CHAPTER 1

INTRODUCTION

Bangladesh is gratified with various fisheries resources for its geographical location and salutary climate conditions. It has vast fisheries potentialities within her boundaries and territory. The entire fisheries can broadly be classified into three categories which are inland capture fisheries, inland aquaculture and marine fisheries. Among them, inland aquaculture is contributing more than 56.24% of the total fish production (DoF, 2018). The water resources are suitable for the culture of different aquatic species. Bangladesh is considered as the suitable place for freshwater species farming due to its favorable biophysical resources.

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are degradable and most of them have toxic effects on many organisms (MacFarlane and Burchett, 2000). Different pollutants of environment, metals are of particular concern, due to their potent toxic effects and their ability to bio-accumulate in aquatic ecosystems (Censi et al., 2006). The contamination of fresh waters with different toxic pollutants has become a matter of concern over the last few decades (Vutukuru, 2005; Dirilgen, 2001). Heavy metals presence in aquatic environments and their accumulations in fish and in the other organisms has been observed over years. Heavy metals are metallic chemical elements that have a relatively high density and toxic or poisonous even at low concentration. These metals are e.g. lead (Pb), Mercury (Hg), Arsenic (As), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and Selenium (Se) (Pandey *et al*, 2014). In aquatic ecosystem, heavy metals are considered among the most serious contaminants due to their high potent to enter and accumulate in food chain (Tam and Wong, 2000; Erdoğrul and Erbilir, 2007).

In the aquatic ecosystem, fish are staying at the top of the food chain and they usually can accumulate heavy metals from food, water and sediment. Some anthropogenic sources including agriculture, shipping, urban and industrial practices, have resulted in disquieting concentrations of heavy metals in aquatic environments. Heavy metal contamination may have demolishing effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et. al., 2007).

Among animal species, fishes are the inhabitants that cannot escape from the pernicious effects of these pollutants (Olaifa et al., 2004). Evaluation of the aquatic ecosystems health fish are widely used because pollutants build up in the food chain and are responsible for ambivalent effects and death in the aquatic systems (Farkas et al., 2002). Heavy metals may alter the physiological activities and biochemical parameters of various fishes both in tissues and in blood (Canli 1995). Accumulation of heavy metals in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Joseph et al., 2012).

Heavy metals enter from contaminated water into fish body via different routes and accumulate in different organ of fish (Olaifa *et al*., 2004). The contaminants which entering the aquatic ecosystem may not directly damage the organisms; however, they can be deposited into aquatic organisms through the effects of bioconcentration, bioaccumulation and the food chain process and eventually threaten the health of humans by food consumption (Joseph et al., 2012).

Different types of enzyme are present in the fish. In this study we investigate the enzymatic variation **(**ATPase and ALP enzyme) in different organ such as liver, kidney, gill and muscle in rohu and catla fish. ATPases are a group of enzymes that catalyze the hydrolysis of a phosphate bond in adenosine triphosphate (ATP) to form adenosine diphosphate (ADP). ATPases are membrane-bound enzymes which involved in regulation of osmotic pressure, cellular volume and membrane permeability in fish (Heath, 1987). In the membrane of the gill epithelium of fish Na+/K+-ATPase are found which plays a direct role in active transport of Na+ and K+ between cell's interior and extracellular fluid (Saravanan et al., 2011). As essential enzyme ATPases play roles in energy conservation, active transport and pH homeostasis. ATPases are important to maintain functional integrity of plasma membrane and in several intracellular functions and are considered to be a sensitive indicator of toxicity. On the other hand, branchial ATPases are closely involved in osmoregulation, acid–base regulation, and respiration of fish. The inhibition of ATPases in gills by, e.g., paper mill effluent could cause disruption of these processes (Parvez et al., 2006). During stress condition ATPase activities are reduced significantly in both muscle and liver tissues and indicating the stress mitigation effect (Ahmmed et al., 2017).

Metabolic enzyme, alkaline phosphates is a significant regulative enzyme in bio-metabolic processes which plays a vital role in digestion, absorption, and transition of nutrients (LIU Wei et al, 2010). As the stability of enzyme activity can control the body's biological metabolism and adaptive capacity (Wang et al, 2001), During the early developmental stages of fish, alkaline phosphates is essential to quantify its activity (LIU Wei et al, 2010).

In FY 2017-18, Total fish production of Bangladesh is 42.77 lakh MT in which aquaculture production contributes 56.24 percent of the total fish production. Bangladesh becomes a self-sufficient country in fish production, with a per capita fish consumption of 62.58 g/day. Fisheries sector contributes 3.57 percent to the national GDP and more than one-fourth (25.30 %) to the agricultural GDP in 2017-18. Near about 11 percent of total population of Bangladesh is engaged in this sector on full time and part time basis for their livelihoods. This sector also has high potential for the perspective of economic development of the country (DOF, 2018).

Nowadays inland aquaculture production shows a moderate increased trend. According to FAO report Bangladesh ranked 3rd in inland open water capture production and 5th in world aquaculture production. In comparison to 2008-09 FY production (10.63 lakh MT), the aquaculture production became more than double in 2017-18 FY (24.05 lakh MT). Indigenous and Indian major carp species such as Rui, Catla and Mrigal has become popular in Bangladesh as suitable aquaculture species over the last decade. In this study, we provided information about accumulation level of As, Cr and Pb in commercially important fish species as Rohu and Catla which cultured in freshwater. The Commercial importance of this fishes is given below:

Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. Its high growth potential, coupled with high consumer preference. *Labeo rohita* contribute a significant portion of the inland fisheries production. About 10.50% of the country's total fish production comes from rohu. Total Production of *Labeo rohita* is about 380221 MT (DOF, 2018).

Catla is a cultured and highly growing species, very delicious food and supply a huge amount of protein for Bangladeshis people. So, its demand is extensive. Its flesh contains 19.2% crude protein, 2-5% fat and 70% water, per gram of its liver-oil contain 583 IV vitamin-A (Rahman, 2005). It contributes 6.40% of the annual production and produces 231878 MT in this year (DoF, 2018).

Various organs of fish uptake heavy metals because of the affinity between them and concentrated at different levels in different organs of the body (Scharenberg *et al*., 1994). Metal accumulation between the different organs of fish depends on the mode of exposure (Alam *et al*., 2002). Metal absorption in fish is carried out via gills surface (water exposure) and digestive tract (diet exposure) (Ptashynski *et al*, 2002). Fish is an important food resource for human consumption, thus assessment of the heavy metal content in fish species is particularly important (WHO/FAO, 2011). About 90% of human health risks related to fish consumption are linked with metal-contaminated fish (Demirak *et al*,2006). Although a particular metal exhibit specific signs of its toxicity but some general signs including gastrointestinal disorders, diarrhea, stomatitis, tremor, hemoglobinuria causing a rust-red color to stool, ataxia, paralysis, vomiting are found (McCluggage 1991). Consequently, monitoring programs and research on heavy metals in inland water samples have become widely important due to concerns over toxic effects of heavy metals in aquatic organisms and to humans through the food chain (Otchere, 2003).

Chattogram is situated in the southern part of Bangladesh which is the largest port city and the coastal city of the country. Different types of land drainages and other coastal pollutants enter the inland water bodies during the rainy season which contains many toxic heavy metals. These heavy metals accumulate into the different cultured species. Due to unawareness and lack of proper management systems: severe toxicity in ecological balance and heavy metal accumulation brings an indispensable loss which encounter in this emerging sector of country's economy. The people who are consumed fish which accumulates the high levels of heavy metals and exposed to this contaminated environment for a long term can cause serious illness and life-threat. Therefore, realizing this significance of the issue assessment of the harmful effects and comparative study of heavy metals accumulation at different organs of fishes are done along with enzymatic variation in investigated organ.

Objectives of the study:

- To assess the heavy metals accumulation in liver, kidney, muscle and gill tissues of two commercially important cultured fishes (Rohu and Catla)
- To draw comparison between ALP and ATPase activities of those organs of fishes.

CHAPTER 2

REVIEW OF LITERATURE

Prior to conduct an experiment it is important to review the previous research works related to the study. The purpose of this chapter is to review the past studies carried by different researchers to the related field. At present the culture practice of Indian major carp has gained much popularity in Bangladesh due to its phenomenal growth, acceptable test and high market demand. The tropics related to accumulation of heavy metals in fresh water fish and enzymatic variations in different organ of fish have been carried out in a number of journal. However, some literatures relevant to the present investigation are available, which are presented below:

2.1 Heavy metals as toxic substance:

Pandey et al. (2014) carried an experiment on heavy metals causing toxicity in animals and fishes. He found that the metal which has a relatively high density and toxic at low quantity is referred as ‘heavy metal’, e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. Some ‘trace elements’ are also known as heavy metals, e.g., copper (Cu), selenium (Se) and zinc (Zn). They are important to maintain the body metabolism, but they are toxic at higher concentrations. The metallic elements which have a relatively high density, and they are poisonous at low quantity. Due to formation of toxic soluble compounds, certain heavy metals become toxic. However, any amount of Pb can result to detrimental effect. Most of the heavy metals are venom, while some metals are less toxic, e.g. bismuth (Bi). Metalloids like As, Pb and Cr may also be toxic.

Chowdhury et al. (1987) conducted an experiment on biological and health implications of toxic heavy metal and essential trace element interactions. Human civilization and a concomitant increase in industrial activity has gradually redistributed many toxic metals from the earth's crust to the environment and increased the possibility of human exposure. Among the various toxic elements, heavy metals cadmium, lead, and mercury are especially prevalent in nature due to their high industrial use. They are cumulative poison, and are toxic even at low dose.

Karthikeyan et al. (2007) studied on influence of pH and water hardness upon nickel accumulation in edible fish *Cirrhinus mrigala*. Heavy metals include a great variety of chemical elements that typically occur in low or trace amounts in the environment but have the potential to provoke toxic effects in organisms.

