CHAPTER-1

INTRODUCTION

In Bangladesh, fish is a popular animal-source food in the diet of millions, both in terms of quantity accounting for approximately 60% of animal protein intake at 18 kg consumed per person per year and frequency of consumption, far exceeding that of any other animal-source food (Belton *et al.*, 2014). Fish is consumed by a larger percentage of populace because of its cheaper price, availability and palatability when compared to other protein sources like goat and chicken meat it is safer and healthier (Astawan and Ikan, 2004). Fish is the most important source of protein for some communities including those who do not consume red meat, the malnourished, immune compromised, pregnant women and nursing mothers. Fish meat is composed of water (66-81%), protein (16-21%), carbohydrates (0.2-25%) and ash (1.2-1.5%) (FAO, 1999) and is considered to have high biological value, due to the contribution of Essential Amino Acids (EAA), as well as, its high levels of polyunsaturated fatty acid such as omega-3 and omega-6 fatty acids, higher than in most meat sold for human consumption. Fish also provides minerals necessary for optimal health (Gjedrem *et al.*, 2012). The American Heart Association recommended eating fish at least twice per week in order to meet the daily requirement of protein and polyunsaturated fatty acids (Kris-Etherton *et al.*, 2002).

Bangladesh is one of the world's leading fish producing countries and the production of fish is 42.77 lakh MT in 2017-18, whereas aquaculture production contributes 56.24 percent (24.05 lakh MT) to total production. After being self-sufficient in fish production for the first time in 2014, Bangladesh has started to get global recognition as one of the biggest fish producers. In 2013-2014 Bangladesh was ranked third in fish production from inland water-bodies, behind China and India according to the report of FAO (FAO, 2016). Average growth performance of this sector is 5.26 percent for last 10 years. It's a great achievement for the country. The continuous effort of the present government for the country's fisheries sector has resulted in such achievement. To ensure continuity of the success, the government will prioritize conservation of jatka (small hilsa), extension of shrimp cultivation, protection of natural fish-breeding grounds and collection of marine fish at a tolerant level (DoF, 2018)

The coastal water of Bangladesh comprises diverse fisheries resources with 475 finfish species (Mazid, 2005). Of these species, only 100 fish species are commercially important including hilsa, pomfret, chanda, tuna, marine catfish, marine eel, jawfish, ribbonfish, bombay duck etc (Quader, 2010). The marine fisheries play significant role in the economy of Bangladesh. More than 11% of the total population of Bangladesh is directly or indirectly involved in this sector for their livelihoods (Fisheries Statistical Yearbook of Bangladesh, 2013). Total production of marine fisheries is 6.55 lakh MT and its contribution to total fish production is 15.31% with growth rate 2.71% (DoF, 2018). At present, the marine fisheries sector contributes about 18% to the country's total fish production. According to the report of FAO, Bangladesh produced 1,13,200 tons of fish from marine and coastal sources and ranked 11th (FAO, 2016). Prime Minister "Seikh Hasina" also sets utmost priority regarding the protection, conservation and biodiversity of marine and coastal resources. She declared the Saint Martin Island and the Sundarbans, the world famous mangrove forest as sanctuaries to develop and protect the fisheries resources as well as biodiversity of that area. The government has also declared a marine reserve covering 698 sq Km in the Bay of Bengal to protect the breeding ground of marine flora and fauna and increase the marine fish production (DoF, 2018).

Fish and fishery products are not only nutritionally important but also important in global trade as foreign exchange earner for a number of countries in the world as they contain high protein content, with little or no carbohydrate and fat value. Fisheries and aquaculture sectors have become the second most important contributors in export earnings of Bangladesh, contributing to 3.74% in national GDP, 2.7% in export earnings and 22.23% in agriculture sector (FAO, 2018). Due to wide range of global market including USA, UK, Japan, Belgium, Netherlands, Thailand, Germany, China, France, Canada, Spain and Italy, the export of marine fish is extended day by day from Bangladesh. Though there are 129 fish processing industries in Bangladesh, only 62 plants have EU approval. So it is very important to maintain and monitor the production and quality of the fish for its acceptance in international trade as well as avoiding the health problems of consumers (DoF, 2011).

Hilsa ilisha is the national fish of Bangladesh. There is a proverb that "hilsa is the king of fishes". Hilsa is the most important open water single species fishery in Bangladesh that occurs in all most all the major river systems estuaries and the sea. About 12.09 percent of the country's total fish production comes from hilsa. Total hilsa production increased from 1.99 lakh MT in 2003- 04 to 5.17 lakh MT in 2017-18. The growth rate of hilsa production is 4.19 percent. It should be mentioned that hilsa has been declared as Geographical Indicator (GI) of Bangladesh. Annual hilsa production in Bangladesh is 284500 MT (DoF, 2018). We earn Tk. 7000 core from hilsa fish export annually. It contributes in national GDP about 1%. Hilsa is an important source of macro and micronutrients and play an important role to provide essential nutrients for the people of Bangladesh. About 50–60 per cent of total hilsa catch of the world is reported from Bangladeshi waters, 20–25 per cent from Myanmar, 15–20 per cent from India and another 5–10 per cent from other countries (e.g. Iraq, Kuwait, Malaysia, Thailand and Pakistan) (Rahman *et al.* 2010).

Harpodon nehereus is mainly known as Bombay duck. This fish is locally known as 'Lotiya Machh' or Loitta' near the coastal areas of Bangladesh (Sarker *et al.*, 2017). Bombay duck is one of the most common and locally available estuarine fish of Chattogram coast and distributed in the inshore shallow water. It is most consumable fish and also provides great source of nutrition to the consumers. Bombay duck contributes about 1.78% of the country's total fish production. The annual Bombay duck fish production in marine fisheries sector is about 75085 MT (DoF, 2018). According to Rahman and Chowdhury (1988), Major portion of Bombay-duck are converted to rope dried product and dried Bombay-duck have commercial importance in south and south-east Asia.

Pampus chinensis or pomfret are found in the muddy bottom near the sea coasts which are quite tasteful and have a high demand in the market. This fish is locally known as 'chanda' or 'rupchanda' fish. The common species found in the Bay of Bengal are Rup chanda, Fali Chanda, Hali Chanda and *Parastromateus niger*. The promfrets contribute a significant portion of the total fisheries resources collected from Bay of Bengal. About 0.28% of the total fish production of Bangladesh comes from *Pampus chinensis*. The annual *Pampus chinensis* fish production in marine sector is about 11899 MT (DoF, 2018).

Now-a-days marine environment is being constantly polluted with chemical pollutants from lithogenic and anthropogenic sources. In the last decades, anthropogenic sources including agriculture, shipping, urban and industrial practices have resulted in disquieting concentrations of chemicals and heavy metals in aquatic environments (Rahman and Chowdhury, 1988). Oil spills can occur from release of crude oil from tankers, offshore platforms, drilling rigs and well and also from spills of refined petroleum product (such as gasoline, diesel etc) and their by-products and heavy fuel used by large ship. Oil spillage into water affects the ecosystem and its component badly. Among marine animal species, fish are the inhabitant that cannot escape from the detrimental effects of these pollutants. This condition is enraged by the lack of natural elimination processes for metals. As a result, metals transfer from one compartment within the aquatic environment to another, including the biota, often with detrimental effects. Marine lives including fish concentrate toxins in their organs and over time the toxins can accumulate and eventually kill the animals (Bhattacharya *et al*., 1994).

The organs of fish that take up heavy metals are the gills, skin, kidney, digestive tract, muscle and liver (Annabi *et al.,* 2013). Heavy metals are a serious source of food contamination and health hazard. The main threats to human health are associated with exposure to arsenic, cadmium, lead, mercury and chromium. Absorption of higher amount of heavy metals than recommended limit through food has been shown to have serious consequences on health such as kidney disease, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and death (Flora *et al.,* 2008).

1.1 Significance of the Study

As a commercial hub and the industrial nerve center of Bangladesh with an estimated population of more than 8.5 million people, Chattogram coastline is at risk of having high levels of heavy metal pollutants due to surrounding activities. Many kinds of industries are situated on Chattogram coast of Bay of Bengal, each discharging its characteristic range of effluents containing heavy metals and other harmful pollutants into the terrestrial and aquatic ecosystems (Manwar, 2001). More important is that the very young stages of fish (larvae and juveniles) grow up in the near shore zone, where the water quality is heavily affected by the raw sewage outfalls. This poses a severe threat to the fish populations of Chattogram whereas many wastewater outfalls discharges untreated sewage into the sea (Rahman and Chowdhury, 1988; Jothi et al., 2018). In view of the potential risks to human being through the food chain and the surrounding activities at the Chattogram coastline, it is important to constantly monitor the pollution status of the marine ecosystem. Hence, this study provides information that is useful towards the status of heavy metals in the Chattogram fishing harbor. Dissemination of these findings will be helpful to the stakeholders or agencies that monitor environmental pollution such as Ministry of Health and Municipalities.

1.2 Aim and Objectives

The aim of this study was to assess the levels of some heavy metals in different organs of fish species caught from Chattogram coastal area, Bangladesh. Public health importance and the hazardous toxic effects of these heavy metals and fish contamination were discussed.

The objectives of the present study was

- To determine the heavy metals (As, Pb and Cr) accumulation in different commercially important marine fishes (*Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha*).
- To develop a comparison among different organs (gill, liver, kidney and muscle) and different species of investigated fishes in terms of heavy metal accumulation.
- To assess their further possible impacts in human.

CHAPTER 2
LITERATURE REVIEW

Before conducting a research by following experimental procedures, it is important to have a look on the previously conducted research activities on the related topics. A review of the literature relevant to the present research work has been given below:

2.1 Environmental Status of the Chattogram Coastal Area

The Bay of Bengal is in the south part of Bangladesh. The coastal length along the Bay of Bengal is about 710 km. The Chattogram coastal environment is facing a large and serious threat due to lack of proper waste disposal and sanitation facilities, raw and partially decomposed sewage and solid wastes from all over the city find their way into the Karnaphuli River and after that into the Sea (Rahman and Chowdhury, 1988). The extent of human impact on the coastal area is directly related to the population density. Hundreds of ships visiting Bangladesh's main port in Chattogram and these foreign and local ships find the Chattogram Port and its outer anchorage a safe dumping area for their waste matter, taking advantage of poor laws and their lax implementation due to logistic support (DoE, 1997). The total number of industries in Chattogram is about 720 of which 370 are responsible for major pollution. The polluting industries of Chattogram are 19 tanneries, 26 textile mills, 1 oil refinery, 1 TSP plant, 1 DDT plant, 2 chemical complexes, 5 fish processing units, 1 urea fertilizer factory, 1 asphalt bitumen plant, 1 paper mill (solid waste disposal hourly 1450 m^3), 1 rayon mill complex, 2 cement factories, 2 pesticide manufacturing plants, 4 paint and dye manufacturing plants, several soap and detergent factories and a lot of industrial units directly discharge toxic effluent without treatment into Karnaphuli river and which directly enter into the sea (FIDH, 2002). Almost 1200 tons of solid wastes are produced daily in the city. The collected solid wastes are dumped in Halishahar and Firinghee Bazar, adjacent to the Karnaphuli River estuary and directly enter the coastal area (Monwar, 2001). The discharge of different industries is generally composed of organic and inorganic wastes. The organic wastes are the wastes and effluents from the tanneries, fish processing units, degradable wood chips, pulps, untreated municipal and sewage (about 40,000 kg BOD daily) etc. The inorganic waster are various acids, bleaching powder, lissapol, hydrogen peroxide, alkali, salts, lime, dyes, pigments, aluminium sulphate and heavy metals used by the different industries. The DDT factory and fertilizer factory are responsible for disposing of DDT, toxic chemicals and heavy metals to the coastal area (Chowdhury, 1994). Ship building/wrecking industry of Chattogram is another unique group for pollution which contributes greater threat to marine environment. This is obligatory in many countries but in Chattogram, ships directly discharge their waste mixtures into the Bay of Bengal (DoE, 1997). Industrial solid wastes in the coastal water in the Chattogram region are very high because most of the industries do not have any treatment plant that is required under the environment law.

