Schizothorax niger* collected from Dal lake during March 2005 to February 2007.

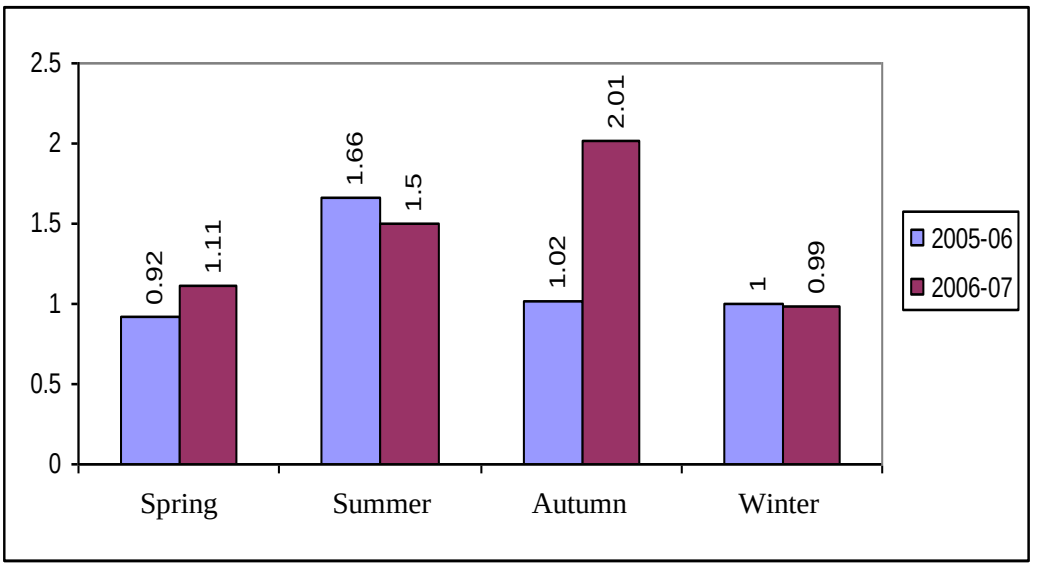


Fig. 74: Showing seasonal estimation of albumin in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

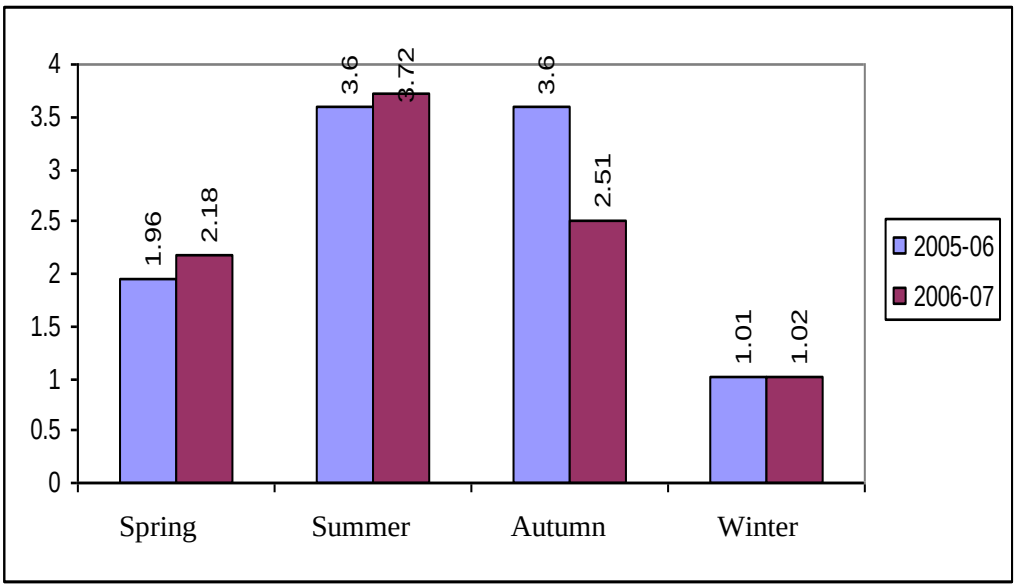


Fig. 75: Showing seasonal estimation of albumin in. *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

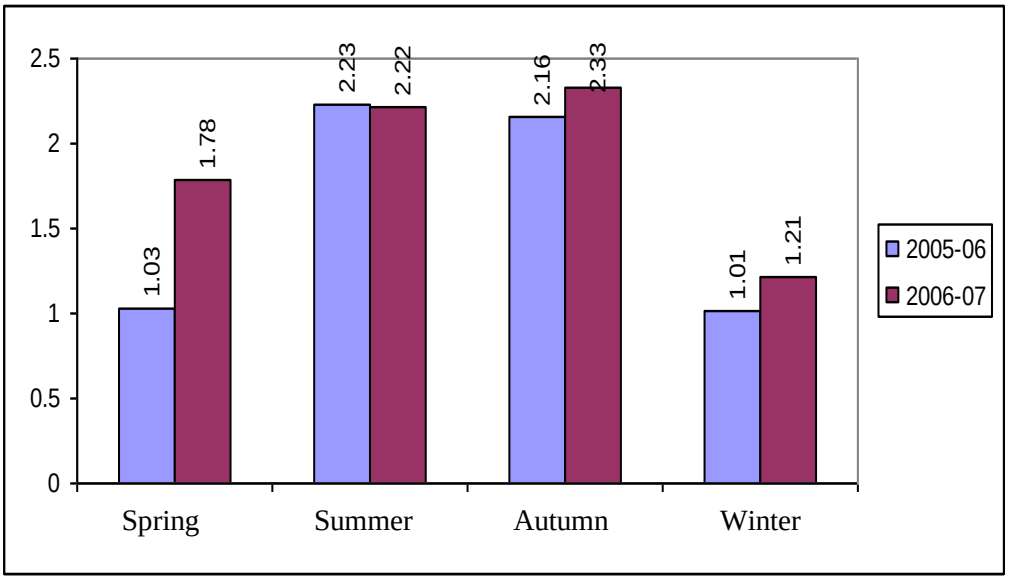


Fig. 76: Showing seasonal estimation of albumin in *Cyprinus carpio* spp collected from River Jhelum during March 2005 to February 2007.

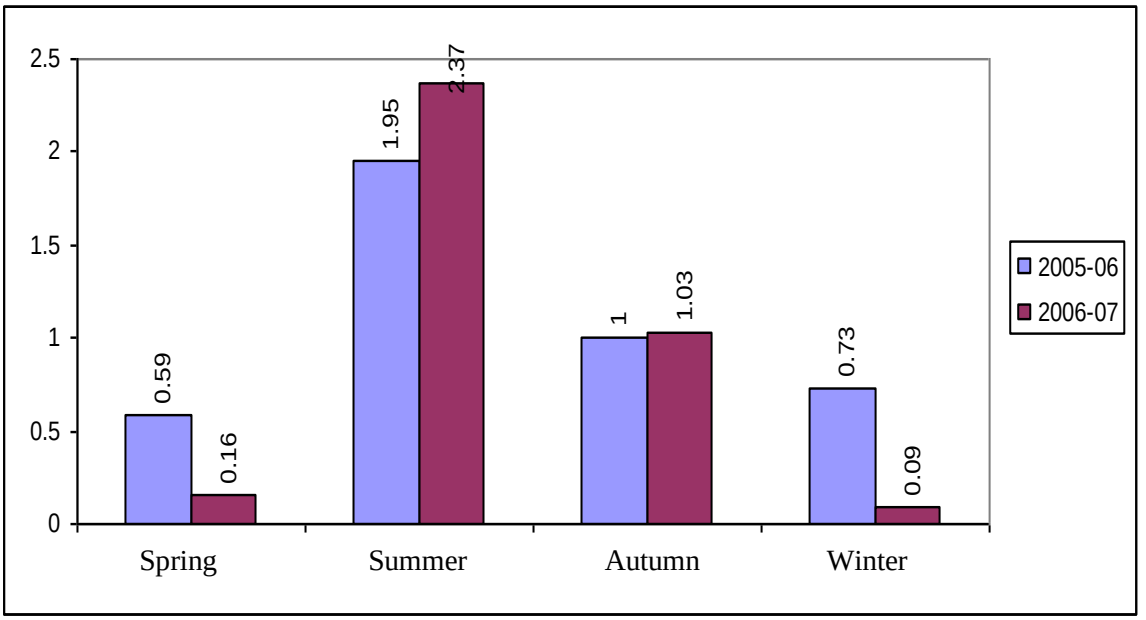


Fig. 77: Showing seasonal estimation of globulin in *Schizothorax niger* collected from Dal lake during March 2005 to February 2007.

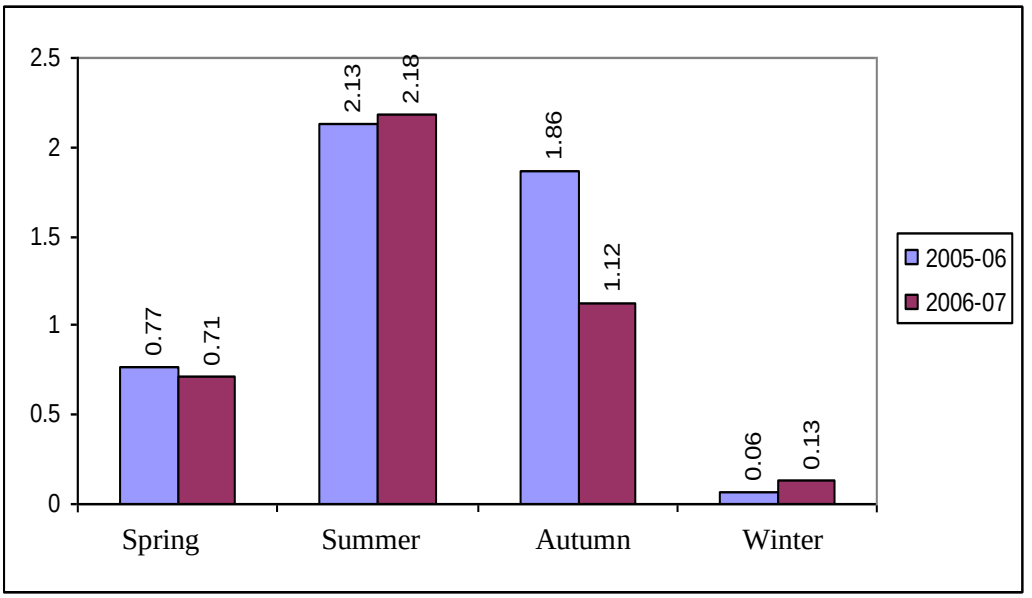


Fig. 78: Showing seasonal estimation of globulin in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

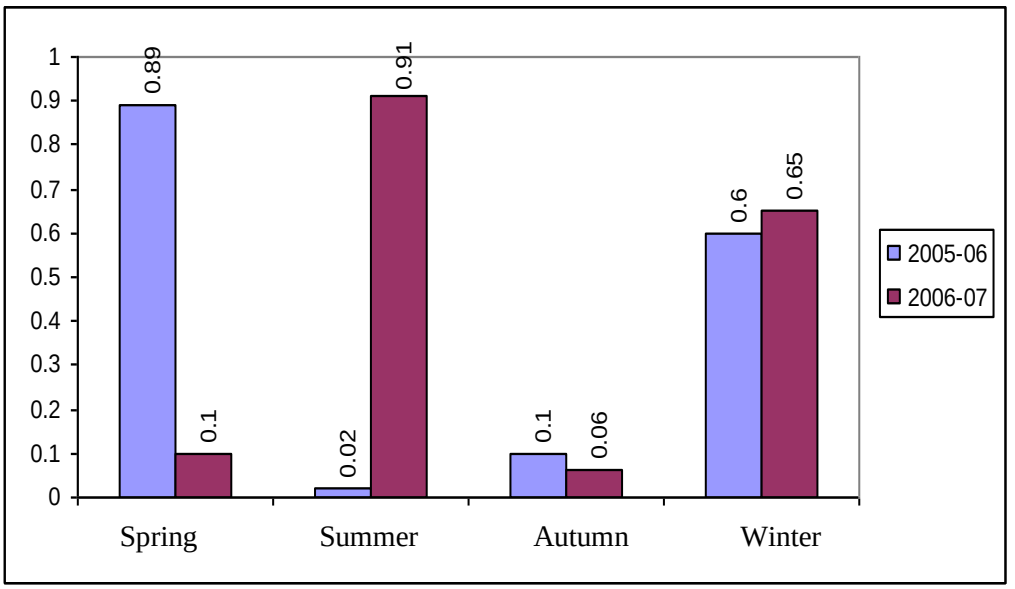


Fig. 79: Showing seasonal estimation of globulin in *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

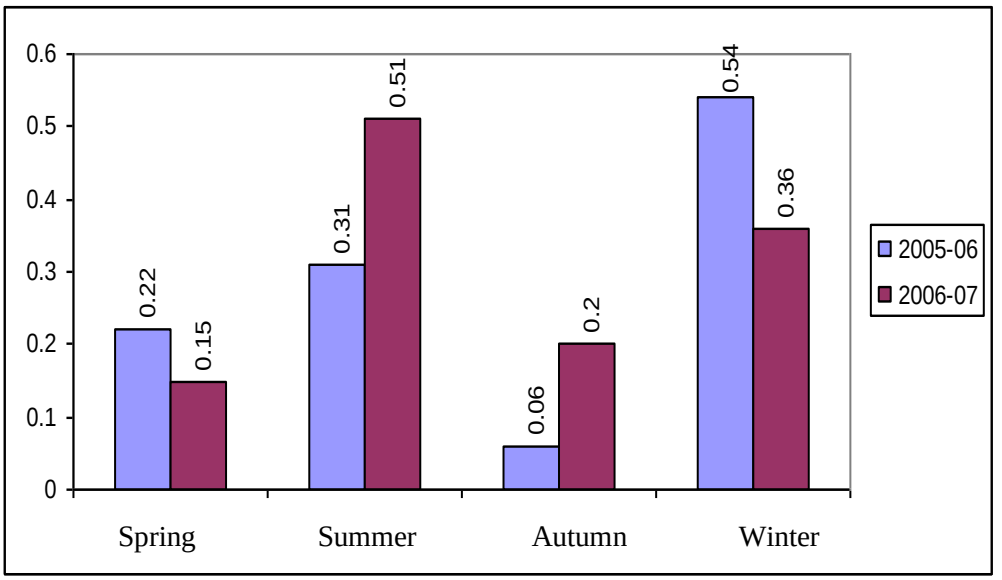


Fig. 80: Showing seasonal estimation of globulin in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007.