Nandi et al. (2012) carried out an experiment lead and cadmium accumulation in fresh water fishes *Labeo rohita* and *Gibelion catla*. Releases of heavy metals into atmosphere contaminate the aquatic bodies. When these metals are absorbed by the living material and ultimately crosses the limit then these metal acts as a toxic substance. Such contamination deteriorates the water quality and leads to high rate of fish mortality.

2.2 Accumulation of heavy metals by fish:

Rauf et al. (2009) conduct an experiment on heavy metal levels in three major carps (*Gibelion catla*, *Labeo rohita* and *Cirrhinus mrigala*) from the river Ravi. At the end of study *Gibelion catla* showed higher levels of metal concentrations than *Labeo rohita* and *Cirrhinus cirrhosus*.

Kousar et al. (2014) studied on heavy metals toxicity and bioaccumulation patterns in the body organs of four fresh water fish species. Various environmental pollutants including metals can cause toxicological effects on aquatic animals especially fish species. They observed that *Gibelion catla* significantly accumulate Ni>As and highest As accumulation was observed in *Ctenopharyngodon idella*, followed by *Cirrhinus cirrhosus* while the same was statistically non-significant between *Labeo rohita* and *Gibelion catla*.

Rajan et al. (1995) conduct an experiment on accumulation of heavy metals in sewage-grown fishes. In the experiment showed results that the metal contents increased in all the fishes including silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio* var. *communis*), catla (*Gibelion catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) when the BOD loading of sewage was increased. There was no relationship between the accumulation pattern of different metals in different fishes and their feeding habits.

Bhuyan et al. (2016) studied on heavy metals status in some commercially important fishes of Meghan River adjacent to Narsingdi District, Bangladesh. A total 32 fish species were analyzed for heavy metals (Zn, Al, Cd, Pb, Cu, Ni, Fe, Mn, Cr, Co) detection. The highest concentration of Cd in *Ompok pabda* (0.12mg/kg); Pb in *Colisa lalia* (5.87); Cu in *Barbodes sarana* (32.44mg/kg); Ni in *Amblypharyngnodon mola* (0.76mg/kg); Cr in *Anabus testudineus* (1.75mg/kg) and Co in *Mystus bleekeri* (0.43mg/kg) during winter and the maximum amount was recorded for Cd in *Ompok pabda* (0.21mg/kg); Pb in *Colisa lalia* (6.75mg/kg); Ni in *Amblypharyngnodon mola* (0.986mg/kg); Cr in Stinging catfish (3.01mg/kg) and Co in *Labeo rohita* (0.70mg/kg) was recorded during winter season.

2.3 Absorption of metals across different organ:

Pandey et al. (2014) studied on heavy metals causing toxicity in animals and fishes. The heavy metals can have toxic effects on different organs. They can enter into water via drainage, atmosphere, soil erosion and all human activities by different ways. With increasing heavy metals in the environment, these elements enter the biogeochemical cycle. The heavy metals can enter from contaminated water into fish body by different routes and accumulate in organisms. These metals can be concentrated at different contents in organs of fish body. These elements could be lethal to aquatic biota and persist in sediments. In the body, the heavy metals enter through respiration, skin and intestinal absorption. The heavy metals after absorption into body can be widely distributed in different organs, including glands.

Kousar et al. (2014) carried a study on heavy metals toxicity and bioaccumulation patterns in the body organs of four fresh water fish species. Metals burden in different organs of *Labeo rohita* was found in the order of liver > gills > kidney > gut > bones > skin > muscles > scales > fins; *Cirrhinus cirrhosus* contain heavy metals in the order of liver > kidney > gills > gut > skin > scales > bones > muscles > fins; *Gibelion catla* accumulates in gills > liver > kidney > gut > bones > skin > muscles > scales > fins and *Ctenopharyngodon idella* contains in the order of liver > kidney > gills > gut > skin > bones > muscles > fins > scales. Heavy metal concentrations were found to decrease in sequence of the *Cyprinus carpio* samples, in the muscle and stomach-intestine as Fe > Cu > Pb> Ni > Cr > Cd; in the gill, heart and liver as Fe > Cu > Ni > Pb > Cr > Cd and in the air sac as Fe > Cu > Ni > Pb > Cd >Cr.

Öztürk et al. (2009) conduct an experiment on determination of heavy metals in fish, water and sediments of Avsar Dam Lake in Turkey. In the end of the experiment the mean concentrations of Cd in the muscle (0.17+0.07), liver (0.79+0.33) and gills (0.15+0.14) of *Cyprinus carpio* samples are found. The mean concentrations of Pb in the muscle (2.14+2.09), liver (3.42+3.23) and gill (3.11+2.50) found in rohu fish. Heavy metal concentrations in the fish samples decreased in the sequence for the muscle as Fe > Cu > Pb > Ni > Cr > Cd, for the gill as Fe > Cu > Ni > Pb > Cr > Cd, for the stomach intestine as Fe > Cu > Pb > Ni > Cr >Cd, for the heart as Fe > Cu > Ni > Pb > Cr >Cd and for the liver as Fe > Cu > Ni > Pb > Cr > Cd.

Nandi et al. (2012) studied on lead and cadmium accumulation in fresh water fishes *Labeo rohita* and *Gibelion catla*. Cadmium and lead accumulation show insignificant variation among the body weight. Cd and Pb accumulation do not very significantly in the different tissues of the fish under study this study. Although according to some authors liver and kidney are the main site of storage for these metals. Low level of Cd induces MT formations but with chronic exposure, Cd binding capacity of MT decreases. Thus low accumulation of the metal in the tissues in possible due to the binding of the metal with thiamin molecules with its subsequent depuration forms the organs. Cd uptake in non-linear and the accumulation factor decreases with is increasing concentration in water Pb.

Vinodhini et al. (2008) studied on bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). The heavy metals such as chromium (Cr), nickel (Ni), cadmium (Cd) and lead (Pb) were analyzed in different organs like gills, liver, kidney and flesh of the control fish surviving in natural water system. The gill is an important site for the entry of heavy metals that provokes lesions and gill damage. They found that lead and cadmium accumulation was relatively higher than other metals in gills. The liver accumulates relatively higher amounts of heavy metals. The higher accumulation in liver may alter the levels of various biochemical parameters in liver. Kidney is the gateway for heavy metal detoxification in body. In kidney tissue, considerable amounts of heavy metals were accumulated. These results also indicate that lead and cadmium strongly accumulated rather than chromium and nickel in kidney flesh is one of the ultimate parts for heavy metal accumulation. The presence of higher amounts of heavy metals in any parts of the body will definitely induce changes in biochemical metabolisms and other induced stresses.

Vinodhini et al. (2008) conduct an experiment on bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). The order of heavy metal accumulation in the gills and liver was Cd > Pb > Ni > Cr and Pb > Cd > Ni > Cr. Similarly, in case of kidney and flesh tissues, the order was Pb > Cd > Cr > Ni and Pb > Cr > Cd > Ni. In all heavy metals, the bioaccumulation of lead and cadmium proportion was significantly increased in the tissues of common carp.

2.4 Effects of different heavy metal pollution on fish:

Davis et al. (2010) studied on elemental toxicity in animals. In this experiment found that nutrient pollutions have been cause outbreaks of fish diseases. The water contaminated with Hg can cause abnormal behavior, slower growth and development, decreased reproduction, and death in fishes. The persistent organic pollutants can cause illness, deformities and deaths in fishes. The high toxic levels of Hg (more than 0.05 ppm in a man weighing about 180 pounds) prevent the body's cells from transporting glucose, thus reducing energy available to body. This can produce convulsion, anorexia, tremor, swollen gum and behavioral disturbances in animals.

Pandey et al. (2014) conduct an experiment on fish cancer by environmental pollutants. The heavy metals are very important pollutants which cause severe toxicity to fishes. The studies performed in various fishes showed that heavy metals may alter the physiological and biochemical functions both in tissues and in blood Carpio. The As compounds, Cd compounds, Ni compounds, crystalline forms of silica, beryllium and its compounds have been said to be chemical carcinogens, resulting into the development of cancer in fishes.

Praveena et al. (2013) studied on impact of tannery effluent, chromium on hematological parameters in a fresh water fish, *Labeo rohita*. Effects of water pollution on animals, available the Cr is present in the tannery effluent, and is known to cause various ill effects. Such health hazards are dependent on the oxidation state of Cr. Its hexavalent form is toxic than the trivalent form. The hematological changes produced on the exposure to sub lethal concentration of Cr have been observed in fresh water fish (*Labeo rohita*). The decrease in hematological parameters suggested that the exposed fishes became anemic due to exposure of Cr. Hence, this heavy metal is toxic which was discharged via the effluents into aquatic environments, and caused severe anemia and alterations in hematological parameters in the rohu fish.

Pandey et al. (2014) conduct an experiment on fish cancer by environmental pollutants. The decreased levels of glycogen, protein and cholesterol have been observed after the administration of potassium dichromate in *L. rohita* fishes.

2.5 Role of heavy metals as a bio-indicator:

Gohil et al. (2010) studied on diversity of fish fauna from downstream zone of river mahisagar, gujarat state, India. Fish diversity of any regime has great significance in assessment of that zone reference to environment and pollution, as well as it contributes to the necessary information for fisheries. Many fishes may be the bio-indicators of environmental pollutants also.