2.2 Wastewater Pollution

Pollution is referred as 'to make foul or unclean or dirty'. Water pollution occurs when a surface of water is seriously affected due to the addition of large amounts of materials to the water. When it is unfit for its intended use, water is considered as polluted. Most of the industries discharge their effluents, whether treated or not, into the marine waters using different designed sewage outfalls. Sometimes, the treated effluents are reused, mainly for irrigation purposes (UNEP and EEA, 1999). The discharge of untreated wastewater into the river or coastal waters is a serious problem for the status of the marine ecosystem. The main source of pollution along Chattogram coastal waters is the discharge of untreated wastewater along the coast. The beaches in front of Chattogram city are polluted by sewage wastes and individual sewage drains ending either on the beach or a short distance from the seashore. This raw sewage water into the sea can cause a number of detrimental effects including untreated sewage affects marine fishes and cause oxygen reduction in seawater, increase in turbidity may affect marine biota, eutrophication (the increase of the nutrient concentration) may cause algal blooms that may be harmful, increasing bacterial growth, shift in fish species composition which encourages the abundance of benthic species rather than pelagic species and poisoning of species by toxic elements (Hilles *et al.*, 2014). This poses a severe threat to the fish populations whereas many wastewater outfalls discharges untreated sewage into the sea.

2.3 Heavy Metal Pollution in the Marine Environment

The rising social concern regarding marine environmental quality can be witnessed in recent years, both on worldwide and local level. Discharge of harmful substances has detrimental effects on the human health, fish health and growth, natural environment and agricultural productivity (Gadzała, 2004). When the purports of marine environmental pollution come to be visible, it is frequently too late to stop them and chronic toxic effects, difficult to observe at the early period of the process, may turn out to be noticeable after many years (Alloway and Ayres, 1998). The aquatic environment is considered to be the main factor for controlling the health and disease case in both human being and animals. The increasing use of the chemical wastes and agricultural drainage systems symbolizes the most hazardous form of chemical pollution mostly heavy metal contamination (Rashed, 2004).

Heavy metals can be defined as a subset of elements that exhibit metallic properties such as hard, opaque, shiny and having a good electrical as well as thermal conductivity. Heavy metal is also defined as those having a specific density of more than 5 g/cm^3 found in all kinds of soils, wind, water and rocks in terrestrial, marine and freshwater ecosystem (Csuros and Csuros, 2002).

The most important heavy metals that cause water pollution including: Arsenic (As), Lead (Pb), Copper (Cu), Lead (Pb), Cadmium (Cd), Mercury (Hg), Nickel (Ni) and Chromium (Cr) as reported by Rashed (2004). When heavy metals find its way into the marine environment, the metal ions can respond with constituents of the water or slow down to the bottom and act in response with the bottom marine sediments. Heavy metals have bigger chance of remaining in solution when complied with chelating compounds such as specific anions whose concentrations are termed by the pH of the surrounding environment. Metals precipitate as oxides/hydroxides at different areas having different pH and the amphoteric components return to solution at greater values of pH. The hydroxide concentration is then of great landmark for the transfer of metals. Other factors also affect the behavior of the metal ions like redox circumstances and the occurrence of adsorbent sediments (Alloway and Ayres, 1998).
The addition of heavy metals in marine environment has direct consequences to the ecology. According to Rashad (2004), some sources of heavy metal are:

- **Natural sources:** Metals are found in the earth (rocks and soil) and are transferred into the water body through natural processes such as weathering and erosion.

- **Industrial sources:** Industrial practices mainly those concerned with the mining and treating process of metal ores, the finishing and plating of metals and the manufacture of metal items. Metal raw materials which are generally used in other industries as pigments in paint and dye manufacture; in the manufacture of leather, rubber, textiles, paint, paper and chromium factories which are built close to water for shipping and workshops effluents.
- **Domestic wastewater:** Domestic wastewater contains considerable amounts of heavy metals and trace metals. The occurrence of heavy metals in domestic formulations such as cosmetic or cleansing agents, decomposed food wastes, chemicals, is regularly unnoticed.
- **Agricultural sources:** Agricultural wastes usually contain residual of pesticides and fertilizers which have traces of metal.
- **Mining runoff and solid waste disposal areas.**
- **Atmospheric pollution:** Acid rains containing trace metals such as suspended particulate matter (SPM) input to the water body will cause the pollution of water with heavy metals.

2.4 Heavy Metals Uses and Sources in the Environment

Heavy metals arise naturally as they are chemical compounds of the lithosphere and are produced into the environment through volcanism and weathering of rocks (Fergusson, 1990). Though, large-scale addition of heavy metals to the water bodies is commonly a result of human contribution (Mance, 1987). Coastal areas are some of the highest sensitive environments and so far they are theme to growing human pressures for the reason that of increasing urbanization, industrial development and recreational activities. Hence, pollution problems are often increased in the coast due to the nearby land-based pollution sources (Wang *et al.*, 2007).

Industrial processes that discharge a range of metals into water bodies include mining, smelting and refining (Denton *et al.,* 2001). Domestic wastewater, sewage sludge, urban runoff, and leachate from solid waste disposal sites are also obvious sources of heavy metals into rivers, estuaries and coastal waters (Mance, 1987). A proportion of the total anthropogenic heavy metal entrance into the sediments in near coast waters, closer to urban and industrial development areas comes from the combustion of fossil fuels. Additional sources including ports, harbors and mooring sites, also exposed to heavy metal inputs accompanying with recreational, commercial and sometimes associated with navy, boating, and shipping activities (Denton *et al.*, 1997). The uses and sources of heavy metal elements selected for this study are described below:

2.4.1 Lead (Pb)

Lead is a non-essential metal and is a well known historical and contemporary contaminant throughout the world. Lead is the most significant pollutant of the heavy metals, and the inorganic forms are absorbed through ingestion by food and water, and inhalation (Ferner, 2001). Once absorbed, lead accumulates in high concentrations in bone, liver, lung, kidney, and spleen of animals and it goes through the blood–brain barrier and the placenta (Goyer and Clarsksom, 2001). Solid and liquid wastes responsible for more than 50% of the lead discharged into the sea. Lead is usually found in ore with zinc, silver and copper and is extracted together with these metals. The main lead mineral is galena (PbS) which contains about 86.6% lead. Inorganic lead is moderately harmful to aquatic biota and ranks behind cadmium, copper mercury and zinc in the order of toxicity to invertebrates. Organolead compounds, particularly the alkyl-lead compounds are considered toxic to any forms of life. The major sources of lead in natural waters including: manufacturing processes and atmospheric deposition. Other sources include domestic wastewaters, sewage and sewage sludge. Lead is reported to be in the 15-50 μg/g range for coastal water and estuarine sediments overall the universe with <25 μg/g in clean coastal sediments. Food can also be contaminated by naturally occurring leads in the soil as well as by lead from sources such as atmospheric fall out or water used for cooking (Denton *et al.*, 1997).

2.4.2 Chromium (Cr)

Chromium, in the crystalline form, is a steel-gray and hard metal characterized by an atomic mass of 51.996. In nature, chromium do not exists as elementary form but exists in earth crust in many oxidation states. Chromium oxidation states range from -2 to +6, but is most frequently found in the environment in the trivalent (+3) and hexavalent (+6) oxidation states (Eisler, 1986).

Ferrochrome, dichromate and sodium chromate are used in different industrial processes (Kakuschke *et al.*, 2005). Ferrochrome is mainly used for the production of stainless steel. The major uses of sodium dichromate are for the production of chromate salts used for tanning leather, mordant dying and wood preservative; and as an anticorrosive. Chromates are produced by a smelting, roasting and extraction process (Goyer and Clarkson, 2001).

In water bodies Chromium is found as Cr (III) and Cr (VI) as water soluble complex anions. Water contaminations occur by atmospheric pollution as major source. Chromium alloy and metal producing industry or cooling towers, industrial waste discharged into the water (electropainting and metal finishing industries) and runoff from urban areas (Eisler, 1986). Sediment is considered an ultimate source for Cr in marine environments but if the ecological conditions are reversed, the sink can become a source by supplying Cr to the interstitial water and underlying seawater. Adsorption of Cr by sediment is salinity dependent. In seawater concentration of Cr may regulate its uptake, accumulation, and toxicity to marine fishes. This situation is further complicated because Cr is affected by redox conditions of the water column (Eisler, 1986).

2.4.3 Arsenic (As)

For hundreds of years, arsenic, in the form of tasteless and odorless metal, has been viewed by the public as the archetype poison. According to Tinwell *et al.,* (1991) arsenic, in the form of arsenite ion, has been classified by the International Agency for Research on Cancer (1987) as a human carcinogen. The dominant form of arsenic in oxygenated marine waters is arsenate (As V). The more harmful and potentially carcinogenic arsenite (As III) rarely accounts for more than 20% of total arsenic in seawater. Marine algae and fishes accumulate arsenate from seawater, reduce it to arsenite, and then oxidize the arsenite to a large number of organoarsenic compounds. The fishes release arsenite, methylarsonic acid and dimethylarsinic acid to seawater. Dissolved arsenite and arsenate are more toxic to marine phytoplankton than to marine invertebrates and fish. Organs of marine invertebrates and fish contain high concentrations of arsenic, usually in the range of about 1 to 100 mg/g dry weight, most of it in the form arsenobetaine. Organoarsenic compounds are transferred to human consumers by seafood products but the arsenic is excreted rapidly, mostly as organoarsenic compounds (Jerry, 1997).

Total natural releases of arsenic to the environment (about 45,000 metric tons/year) are just about 1.5 times the estimated emissions of arsenic to the environment from human activities (28,000 metric tons/year) (Chilvers and Peterson, 2009).

Atmospheric arsenic contributes little to the arsenic budget of the ocean. Important sources of arsenic in surface waters of the ocean are riverine inputs and upwelling of deep ocean water enriched in arsenic (Cutter and Cutter, 1995). Thus, human activities contribute very little to the arsenic budget of the open ocean, but may be important in coastal waters receiving arsenic-contaminated drainage from the land.

Several countries have regulations for the maximum permissible limits (MPC) of arsenic in seafood consumed by humans. These regulatory limits range from 0.1 mg/kg (Venezuela) to 10 mg/kg (Hong Kong) (Edmonds and Francesconi, 1993).