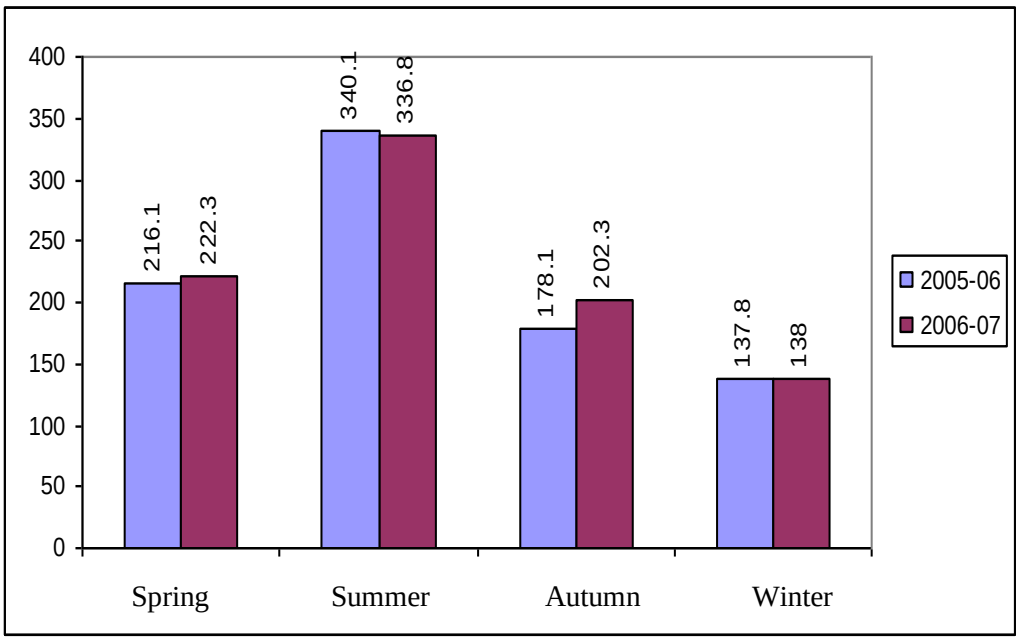


Fig. 81: Showing seasonal estimation of glucose in *Schizothorax niger*. collected from Dal lake during March 2005 to February 2007.

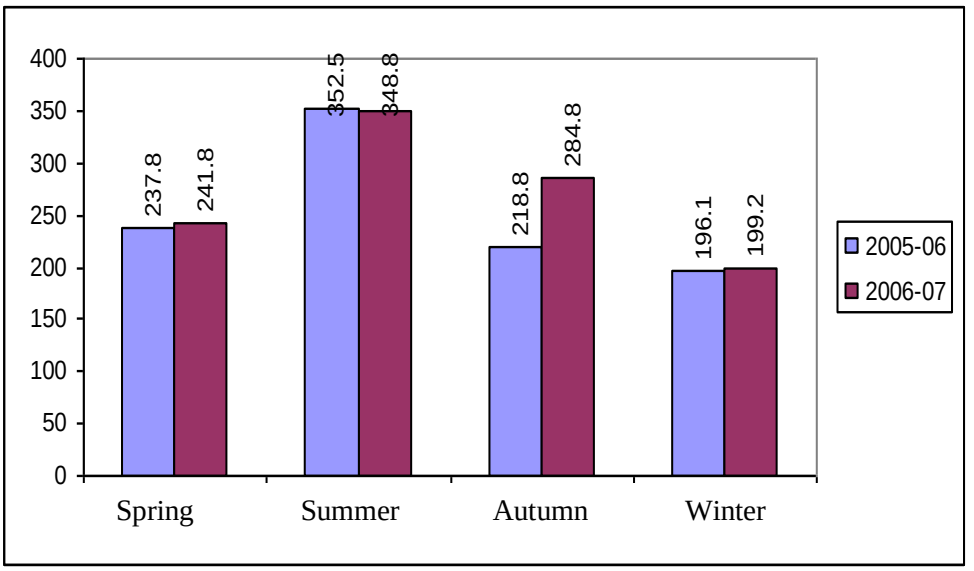


Fig. 82: Showing seasonal estimation of glucose in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

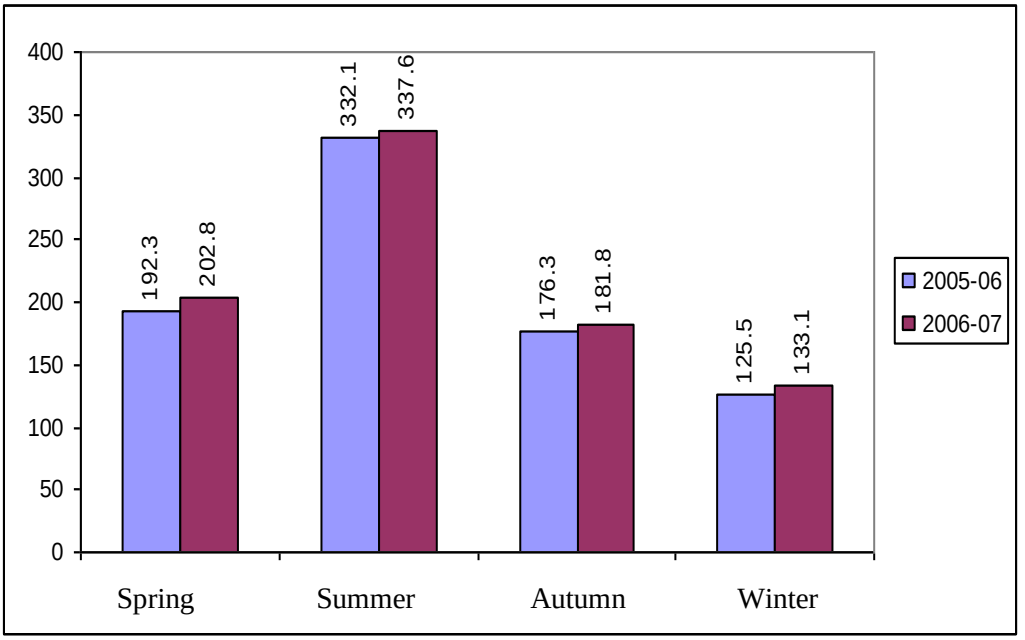


Fig. 83: Showing seasonal estimation of glucose in *Schizothorax niger*. collected from River Jhelum during March 2005 to February 2007.

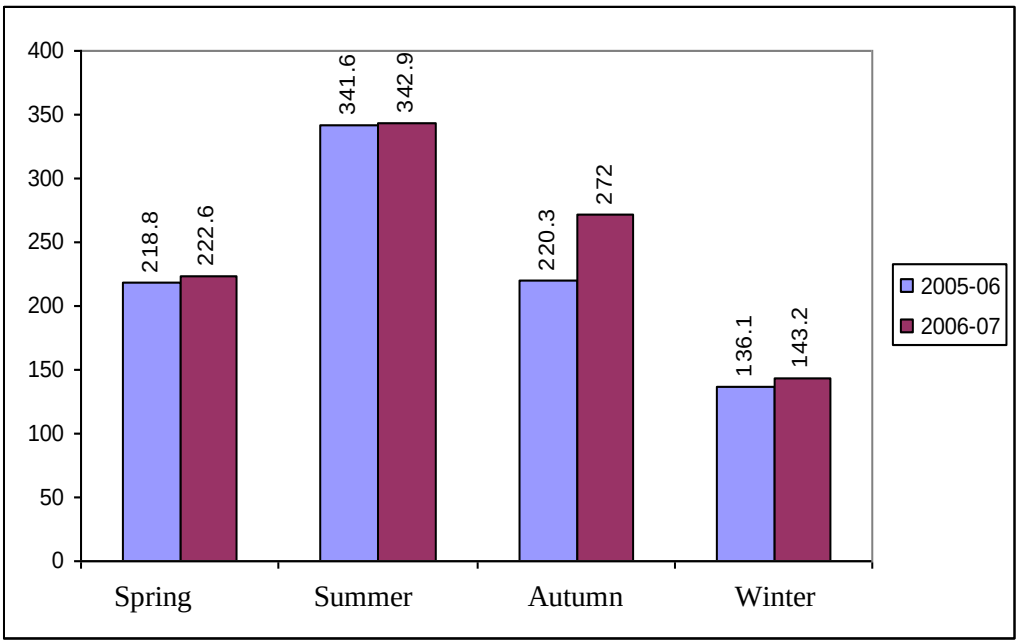


Fig. 84: Showing seasonal estimation of glucose in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007

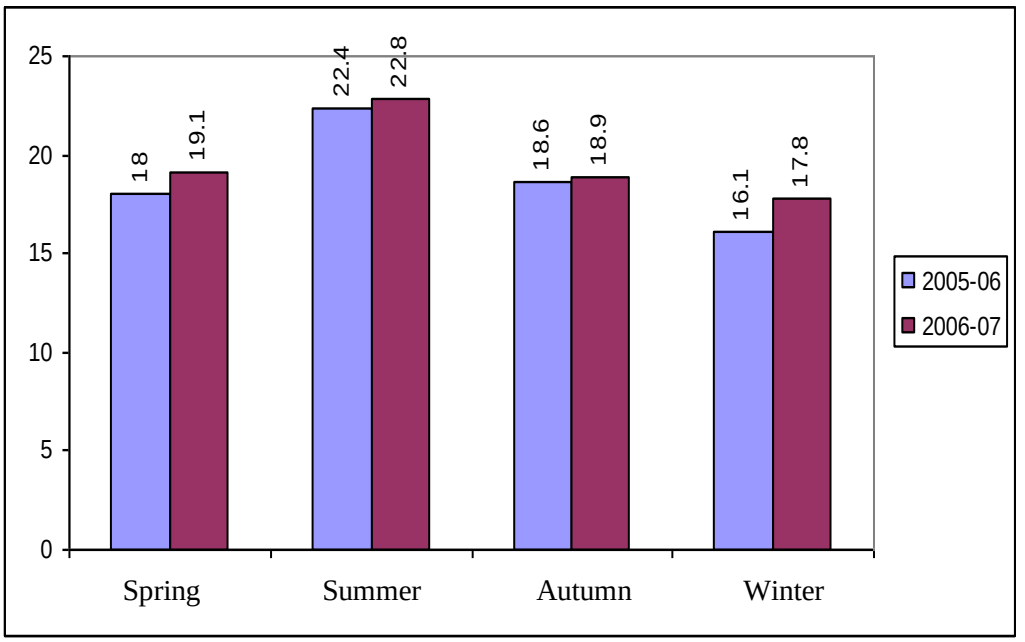


Fig. 85: Showing seasonal estimation of urea in *Schizothorax niger*. collected from Dal lake during March 2005 to February 2007.

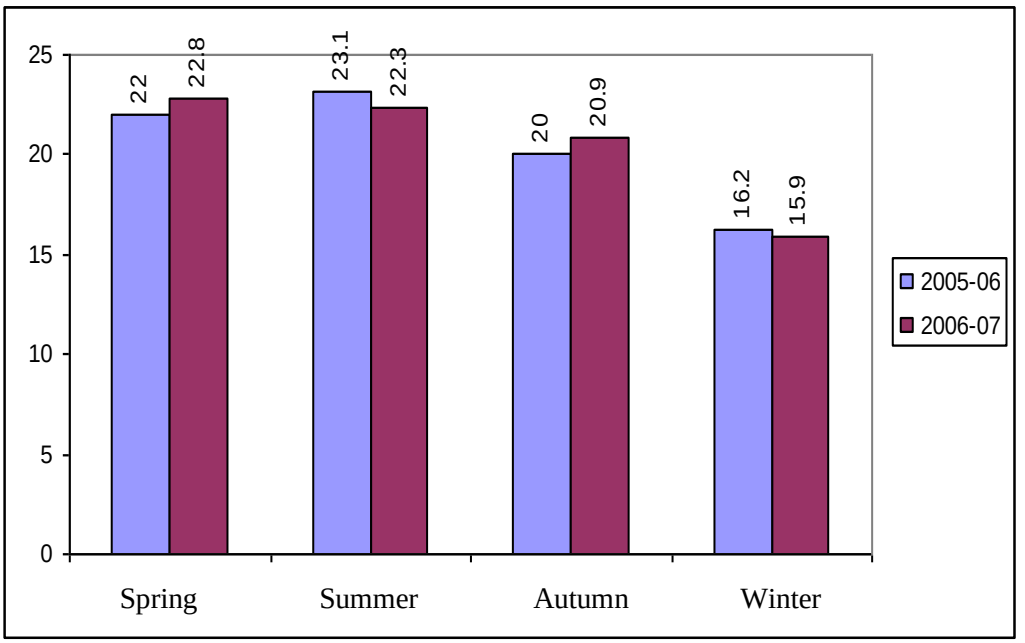


Fig. 86: Showing seasonal estimation of urea in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

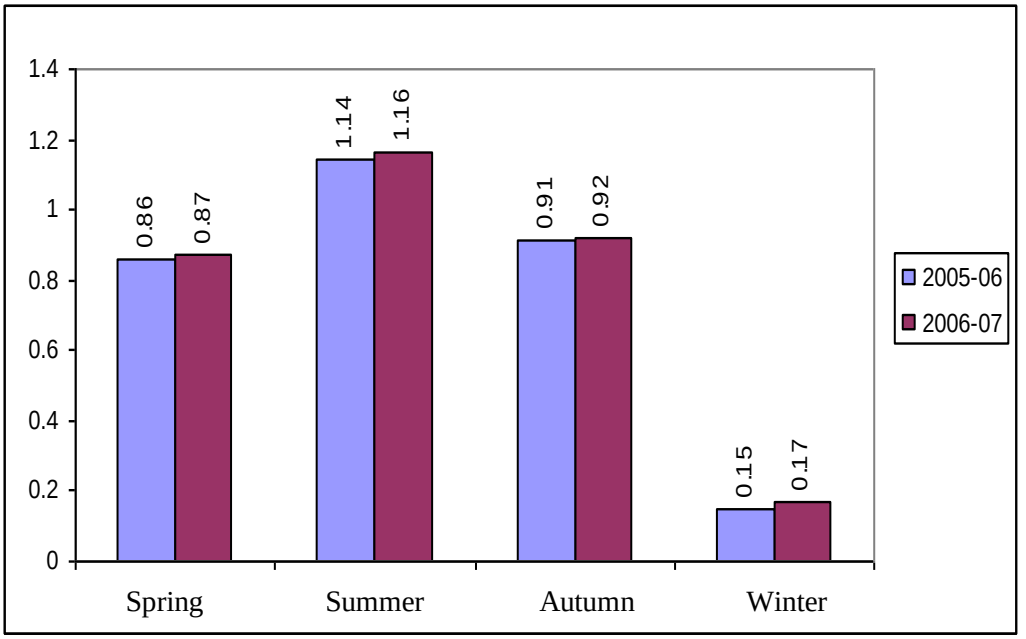


Fig. 87: Showing seasonal estimation of creatinine in *Schizothorax niger* collected from Dal lake during March 2005 to February 2007.