Authman et al. (2015) carried an experiment on use of fish as bio-indicator of the effects of heavy metals pollution. The experiment showed that fish assimilate Cr by ingestion or by the gill uptake tract and accumulation in fish tissues, mainly liver, occurs at higher concentrations than those found in the environment. The overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic, physiologic activities and could impair the growth and behavior of fish. Tilapia exposed to sub lethal Cr showed histological alterations in the liver (congestion of blood vessels; fat accumulation; increase in melano-macrophage centers and necrosis), gills (hyperplasia of primary lamellar epithelium), ovaries (deposits in interstitial tissue) and testes (hypertrophy and vacuolation of spermatocytes). Acute poisoning by chromium compounds causes excess mucous secretion, damage in the gill respiratory epithelium and the fish may die with symptoms of suffocation. Kidney is a target organ for chromium accumulation.

Olojo et al. (2005) studied on histopathology of the gill and liver tissues of the African catfish, *Clarias gariepinus* exposed to lead. At the end of the experiment found that hepatocyte vacuolization, hepatic cirrhosis, necrosis, shrinkage, parenchyma degeneration, nuclear pyknosis and increase of sinusoidal spaces were the distinct changes observed in the liver of lead-exposed fish.

Parashar et al. (1999) studied on histopathological analysis of sub lethal toxicity induced by lead nitrate to the accessory respiratory organs of the air breathing teleost, *Heteropneustes fossilis* (Bloch). Acute lead toxicity is initially characterized by damaging gill epithelium and ultimately suffocation. Two types of structural alterations of gill, compensatory responses and direct deleterious effects were observed in chronic lead exposed fish.

2.6 Environmental aspects of heavy metal:

WHO (World Health Organization) (2000) carried out an experiment on hazardous chemicals in human and environmental health. Heavy metals are of particular concern in the environment since they exhibit both toxicity and persistence and are known to bioaccumulation in the food chains. They also have deleterious effects on both plant and animal life, in addition to risk on human health. Both the beneficial and toxic effect of metals is influenced by other elements in the environment and biological factors. Toxicity can often be lessened or even concentrated by biological adaptation.

Nayar et al. (2006) studied on tree canopies air pollution and plants. Heavy metal accumulations in aquatic ecosystems show that they are accumulated either in aquatic organisms or in the sediments. In estuarine ecosystem, sediments are not only functioning as heavy metal scavenger, but also as one of potential sources for heavy metals to the ecosystem. Due to the capability of aquatic organisms to accumulate heavy metals, there is a possibility of heavy metals to exert their toxic effect towards the organisms.

Cukrov et al. (2011) carried out an experiment on a recent history of metal accumulation in the sediments of Rijeka harbor, Adriatic Sea, Croatia. They found that metals incorporated into sediments they are not readily available to aquatic habitats, but changes in physicochemical conditions like pH, temperature, salinity, redox potential and organic ligand concentrations can help dissolution of metals from a solid phase. Thus, environmental conditions of an area largely determine the bioavailability, mobility and toxicity of metals.

Pandey et al. (2014) studied on heavy metals causing toxicity in animals and fishes. In this experiment described that heavy metals are accumulated in living organisms when they are taken up, and stored faster than they are broken down or excreted. The three most pollutant heavy metals have been reported include Pb, Hg and Cd badly affect the environment. ‘Heavy metals toxicity’ has been reported to be caused by different means; e.g., from contamination of drinking-water (Pb pipes), high ambient air concentrations near emission sources, or from food chain. The heavy metals are poisonous since they bio-accumulate. Exposure of Pb can cause many effects depending on level and duration of Pb.

2.7 Detrimental effects of arsenic, lead and chromium on human:

Rahman et al. (2012) study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. Food consumption is the main pathway for human exposure to heavy metals compared to other routes such as inhalation and dermal contact. Fish is widely consumed and a main source of nutrition in many coastal communities. Although fish is highly nutritious, higher consumption rate can have significant deleterious effects on human health because of bioaccumulated toxic metals beyond the safe limits.

Jordan et al. (2013) observed that both humans as well as animals are daily contaminated by the heavy metals. The heavy metals like Hg and light metals like Al are poisoning us and animals every day in vaccines, from environmental pollution in air and water, and from the foods. The heavy metals are also being sequestered in the long bones of food animals in our country due to use of leaded fuels in the automobiles.

Pandey et al. (2014) conduct an experiment on heavy metals causing toxicity in animals and fishes. Water-soluble inorganic As is readily absorbed from digestive system. Inorganic forms of As are particularly toxic. It causes irritation to lung, stomach and intestine, skin disturbances, and decreased formation of RBCs and WBCs. Very high concentrations of inorganic As can cause infertility, skin disturbances, decreased resistance to infections, heart disruptions, brain damage and death. Lead can enter the body through ingestion and inhalation. Its maximum allowable levels may be 5 µg/L (in bottled water) to set elemental impurities limit. It can cause disruption of biosynthesis of Hb, anaemia, kidney damage, reproductive/fertility problems and brain or nervous system damage. The Cr has been reported to be used in metal alloys and pigments for paints, cement, paper, rubber and other materials. The low level Cr can irritate skin and can produce ulcer. Its chronic exposure can produce kidney and liver damage. The Cr can also damage to circulatory and nerve tissues. In aquatic animals, it is normally accumulated and can cause toxicity to eating fish.

2.8 Importance of enzymatic activities in fish especially ATPase and ALP:

Liu et al. (2010) carried out an experiment on digestive enzyme and alkaline phosphatase activities during the early stages of fish development. The activity of digestive enzymes is an important indicator of digestive physiology in fish species. The level of digestive enzyme activity in fish determines the capacity of digestion and absorption of nutrients, which influences the speed of growth and development in fish.

Ahmmed et al. (2017) conducted an experiment on biochemical impacts of salinity on the catfish, *Heteropneustes fossilis* (Bloch, 1794), and possibility of their farming at low saline water and found that ALP and ATPase activities were reduced significantly in both muscle and liver tissues at higher salinity, indicating the stress mitigation effect.

Stettner et al. (2013) studied on sulfatides are required for renal adaptation to chronic metabolic acidosis. ATPase is arguably the most important enzyme in the animal cell plasma membrane, but the role of the membrane in its regulation is poorly understood. In all animal cells, the major role of the Na^+-K^+-ATPase is to maintain ion electrochemical gradients across the plasma membrane. It has been estimated that this action accounts for about 25% of standard metabolic rate making the pump energetically among the most important proteins in the cell. In vertebrate osmoregulatory epithelia, this role is modulated to meet the demands of ionic homeostasis.

Sarma et al. (2010) studied on prospects of fish culture in the broad bed and furrow system in Andaman. In this study he found that ATPase provides the fish immediate energy and reduction in this enzyme might have significantly affected the fish.

Parvez et al. (2006) conduct an experiment on decreased gill ATPase activities in the freshwater fish *Channa punctata* (Bloch) exposed to a diluted paper mill effluent. Determination of adenosine triphosphate (ATPase) of muscle and liver tissue could be an important biomarker to assess the condition of fish in stressed condition.

2.9 Enzymatic variation in fish due to heavy metal accumulation:

Ahmmed et al. (2017) conducted an experiment on biochemical impacts of salinity on the catfish, *Heteropneustes fossilis* (Bloch, 1794), and possibility of their farming at low saline water. The liver tissue produces more ALP than other organs of fish, but the production of ALP is inhibited in stress condition. As a potential stress indicator, ALP activity was level was found to be reduced in liver tissue at 9 ppt salinity. Madhuban and Kaviraj (2009) also found a trend of reduced ALP activity in stressed condition of Clarias. So, the inhibition of ALP in *H. fossilis* might be due to continuous exposure to higher salinity resulting in decreased amount of the enzyme ALP. Reduction in ALP activity in liver may happen due to sudden changes in metabolism to supply additional energy in hyperosmotic condition.

Atli et al. (2007) conducted an experiment on enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus*. Freshwater fish *Oreochromis niloticus* were exposed to Cd, Cu, Zn and Pb and responses of several enzymes were determined subsequently. Liver catalase (CAT) activity was influenced by Cd and Pb exposures. Liver alkaline phosphatase (ALP) activity was first stimulated at lowest (5 μM) exposure concentration, while there were significant inhibitions at higher (10 μM) exposure concentration. Na, K-ATPase activity in the gill and intestine was inhibited by all the metal exposures. Similarly, muscle Ca-ATPase activity was inhibited by all the metal exposures, except Cu exposures. This study indicated that enzymatic systems may be used as a sensitive bio-indicator of metal contamination in aquatic systems.

Pandi et al. (2009) studied on hepato toxicity of lead in Indian major carp *Labeo rohita* (Rohu). It was concluded that the protein content varies significantly in the lead exposed fishes. It is found that the structural level changes in the proteins and marker enzymes due to lead toxicity.

CHAPTER 3

MATERIALS AND METHODS

Materials and methods is an indispensable and integrated part of any scientific research. Accurate methodology enables the researcher to collect valid and reliable information as well as to analyze the information properly in order to achieve a good conclusion. Thus the methodology is important to attain the objective of the research.

3.1 Study area

The experiment was conducted in the laboratory of Applied Chemistry and Chemical Technology department, Chattogram Veterinary and Animal Sciences University, Chattogram. It was provided all the necessary facilities for conducting the experiment smoothly.

3.2 Sample collection process

Two fish species Rohu (*Labeo rohita*) and Catla (*Gibelion catla*) were collected from the four fish farm which are located on the coast of Chattogram at different times. Then the collected fish species were brought to the laboratory of Applied Chemistry and Chemical Technology department, Chattogram Veterinary and Animal Sciences University. Fish samples were stored in plastic bags at -20º C until dissection. Total length (cm) and weight (g) of fish samples were measured carefully and recorded in every time.

Rohu (*Labeo rohita*)

Catla (*Gibelion catla*)

Plate -1: Collected fish sample

Plate-2: Fish stored in freezer **Plate-3: Measuring the length** **Plate–4: Weighing the fish**

3.3 Dissection of fish sample

Each of the collected fish sample was dissected and collected its gill, muscle, liver and kidney tissues. The collected gill, muscle, liver and kidney tissues were kept into the plastic jar as sample.