2.5 Fish as Bioindicators of Water Pollution

A bioindicator is an organism or a part of an organism or a community of organisms that contains information on the quality of the environment (Markert, 1994). Thus, bioindicators should help to describe the quality of environment, to detect and assess human impacts due to environmental hazard and to evaluate restoration or remediation measures. Despite rising several steps of many industrialised countries to reduce toxicants and heavy metals from industrial and motor vehicle exhausts, our ecosystems still contain harmful concentrations of an increasing number of chemicals. They can transfer to water and sediments from which they can be remobilised after changing their physico-chemical state and many of these substances persist for decades (Bryan and Langston, 1992).

Fish are one of the symptomatic factors in water bodies, particularly for the identification of the lethal hazards from human utilizations. Heavy metals taken up by a marine fish are distributed to different organs of the fish, because of the chemical assemblage between them. The selection of aquatic biota depends on several factors such as heavy metal accumulating potential of the organism, motility, economic value along others. Fish are the often preferred as biota due to their big size and because of size, they can be easily indefinable and their ability to accumulate metals, long lifespan, easy to be sample and optimum size for analysis. Therefore fish are valuable as sentinel species and bio-monitor of heavy metal pollution and water quality as they can help to understand the risk to the aquatic ecosystem and to humans (Authman *et al.*, 2015).

2.6 Bioaccumulation and Metabolism of Heavy Metals

Most environmental transformations of metals appear to occur in the soil, sediments, plants, animals and in zones of biological activity in the oceans. Among environmental pollutants, metals are of particular concern, because of their potential toxic effect and ability to bioaccumulate in aquatic ecosystems. Bioaccumulation means an increase in the degree of a chemical element in a living organism over time. Three major modes of bioaccumulation of heavy metals species have been found to occur in the environment: redox transformation between metallic ions, the reduction and methylation and the biosynthesis of organo-arsenic compounds. There is biogeochemical cycling of compounds formed by these processes (Censi *et al.,* 2006). According to Allen (2002) the biomethylated forms of metals are subject to oxidation and bacterial demethylation back to inorganic forms. Heavy metals in water can undergo a complex series of transformations, including redox reactions, ligand exchange and biotransformation. Several studies have shown that the accumulation of heavy metals in fish species is mainly dependent upon the needs, sex, size and molt of marine animals and also upon the water concentration of metals and the exposure period. Other environmental factors such as salanity, P^H, hardness and turbidity also play significant roles in metal accumulation. In aquatic environments, several species of microorganisms make metals biologically available to organisms. Seasonal variations in temperature and water levels could have strong effects on metal concentration and speciation in water due to changes in microbial uptake (Canl *et al.*, 2003).

Heavy metal concentrations in aquatic environment are usually monitored by determining their concentrations in water, sediments and marine fishes (Camuso *et al.,* 1995). These heavy metals generally exist in low levels in water and attain considerable concentration in sediments and biota (Naminga and Wilhm, 1976). Heavy metals including both essential and non essential metals have a exceptional significance in ecotoxicology, since they are highly persistent and all have the toxic effect to living organisms. Small fish become contaminated with the accumulated substances. Predatory fish again, general show higher levels of pollutants than their prey. In the end man, consuming the fish, inevitably suffers from the results of an enrichment taken place at each trophic level, where less is extracted than ingested (Forstner and Wittmann, 1983).

Water organisms, fish and shellfish heap up metals to concentrations much higher than accumulating in water and sediments. Marine fishes develop a protective measure against the dangerous impacts of heavy metals and other contaminants that cause destructive changes like oxidative pressure in the Cichlidae domains are greatly known and highly frugal to fish body (Abouel-Naga *et al.,* 2005). Therefore, it is important to monitor all potential contaminations of the ecosystem and its effect on food webs to ensure the fish quality and safety.

2.7 Heavy Metal Uptake by Fish Organs

Heavy metals enter fish through five main routes such as via food, non-food particles, gills, oral consumption of water and the skin. On absorption, the pollutant hazard is carried in blood stream to either a storage point or to the liver for transformation and/or storage. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat, which is an extra hepatic tissue (Nussey *et al.*, 2000). The concentration of polluted elements in any fish organs depends on its rate of absorption and the dynamic processes associated with its elimination by the fish. Some researchers also researched that active fish tissues, like liver and gill, had higher heavy metal accumulated abilities than fish muscle. The data obtained by many authors showed that heavy metals display different affinity to various organs. The major part of total body loads accumulated at different amounts of metals in the water and at various exposure times are found in liver, kidney and gills (Al-Mohanna, 1994; Kock *et al.*, 1998). High metal concentrations in the digestive tract of fish are related to the dietary uptake route. Some authors observed also considerable concentrations of metals in the digestive tract of fish from natural water bodies (Giguere *et al.,* 2004). So, it is necessary to evaluate the heavy metal and metalloid distribution in different organs of fishes, and to compare the corresponding health risks associated with it.

2.7.1 Fish Liver

The liver plays an important role in entrance and detoxification of heavy metal (Yousafzai, 2004). Fishes are known to contain metallothioneine proteins (Akan *et al*., 2012) which have high affinities for heavy metals and in doing so, accumulate and regulate these metals in the liver. Metallothioneine proteins bind and detoxify the metal ion (Carpene and Vasak, 1989). Liver is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. The liver is a good monitor of water pollution with heavy metals since their concentrations accumulated in this organ are often proportional to those present in the environment. Liver plays important role in metabolism to maintain energy level and structural stability of the body (Nussey *et al.,* 2000). Metal levels in the liver rapidly increase during exposure and accumulate high for a long time of depuration, when other organs are already cleared (Jezierska and Witeska, 2006). The presence of heavy metals in liver tissue often causes oxidative stress and increases of reactive oxygen species (ROS) through induction of O_2 and transformation into superoxide, H_2O_2 (hydrogen peroxide) and OH^- (hydroxyl radical). The hydroxyl radical is able to interact with many compounds. These radicals could transfer energy to other molecules including vital molecules. This may cause damage such as DNA single (SSB) and double strand breaks (DSB), micronuclei, chromosome aberrations in hepatocytes and other cells (Frenzilli *et al.,* 2009). Among other cell injuries caused by free radicals from heavy metals in hepatocytes is lysosomal damage. Fish hepatocytes often have a few numbers of lysosomes and during heavy metal accumulation the number and size of the lysosmes increase to store pollutant and lipids. This results in more damage to hepatocytes (Köhler, 1991; Myers *et al.*, 1987).

2.7.2 Fish kidney

Kidney of fish species are considered as a good indicator of pollution too. During depuration, kidney heavy metal levels remain high or may even increase for some time, which is related to the function of kidneys as excretory organs (Bukola *et al.,* 2015). Kidney is a target organ of heavy metal toxicity. The renal damage by heavy metals depends on the nature, the dose, route and duration of exposure. Both acute and chronic intoxication have been demonstrated to cause nephropathies, with various levels of severity from tabular dysfunctions to severe renal failure leading occasionally to death of fishes (Barbier and Tauc, 2005).

2.7.3 Fish Gill

The gills are considered the main site of entry for the dissolved metals and could also be a significant influence in the total metal levels of the gill. Gills are metabolically active parts that can accumulate heavy metals in higher level. Gills carry out three main functions, viz. gas exchange, ion regulation and excretion of metabolic waste products (Spicer and Weber, 1991). They are sensitive to any change of water components and serve as a good indicator of water quality since gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants. Fish gill surface consists of an epithelial membrane which primarily contains phospholipids covered by a mucous layer (Bolis *et al.,* 1984). Fish gill surface is negatively charged and thus provides a potential site for gill-metal interaction sites for positively charged metals (Reid and McDonald, 1991). The accumulation of metals in gill tissue is usually associated with structural damage to the gill epithelium as well as impaired respiratory and osmoregulatory function. These effects have often been cited as the acute mechanism of metal toxicity (Burton *et al.,* 1972).

2.7.4 Fish Muscle

The muscles provide the power for swimming of fishes. The muscles allow the fish to move in any direction and constitute up to 80 % of the fish itself. Muscle is the most commonly consumed portion of fish (Bashir and Alhemmali, 2015). Muscle is not a metabolically active tissue. Fish muscle is a poor indicator of heavy metal concentration in fish species (Palaniappan and Vijayasundaram 2009). In case of severe water pollution heavy metal tends to accumulate into the fish muscle system and causes many health hazards for human being by entering into the food chain. While, the concentration of heavy metal in fish muscle is always low compared to the other tissues of fish due to their low metabolic activity (Bashir and Alhemmali, 2015), however, it is still important to compare muscles in order to determine the safe levels. This is because the consumption of fish muscle is the greatest mass compared to the other part of the fish. The higher intake of heavy metal in our body can cause bad impact towards health (Joseph *et al.,* 2010).

2.8 Toxic Effects of Heavy Metals Contamination on Public Health

It was showed by Smith (1986) that the toxicity of a metal is commonly defined in terms of the concentration binding to cause a severe response especially death or a sublethal response. The low concentrations of essential metals can be as harmful as high concentrations. Heavy metals can be the reason of serious health effects with different signs depending on the nature and the amount of the metal consumed

(Adepoin-Bello and Alabi, 2005). Environmental contamination by heavy metals influences human health negatively. Their removing process from environment is difficult because of the persistence and non-degradability of heavy metals. The accumulated metal contents in marine sediments can affect the distribution and composition of benthic groupings and this may be accompanying to high concentration noted in living organisms (Pempkowink *et al.,* 1999). The marine environmental pollution due to toxic metals is becoming a worldwide concern. As a result of the increasing concern with the possible effects of the metallic contaminants on human health and the environment, the research on essential, useful and health phases of trace metals in the environment is increased. Concentrations of essential elements in marine fishes are normally homeostatically-controlled, with uptake from the environment regulated according to healthful demand. Effects on the marine biota are noticeable when this guideline mechanism breaks down as a result of either lacking (deficiency) or additional (toxicity) metal (Duffus, 2002). The effects of heavy metal elements selected for this study on human health is outlined as bellow:

Lead is concentrated by benthic bacteria, freshwater plants, invertebrates and fish species (DWAF, 1996). The chronic effect of lead on human includes neurological disorders, especially in the fetus and in children. Children are more sensitive to lead because they absorb more lead than adults. This can causes behavioral changes and impaired performance in IQ tests (Needleman, 1987). The most affected systems by lead are the nervous, the cardiovascular, hematologic and renal. Lead chronic toxicity in human produce low attentional capacity, epigastric pain apathy, irritability, constipation, vomit, convulsions, coma and death. It has been classified as carcinogenic for 2B Group humans while inorganic lead compounds have been classified as carcinogenic for 2A Group humans, mainly related to stomach cancer (IARC, 2014). A notably serious effect of lead toxicity is its teratogenic effect. Lead toxicity also causes inhibition of the synthesis of haemoglobin, affects enzyme activity in the blood and the transport of oxygen around our bodies. It also accumulates in our bones (NIFES, 2016).