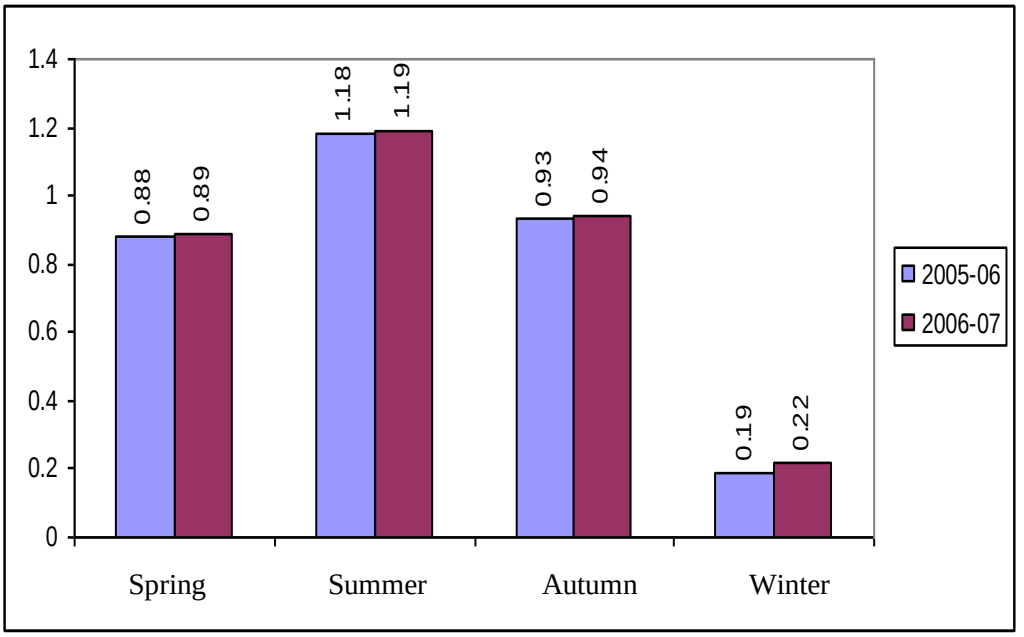


Fig. 88: Showing seasonal estimation of creatinine in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

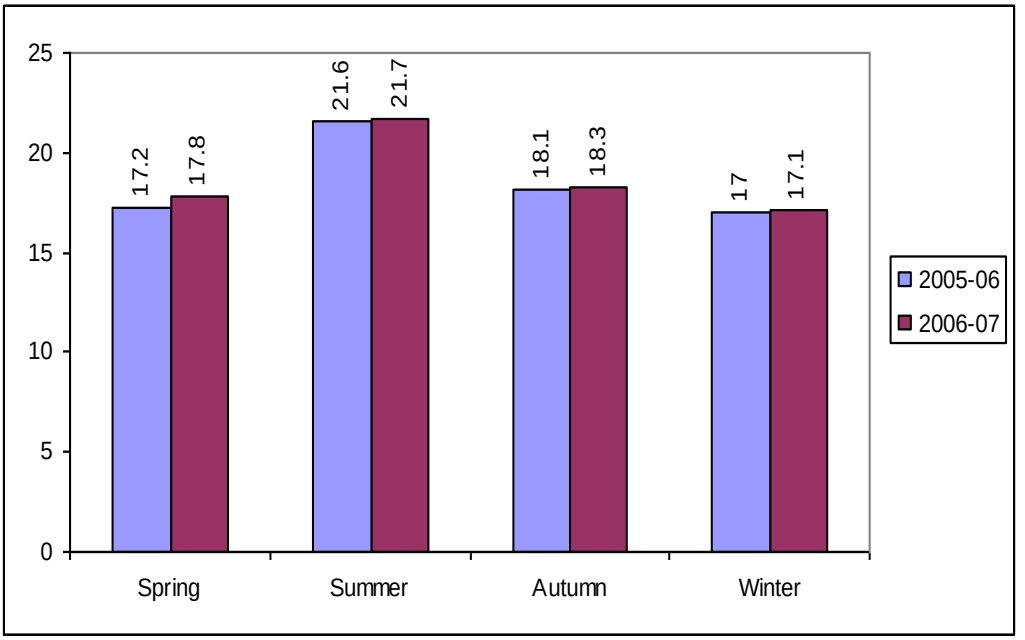


Fig. 89: Showing seasonal estimation of urea in *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

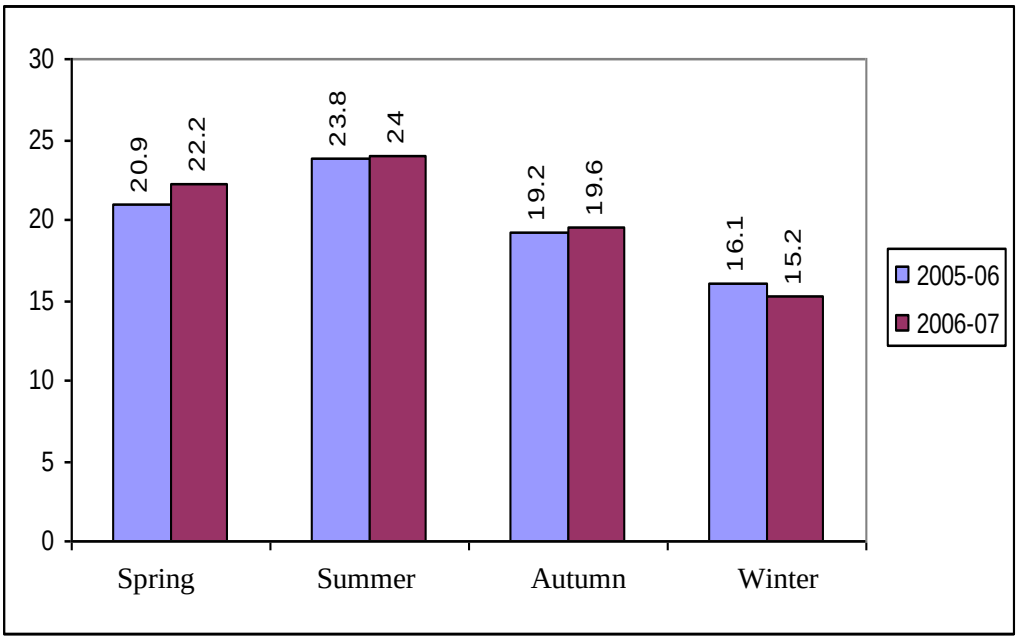


Fig. 90: Showing seasonal estimation of urea in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007.

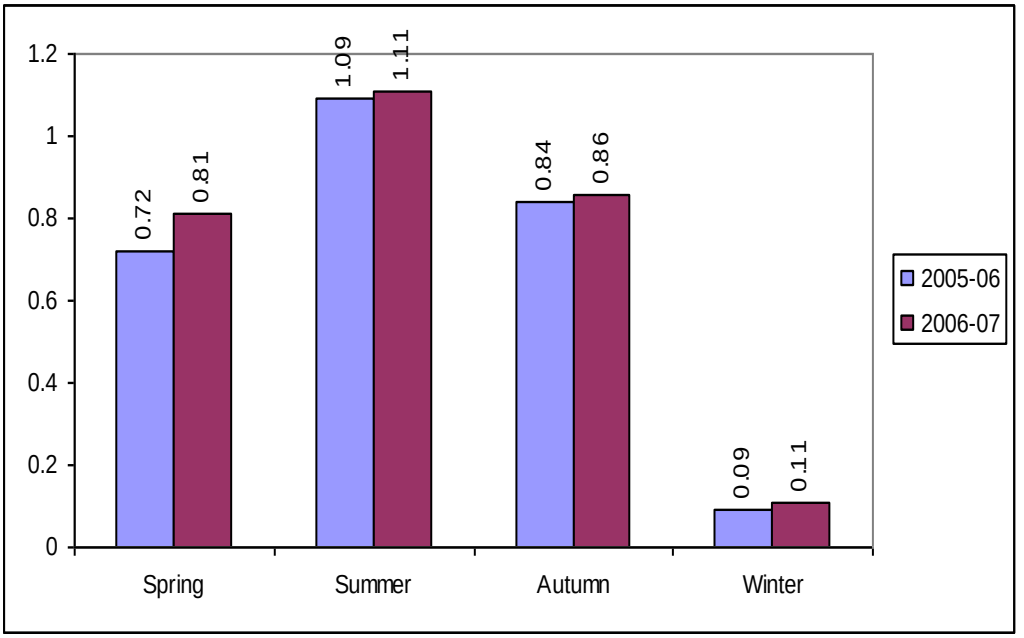


Fig. 91: Showing seasonal estimation of creatinine in *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

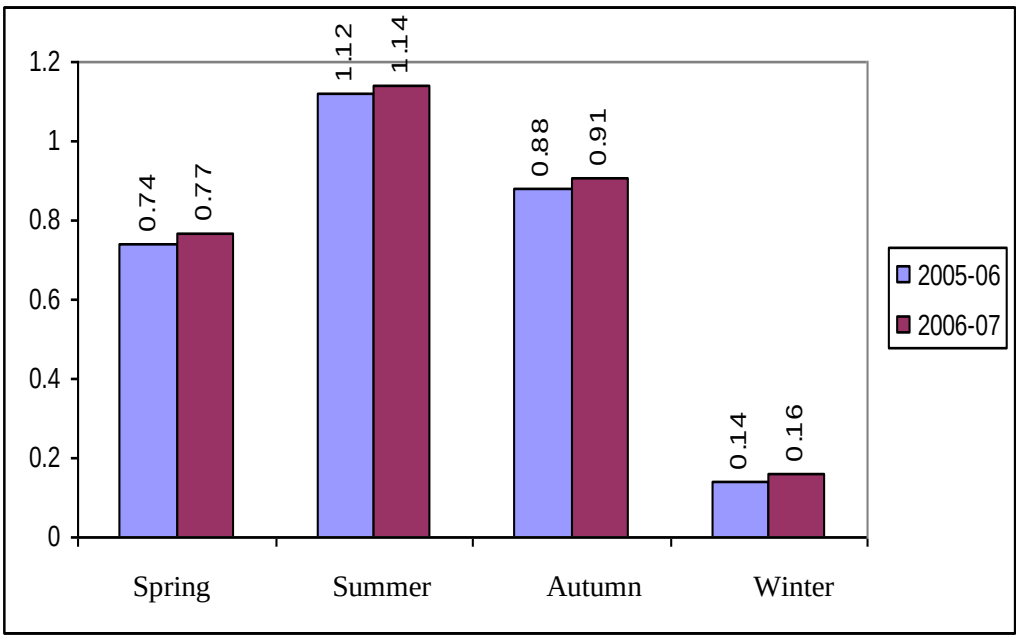


Fig. 92: Showing seasonal estimation of creatinine in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007.

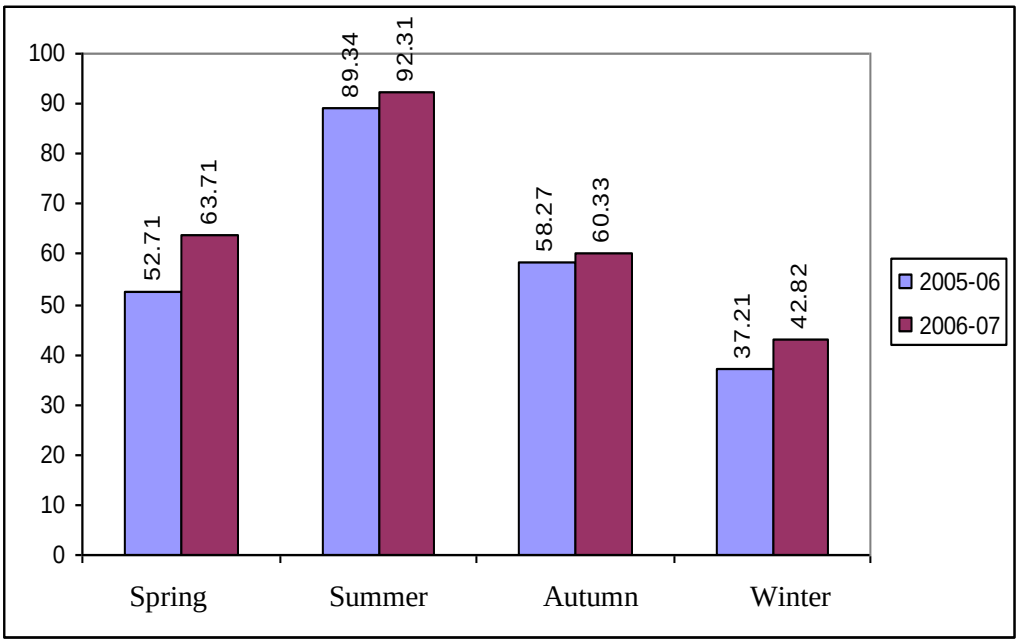


Fig. 93: Showing seasonal estimation of cholestrol in *Schizothorax niger* collected from Dal lake during March 2005 to February 2007.

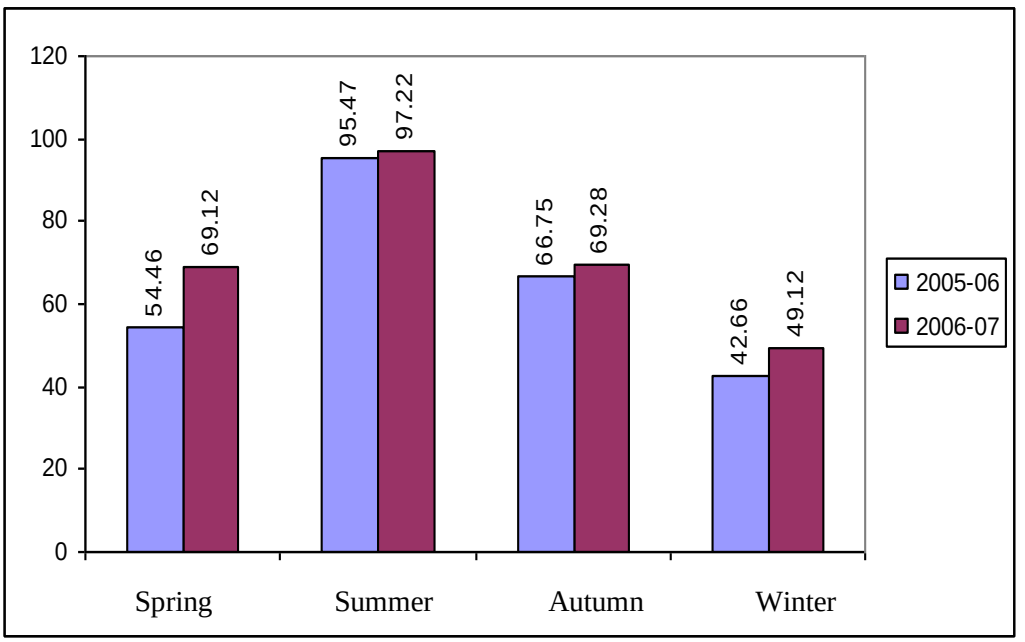


Fig. 94: Showing seasonal estimation of cholestrol in *Cyprinus carpio* spp. collected from Dal lake during March 2005 to February 2007.

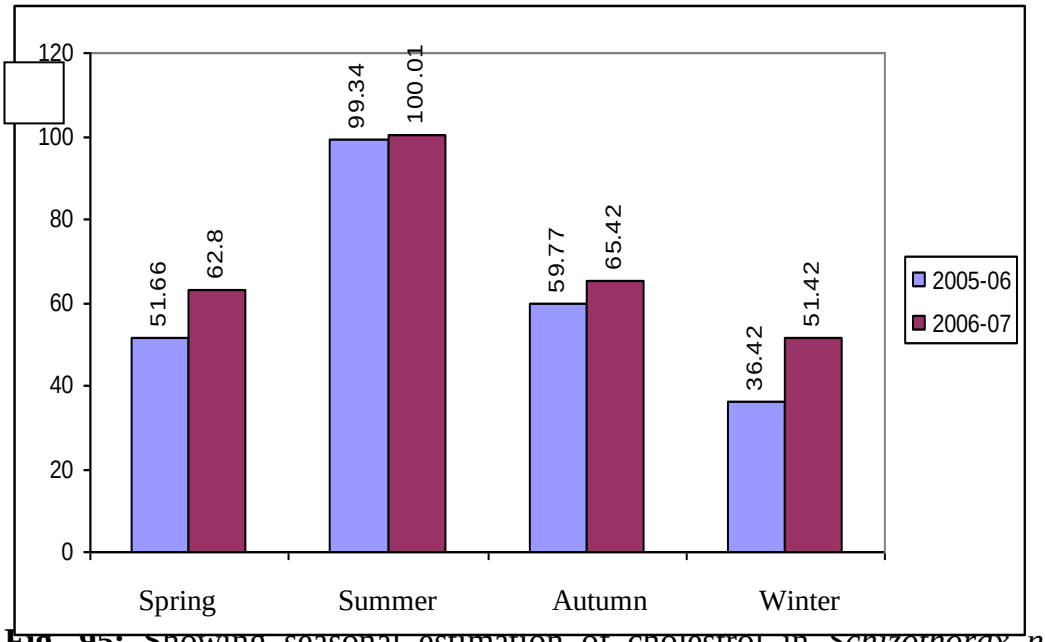


Fig. 95: Showing seasonal estimation of cholestrol in *Schizothorax niger* collected from River Jhelum during March 2005 to February 2007.