Plate – 5: Dissection of fish sample

Plate – 6: Dissected fish

Plate – 7: Collection of fish organ

Plate -8: Fish organ

3.4 Preparation of the sample for digestion

All sample preparation and analysis method was carried out according to the procedure described by UNEP Reference Methods (1984).For further analysis the prepared samples were stored in 10% formalin solution.

Plate -9: Sample kept into formalin

Plate -10: Marking sample

Plate -11: Sample preparation for drying

Plate -12: Sample kept into hot air oven

Plate -13: Drying in hot air oven **Plate -14: Dried fish organ**

3.5 Digestion of the sample

The selected sample tissues were digested with concentrated nitric acid and perchloric acid (2:1 v/v) at 60 ºC for 3 days. By using a microwave digester all samples were diluted with double distilled water. After acid digestion, all samples were analyzed for three heavy metals (As, Pb and Cr) by atomic absorption spectrometry (Phillips AAS with double beam and deuterium background corrector).

Plate -15: Weighing of dried sample **Plate -16: Acid taken by pipette** **Plate -17: Acid mixing with sample**

Plate -18: Set up tube for microwave digestion

Plate -19: Sample taken into test tube

3.6 Heavy metal analysis

Lead (Pb), Arsenic (As) and Chromium (Cr) were analyzed in a graphite furnace (GBCGF 3000 with Zeeman background corrector) with an auto sampler. For determining each metal digested samples were analyzed three times. The standard addition method was used to correct for matrix effects (If any). Standard solutions prepared from commercial materials were used to calibrate the instrument. Analytical blanks were run in the same way as the samples and determined using standard solutions prepared in the same acid matrix.

Plate -20: Sample prepared for analysis

Plate – 21: AAS used for metal analysis

3.7 Sample preparation for enzyme analysis

To determine the enzymatic activity different investigated organ including liver, kidney, gill and muscle were dissected out from the fish. Each of the dissected part was mixed in chilled sucrose solutions (0.25 M) separately with a mechanical tissue homogenizer (WN-AD200LPN, China) to prepare 5% homogenate. The homogenate was centrifuged at 3000 g for 15 min using a laboratory centrifuge (centrifuge 5702R, Germany); the supernatant was collected and stored at -20°C for further analysis within 1–2 days.

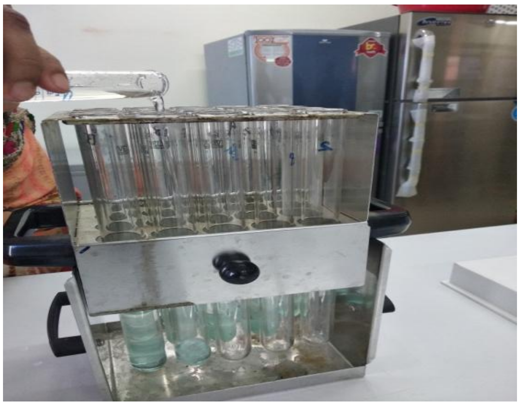

Plate – 22: Preparation of sample for enzyme analysis

3.7.1. Alkaline phosphates (ALP) analysis

ALP (alkaline phosphates) activity of liver, Kidney and was assayed using the standard method stated by Garen(1960).In the muscle ALP (alkaline phosphates) activity was determine by using the standard method narrated by Levinthal (1960). To prepare the solution 0.1 mL of 0.1 M magnesium chloride, 0.2 mL of HCO3 buffer, 0.05 ml tissue homogenate, 0.1 mL of 0.1M para-nitrophenyl phosphate and 0.5 mL of distilled water was used. Water bath is used to incubate the mixture at 37°C for 20 min. Optical density was recorded at 410 nm using UV–visible spectrophotometer (LT2900, Germany).

Plate – 23: Water bath used to incubate the mixture

3.7.2. ATPase enzyme analysis

ATPase enzyme activity was determined to follow the standard method stated by Post and Sen (1967). A mixture was prepared with 100 mM Nacl, 3 mM magnesium chloride, 0.1 M HCl buffer, 20 mM potassium chloride, 0.1 mL tissue homogenate and 5 mM ATP which was used. The reaction mixture was incubated for 15 min, and 10% TCA was used to stop the reaction. Optical density was maintained at 660 nm (Fiske & Subbarow 1925).

Plate – 24: Incubate used for the incubation

3.8 Statistical analysis

All the data collected during the experimental period and laboratory test were recorded and preserved in computer. The data obtained in the experiment was analyzed by using SPSS statistical package program. One-way analysis of variance (ANOVA) and Duncan multiple range test were used to evaluate whether metal concentrations varied significantly between species & different organs. The comparative accumulation of Lead (Pb), Arsenic (As) and Chromium (Cr) in each species was demonstrated by using Microsoft Excel. A 5% level of significance was used ($P < 0.05$).

CHAPTER 4

RESULTS

This chapter presents the findings of heavy metal concentrations in rohu and catla fish along with evaluation of enzymatic activities (ATPase and ALP) in examined organs.

Concentration of As, Pb and Cr in gills, livers, kidneys and muscles of rohu fish:

4.1 Different heavy metals concentration in gills of rohu fish

The values of heavy metal concentration (As, Pb and Cr) in gills of cultured rohu are shown in (Fig. 1). Among these heavy metals, lead concentration was the highest (0.021 ppm) where Chromium concentration was the lowest (0.001 ppm) in gills of cultured rohu fish. The values of Arsenic and chromium (0.006 ppm and 0.001 ppm) are significantly different compared to lead in gills of examined cultured rohu fish.

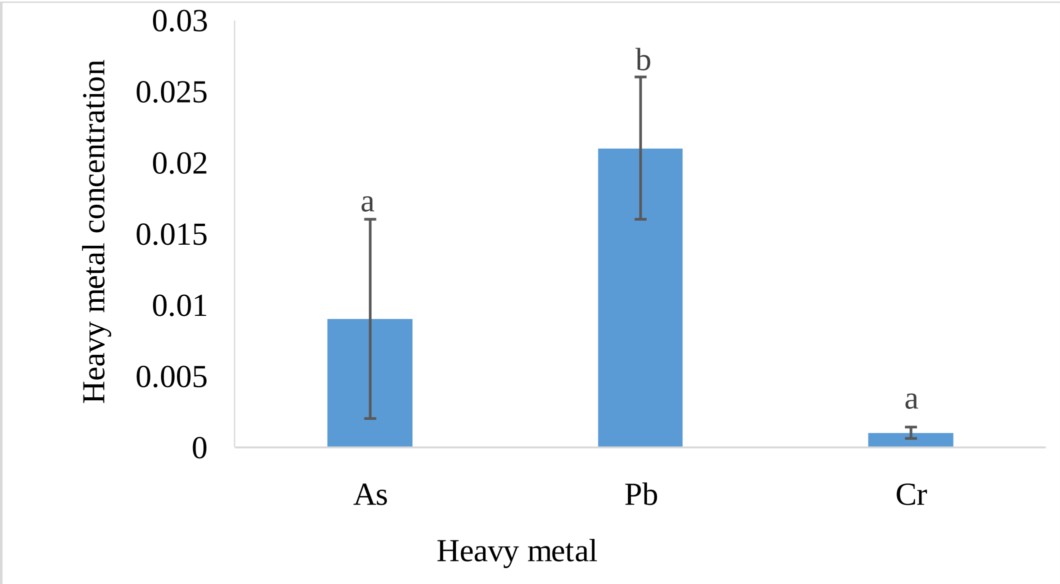

Figure 1: Different heavy metals concentration in gills of cultured rohu fish

4.2 Different heavy metals concentration in liver of cultured rohu fish

The values of lead were found to be the highest in liver (0.021 ppm) followed by arsenic(0.009 ppm) and chromium (0.001 ppm) respectively. The values of lead(0.021 ppm) are significantly higher than chromium (0.001 ppm) whereas the recorded values of arsenic(0.009 ppm) in liver are not statistically different in comparison with the lead(0.021 ppm) but significantly varied with the chromium (0.001 ppm) and in liver of rohu fish.

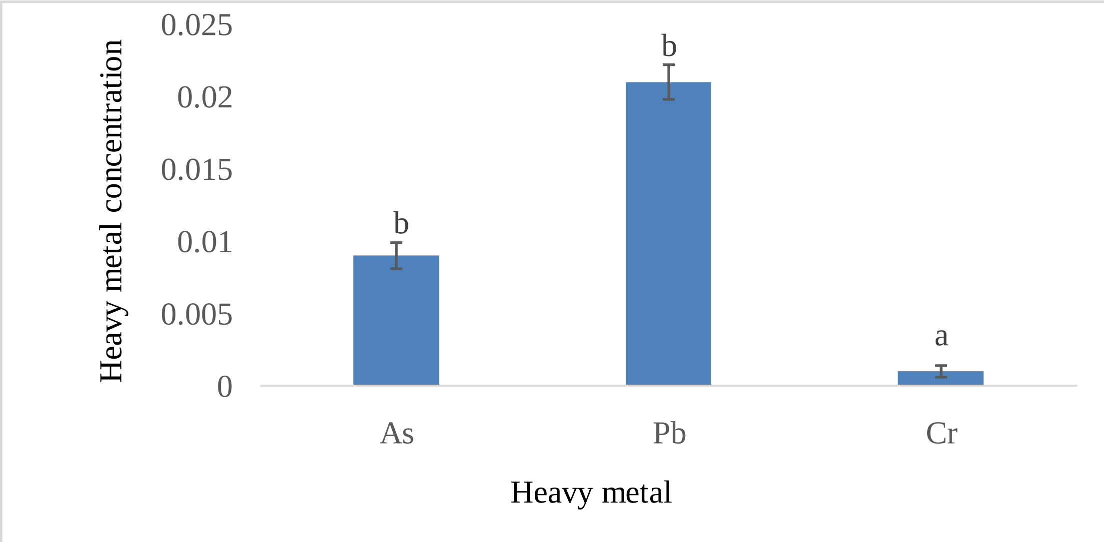

Figure 2: Different heavy metals concentration in liver of cultured rohu fish

4.3 Different heavy metals concentration in kidney of cultured rohu fish

The recorded values are shown below the fig. 3. The concentration of Pb was found to be the highest (0.028 ppm) followed by As (0.012 ppm) and Cr (0.004 ppm) respectively. The obtained values of different heavy metals in kidney of cultured rohu are significantly varied among each other statistically.