Arsenic especially its inorganic forms are significant for contaminants. This element is cumulated in large quantities in food of marine origin (ATSDR, 2000). The International Agency for Research on Cancer (IARC) has classified arsenic to Group I as substance carcinogenic to humans. Inorganic arsenic compounds are also genotoxic and neurotoxic mainly for the developing central nervous system of foetus, infants and young children. In addition, arsenic causes cardiovascular diseases, peripheral vascular disorders, anaemia, immune and reproductive system disorders together with an impairment of metabolic cellular processes through enzymatic inhibition (Tchounwou *et al.,* 2004; ATSDR, 2000). Ingested As may go through the placental barrier affecting the fetus, it is transported in the blood joined to the red cells and it is distributed throughout the body. The signs of As toxicity are skin changes and wart or callus formation on palms or soles, along interspersed hyperpigmentation areas on the face, neck, and back, vomit, diarrhea, cramps, salivation, fever, cardiovascular disorders, and it may lead to death . Arsenic toxicity mainly related to related with lung, kidney, bladder, and skin cancer (Kakkar and Jaffery, 2005).

Chromium is an essential micronutrient for fish, animals and plants. Fish are usually more resistant to Cr than other aquatic organisms. They can be affected sub-lethally when the concentration increases (Krishna *et al.*, 2014). The average level of Cr in marine water has been reported between kidney failure; in the lungs, it causes fibrosis, cardiovascular system risks due to the increment of cholesterol and free fatty acids in the blood, increasing the risk of aortic and coronary atherosclerosis, although the mechanisms and Cr relation to dyslipidemia which is mainly due to the reduction of the High Density Lipoproteins (HDL) and to the high triglyceride and HDL proportion. Furthermore, Cr also affects children central nervous system causing neurological disorders, learning problems and hyperactivity. It has been classified as carcinogen for group 1 humans and it has been mainly related to lung, prostate, pancreas, kidney, and bladder cancer (IARC, 2014).

2.9 Previous Works Done Relevant to the Present Study

In many studies, fishes were the subject of investigation on heavy metal accumulations and monitoring programs in seas or fresh water, due to their importance in human nutrition (Sioen *et al.,* 2007). Such interest aimed at ensuring the safety to the food supply and minimizing the potential hazard effect on human health. Some of the important documented contributions relevant to the present study are as follows:

Mukherjee *et al.,* (2011), studied concentration of Cadmium, Mercury and Arsenic in muscle tissue of six marine fish species collected from north eastern Bay of Bengal, India. There was considerable variation of arsenic levels among the fish species from Bay of Bengal. Comparatively higher concentration of arsenic was observed in *Hilsa Ilisa*. They reported that the observed average concentration under in different species was *Harpadon nehereus* > *Formio niger* > *Hilsa ilisha* > *Rastreliger kanagurta* > *Pampus argentius* >*Daysciaena albida*. The result of this study revealed that consuming fish from north eastern Bay of Bengal may not have harmful effects because observed values of dietary intake of heavy metals were far below the permissible PTWI and PTDI limits for human consumption.

Jothi *et al.,* (2018) investigated the concentration of heavy metals in fish and water samples collected from different locations of Chattogram District, Bangladesh. The concentrations of heavy metals including Cr, Cu, Ni, Pb and Fe in sea water and marine fishes (pama croaker, bombay duck and rat-tail anchovy) were detected by using Air / Acetylene Flame Atomic Absorption Spectrophotometer. The distribution of heavy metals in fish samples analyzed were in the order of magnitude as Fe > Cr> Cu> Pb> Ni. Among all samples, 17% fish samples had higher amount of Cr whereas acceptable limit is 15.0 mg/kg for fish. Mean concentrations of Pb in all fish samples were 17% below detection level, 33% optimum and 33% higher than the maximum permitted concentrations (1.5 mg/Kg). Fe in all fish samples were 33% below, 17% optimum and 50% higher than the maximum permitted concentrations (43 mg/Kg) recommended by FAO/WHO.

Chakraborty *et al., (*2016) analyzed the concentrations of zinc, copper, cadmium and lead in the muscle of the Hilsa fish in the lower stretch of the river Ganga and coastal west Bengal during April, 2015. Heavy metals in the muscle of the investigated fish species were compared with the permissible levels for human consumption. They recommended that all the selected heavy metals except Pb were below the permissible level confirming the species mostly suitable for human consumption.

Concentrations of five heavy metals, arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) were determined in water, sediments and in marine fish, the Indo-Pacific king mackerel by Sujatha *et al.,* (2016). The samples were collected near the seashore of the Bay of Bengal from five different locations in North Tamilnadu. The maximum concentrations of heavy metals observed in fish were arsenic (0.382 mg/Kg), cadmium (0.441 mg/Kg), chromium (0.711- mg/Kg), lead (0.673 mg/Kg) and mercury (0.08 mg/Kg). Maximum heavy metal concentrations in water are arsenic (0.03 mg/L), chromium (0.046 mg/L), lead (0.015 mg/L) and mercury (0.016 mg/L). So, all metals were higher than the safety values.

In India, Oza and Muralidharan (2018) performed a study to estimate the levels of lead in liver, muscle, gill and brain tissues of *Harpodon nehereus* during different seasons of year 2016-2017 collected from Sassoon dock, Mumbai coast of Maharashtra. The concentration of lead in the work was found to be more than the maximum permissible limits in gill and liver tissues of fish. They reported that during the pre-monsoon season it was found to be higher than the acceptable values for human consumption set by FAO in all the selected tissues. Results showed that the highest concentration of lead in muscle, liver and gill tissue was also obtained during the post monsoon period.

Concentrations of Cr, Pb, Mn, Fe, Cd, Hg and As in muscle tissue of fish species collected from North East coast of India were determined by Bhupander Kumar *et al*, 2012. The bioaccumulation of Fe, Pb, Cr and Mn was predominant followed by As, Hg and Cd in muscle tissue of coastal fishes. The concentration of heavy metals was species specific and significantly different. They reported that comparatively higher concentrations of heavy metals were accumulated in *Trichiurus trichiurus*, *Pampus argentius*, *Harpadon nehereus* and *Arius* sp. *f*ollowed by *Daysciaena albida*, *Formio niger*, *Hilsa ilisha* and *Rastrelliger kanagurta.* The order of heavy metal concentration was observed as: Fe>Pb>Cr>Mn>As>Hg>Cd. Concentration of Mn in fish tissue was higher than WHO/FAO guideline values, but other metals were lower than certified values.

A study was conducted to determine the accumulation of heavy metals (zinc, copper, iron, cadmium and lead) in various organs of four commonly consumed fish (*Euthynnus affinis*, *Pampus Chinensis*, *Descapterus macrosoma* and *Leiognathus daura*), prawn (*Fenneropenaeus indicus*) and crab (*Portunus pelagicus*) of Tok Bali Port, Kelantan, Malaysia. Health risk was assessed using estimated daily intake and target hazard quotients. Although the concentrations of all the heavy metals in all fish, prawn and crab species were lower as per Malaysian Food Act, but the concentrations showed remarkable differences among the species and organs. The concentration of heavy metals in the gill was the highest of all fish species followed by in the liver and flesh. The total accumulation of heavy metals was maximum in *Euthynnus affinis* followed by *Leiognathus daura, Descapterus macrosoma* and *Pampus chinensis* of the fish species (Salam *et al.,* 2019).

In a study, concentration of Chromium, Lead, Zinc and Mercury were determined in the muscles, gills and liver of ten fish as well as three specimens of crustaceans and two Specimens of squids collected from Jeddah coastal water. The obtained results declared that, the average concentrations of heavy metals were as follows: Cr (0.098, 0.20, 0.106), Pb (0.3, 0.257, 0.196), Zn (3.00, 7.390, 4.999) µg/g wet weight in the muscle, gills and liver, respectively. While, the concentration of Hg was invariably undetectable in all samples of different organs of the collected fish species. The average concentration of Cr, Pb, Zn and Hg in the soft part of the investigated crustaceans and squids were relatively higher compared with the muscle tissues in the examined fish species (Younis *et al.,* 2014).

Concentrations of 10 heavy metals (Cu, Zn, Cd, Pb, Fe, Mn, Cr, Se, As, and Hg) were determined by Khalid *et al., (*2015) in different organ tissues of four selected common red sea fish species viz., *Pampus chinensis*, *Cetoscarus pulchellus*, *Plectorhynchus schotaf and Epinephelus spp*. There was a highly significant (P< 0.01) difference among the 4 fish species and between organs for the accumulation of all 10 metals. The concentration of Pb was highest closely followed by Zn, whereas Cr was detected in the lowest concentration. The liver accumulated the highest concentration of metals and muscles had the concentration of all studied metals. It has been observed that *Pampus Chinensis* species accumulated the highest concentration of total analyzed elements in this study, which indicate that this species have more potential to accumulate all of metals in each tissue.

In Pakistan, a study was conducted by Mahboob *et al.,* (2016) which was aimed to evaluate the effect of heavy metals on an important tissue of two fish species *Cyprinus carpio* and *Wallago attu*, sampled from Mianwali District, Pakistan. The concentration of selected heavy metals Pb, Fe, Cr, Cu in gills, muscles, kidney and liver was compared with an International standard of food fish. The overall metal concentrations among different weight categories in *Cyprinus carpio* were in the order of Fe > Cu > Cr >Pb. In *Wallago attu* the overall accumulation of these metals were, in order of Fe > Cu > Cr > Pb. The order of accumulation of metals in gills and muscle of *Cyprinus carpio* was Fe > Cr > Pb > Cu; kidney and muscles of *Wallago attu* was Fe > Cr > Cu > Pb; liver Fe > Cu > Cr > Pb. There was a significant difference in the accumulation of heavy metals in different organs of both species ($p<0.01$). All studied heavy metals except Cr were within permissible limits described by various international agencies like WHO, FAO and FEPA in edible tissues of *Wallago attu* and *Cyprinus carpio.*

To investigate the occurrence of heavy metals in marine fish species from the South China Sea, 14 fish species were collected along the coastline of Hainan China and examined for species- and tissue-specific accumulation. Levels of Cu, Zn, Cd and Cr were found to be higher in the liver and gills than in muscle, while Pb was preferentially accumulated in the gills. Differing from other heavy metals, As did not exhibit tissues pecific accumulation. Human dietary exposure assessment suggested that the amounts of both Cr and As in marine wild fish collected from the sites around Hainan, China were not compliant with the safety standard of less than 79.2 g d-1 for wild marine fish set by the Joint FAO/WHO Expert Committee on Food Additives (Jin-Ling *et al.*, 2015).

CHAPTER 3
MATERIALS AND METHODS

3.1 Sample Collection

The samples (three individuals of each three species) were collected from the caught fishes by fishermen's nets from the Chattogram coast of Bay of Bengal and some were collected from local market at three different times. Then the collected fish species were transported 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. The total length (cm) and weight (g) of fishes was measured in every case.