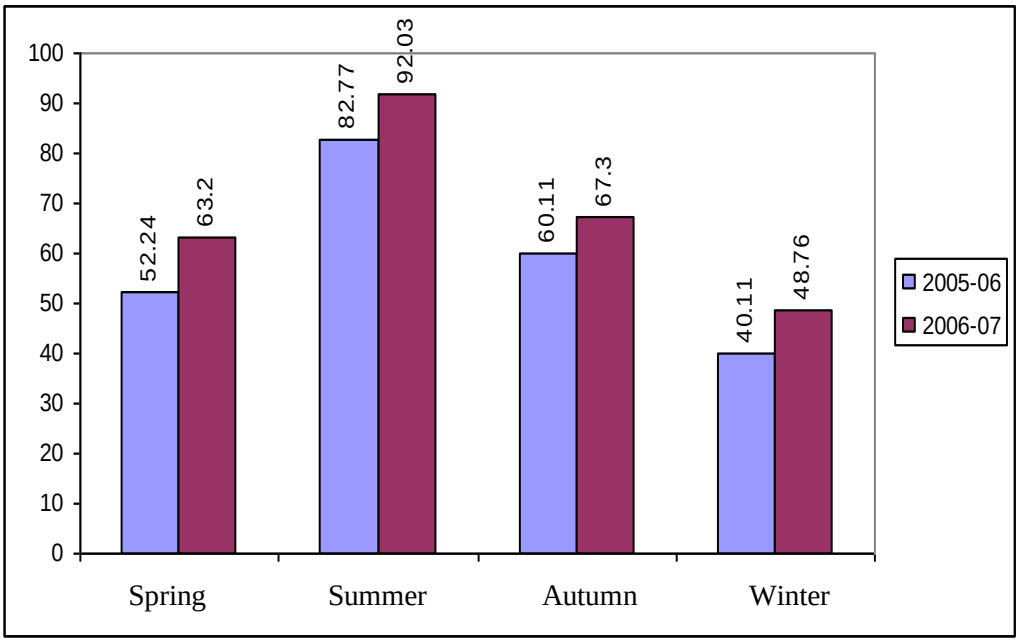
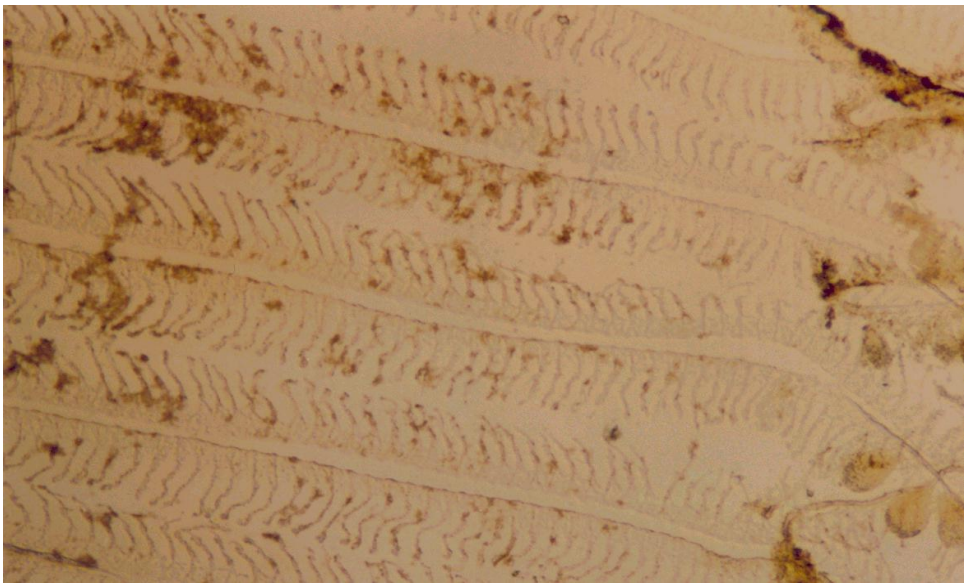


Fig. 96: Showing seasonal estimation of cholestrol in *Cyprinus carpio* spp. collected from River Jhelum during March 2005 to February 2007.

Plate - 1



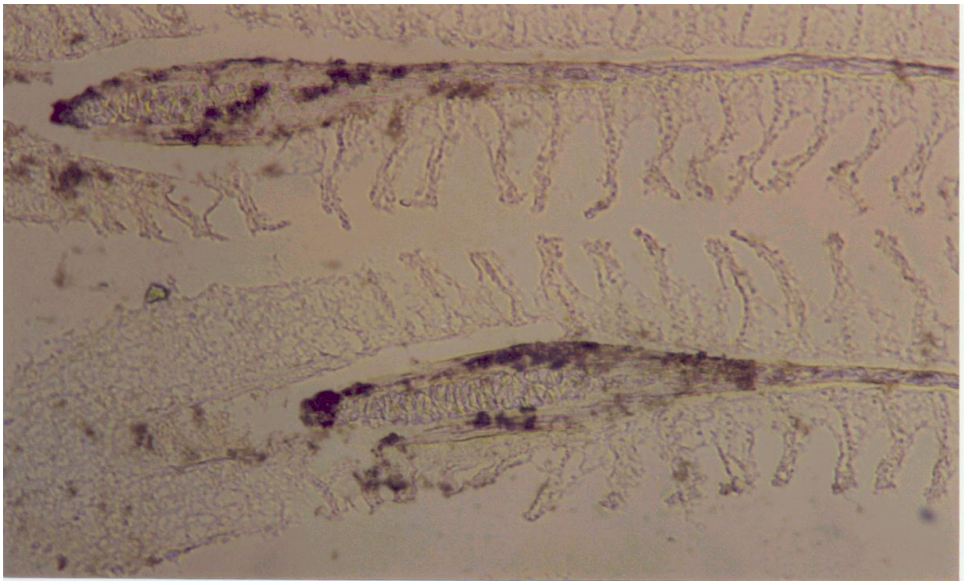
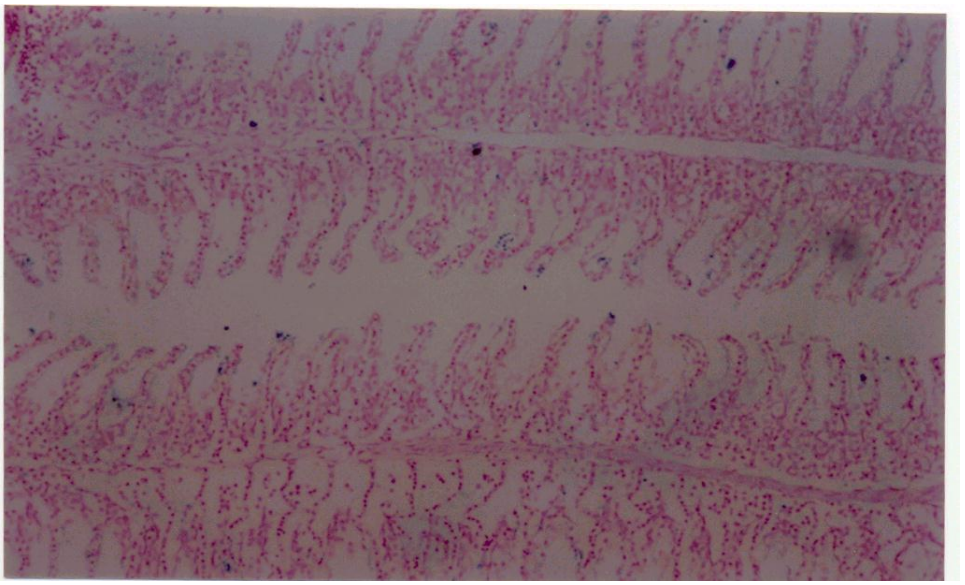


Plate - 2



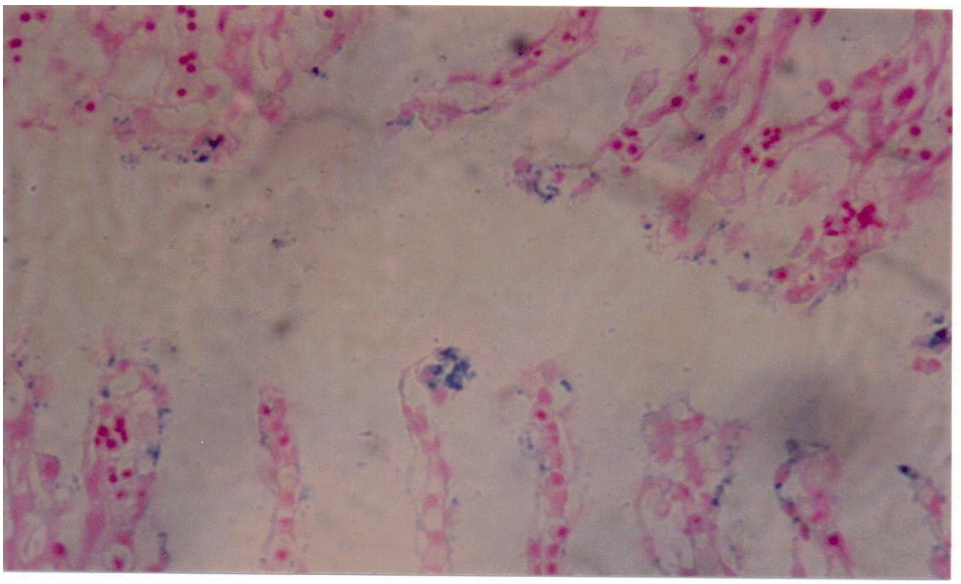
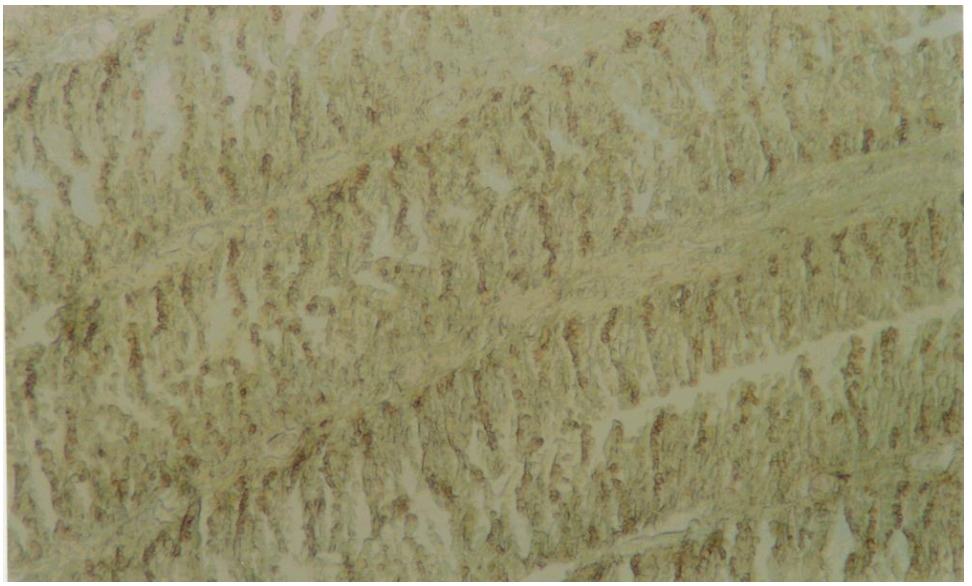
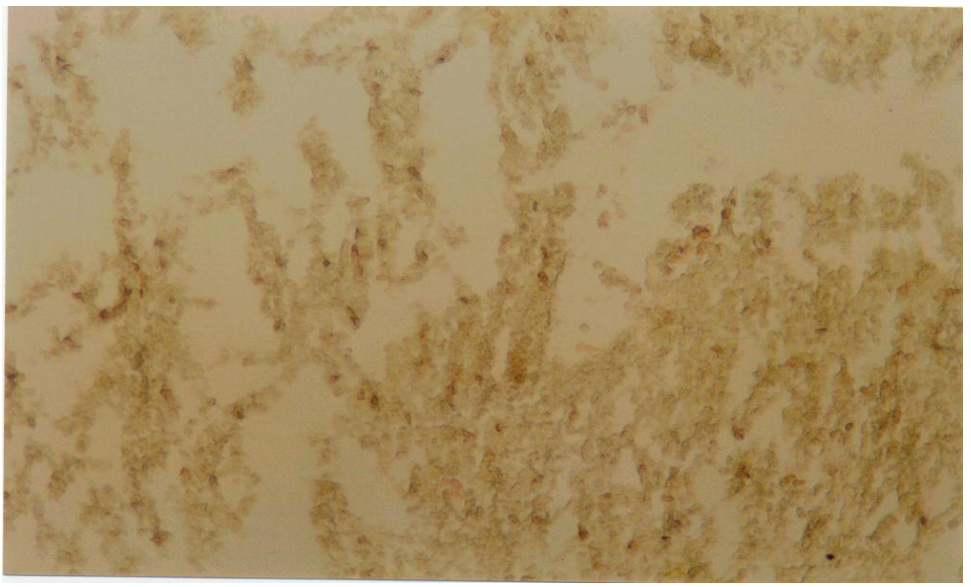


Plate - 3





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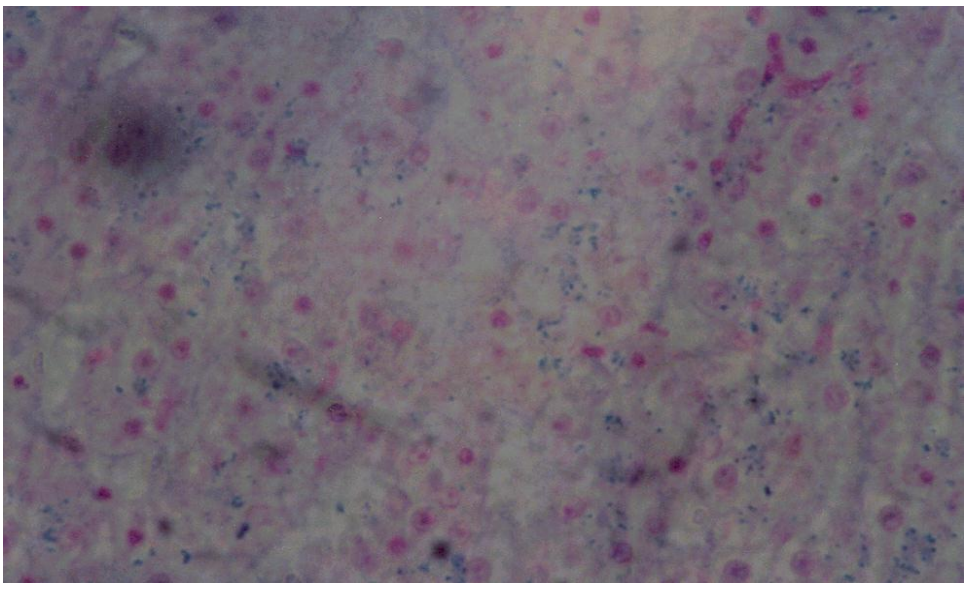
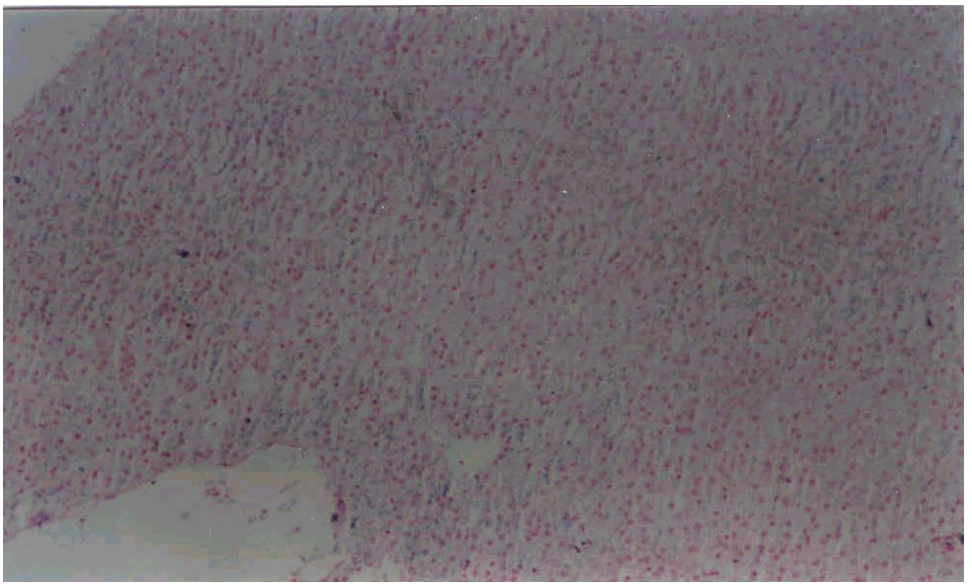