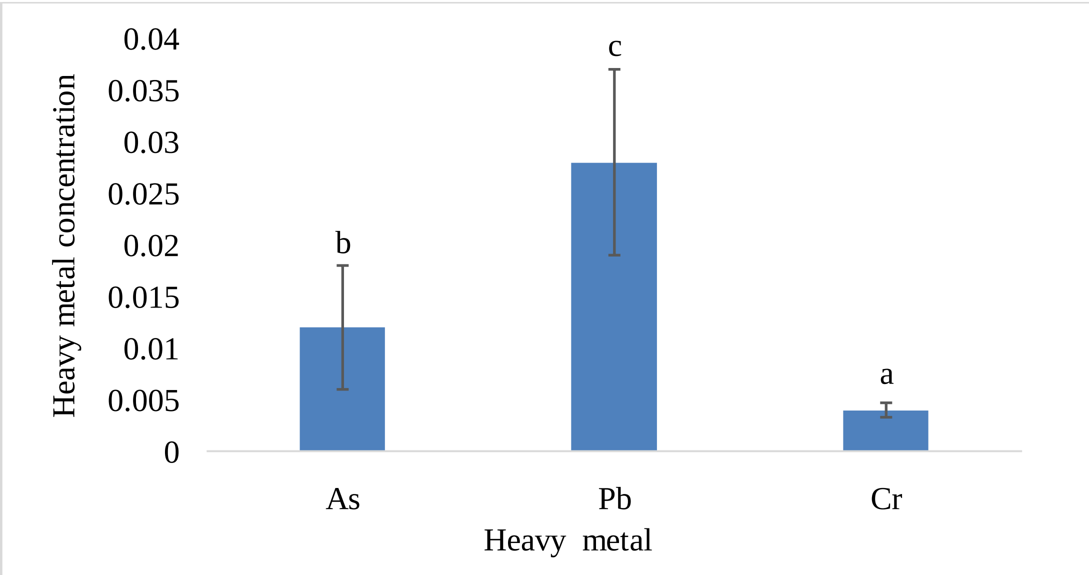

Figure 3: Different heavy metals concentration in kidney of cultured rohu fish

4.4 Different heavy metals concentration in muscles of cultured rohu fish

Among the investigated heavy metals (As, Pb, Cr), lead concentration (0.008 ppm) was the highest in muscles of rohu fish followed by the concentration of chromium (0.006 ppm) and Arsenic (0.004 ppm) though the values are not statistically significant.

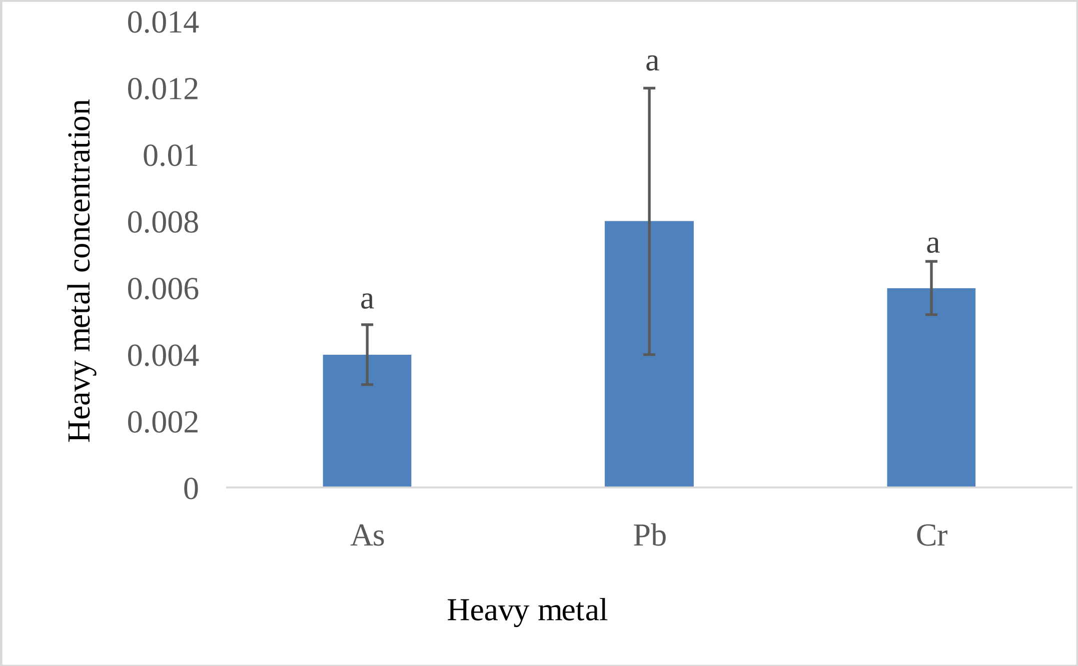

Figure 4: Different heavy metals concentration in muscles of cultured rohu fish

Concentration of As, Pb and Cr in gills, livers, kidneys and muscles of cultured Catla:

4.5 Different heavy metals concentration in gills of cultured catla fish

The Arsenic concentration was recorded as the highest (0.021 ppm) in gills of catla followed by Lead and Chromium (0.018 ppm and 0.002 ppm). The values of Arsenic and Lead (0.021 ppm and 0.018 ppm) are significantly different in comparison with Chromium in gills of cultured catla.

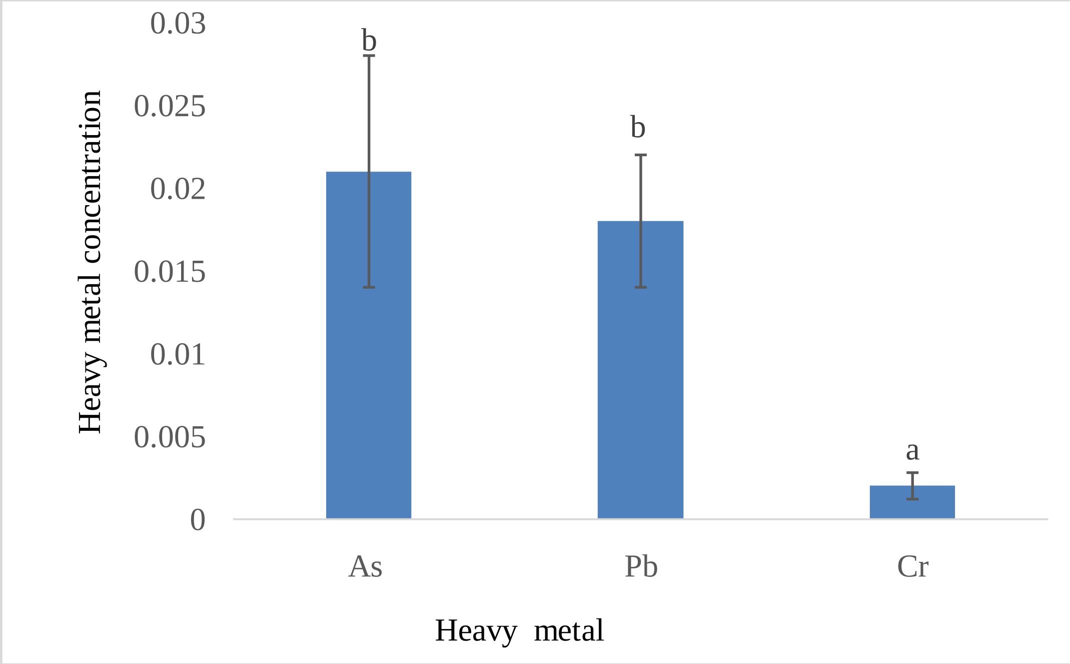

Figure 5: Different heavy metals concentration in gills of cultured catla

4.6 Different heavy metals concentration in livers of cultured catla fish

The values of lead were found to be the highest in liver of catla (0.020 ppm) followed by Arsenic (0.019 ppm) and Chromium (0.001 ppm) respectively. The values of Chromium varies from other two heavy metals.