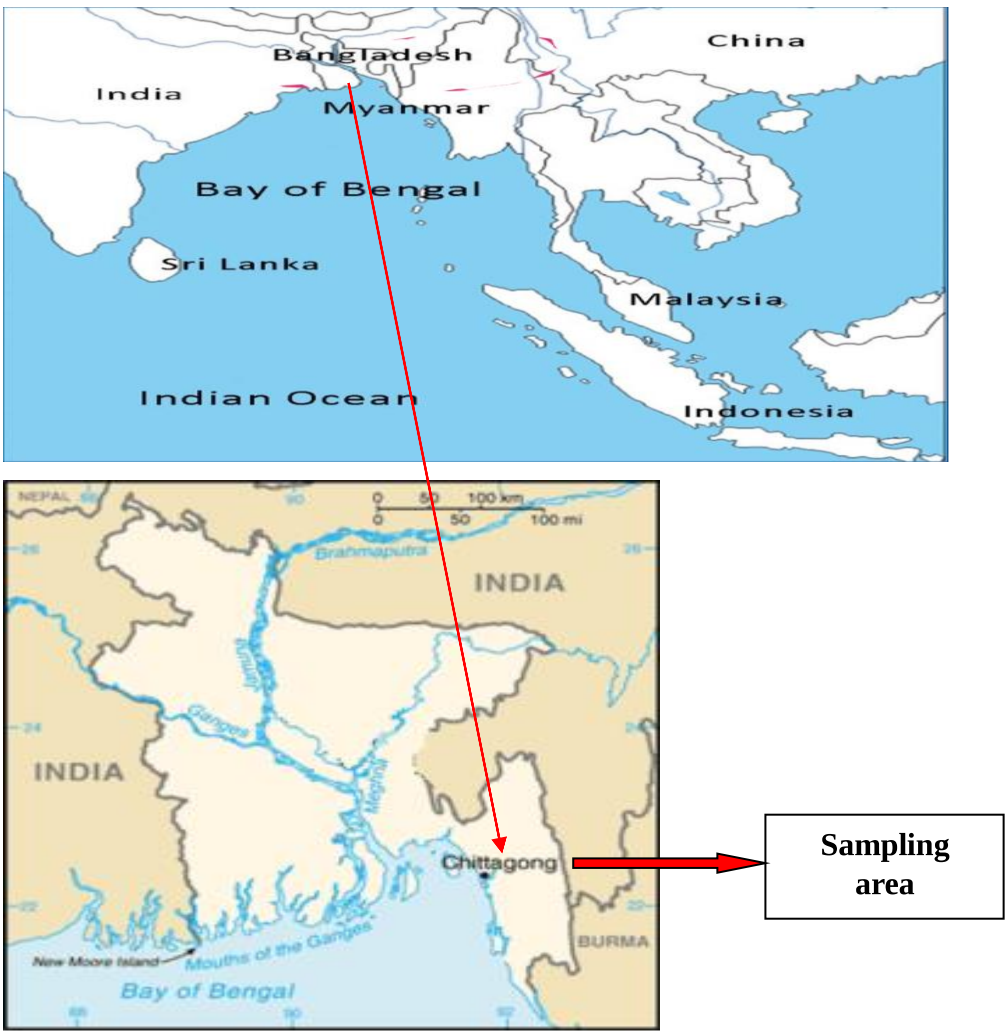

Figure 1: Sampling Area

3.2 Dissection and Preparation for Digestion:

Each of the collected fishes was dissected for its muscle, gill, liver and kidney tissues. All the final sample preparation was carried out according to the procedure described by UNEP Reference Methods (1984).

3.3 Heavy Metal Analysis:

Heavy metals (As, Pb and Cr) were analyzed in a graphite furnace (GBCGF 3000 with Zeeman background corrector) with an auto sampler.

3.3.1 Apparatus

A) Atomic Absorption Spectrophotometer (AAS) (Phillips AAS with double beam and deuterium background corrector).
B) Hollow cathode or electrodeless discharge lamps.
C) Microwave oven.
D) Teflon digestion vessels 100ml, withstanding a pressure of at least 1.4 MPa.
E) Volumetric flask.
F) Funnels.
G) Plastic bottles.
H) Drying oven.

3.3.2 Reagents

A) Deionized water.
B) Nitric acid 65% (w/w).
C) Perchloric acid 30% (w/w).

3.3.3 Procedure

A) Pre-treatment: For analyzing metals, fish organs were preserved with 10% formalin solution after dissection.
B) Drying: Fish organs were then dried in drying oven at 105°c to constant weight. The various organs of each species collected were pooled and milled with a mortar and pestle. They were put in dry labeled plastic containers and stored in desiccator until digestion.
C) Digestion: 0.75 g dry fish organs are weighted into digestion vessel. Then 5 ml 65% HNO_3 and 2ml 30% perchloric acid were added. Vessels were then closed and placed in holder. Vessel holder then placed in digestion chamber

and exposed to defined program parameters 250 watt for 3 min, 630 watts for 5 min, 500 watts for 22 min and final 0 watts for 15 min. Then removed digestion vessels from digestion chamber and cooled thoroughly before opening them. Vessels were then opened transferred to 25 volumetric flask and dilute to mark with deionized water. Then solution transferred to plastic tube.

D) AAS determination: The concentration of Pb, Cr and As were determined by flame techniques in AAS.

E) Calibration curves: A calibration curve is used to determine the unknown concentration of an element. The instrument is calibrated using several solutions of known concentrations. A calibration curve is produced which is continually rescaled as more concentrated solutions are used the more concentrated solutions absorb more radiation up to a certain absorbance. The calibration curve shows the concentration against the amount of radiation absorbed. Calibration curves were plotted for each of metal standard solution.

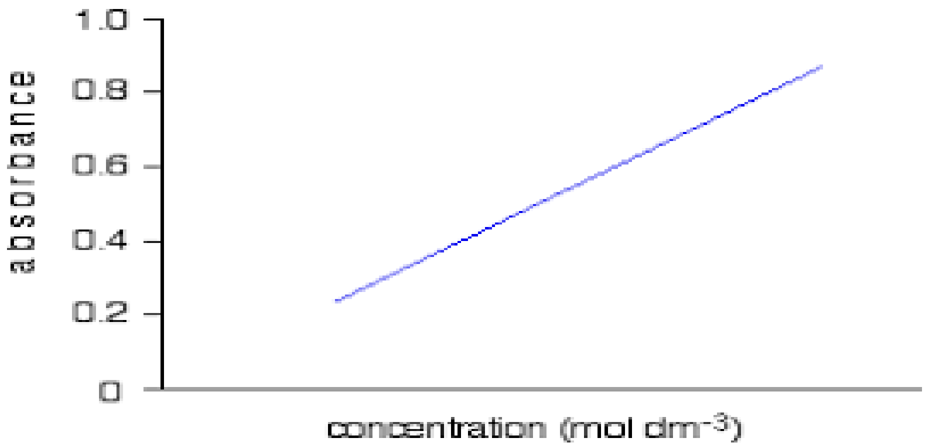

Figure 2: Calibration Curve of detecting unknown metal concentration

When sample solution fed into the instrument and the unknown concentration of the element then displayed on the calibration curve. All digested samples were analyzed three times for each metal. The instrument was calibrated with standard solutions prepared from commercial materials. Analytical blanks were run in the same way as the samples and determined using standard solutions prepared in the same acid matrix.

3.4 Data Analysis:

Statistical analysis of data was carried out using SPSS statistical package program. One-way analysis of variance (ANOVA) and Duncan multiple range tests were used to assess whether metal concentrations varied significantly among species & organs. The comparative accumulation of Pb, Cr and As in each species was demonstrated by using Microsoft Excel software. A 5% level of significance was used.

CHAPTER-4

RESULTS

This chapter presents the findings of heavy metal concentrations in three selected fish species (*Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha*).

4.1 Organ-wise Lead (Pb) Concentration:

When we consider the organs of all three fishes, highest concentration of Lead was estimated in Kidney (0.041467 ppm) and the second best value was recorded in gill (0.041422 ppm). The recorded values in Liver (0.014878 ppm) and Muscle (0.020822ppm) were significantly differed from the above mentioned two organs.

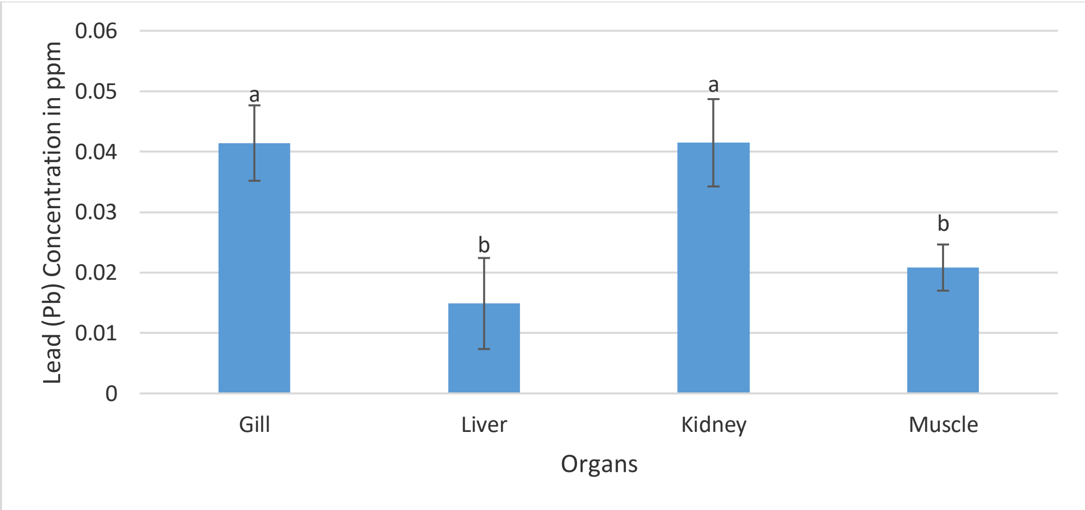

Figure 3: Lead Concentration in Fish Organs and Muscle.

4.2 Organ-wise Chromium (Cr) Concentration:

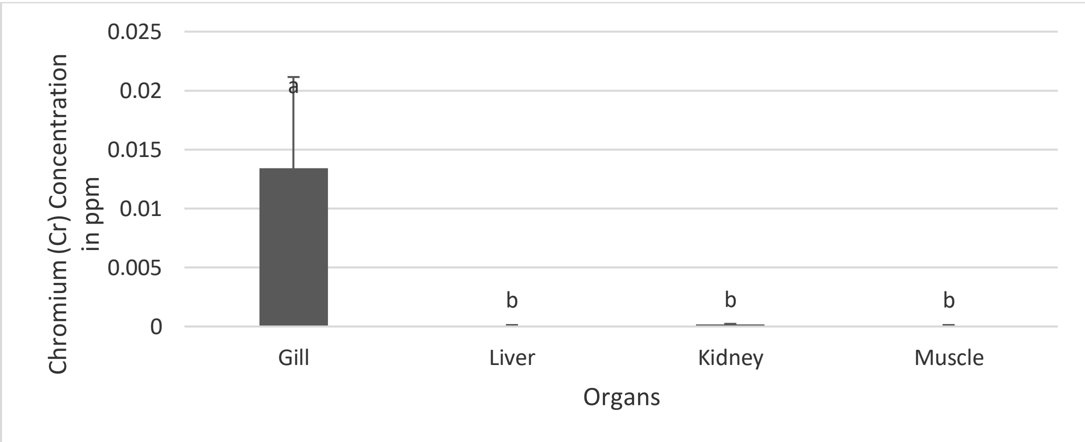

Figure 4: Chromium Concentration in Fish Organs and Muscle

The chromium concentration was found highest in gill depicting a value of 0.013422 ppm with a very lower value in liver, kidneys and muscles resembling 0.000089ppm, 0.000167ppm and 0.000089 ppm respectively which are statistically significantly different in comparison with gill.