Plate - 5



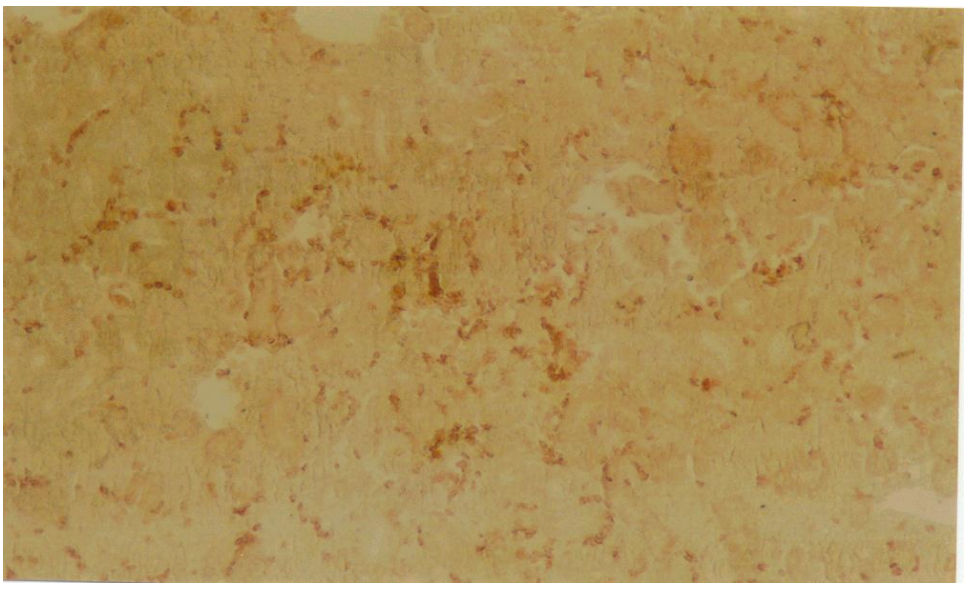
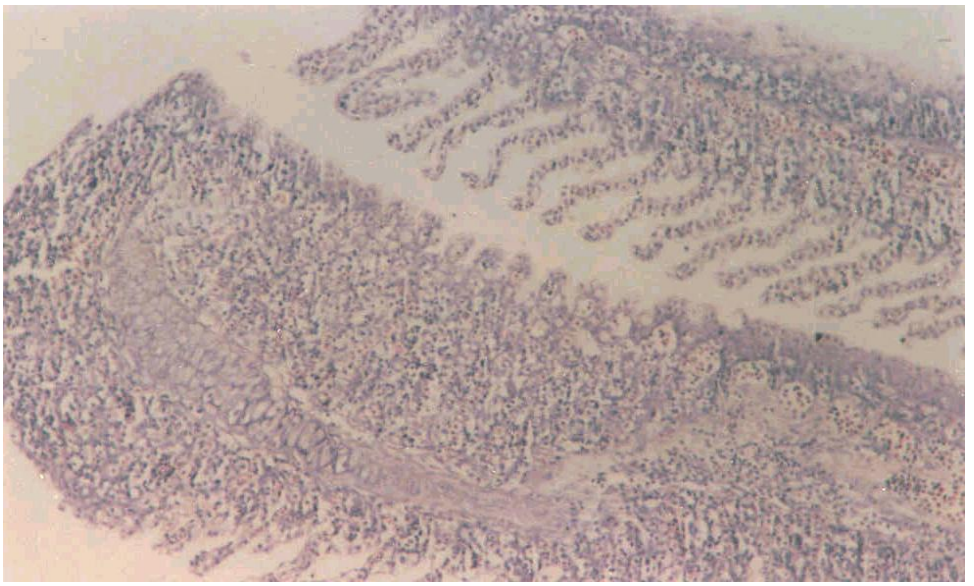


Plate - 6

Plate – 7



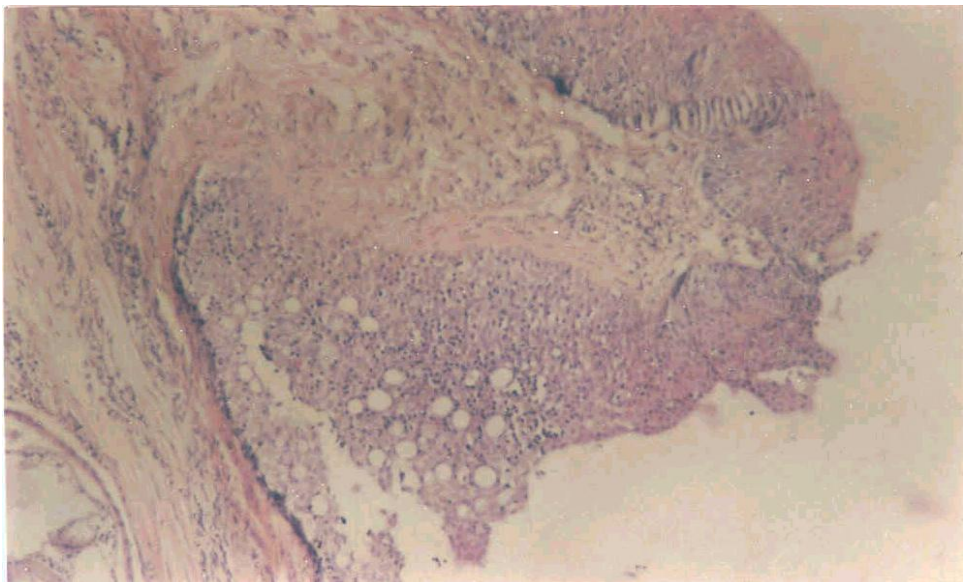
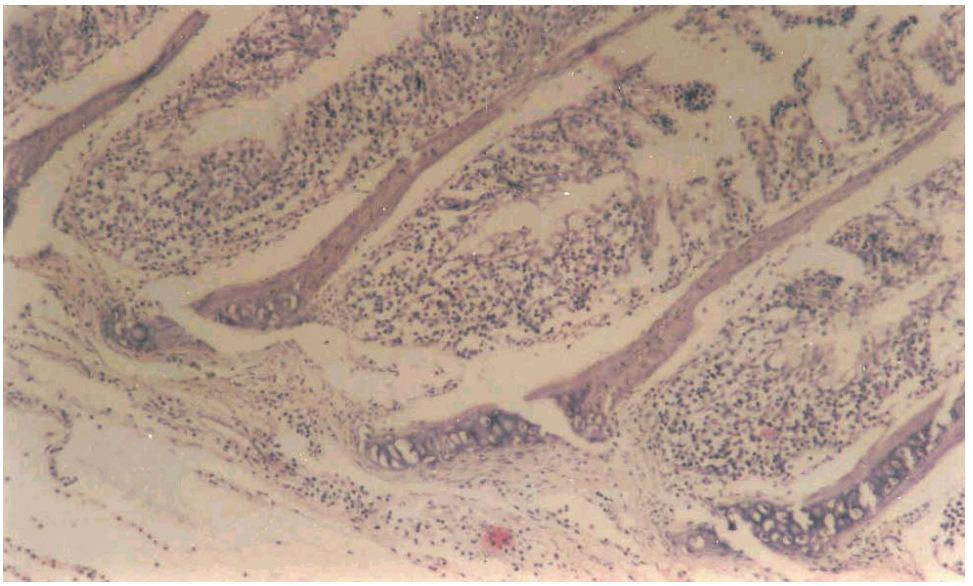


Plate - 8



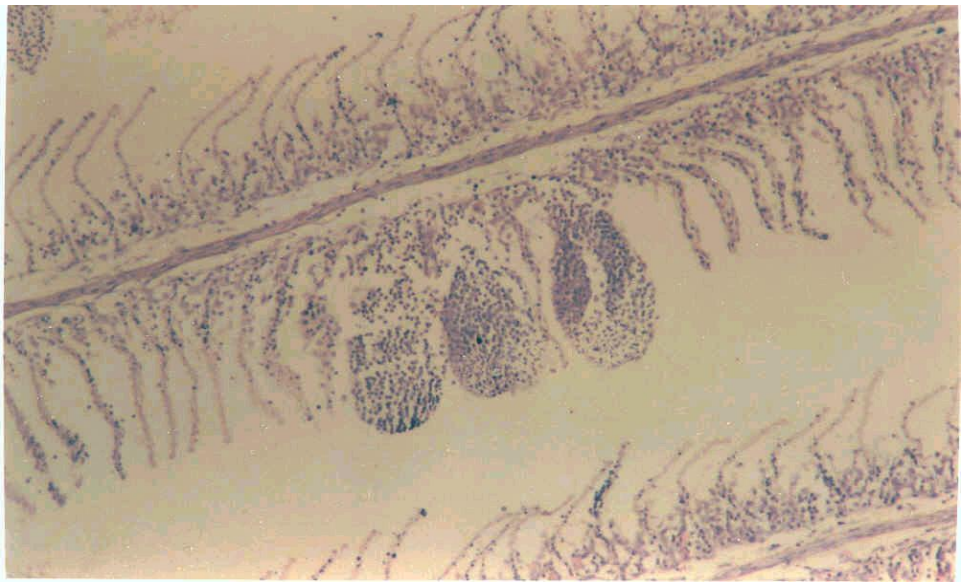
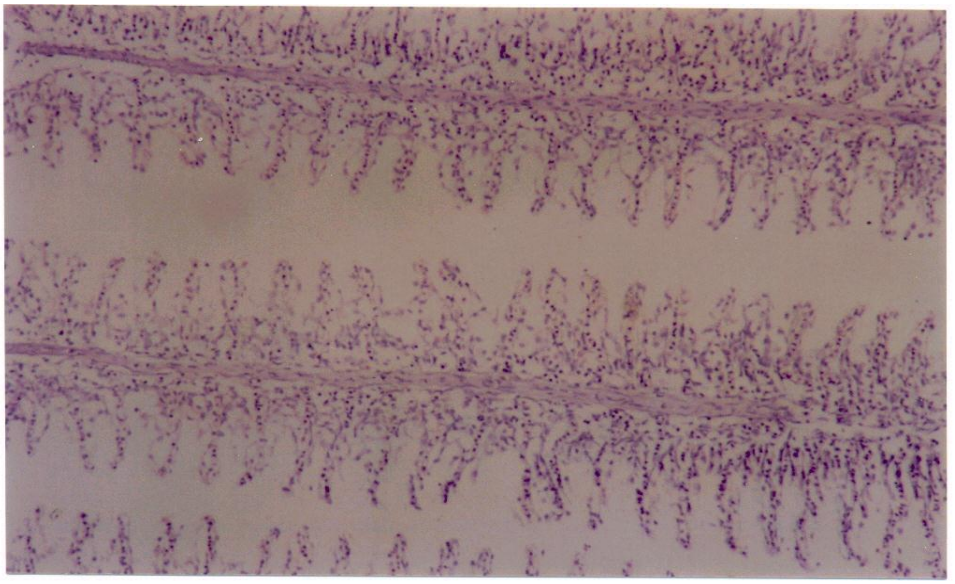
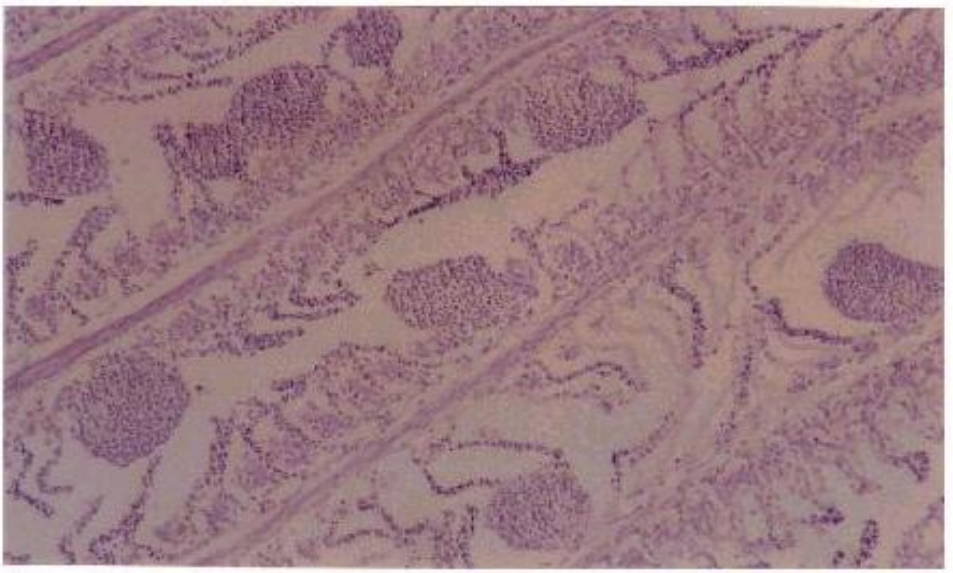


Plate - 9





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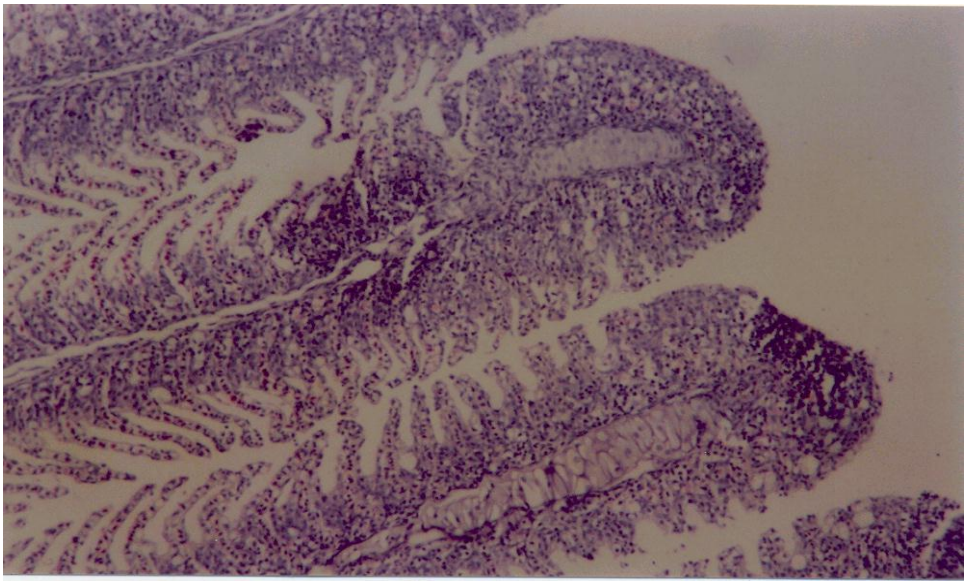