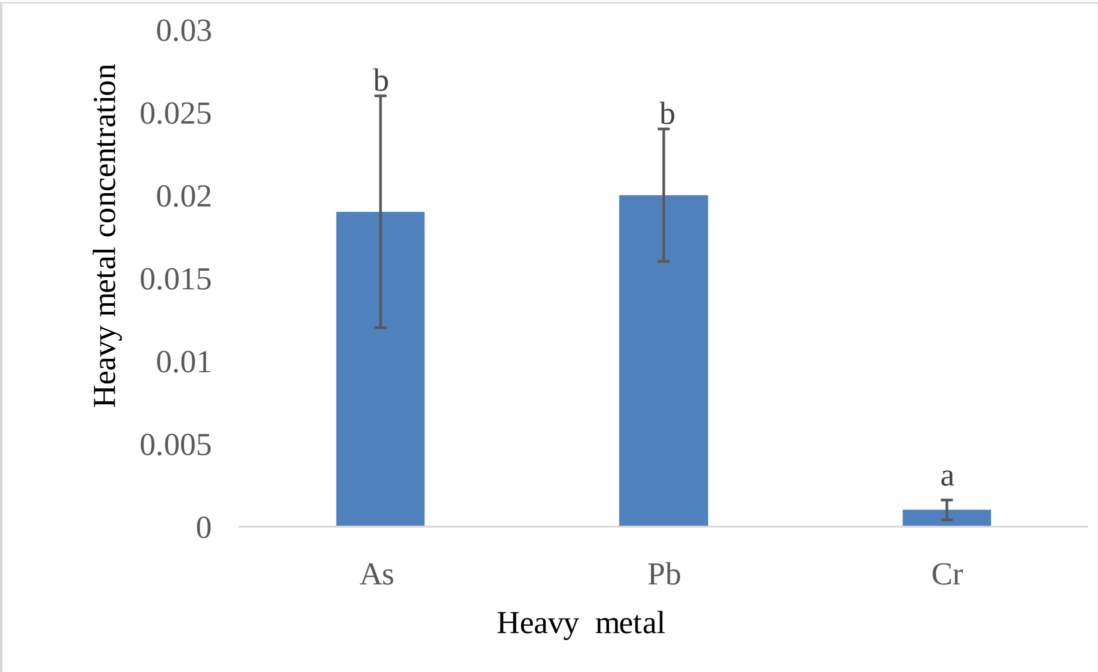

Figure 6: Different heavy metals concentration in livers of cultured catla

4.7 Different heavy metals concentration in kidneys of cultured catla fish

The following graph shows that the concentration of lead was the highest (0.026 ppm) with a significantly lower value of Arsenic (0.010 ppm) and chromium (0.005 ppm). The value of arsenic in kidney is not statistically different from chromium but varied from lead.

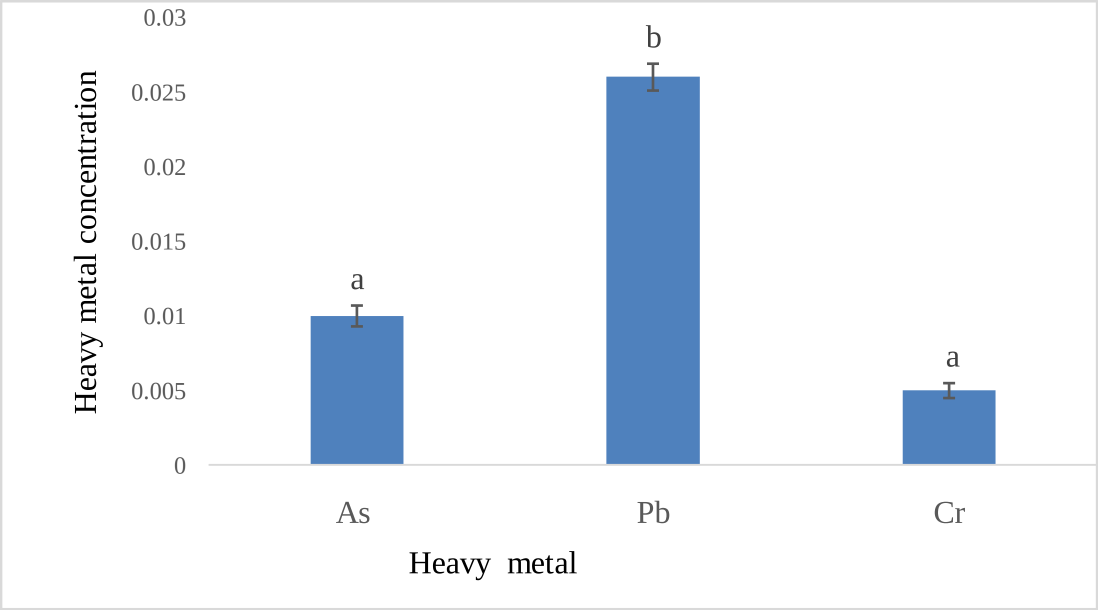

Figure 7: Different heavy metals concentration in kidneys of cultured catla

4.8 Different heavy metals concentration in muscles of cultured catla fish

Among the investigated heavy metals (As, Pb, Cr), lead concentration (0.00 6ppm) was the highest in muscles of catla followed by the concentration of chromium(0.003 ppm) and Arsenic (0.002 ppm) though the values are not statistically significant ($P < 0.05$).

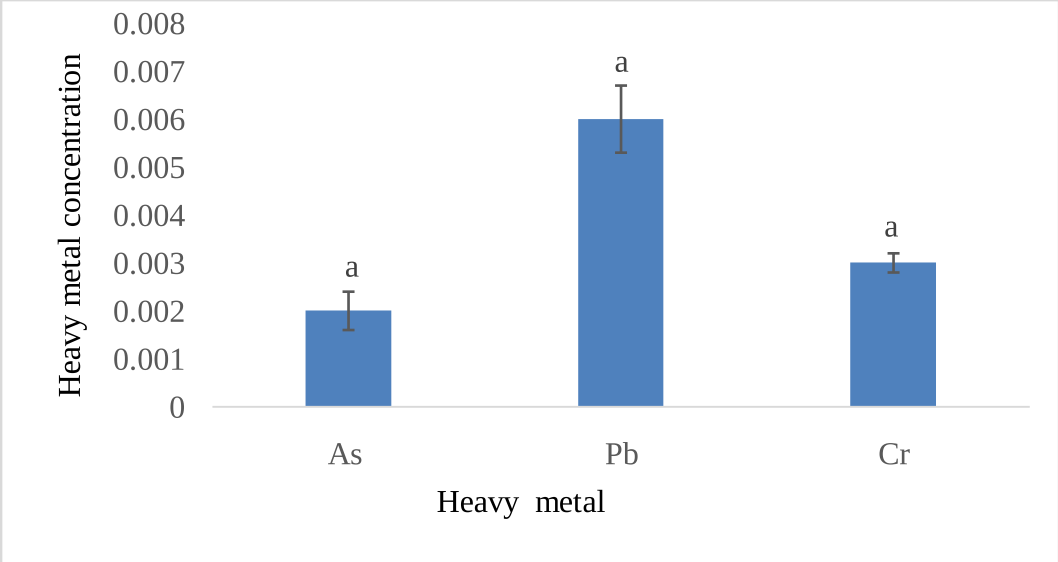

Figure 8: Different heavy metals concentration in muscles of cultured catla

4.9 ATPase activity in different investigated organs of cultured rohu fish

The graph explain the ATPase activities of different examined organs such as gills, liver, kidney and muscle of rohu fish. From the findings, it is observed that the ATPase enzymatic activities was found to be the highest in kidneys with insignificant difference for the values obtained in livers. The figure also suggests that the lowest ATPase activity was recorded in muscle which was statistically significant varied with the above two organs along with the values obtained for gills of rohu fish.

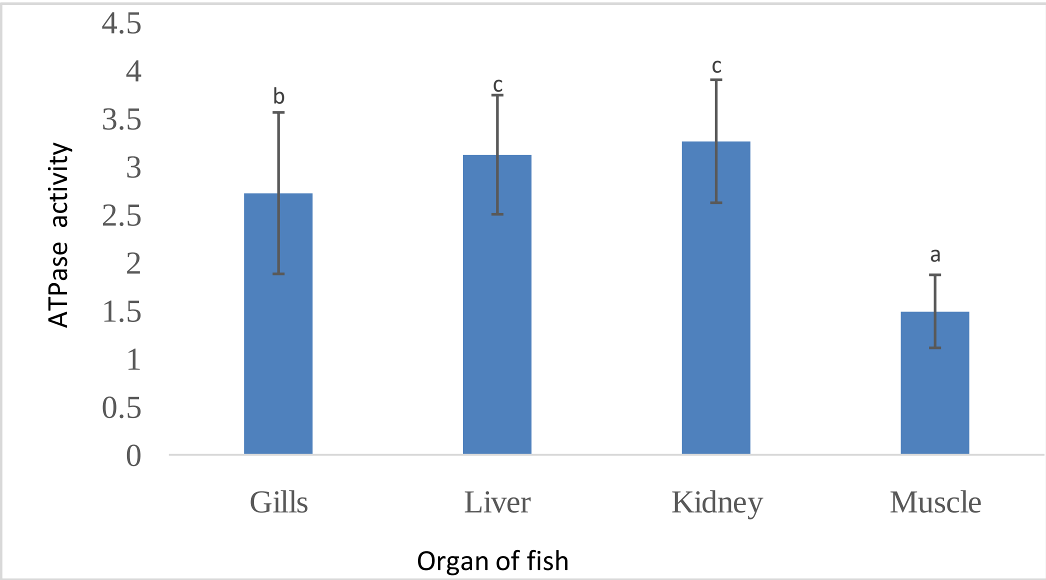

Figure 9: ATPase activity as µg of phosphorus mg protein^{-1} per min (37°C) in different organs of cultured rohu fish

4.10 ATPase activity in different organs of cultured catla fish

The recorded values of ATPase activities in different organs of cultured catla fish are in a line of match with the results obtained for rohu fish depicting the highest in kidneys followed by livers, gills and muscles respectively. The values here for gills are significantly indifferent from liver and kidney. But the lowest value of muscle significantly varied from gill, liver and kidney.