4.3 Organ-wise Arsenic (As) Concentration:

The concentration of arsenic (0.046256 ppm) was found highest in kidney with insignificant statistical difference liver (0.043722 ppm). The lowest value observed in muscle (0.021133 ppm) with significant difference from above mentioned two whereas the recorded value in gill are in a middle position statistically in comparison with the above three organs (0.040033ppm)

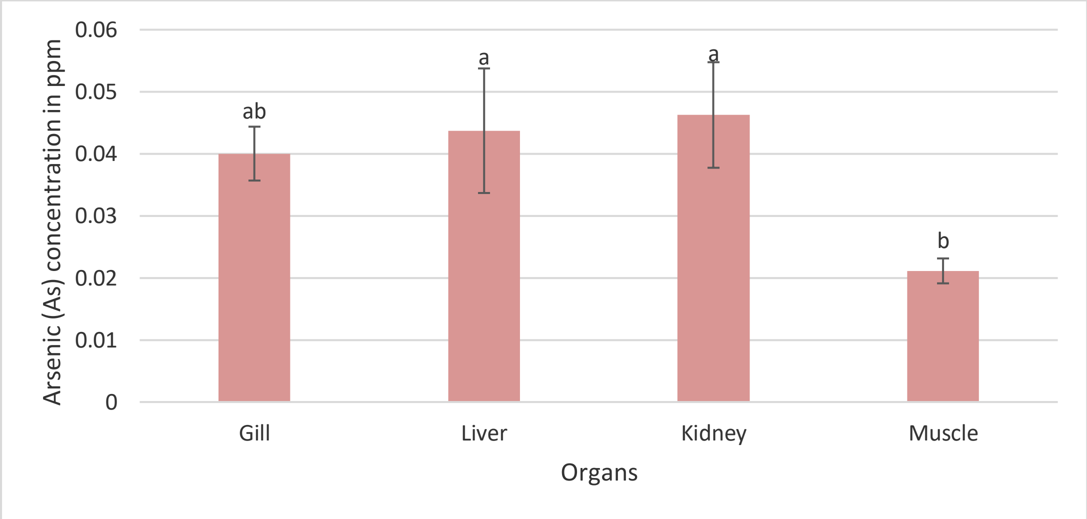

Figure 5: Arsenic Concentration in Fish Organs and Muscle.

4.4 Species-wise Lead (Pb) Concentration:

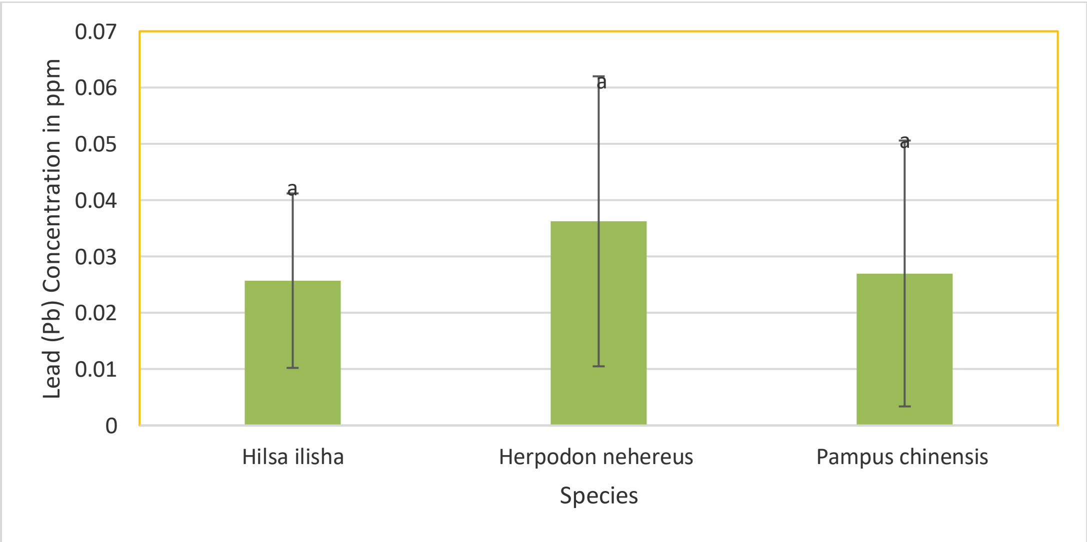

Figure 6: Lead Concentration in Different Marine Fish Species.

The values of lead were found to be the highest in *Herpodon nehereus* (0.036250 ppm) followed by in *Pampus chinensis* (0.026983 ppm) and in *Hilsa ilisha* (0.025708 ppm) respectively. The obtained data base was not statistically significantly varied.

4.5 Species-wise Chromium (Cr) Concentration:

The concentration of chromium in different fishes was found to be lower than the values of lead with maximum to be recorded in *Herpodon nehereus* (0.004725 ppm). The values were not significantly different in other two species- *Pampus chinensis* and *Hilsa ilisha* respectively (0.004375 ppm and 0.001225 ppm).

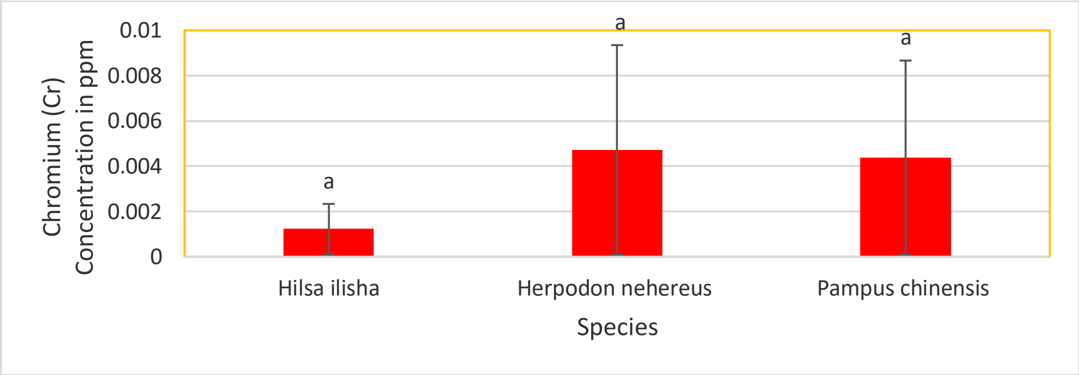

Figure 7: Chromium Concentration in Different Fish Species.

4.6 Species-wise Arsenic (As) Concentration:

The Arsenic concentration was also recorded highest in *Herpodon nehereus* (0.049258 ppm) followed by *Pampus chinensis* (0.032567 ppm) and *Hilsa ilisha* 0.031533 ppm). The concentration of Arsenic was found to be higher than Lead and Chromium.

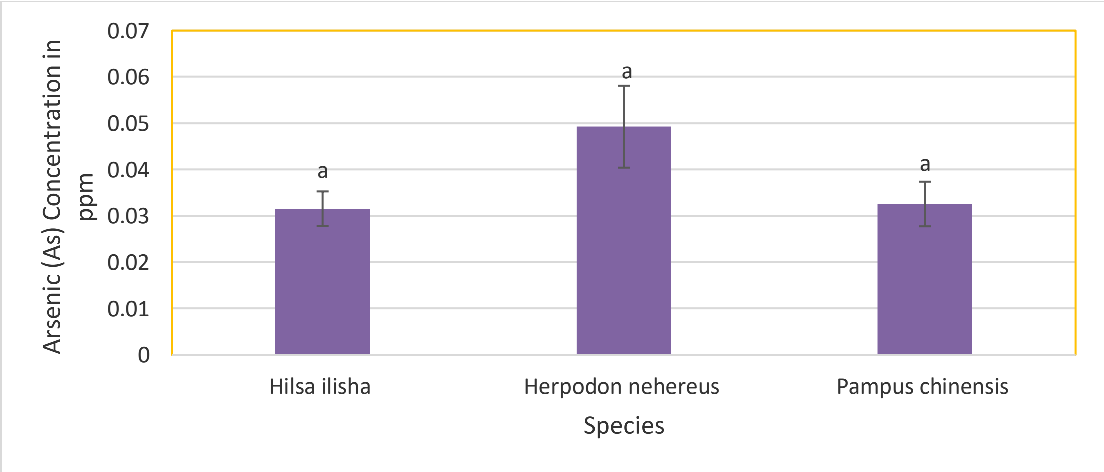

Figure 8: Arsenic Concentration in Different Marine Fish Species.

4.7 Different Heavy Metal Concentration in *Hilsa ilisha*:

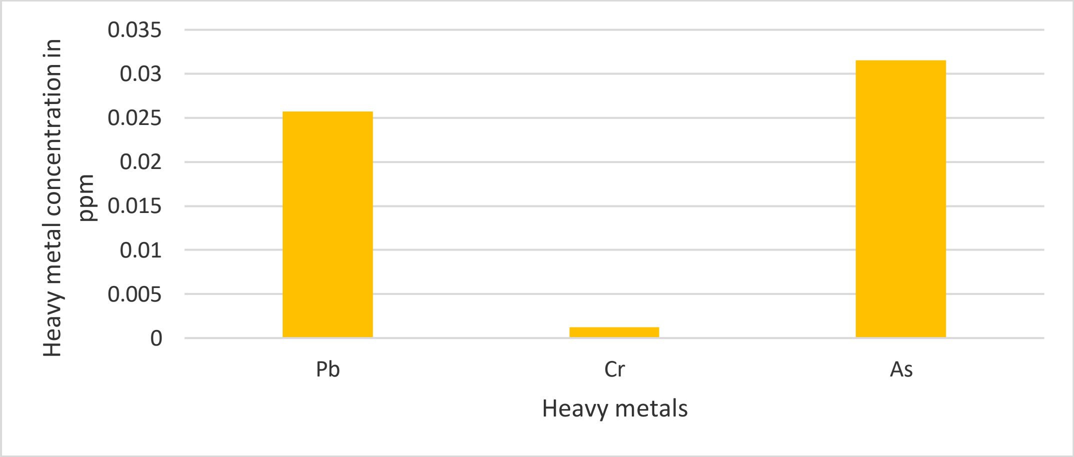

Figure 9: Different Heavy Metal Concentration in *Hilsa ilisha*

The recorded values in *Hilsa ilisha* revealed that the higher concentration is observed in case of arsenic (As) with a mean value of 0.031533 ppm which is higher than the recommended value of 0.01 ppm (WHO/FAO, 2005). The mean average value of Chromium was the least among the three investigated heavy metals (0.001225 ppm) which is far lower than the standard value (0.1 ppm) of Chromium (WHO/FAO, 2005). The average value of lead was observed (0.025708 ppm) which is also lower than the standard value of 0.3 ppm by WHO/FAO (2005).