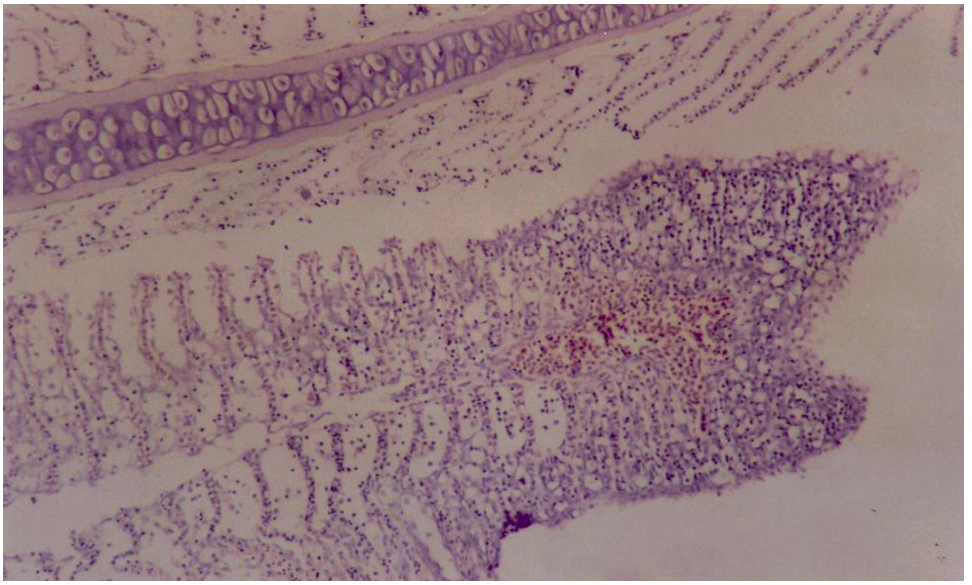

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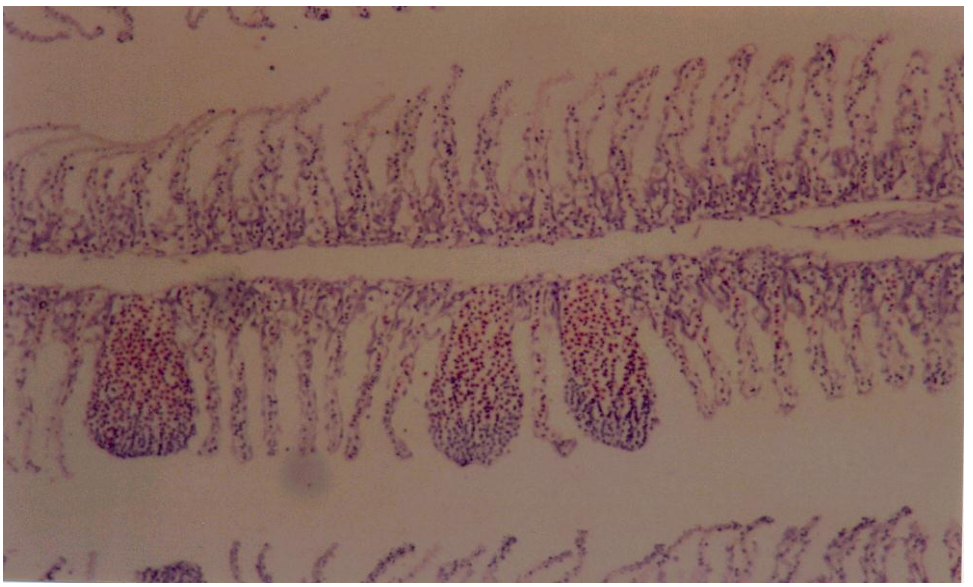

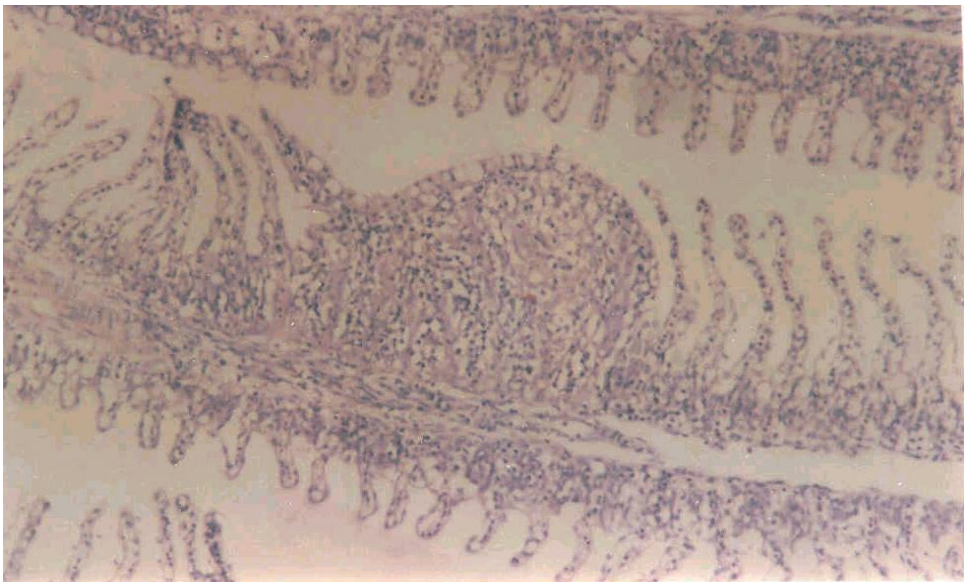

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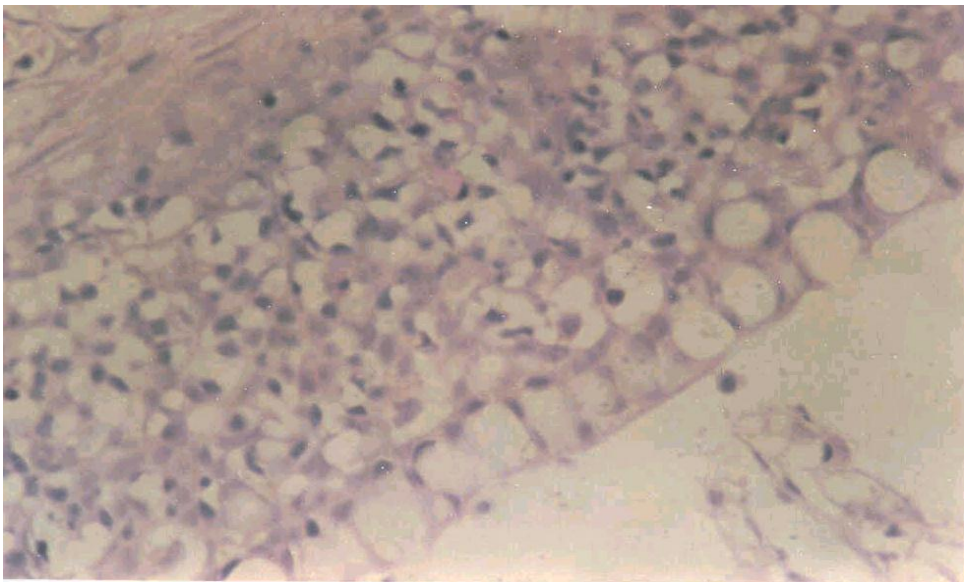

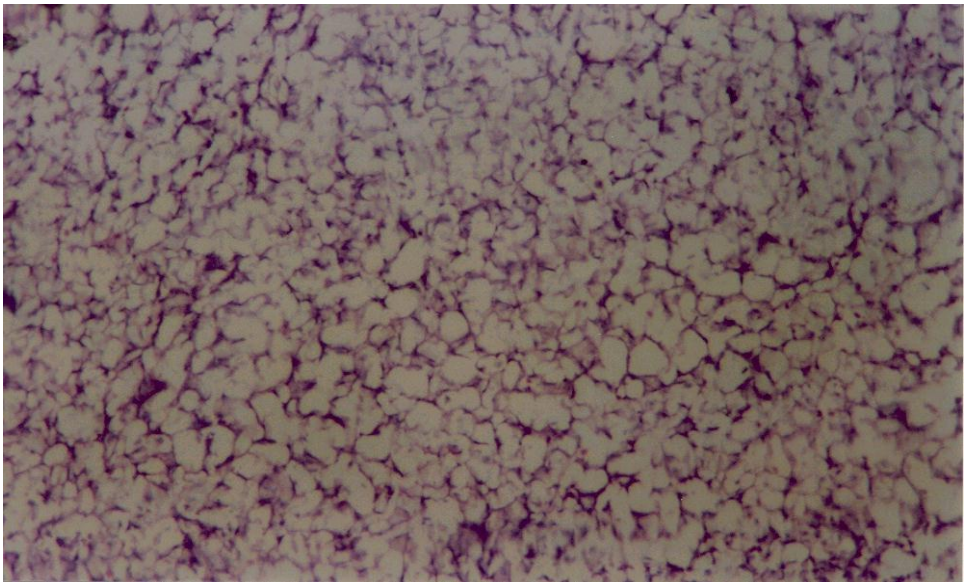

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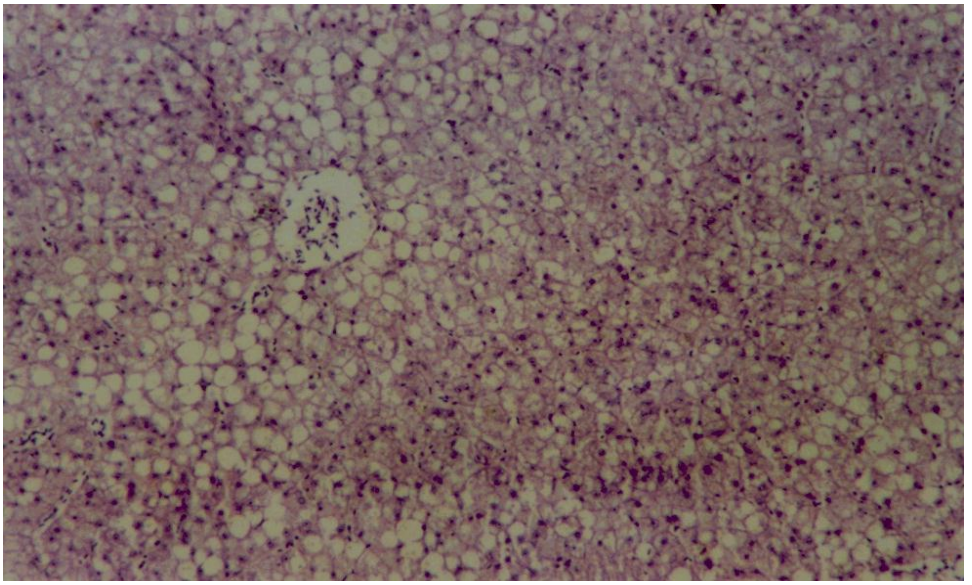

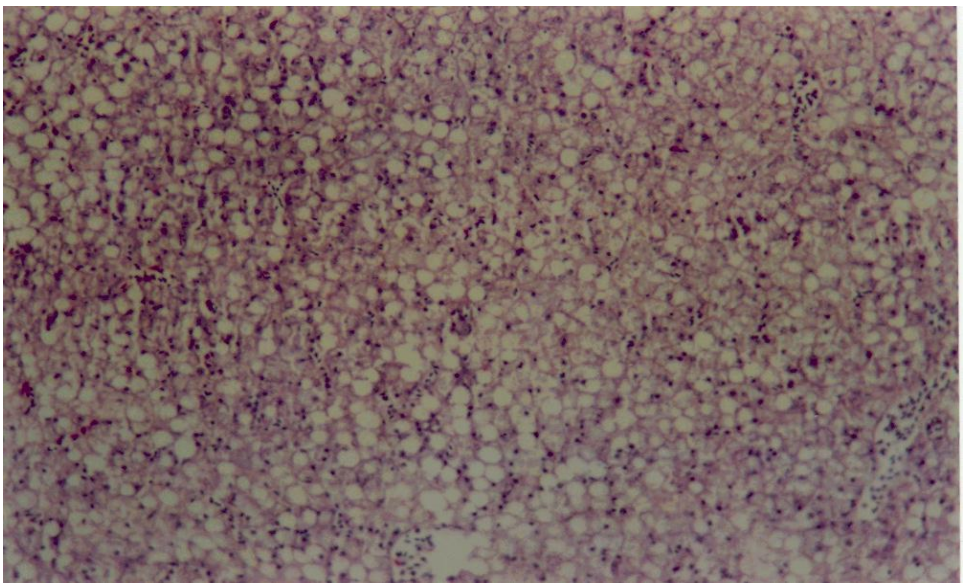

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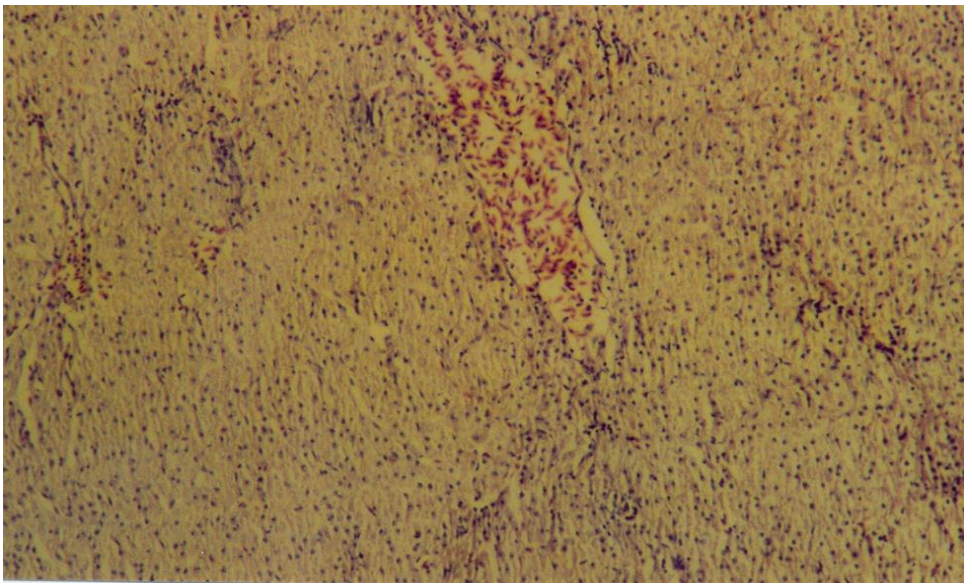