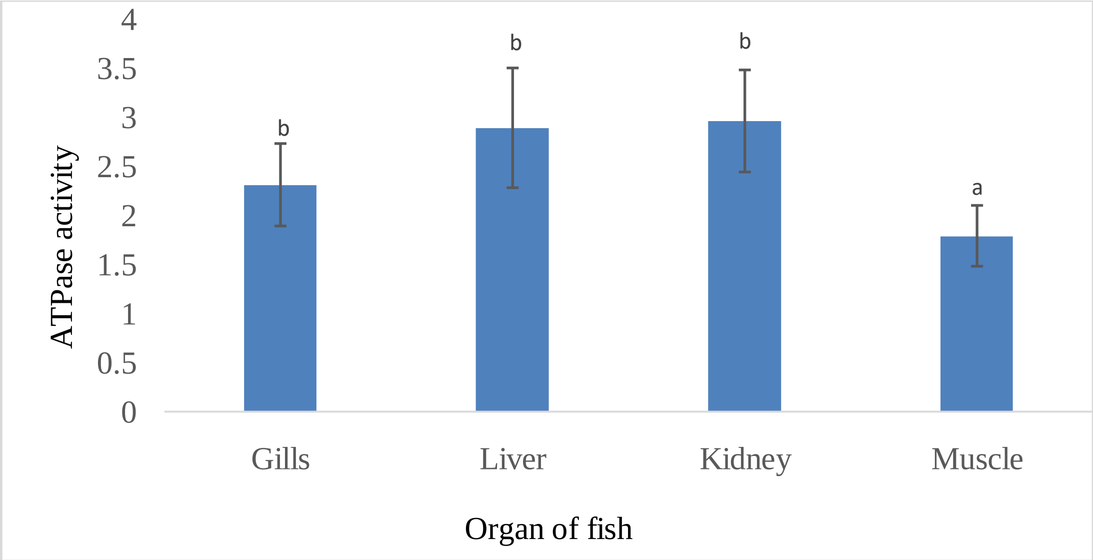

Figure 10: ATPase activity as µg of phosphorus mg protein^{-1} per min (37°C) in different organs of cultured catla

4.11 ALP activity in different organs of cultured rohu fish

The concentration of ALP activity was found highest in kidney (29 ppm) with insignificant statistical difference for that of liver (26 ppm) in rohu fish. The lowest value observed in muscles (10 ppm) with significant difference from kidney and liver whereas the recorded values in gills are not statistically different from muscle (10 ppm).

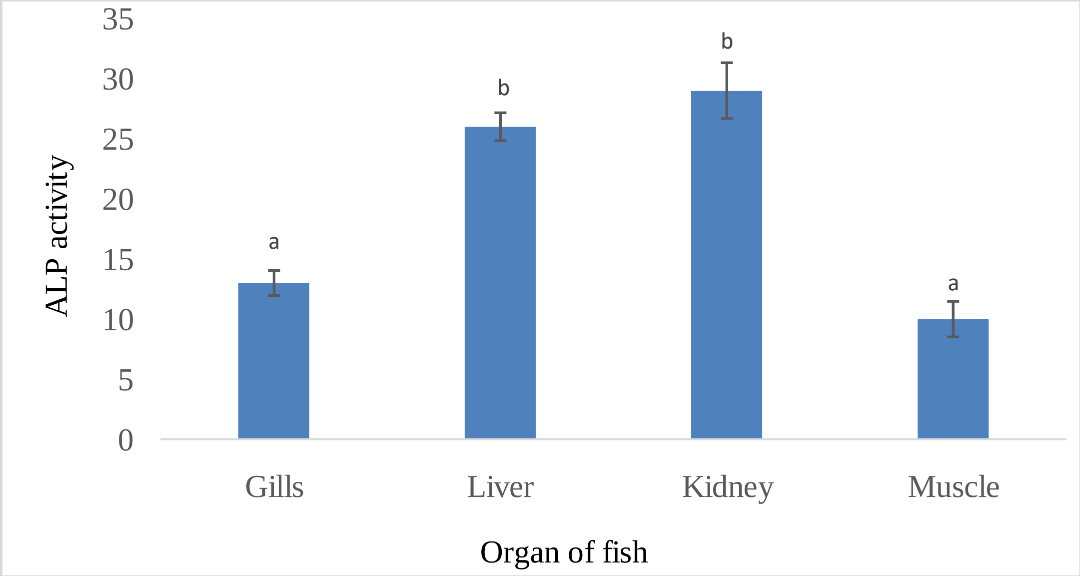

Figure 11: ALP activity as n moles of para-nitrophenol mg protein^{-1} (37°C) in different organs of cultured rohu fish

4.12 ALP activity in different organs of cultured catla fish

The concentration of ALP activity was found highest in kidney (28 ppm) with insignificant statistical difference for that of liver (27 ppm) in catla fish. The lowest value observed in muscles (09 ppm) and gills (12 ppm) with significant difference from above mentioned two organs whereas the recorded values in muscle and gills are not statistically different from each other.

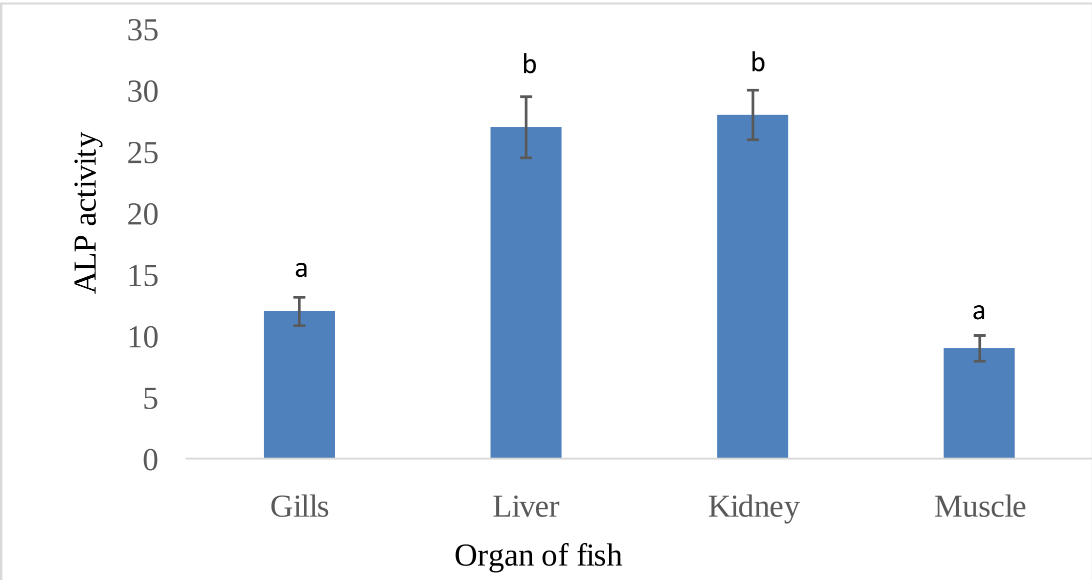

Figure 12: ALP activity as n moles of para-nitrophenol mg protein^{-1} (37°C) in different organs of cultured catla

4.13 Average concentration of different heavy metals in organs of cultured rohu and catla fish

The data values found in rohu stated that concentration of Arsenic (As) with a mean value of 0.008 ppm which is lower than the recommended value of 0.01 ppm (WHO/FAO, 2005). The average value of lead (Pb) was observed (0.0195 ppm) which is also lower than the standard value of 0.3 ppm by WHO/FAO (2005). The mean average value of Chromium (Cr) was the least among the three investigated heavy metals (0.003 ppm) which is far lower than the standard value (0.1 ppm) of Chromium (WHO/FAO, 2005).

Table 1: Average concentration of different heavy metals in organs of cultured rohu fish with recommended limit

Heavy metals	Rohu fish	WHO/FAO limit
As	0.008 ppm	0.01 ppm
Pb	0.0195 ppm	0.3 ppm
Cr	0.003 ppm	0.1 ppm

The values in catla demonstrated that the higher concentration is observed in case of Arsenic (As) with a mean value of 0.013 ppm which is higher than the recommended value of 0.01 ppm (WHO/FAO, 2005). The average value of lead(Pb) was observed (0.0175 ppm) which is also lower than the standard value of 0.3 ppm by WHO/FAO (2005).The mean average value of Chromium(Cr) was the least among the three investigated heavy metals (0.00275 ppm) which is far lower than the standard value (0.1 ppm) of Chromium (WHO/FAO, 2005).

Table 2: Average concentration of different heavy metals in organs of cultured catla fish with recommended limit

Heavy metals	Catla fish	WHO/FAO limit
As	0.013 ppm	0.01 ppm
Pb	0.0175 ppm	0.3 ppm
Cr	0.00275 ppm	0.1 ppm

CHAPTER 5

DISCUSSION

This study was set to investigate the heavy metal concentrations in commercially important cultured fish species such as rohu and catla which are collected from the different farm of Chattogram coast. The investigated fishes as Rohu and Catla are cultured widely around our country and consumed by all. It has been reveal that the level of contaminants in fish depends on different things such as their feeding habit, habitats, duration of exposure of fish to contaminants, age, size and concentrations of contaminants in water bodies. The result of the study on concentration of heavy metal accumulation in different fish organ and enzymatic activities in examined organ are discussed below and compared with the findings of other authors in the relevant field.

Heavy metals persist in the environment long time because these are non-biodegradable substance and may become concentrated up to the food chain (Eja *et al*., 2003). Accumulation of heavy metals occurred in living organisms when they are taken up, and stored faster than they are broken down (metabolized) or excreted. They enter into the water body through industrial and consumer materials, or even from acidic rain breaking down soils and releasing heavy metals into different water body (Pandey *et al*., 2014).

The levels of heavy metals in fish vary species to species and different aquatic habitats. The concentration of heavy metal varied among different organs of a species as well as between species (Canli and Atli, 2003). Among different accomplished values of heavy metals, we found that Pb concentrations in different tissues of the empirical fish species were much higher than any other concentration of other heavy metals whereas Cr showed lowest values in almost all of the experimental organs. Previous study showed that different fish species contained various concentrations of metal levels in their tissues that might be related to the metabolic activities, ecological needs, swimming behaviors (Kalay *et al.* 1999).

Previous study showed that gills, liver, kidney and muscle of *Gibelion catla, Labeo rohita and Cirrhinus cirrhosus* which are examined for the accumulation of metals that are significantly different due to their ability to concentrate heavy metals. However, catla showed significantly higher tendency for the accumulation of all metals in its body than other two fish species (Javed, M., 2003).