4.8 Different Heavy Metal Concentration in *Herpodon nehereus*:

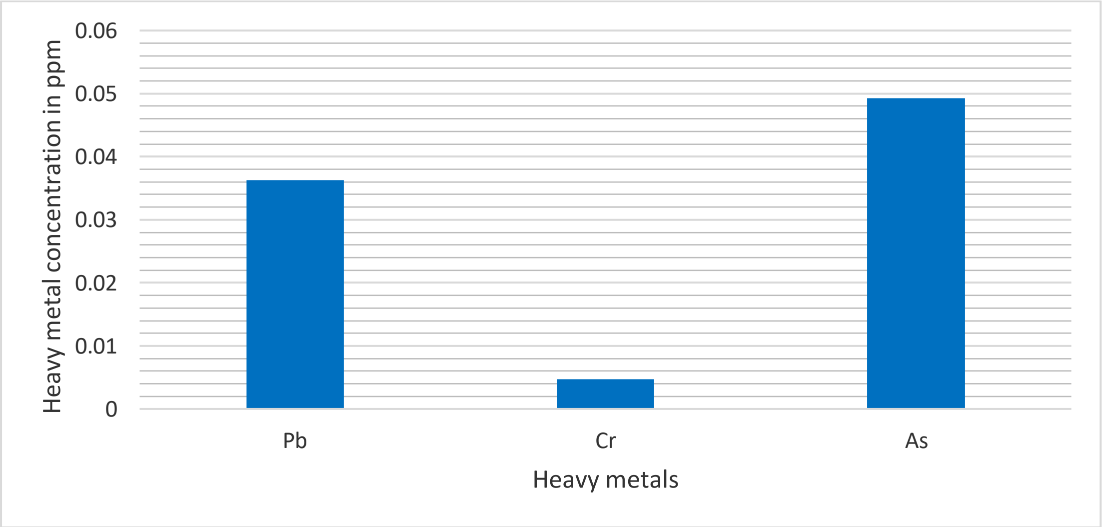

Figure 10: Different Heavy Metals Concentration in *Herpodon nehereus*

The recorded values in *Herpodon nehereus* expressed that the higher concentration is observed in case of arsenic (As) with a mean value of 0.049258 ppm which is higher than the recommended value (0.1 ppm). The mean average value of Chromium was the least among the three investigated heavy metals (0.004725 ppm). The average value of lead was observed (0.031533 ppm). The values of Cr and Pb are lower than the standard value (0.1 ppm and 0.3 ppm respectively) issued by WHO/FAO (2005).

4.9 Different Heavy Metal Concentration in *Pampus chinensis*:

The observed values in *Pampus chinensis* demonstrated that the higher concentration is observed in case of arsenic (As) with a mean value of 0.032567 ppm which is higher than the recommended value 0.01 ppm. The mean average value of Chromium was the least among the three investigated heavy metals (0.004375 ppm) which is far lower than the standard value of 0.1 ppm. The average value of lead was observed (0.032567 ppm) which is also lower than the standard value (0.3 ppm) given by WHO/FAO (2005).

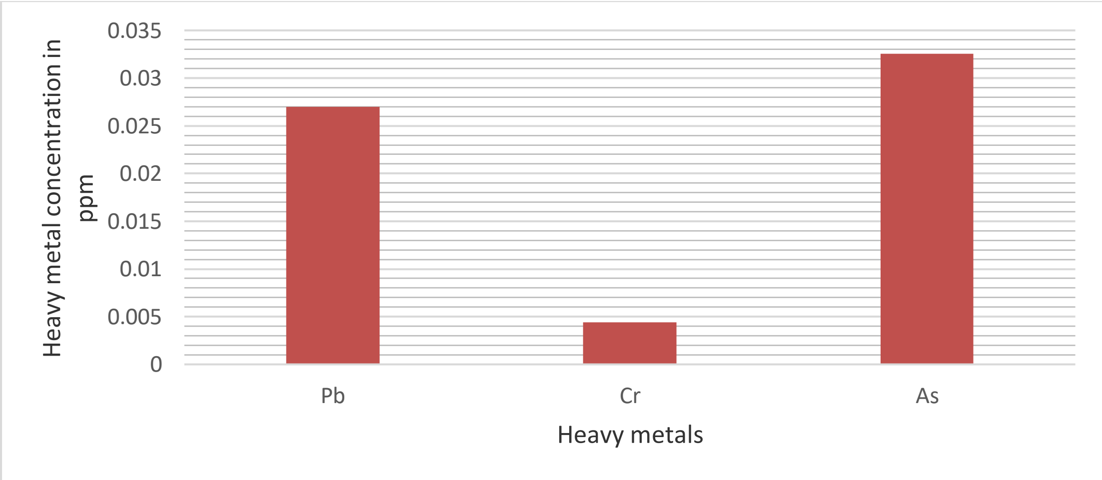

Figure 11: Different Heavy Metals Concentration in *Pampus chinensis*.

4.10 Species –Wise Different Heavy Metal Concentration:

Among all the three investigated heavy metals arsenic was found to be the highest in all three investigated species. The results revealed as the values of arsenic is higher than the recommended values of human intake in all the three cases of fish, it is of high priority to undertake necessary actions. The recorded values in case of lead and chromium are within the safety levels.

Table 1: Heavy Metal Concentrations in Different Fish Species

Species	Lead (Pb)	Chromium (Cr)	Arsenic (As)
Hilsa ilisha	0.025708[a]	0.001225[a]	0.031533[a]
Herpodon nehereus	0.036250[a]	0.004725[a]	0.049258[a]
Pampus chinensis	0.026983[a]	0.004375[a]	0.032567[a]
WHO limit	0.3 ppm	0.1 ppm	0.01 ppm

NOTE: WHO= World Health Organization. Results are means of triplicates. Mean followed by different superscript in each column and row are significantly different by the Duncan multiple range test ($p<0.05$).

4.11 Organ –Wise Different Heavy Metal Concentration:

In terms of organ the heavy metals are mostly concentrated in the kidney tissues. But the concentration in muscle is found to be the lowest which is a positive finding as we consume the muscles widely even though the concentration of Arsenic in muscles was recorded higher than the safety values.

Table 2: Heavy Metal Concentration in Different Fish Organs

Organs	Lead (Pb)	Chromium (Cr)	Arsenic(As)
Gill	0.041422[a]	0.013422[a]	0.040033[ab]
Liver	0.014878[b]	0.000089[b]	0.043722[a]
Kidney	0.041467[a]	0.000167[b]	0.046256[a]
Muscle	0.020822[b]	0.000089[b]	0.021133[b]

NOTE: Results are means of triplicates Mean followed by different superscript in each column and row are significantly different by the Duncan multiple range test ($p<0.05$).

CHAPTER-5
DISCUSSION

This study provided valuable information of As, Cr and Pb distribution in different organs (gill, liver, muscle and kidney) of various fishes (*Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha*) captured from Chattogram coast of Bay of Bengal. These fish species were selected as they are most commonly consumed and commercially important marine fish in Bangladesh. Cr, Pb and As were chosen because these are common detrimental elements found in industrial discharges and have elaborate biological response if bioaccumulated even at low concentration. This study also investigated the relationships among heavy metal concentrations in *Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha*. It was observed that there was no statistical difference in case of heavy metals (Pb, As and Cr) for all three fish species (Table 1). The correlation among the heavy metal concentrations in different organs of fish species was statistically analyzed. The presence of three heavy metals was statistically varied in different organs of fish species (Table 2).

Essential metals and non-essential metals have been demonstrated to accumulate along the trophic chain in coastal water ecosystems. Non-essential metals do not have any metabolic function although, as a consequence to their bioaccumulation in fish, these metals can be toxic for humans, even at very low concentrations (Anwar *et al.*, 2009). The heavy metals concentration in fish is important both with respect to nature management and human consumption.

In fish samples, according to analysis results, the following findings were obtained for the concentration ranges of the metals: Cr: 0.001225– 0.004725 ppm; Pb: 0.025708–0.036250 ppm; As: 0.031533-0.049258 ppm. Heavy metal concentrations in *Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha* were decreased in the sequence of As> Pb> Cr. Heavy metal concentrations varied among different species as well as between species. Among different observed values of heavy metals, we found that arsenic concentration in different experimental fishes were much higher than any other values whereas Cr showed lowest values in all experimental fishes (Table 1). This finding was being Contradictory to the work of (Minareci *et al.*, 2009).

Concentrations of Cu, Zn, Mn, Fe, Cr, Pb and As in muscle tissue of fish species collected from North East coast of India were determined by Bhupander *et al.,* (2012). They found higher concentration of Arsenic accumulation was observed in *Harpadon nehereus* (0.91 µg g-1), Arius sp. (1.22 µg g-1) and *Formio niger* (0.93 µg g-1). however, lower concentrations were accumulated by *Hilsa ilisa* (0.05 µg g-1) which is similar to this study.

The Chattogram coastal water might be highly polluted with Arsenic. It could be associated to effluent discharges from a major industrial and siderurgic plant near the sea and to agro-industrial activities, mainly run off from agricultural soils where phosphate fertilizers are used and other waste. The maximum permitted arsenic concentrations of fish tissues was exceeded WHO and FAO limit in most cases, indicating the low water quality of Chattogram coastal area. The average concentration of arsenic in muscle of three fish species is 0.021133 which is higher than the standard values of arsenic given by WHO/FAO (Figure 5). Arsenic is a great threat to life if present in substantial quantity and has no known function in biochemical processes. Once ingested by fish, arsenic is absorbed from GI tract and enters circulation. Arsenic rapidly leaves the blood and is distributed to other tissues (Kakkar and Jaffery, 2005).

Another important result of this study was a high As concentration in the kidney and liver, 0.046256 ppm and 0.043722 ppm, respectively (Figure 5). Arsenic concentration in liver and kidney found statistically different from gill and muscle arsenic concentration. This might be related to the feeding behavior of fish species which feeds on decayed organic matter. Fishes normally endure to high pollutant exposure as they can develop pollutant-sequestering detoxifying systems which make the metal relatively inert and not toxic to the organism (Weis, 1989).

In case of all three fishes, highest lead concentration was estimated in kidney (0.041467ppm) and the second best value was recorded in gill (0.01422ppm). The recorded values in Liver (0.014878 ppm) and Muscle (0.020822ppm) were significantly different from the kidney and gill (Figure 3). When lead accumulates in the human body, it replaces calcium in bones (DHSS, 1980). Oza and Muralidharan (2018) found lead concentration more than the maximum permissible limits in gill and liver tissues of fish, *Harpodon nehereus* in the west coast of India during the monsoon season. During the pre-monsoon season it was also found to be higher than the acceptable values for human consumption set by FAO in all the selected tissues.

A study was conducted by Kebede and Wondimu (2004) reported higher lead concentrations in the gills compared with the muscles in O. niloticus. In fish, kidneys are considered to be the target site for contaminant uptake because of their anatomical and physiological properties that maximize absorption efficiency from water. The findings of this study are contrary to (Farombi *et al.*, 2007) who reported that lead was greater in liver as compared to the gills and kidney.

For all three marine fishes, chromium concentration was found highest in gill with a value of 0.013422 ppm with very lowest values in liver, kidneys and muscles resembling 0.000089ppm, 0.000167ppm and 0.000089 ppm respectively which are statistically different in comparison with gill. An MSDS (2006) report showed that in fish in general, accumulates chromium primarily in the gill, liver, kidney and bone. The adsorption of metals onto the gill surface could also be an important influence in total metal levels of the gill (Canli and Furness, 1993). This observation agrees with those of Amundsen *et al.,* (1997) freshwater fish species from the border region between Norway and Russia.