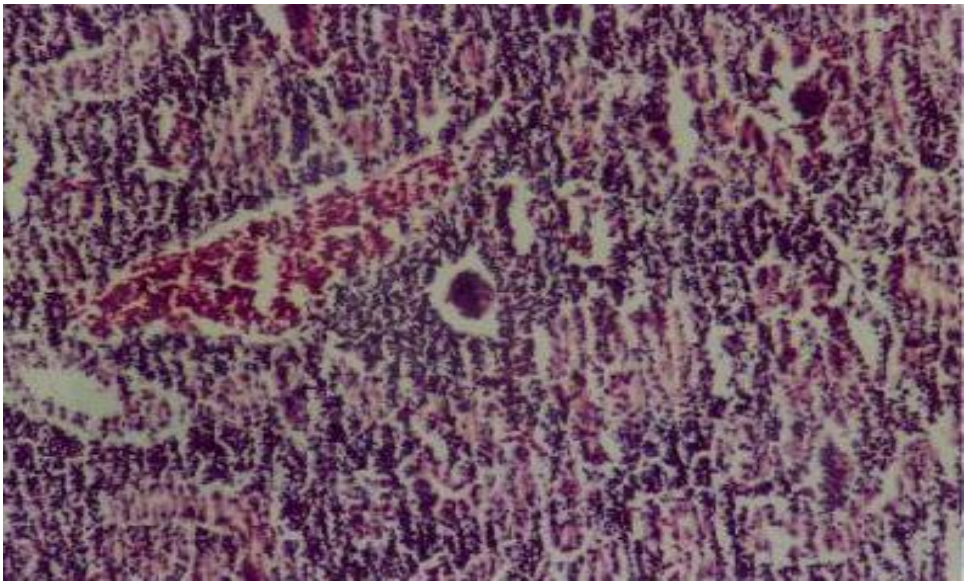

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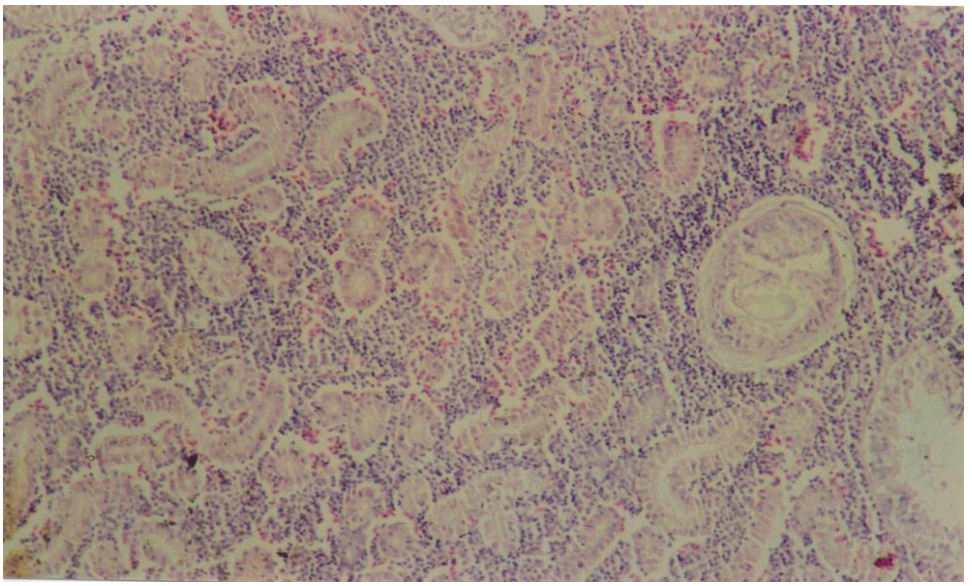

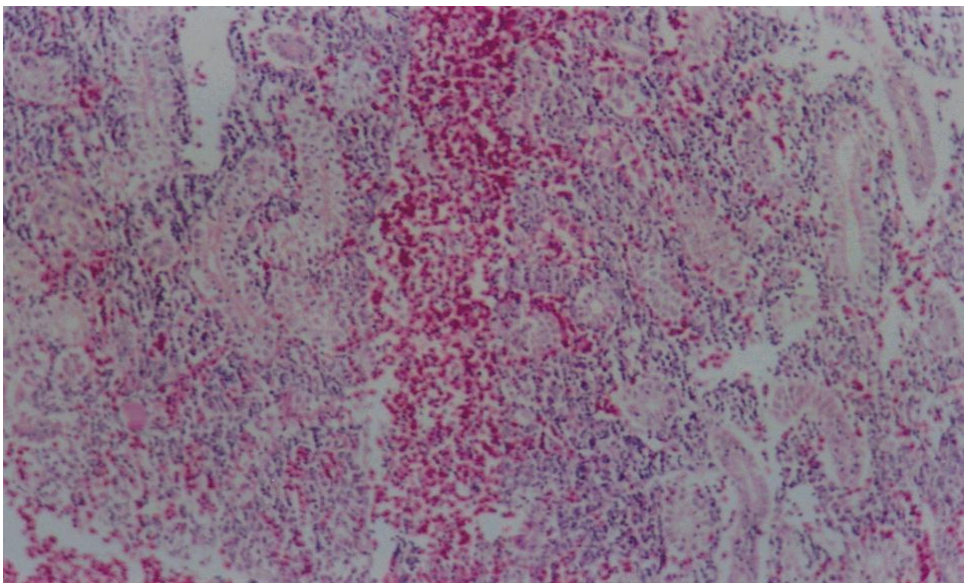

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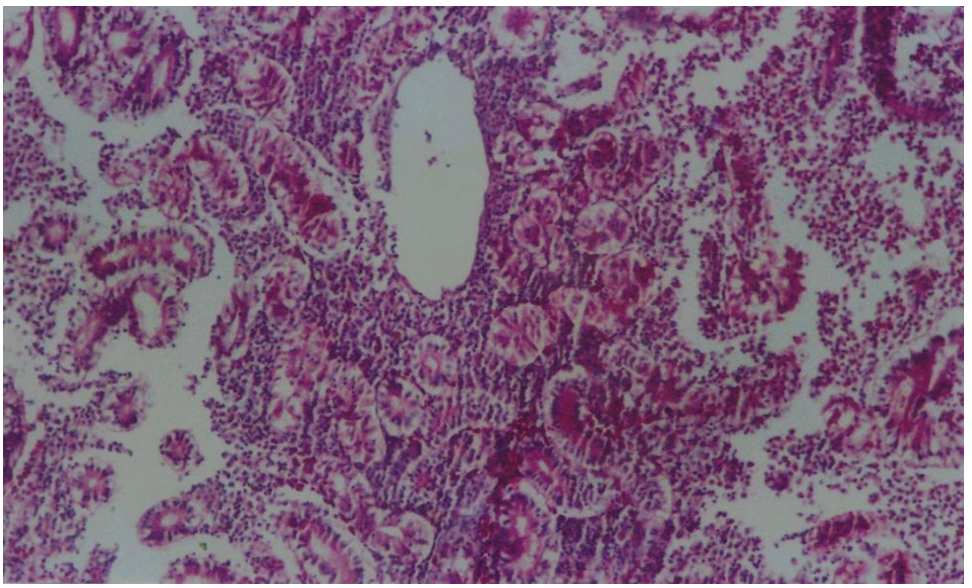

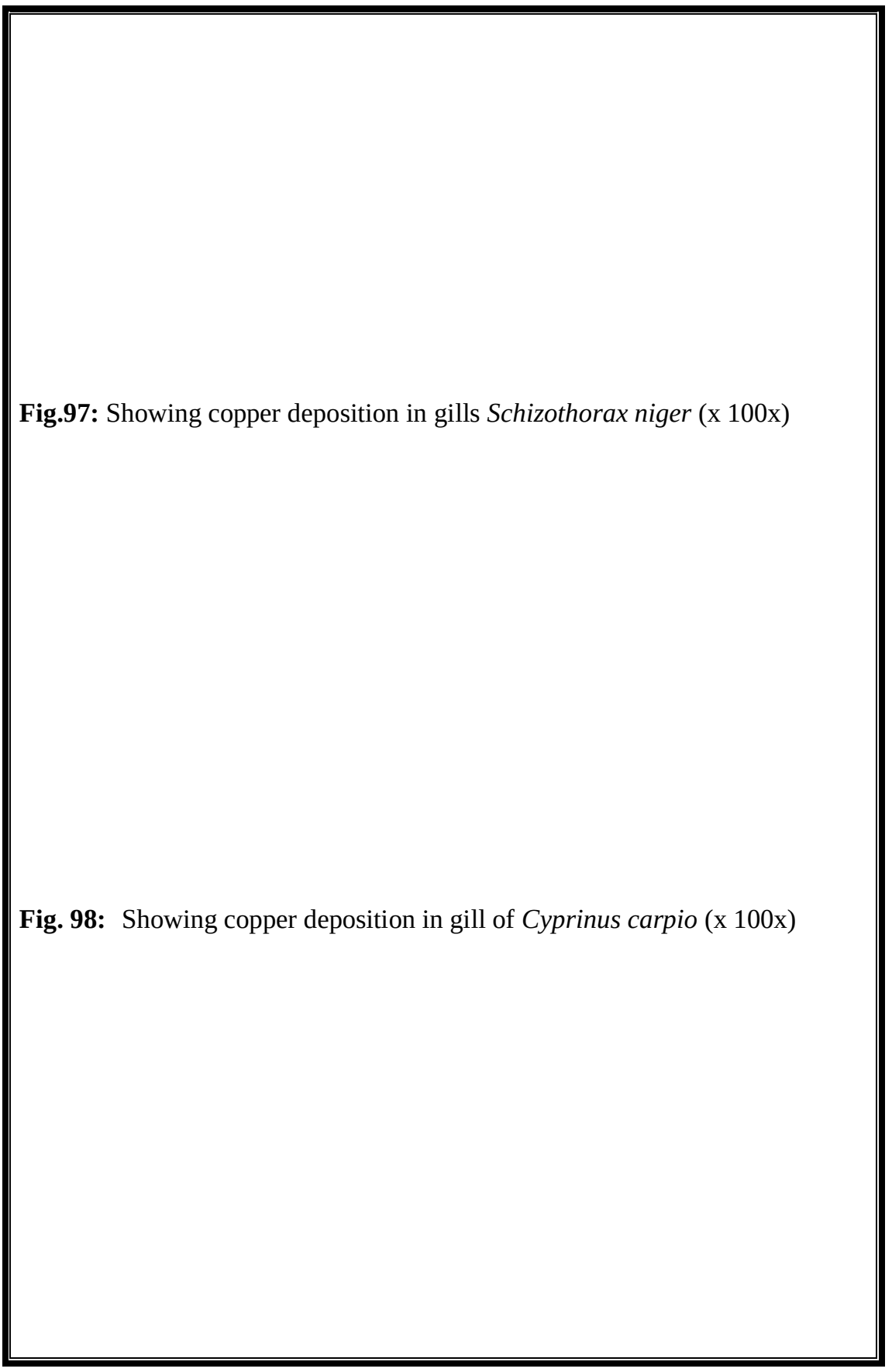

Fig.97: Showing copper deposition in gills *Schizothorax niger* (x 100x)

Fig. 98: Showing copper deposition in gill of *Cyprinus carpio* (x 100x)

Fig.99: Showing copper deposition in gill filaments of *Cyprinus carpio* (x 100x)

Fig.100: Showing copper deposition in gill filaments of *Schizothorax niger* (x 400x)

Fig.101: Showing copper deposition in gill filaments of *Cyprinus carpio* (x 100x)

Fig.102: Showing zinc deposition in liver of *Schizothorax niger* (x 100x)

Fig.103: Showing zinc deposition in liver of *Schizothorax niger* (x 100x)

Fig.104: Showing iron deposition in liver of *Cyprinus carpio* (x 400x)

Fig.105: Showing zinc deposition in liver of *Cyprinus carpio* (x 100x)

Fig.106: Showing zinc deposition in kidney of *Cyprinus carpio* (x 100x)

Fig.107: Showing copper deposition in liver of *Cyprinus carpio* (x 100x)

Fig.108: Showing copper deposition in kidney of *Cyprinus carpio* (x 100x)

Fig.109: Showing severe hyperplasia and fusion of gill lamellae in *Cyprinus carpio* (x 100x)

Fig. 110: Showing hyperplasia and vacuolation of the gill racker in *Cyprinus carpio* (x 100x)

Fig. 111: Showing hyperplasia of basal epithelium in the primary lamellae of *Schizothorax niger* (x 100x).

Fig. 112: Showing club formation of the secondary lamellae of *Cyprinus carpio* (x 100x).

Fig. 113: Showing loss of epithelium in *Schizothorax niger* (x 100x)

Fig. 114: Showing club formation of secondary lamellae in *Schizothorax niger* (x 100x)

Fig. 115: Showing hyperplasia and fusion of secondary lamellae in Cyprinus carpio (x 100x)

Fig. 116: Showing fusion and vacculation of secondary lamellae in *Schizothorax niger* (x 100x)

Fig. 117: Showing telangietatisis of secondary lamellae in *Cyprinus carpio* (x 100x)

Fig. 118: Showing hyperplasia and vacuolation in *Schizothorax niger* (x 100x)

Fig. 119: Showing high magnification of gill lamellae with hyperplasia and vacuolation in *Schizothorax niger* (x 1000x)

Fig. 120: Showing severe congestion and degenerative changes in hepatocytes in *Cyprinus carpio* (x 100x)

Fig. 121: Showing congestion and degenerative changes in *Cyprinus carpio* (x 100x)

Fig. 122: Showing kupffer cell hyperplasia in *Schizothorax niger* (x 100x)

Fig. 123: Showing degenerative changes in kidney of *Schizothorax niger* (x 100x)

Fig. 124: Showing atrophy of glomerulus in *Cyprinus carpio* (x 100x)

Fig. 125: Showing hypertrophy and hyperplasia in kidneys of *Schizothorax niger* (x 100x)

Fig. 126: Showing severe congestion in kidneys of *Schizothorax niger* (x 100x)

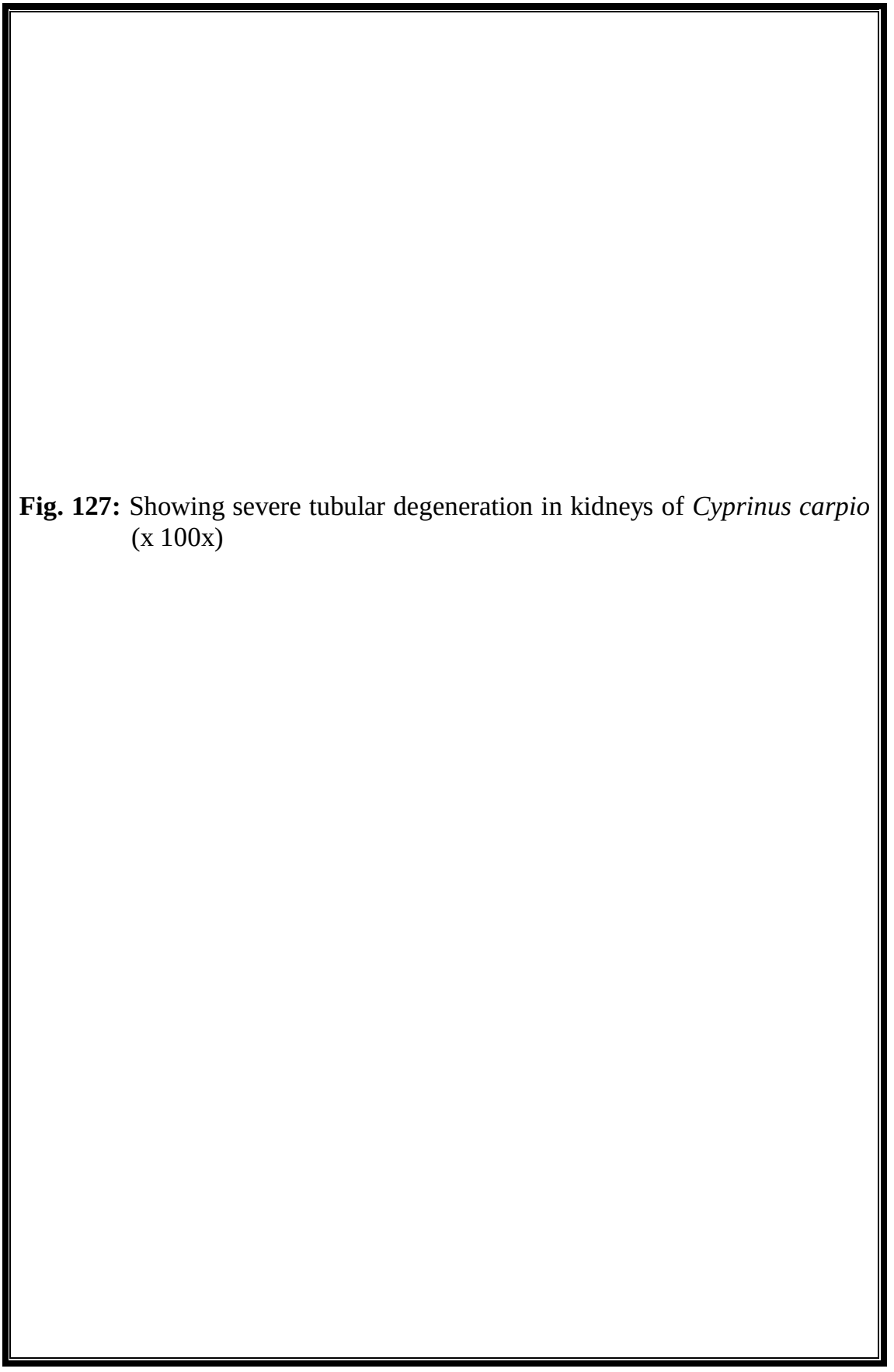

Fig. 127: Showing severe tubular degeneration in kidneys of *Cyprinus carpio* (x 100x)

In the present study, the fishes viz. *Schizothorax niger* and *Cyprinus carpio* spp. inhabiting the water bodies of Kashmir valley particularly Dal lake and River Jhelum are constantly exposed to a wide variety of metals by way of geochemical processes and large scale releases into the aquatic environment by anthropogenic activities. Subsequently, these aquatic animals get victimized by various pollutants especially the untraced metals leading to biochemical, physiological and histological alterations of these economically important hosts.