El-Shahawi and Al-Yousab (1998) reported that in fish, the levels of nickel, cobalt, lead and chromium was found to follow the order: liver > skin. Metal levels found in liver and skin followed the sequence: chromium > lead > nickel > cobalt. However, during this study the rohu fish organs accumulated As and Pb followed the order: kidney > liver > gills > muscle while Cr showed: muscle > kidney >liver >gill. In catla fish organ accumulated As to follow the order gills > liver > kidney > muscle while for lead accumulation in fish was: kidney > liver >gill > muscle. The concentration of Cr found in the catla fish follow the order: kidney > muscle >gill >liver.

it's very difficult to compare the metal concentrations even between the same tissue in different species because of many factors such as aquatic environments, concerning the type and the level of water pollution, feeding habits whether omnivorous or carnivorous, and level of fish presence in water, whether pelagic or benthic fish etc.

Heavy metal concentrations were found to decrease in sequence of the *Cyprinus carpio* samples, in the muscle and stomach-intestine as Fe > Cu > Pb> Ni > Cr > Cd; in the gill, heart and liver as Fe > Cu > Ni > Pb > Cr > Cd and in the air sac as Fe > Cu > Ni > Pb > Cd >Cr (Kousar et al. 2014). Kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids and besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotic to which the fish may be exposed. It was evident from our study that heavy metal concentration in kidney of Rohu and Catla fish as Pb > As > Cr. In liver of both fish metal concentration found in the order of Pb > As > Cr and in the muscle of both fish it found that Pb > Cr > As.

Gills are considered to be the potential site for contaminant uptake in fish because of their anatomical and physiological properties that maximize absorption efficiency from water (Ekeanyanwu 2010). However, it was evident from our study that, in general kidney and liver were the sites of maximum accumulation for the Pb and As while gill was the overall site of least metal accumulation in both species. The metal concentrations in the gill of Rohu and Catla fish has in the decreasing order of Pb>As>Cr and As >Pb > Cr respectively.

Between two fish species, the variation of metals concentration in the muscle of Rohu and Catla fish are found in the order of Pb > Cr >As and Pb >Ar > Cr respectively. In average Cr concentrations have found to be lower in rohu fish gills and liver than other two organs and in case of catla Cr also found lower in liver and gills. The low concentrations of all metals were found both species muscle relatively.

In catla fishes, As overpass the recommended value provided by FAO/WHO whereas Pd and Cr are far lower than the guideline value. In case of Rohu fish, experimented value of As, Pb and Cr are within the recommended value provided by FAO/WHO. Bashir *et al.* (2015) found that muscle contained the minimum concentrations of trace metals among all the tissues which support our findings. Among different organs, highest concentration of metals were observed in kidney tissues but the lowest concentration in muscles was found which is a positive finding as we mainly consume the muscles portion of fish widely. Therefore, we can conclude that our findings are agreed upon previous studies.

However, all these concentrated metals in different parts of fish body could be concentrated into human body, if they are consumed. In Bangladesh, water sources are getting more polluted day by day and thus these HMs from polluted water bodies are getting more concentrated in those fish living in that areas. There is another way of concentrating HMs in fish body through the feed they are reared with and for farmed fish; it is our lacking that we don’t care about those HMs from artificial feed getting introduced into fish body parts. If all these bad practices live long and if so happened in this way there will be a lot of reason to worry about as well as there will be a great threat for human health. The results found in the present study are just a warning for us and our future generation and it is of high priority to undertake necessary actions regarding safety and environmental friendly discharge of arsenic. The recorded values in case of lead and chromium were within the safety levels and considered safe for human consumption, but we need continuous monitoring for maintaining this level.

Enzymes activities including Adenosine triphosphatases (ATPase) and alkaline phosphatase (ALP) were evaluated indifferent important fish organs (liver, kidney, gills and muscle) in present study. By knowing the enzymatic activities in an organism, we can easily identify disturbances in its metabolism. Adenosine triphosphate (ATP) is a complex organic chemical that provides energy to drive many processes in living cells which includes contraction of muscle, propagation of nerve impulse, and synthesis of chemical. ATP is referred to as the "molecular unit of currency" of intracellular energy transfer in all form of life. ATP is a precursor to DNA and RNA, and is also used as a coenzyme. These enzymes release energy and helps in osmoregulatory activities (Bhanu & Philip 2011). All living cell contain ATP, and it should be possible to look for changes in ATP levels in water as an indication of biological contamination. Adenosine triphosphate (ATPase) determination in fish in liver and muscle tissue could be an important biomarker to evaluate the condition of fish in stressed condition (Parvez et al., 2006).Under the stress condition ATPase activity reduced in fish (Begum and Vijayaraghavan 1995).

In the present study, The ATPase activity in different organs of rohu fish decreasing the order: kidney > liver >gill > muscle. On the hand, ATPase activity in different organs of Catla fish is in the order: kidney > liver > Gill > Muscle. In the both fish we found that ATPase activity was highest in kidney and lowest in muscle. Kidney mitochondria have a greater capacity for ATP hydrolysis (Weiner MW). Enzyme analysis in different organs of fish such as muscles, kidney, liver, heart and gills provides vital information about the organism’s internal environment. Physiological and biochemical processes disrupt when enzyme activities changes. A major biochemical response to the effect of a toxicant in fishes is the inhibition of activities of different enzyme activities (Neelima et. al., 2016). The result found in the present study is in agreement with the previous studies reporting.

Alkaline phosphatase is an enzyme found throughout the whole body but it is mainly found in the bones, kidneys, liver and digestive system. ALP catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters. It is a ubiquitous plasma membrane-bound enzyme which employed to assess the integrity of plasma membrane and endoplasmic reticulum. The liver and biliary tracts are the sources of alkaline phosphatase (Mohamed et al., 2019). ALP is found more in liver than other organs of fish but ALP production inhibited due to stress condition (Rao 2006). ALP used as potential stress indicator. Reduction in ALP activity in liver may happen due to sudden changes in metabolism to supply additional energy in hyper osmotic condition.

The activities of ALP enzyme in different organs of Rohu are in the decreasing order of: kidney > liver > gill > muscle. The liver tissue produces more ALP than other organs of fish, but the production of ALP is inhibited in stress condition (Rao 2006).In case of catla fish, the activities of ALP enzyme in different organs are in the decreasing order of: kidney > liver > gill > muscle. Kidney was found to be a organ in with the highest ALP activity. So our findings are consistent with previous studies. We found the present study, Kidney produces more ALP then liver, gill, muscle respectively. The finding is very close to the results of the present study.

Enzymes are the vital biochemical components which control metabolic process of organisms because of that reasons slight variation of enzyme activities in organisms would affect more. Enzymes are biochemical macromolecules that control metabolic processes of the organisms, ion transport, regulate cell volume (Grosell et al., 2003). Therefore, it is important to monitor the activities of this enzyme and their levels in various organs of fish.

Chapter 6

CONCLUSION

The heavy metals such as Arsenic (As), Cadmium (Cd), Lead (Pb), Chromium (Cr) and Mercury (Hg) are most venom to all animals, fishes, human beings and also environment. Heavy metals when exceed the tolerance level may cause severe toxicity. Though some heavy metals are essential for animals and several other organisms, all heavy metals exhibit their toxic effects through metabolic interference and mutagenesis. The Pb, As, Cr and Hg causes severe toxicity in all. Fishes are not the exception and they may also be highly polluted with heavy metals, leading to serious problems and ill-effects. The heavy metals can have toxic effects on different organs. They can enter into water via drainage, atmosphere, soil erosion and all human activities by different ways. With increasing heavy metals in the aquatic environment ,these elements bio-accumulate into the food chain leading to toxicity in animals, including fishes. Many researcher observed that high concentrations of As in kidney and liver which disrupts the normal metabolism and other physiological process of fish. From present study we found the As is high in catla fish than rohu. On an average other metals found the lower concentration from the permissible limits in case of both fish. Chromium concentration was the lowest whereas Arsenic was the highest. Kidney and liver is the most susceptible organ of fish to accumulate the toxic heavy metals. The findings of this present study stated that consumption of Indian major carp (Rohu and Catla) from the cultured pond may not be detrimental to consumers because finding values of heavy metals (except arsenic) were below the permissible limits issued by FAO/WHO for human consumption. It is a matter of concern that Arsenic found higher than certified level which is harmful to human health. To reduce the level of heavy metals concentration from fish and other organisms close monitoring of aquatic environment is a must. We also investigated the enzymatic activities(ATPase and ALP) of rohu and catla fish which involved in the osmoregulation, respiration, digestion, absorption, transition of nutrients. ATPase activities found highest in rohu fish than catla. In both fishes kidney has highest ATPase activitities and muscle show the lowest ATPase activities. ALP activities were higher in all organs of rohu fish except liver than catla fish. In case of both fish muscle has lowest and kidney has highest ALP activities in present study. The information about the mechanism of ATPase and ALP activities in various fish and different organ of fish not enough yet for this reason the more research required on this topics.

CHAPTER 7

RECOMMENDATION AND FUTURE PERSPECTIVES

Although a qualitative approach was followed to explore the objectives of the research, there are some limitations of the study which can be minimized by following the recommendation:

- Similar studies may be performed to check contamination with other toxic heavy metals such as copper, nickel, mercury, cadmium, cobalt and Zinc in cultured fish.

- Periodical monitoring of heavy metal concentration in cultured fish is important because cultured fish accumulates the heavy metals which effects on the fish.

- Environment quality of the cultured area must be under control and discharging of industrial waste and other pollutants into the cultured area should be prevented.

- The mechanism of ATPase and APL activities in various fish and different organ of fish still not enough because of this reason the more research required.

- Variation in ATPase and ALP activities due to heavy metal exposure are needed to determine to reveal any deformities in enzyme activities.

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