In the first set of research study, the concentration of metals particularly copper, zinc, iron and manganese in Dal lake and River Jhelum were checked seasonally for a period of two years by Atomic Absorption Spectrophotometer. The concentration of copper ranged from 1.020 to 1.070 ppm in Dal lake and 1.002 to 1.006 ppm in River Jhelum throughout the study period. However, the maximum concentration of copper in both the water bodies was found to be highest in summer of 2006-2007. For iron, the concentration ranged between 0.110 to 0.191 ppm in Dal lake and 0.129 to 0.168 ppm in River Jhelum. Like copper, the highest values for iron were found in summer season of 2006-2007. The zinc concentration ranged between 0.150 to 0.542 ppm in Dal lake and 0.100 to 0.483 ppm in River Jhelum. The highest values were also found in summer season of 2006-2007.

The concentration for manganese ranged between 0.021 to 0.822 ppm in Dal lake and 0.0056 to 0.053 ppm in River Jhelum with highest values in summer of 2006-2007 like other metals.

In the second set of research study, the accumulation of these metals in different tissues viz. gills, liver, kidneys and muscles of *Schizothorax niger* and *Cyprinus carpio* spp. estimated by Atomic Absorption Spectrophotometer. In Dal lake, the concentration of copper in gills of *Schizothorax niger* varied from 13.52 to 21.84 ppm and 16.83 to 25.99 ppm in *Cyprinus carpio* spp. throughout the study period. The highest concentration of copper in gills of both the fishes was found in summer season of 2006-2007. However, in River Jhelum the concentration of copper in the gills of *Schizothorax niger* ranged from 10.13 to 19.54 ppm and 14.82 to 23.24 ppm in *Cyprinus carpio* spp. throughout the study period. The maximum accumulation of copper in gills of *Schizothorax niger* and *Cyprinus carpio* spp. in River Jhelum was found in summer season of 2005-2006 and 2006-2007 respectively.

The accumulation of copper in the liver of *Schizothorax niger* varied from 66.77 to 81.63 ppm in Dal lake and 63.69 to 79.52 in River Jhelum. However, in *Cyprinus carpio* spp. the accumulation of copper varied from 99.41 to 139.22 in Dal lake and 131.99 to 97.62 ppm in River Jhelum. The maximum concentration of copper in liver of both the fishes in both the water bodies was found to be highest in summer season of 2006-2007.

In kidneys, the concentration of copper in *Schizothorax niger* and *Cyprinus carpio* collected from Dal lake varied from 64.61 to 78.90 ppm and 96.52 to 132.83 ppm respectively. However, the concentration of copper in kidneys of *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum showed values ranging from 62.54 to 77.64 and 94.33 to 129.62 ppm

respectively. The highest values were also found in summer season of 2006-2007.

In muscles, the accumulation of copper in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake ranged between 07.81 to 12.72 ppm and 09.99 to 17.58 respectively with highest values observed during summer season of 2006-2007. However, in River Jhelum, the accumulation of copper in muscles of *Schizothorax niger* and *Cyprinus carpio* spp. ranged between 06.33 to 12.72 ppm and 08.24 to 16.27 ppm respectively. The highest value of copper in *Schizothorax niger* and *Cyprinus carpio* spp. was observed in summer seasons of 2005-2006 and 2006-2007 respectively.

In Dal lake, the concentration of zinc in gills of *Schizothorax niger* varied from 52.11 to 72.44 ppm and 56.92 to 87.25 ppm in *Cyprinus carpio* spp. throughout the study period. The highest concentration of copper in gills of both the fish hosts was found in summer season of 2006-2007. However, in River Jhelum, the concentration of zinc in the gills of *Schizothorax niger* ranged from 50.60 to 69.24 ppm and 54.79 to 86.43 ppm in *Cyprinus carpio* spp. throughout the study period. The maximum accumulation of copper in gills of *Schizothorax niger* and *Cyprinus carpio* spp. in River Jhelum was found in summer season of 2005-2006 and 2006-2007 respectively.

The accumulation of zinc in the liver of *Schizothorax niger* varied from 73.81 to 97.84 ppm in Dal lake and 71.99 to 95.43 in River Jhelum. However, in *Cyprinus carpio* spp. the accumulation of zinc varied from 111.35 to 152.61 in Dal lake and 109.98 to 141.81 ppm in River Jhelum. The maximum concentration of zinc in liver of both the fishes in both the water bodies was found to be highest in summer season of 2006-2007.

In kidneys, the concentration of zinc in *Schizothorax niger* and *Cyprinus carpio* collected from Dal lake varied from 88.77 to 101.99 ppm and 119.84 to 159.32 ppm respectively. However, the concentration of zinc in

kidneys of *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum showed values ranging from 80.42 to 99.84 and 116.31 to 148.92 ppm respectively. The highest values were also found in summer season of 2006-2007.

In muscles, the accumulation of zinc in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake ranged between 29.93 to 39.72 ppm and 34.46 to 44.52 respectively with highest values observed during summer season of 2006-2007. However, in River Jhelum, the accumulation of zinc in muscles of *Schizothorax niger* and *Cyprinus carpio* spp. ranged between 27.18 to 38.12 ppm and 32.17 to 43.44 ppm respectively. The highest value of zinc in *Schizothorax niger* and *Cyprinus carpio* spp. was observed in summer seasons of 2005-2006 and 2006-2007 respectively.

In Dal lake, the concentration of iron in gills of *Schizothorax niger* varied from 130.10 to 192.52 ppm and 153.72 to 201.61ppm in *Cyprinus carpio* spp. throughout the study period. The highest concentration of copper in gills of both the fishes was found in summer season of 2006-2007. However, in River Jhelum the concentration of iron in the gills of *Schizothorax niger* ranged from 127.52 to 187.46 ppm and 151.92 to 199.52 ppm in *Cyprinus carpio* spp. throughout the study period. The maximum accumulation of iron in gills of *Schizothorax niger* and *Cyprinus carpio* spp. in River Jhelum was found in summer season of 2006-2007.

The accumulation of iron in the liver of *Schizothorax niger* varied from 204.92 to 296.51 ppm in Dal lake and 208.44 to 294.62 in River Jhelum. However, in *Cyprinus carpio* spp., the accumulation of iron varied from 290.32 to 392.21 in Dal lake and 281.81 to 381.43 ppm in River Jhelum. The maximum concentration of iron in liver of both the fishes in both the water bodies was found to be highest in summer season of 2006-2007.

In kidneys, the concentration of iron in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake varied from 200.99 to 292.61 ppm and 286.75 to 388.69 ppm respectively. However, the concentration of iron in kidneys of *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum showed values ranging from 204.32 to 289.77 and 278.66 to 378.66 ppm respectively. The highest values were also found in summer season of 2006-2007.

In muscles, the accumulation of iron in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake ranged between 40.88 to 49.23 ppm and 51.67 to 62.71 respectively with highest values observed during summer season of 2006-2007. However, in River Jhelum, the accumulation of iron in muscles of *Schizothorax niger* and *Cyprinus carpio* spp. ranged between 31.90 to 48.16 ppm and 49.22 to 61.07 ppm respectively. The highest value of iron in *Schizothorax niger* and *Cyprinus carpio* spp. was observed in summer seasons of 2006-2007.

In Dal lake, the concentration of manganese in gills of *Schizothorax niger* varied from 02.71 to 11.55 ppm and 05.99 to 13.21 ppm in *Cyprinus carpio* spp. throughout the study period. The highest concentration of manganese in gills of both the fishes was found in summer season of 2006-2007. However, in River Jhelum the concentration of manganese in the gills of *Schizothorax niger* ranged from 01.54 to 10.66 ppm and 04.76 to 12.01 ppm in *Cyprinus carpio* spp. throughout the study period. The maximum accumulation of manganese in gills of *Schizothorax niger* and *Cyprinus carpio* spp. in River Jhelum was found in summer seasons of 2006-2007.

The accumulation of manganese in the liver of *Schizothorax niger* varied from 01.13 to 08.30 ppm in Dal lake and 0.87 to 06.73 in River Jhelum. However, in *Cyprinus carpio* spp., the accumulation of manganese varied from 03.92 to 11.72 in Dal lake and 0.97 to 08.95 ppm in River

Jhelum. The maximum concentration of manganese in liver of both the fishes in both the water bodies was found to be highest in summer season of 2006-2007.

In kidneys, the concentration of manganese in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake varied from 0.84 to 06.95 ppm and 01.32 to 09.34 ppm respectively. However, the concentration of manganese in kidneys of *Schizothorax niger* and *Cyprinus carpio* spp. collected from River Jhelum showed values ranging from 0.51 to 03.61 and 0.57 to 06.74 ppm respectively. The highest values were also found in summer season of 2006-2007.

In muscles, the accumulation of manganese in *Schizothorax niger* and *Cyprinus carpio* spp. collected from Dal lake ranged between 0.09 to 03.92 ppm and 0.06 to 06.82 respectively with highest values observed during summer season of 2006-2007. However, in River Jhelum, the accumulation of manganese in muscles of *Schizothorax niger* and *Cyprinus carpio* spp. ranged between 0.05 to 01.00 ppm and 0.24 to 03.43 ppm respectively. The highest value of manganese in *Schizothorax niger* and *Cyprinus carpio* spp. was observed in summer seasons of 2006-2007.

In the third set of research study, the subsequent effects of metals on the biochemical parameters viz. total protein, albumin, globulin, glucose, serum urea, serum creatinine and total cholesterol of fishes collected from Dal lake and River Jhelum were estimated seasonally. The estimation of total protein in *Schizothorax niger* varied seasonally from a low concentration of 1.11 g/dl in winter (2006-2007) in Dal lake to a maximum value of 4.32 g/dl in summer season (2006-2007) in Dal lake. However, for *Cyprinus carpio* spp., the total protein values varied from a minimum of 1.05 g/dl in winter season (2005-2006) in Dal lake to a maximum value of 3.79 g/dl in summer season (2005-2006) in Dal lake.

The estimation of albumin in *Schizothorax niger* varied seasonally from a low concentration of 1.01 g/dl in winter (2005-2006) in River Jhelum to a maximum value of 3.72 g/dl in summer season (2006-2007) in Dal lake. However, for *Cyprinus carpio* spp., albumin values varied from a minimum of 0.92 g/dl in spring season (2005-2006) in Dal lake to a maximum value of 2.23 g/dl in summer season (2005-2006) in River Jhelum.

The estimation of globulin in *Schizothorax niger* varied seasonally from a low concentration of 0.06 g/dl in autumn (2005-2006) in River Jhelum to a maximum value of 2.37 g/dl in summer season (2006-2007) in Dal lake. However, for *Cyprinus carpio* spp., globulin values varied from a minimum of 0.06 g/dl in autumn season (2006-2007) in River Jhelum to a maximum value of 2.13 g/dl in summer season (2005-2006) in Dal lake.

The estimation of glucose in *Schizothorax niger* varied seasonally from a low concentration of 125.5 g/dl in winter (2005-2006) in River Jhelum to a maximum value of 340.1 g/dl in summer season (2005-2006) in Dal lake. However, for *Cyprinus carpio* spp., the glucose values varied from a minimum of 136.1 g/dl in winter season (2005-2006) in River Jhelum to a maximum value of 352.5 g/dl in summer season (2005-2006) in Dal lake.

The estimation of serum urea in *Schizothorax niger* varied seasonally from a low concentration of 16.11 g/dl in winter (2005-2006) in Dal lake to a maximum value of 22.8 g/dl in summer season (2006-2007) in Dal lake. However, for *Cyprinus carpio* spp., serum urea values varied from a minimum of 15.2 g/dl in winter season (2006-2007) in River Jhelum to a maximum value of 24.0 g/dl in summer season (2006-2007) in River Jhelum.

The estimation of serum creatinine in *Schizothorax niger* varied seasonally from a low concentration of 0.09 g/dl in winter (2005-2006) in River Jhelum to a maximum value of 1.16 g/dl in summer season (2006-2007) in Dal lake. However, for *Cyprinus carpio* spp., serum creatinine values

varied from a minimum of 0.14 g/dl in winter season (2005-2006) in River Jhelum to a maximum value of 1.19 g/dl in summer season (2006-2007) in Dal lake.

The estimation of serum cholesterol in *Schizothorax niger* varied seasonally from a low concentration of 36.42 g/dl in winter (2005-2006) in River Jhelum to a maximum value of 100.01 g/dl in summer season (2006-2007) in River Jhelum. However, for *Cyprinus carpio* spp., serum cholesterol values varied from a minimum of 40.11 g/dl in winter season (2005-2006) in River Jhelum to a maximum value of 98.22 g/dl in summer season (2006-2007) in Dal lake.

In the fourth set of research study, the accumulation of metals in tissues of fishes were analyzed histochemically inconsistent with biochemical study. The same tissues were then subjected to histological/ histopathological studies so as to check their deleterious effects histomorphologically.

In this study, the enormous amounts of metals were observed in summer seasons in all the tissues of fishes particularly the liver followed by kidneys and gills. Muscles of both the fishes were found to possess negligible amount of metals. However, least concentrations of metals were observed in winter/ spring seasons.

After localization of metals in tissues of fishes, the same tissues were checked for histomorphological alterations. The liver of both the fishes collected from Dal lake and River Jhelum showed congestion and degenerative changes that varied from mild in winter season to severe vascular degeneration in summer seasons. The general changes observed in gills included congestion, oedema, hyperplasia and hypertrophy. However, the severity of histomorphological alterations were observed in gills of *Cyprinus carpio* spp. collected from Dal lake during summer season. The kidneys showed atrophy of glomerulus, hyperplasia and hypercellularity. The

mild changes in kidneys were observed in *Schizothorax niger* during winter seasons and severe degenerative changes in *Cyprinus carpio* spp. during summer seasons.

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