The data in Table 2 shows that metal concentrations in the kidney were highest among different tissues of marine fishes following the order: Kidney> liver > gill > muscle. The total mean of three metal concentrations (mg/kg in wet weight basis) in kidney was 0.031 ppm whereas it was only 0.0140 ppm in muscle tissues. Contradictory results were obtained by Taghavi Jelodar *et al.*, (2011). They found that the mean concentrations of Cr and Pb in the muscle higher than other organs of *Liza aurata* from southern part of the Caspian Sea, were 1070 and 2600 μg/kg respectively. In another study was performed in Persian Gulf near the Iran coast, by Hossein Khezri *et al.,* (2014), Cr and Pb concentrations in the fish muscle were observed between 36-107 μg/kg and 264-1188 μg/kg respectively. Muscles often contain the lowest concentrations (Chen *et al.,* 2004; Alcorlo et al., 2006), which is of special relevance for human health, since muscles constitute the edible part of the fish. According to Goel (1996) heavy metals are accumulated by the digestive system through ingested food, particulates and water, so a high concentration might be found in kidney. although many organisms can tolerate high concentrations of essential or non-essential metals by accumulating them at non-active sites like bone, feathers or exoskeleton, scales, gills and intestine of fishes.

Herpodon nehereus showed high accumulation of arsenic in liver (0.271991ppm) but *Pampus chinensis* and *Hilsa ilisha* (0.249 ppm and 0.02243 ppm) showed high accumulation in kidney (Appendix E and G). It is well known that considerable metallothionein induction occurs in the liver tissue of fishes. A report revealed that heavy metals induce the synthesis of metal-binding protein metallothionein in the liver of *Oreochromis niloticus*. Most of metals or metalloids studied so far exhibit the highest concentrations in the liver or kidneys of fish species (Cheung *et al.,* 2004).

The difference in the levels of accumulation in different organs of a fish can be attributed to the differences in the physiological role of each organ. Several factors such as regulatory ability, behavior, ecological needs, swimming behaviors and the metabolic activities among different fish species may play a significant role in the accumulation differences in the different organs (Kalay *et al.,* 1999). Also the chemical nature of the metals ionic strength and pH tends to be a main variable in the accumulation process. In acidic conditions, there are enough hydrogen ions to occupy many of the negatively charged surfaces and little space is left to bind heavy metals, hence more heavy metals remain in the soluble phase. The soluble form of metals is thought to be more toxic because it is more easily transported and more readily available to aquatic organism (Ishaq *et al.,* 2011).

It was observed that *Herpodon nehereus* showed the highest metal accumulation value compared to the other two species (Table 1). With the highest level of three heavy metals in *Herpodon nehereus,* the lowest lied in the *Hilsa ilisha*. Heavy metal concentrations were decreased in the following order: *Herpodon nehereus >Pampus chinensis* > *Hilsa ilisha.* As *Herpodon nehereus* occupies a higher trophic level of the food chain, the bioaccumulation process may increase the concentration of heavy metals in their bodies. According to Goel (1996) the bio- accumulation mechanism involves uptake of the heavy metal with food taken by marine fishes. The food is digested, assimilated and excreted but the heavy metal accumulates in the organs of the fishes. As the assimilation efficiency of each trophic level is not more than 10%, each higher trophic level consumes 10 times more than it's immediately preceding trophic level to gain the same weight. This results 10-fold increase in concentration of heavy metals as the food passes through from one trophic level to another.

Hilsa ilisha revealed higher concentration of Arsenic (As) with a mean value of 0.031533 ppm which exceeded the recommended value of 0.01 ppm (WHO/FAO, 2005). The average value of lead was observed (0.025708 ppm) which is also lower than the standard value of 0.3 ppm by WHO/FAO (2005). A previous study has found that the content of arsenic in the liver is higher than that of muscles (Chen *et al*., 2004). Because fish muscle does not have direct contact with the arsenic and the muscle is not active part of detoxification. The study on toxicity of fish has shown that arsenic is absorbed primarily through gills in the aqueous phase, and thus the accumulation is higher in the gills (Ventura-Lima *et al*., 2009). Chromium plays an important role in body function (metabolic function, co-factor of insulin) in trace amount but it turn to be toxic when it exceeds the tolerance limit. The daily requirement of chromium for adult is estimated between 0.02 to 0.5 mg/day. The permissible limit of chromium in fish is 0.1 ppm (WHO/FAO, 2005). The mean average value of Chromium in *Hilsa ilisha* was the least among the three investigated heavy metals (0.01225 ppm) which is far below the permissible value of Chromium (Table 1).

The recorded values in *Herpodon nehereus* expressed that the higher concentration is observed in case of Arsenic (As) which is higher than the recommended value. The mean average value of Chromium was the least among the three investigated heavy metals which is far lower than the standard value of Chromium. The average value of lead was also lower than the standard value.

Pampus chinensis showed the concentration of Arsenic (As) with a mean value of 0.032567 ppm which is higher than the recommended value. The mean average value of Chromium was the least among the three investigated heavy metals which is far lower than the standard value of Chromium. The average value of lead was observed 0.032567 ppm (Table 1).

In this study, the heavy metal accumulation pattern of gill as pb>As>Cr but for the liver, muscle and kidney as As>Pb>Cr (Table 2). Lead concentration in gill and kidney significantly varied from liver and muscle (Figure 3). For arsenic, there is no significant variation among gill, liver and kidney (Figure 5).

This study found that the accumulation level of arsenic in different organs of fishes was higher than the standard reference values recommended by WHO and FAO. High concentrations were found in non-edible portions of fish body. But this threatened concentration of metals can retard fish development Fish development can be affected by the presence of heavy metals in water and especially the early life stages such as hatching time, larval development and juvenile growth as they are more sensitive than the mature stages (Weis and Weis 1989; Friedmann *et al.*, 1996). Friedmann *et al.*, (1996) showed that even low levels of As inhibited growth of *juvenile walleye* and *Stizostedion vitreum*. Weis and Weis (1989) also indicated that both essential and non-essential metals could alter embryonic development of fish embryos causing retardation of normal development, disability of organs or mortality. However, fish growth and its relationship with metal concentration in the aquatic environment should be assessed occasionally in the field to better understand the effects of metals on fish development and the current situation of population dynamics.

CHAPTER-6

CONCLUSION

The Bay of Bengal is being considered with vast biodiversity and it is capable of supporting various fish species including *Herpodon nehereus*, *Pampus chinensis* and *Hilsa ilisha*. But in recent years, the impact of the wastes discharged in Bengal Sea has been significant due to the high effluent discharge from ship breaking yard and industrial effluents. As being a widely exposed coast of the Bay of Bengal, Chattogram region is affected by numerous numbers of heavy metals. Heavy metals are considered particularly dangerous to human health because, in the preparation of food, they do not decompose; on the contrary, their concentration tends to bioaccumulate. A long term consumption of heavy metal containing food above tolerance limit has a hazardous impact on human health. Even if there were in small amounts, the presence of these heavy metals can generate worries due to their cumulative effect in the consumers. The results of this study revealed that consuming fish from the Chattogram coast of southern Bay of Bengal may not have harmful effects because edible part (muscle) of fishes are not heavily burdened with metal and most of the concentration below the standard limits given by WHO and FAO. However, arsenic in all fish tissues was higher than certified level so it is a matter of concern in fish accumulation, on the contrary, concentration of chromium measured in all the tissues of the species studied generally lower than the levels issued by WHO/FAO. From the findings of this study, it was concluded that the fish kidney exhibited highest accumulation of heavy metals (As and Pb) in *Herpodon nehereus* , *Pampus chinensis* and *Hilsa ilisha.* Fish gill showed highest accumulation of Cr in three investigated fish species which is lower than the standard value of Cr. The current research has already revealed that arsenic concentration is far above from the safety levels while the concentration of lead is also approaching. So, the immediate safety and strict regulatory actions on environment friendly discharge heavy metal is mandatory from the government organizations and also from non-government responsible personnel and organizations. As far as the importance of fish in the human diet is concerned, it is necessary that the biological monitoring of water and fish should be done periodically to ensure the safety of fish and seafood consumption.

CHAPTER-7

RECOMMENDATION AND FUTURE PERSPECTIVES

Although all results of heavy metal concentrations studied showed that regular consumption of the three fish species did not cause so much harmful effect on human health, the following recommendations should be taken into consideration:

- More intensive sampling and analysis including sampling of marine fish species of different heights, ages from different sections of the Chattogram districts may be carried out which could better describe the heavy metal quality of fish species.
- It is recommended to conduct a comprehensive assessment program along Chattogram coast to study and identify the major sources causing pollution in the marine waters and fishing harbor.
- Similar studies may be performed to monitor the contamination with other toxic heavy metals such as cadmium, mercury, cobalt, zinc, copper and manganese in commercially important fish species, coastal water and sediment.
- Since *Herpodon nehereus* from chattogram coastal area showed the highest concentration of arsenic, the environment quality of that region must be checked and discharge of waste water without treatment into the sea should be prevented.
- It is recommended to conduct monitoring for commercial fishes in Chattogram strip markets periodically to ensure that the concentrations of metals remain within the prescribed worldwide limits.
- More intensive study needs to be conducted in order to determine the bio-accumulation of heavy metals in fishes from study area.
- The study on health risk associated with dietary intake of excess heavy metals by the consumer is a vital and integral part of regulatory processes. These types of study need to be conducted for understanding the toxic effects of heavy metals in human body.
- To preserve the unpolluted state of the Chattogram fishing harbor, it remains important that continuous monitoring of heavy metals, hydrocarbons and pesticides concentrations is recommended to be conducted regularly.

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Appendix A: Fish Samples of the Study Collected From Chattogram Coastal Area

Herpodon nehereus

Hilsa ilisha

Pampus chinensis

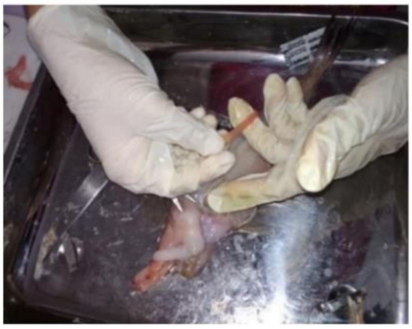
Measuring Length and Weight of Fishes

Appendix B: Dissection and Preparation for Digestion

Hilsa ilisha Dissection

Herpodon nehereus Dissection

Pampus chinensis Dissection



Dissection of Fishes

Fish Organs after Dissection

Storing Fishes

Dipping fish organs into 10% formalin solution

Removing organs from formalin

Drying fish organs

Dried fish organs

Taking the Weight of Dried Fish Organs

Appendix C: Digestion and Heavy Metal Analysis

Adding digestion acid mixture into sample

Digestion chamber

Pouring sample into tube after digestion

Sampling and labeling

AAS used for metal analysis

Appendix D: Calculation For Weight and Length Of Fish Species

Herpodon nehereus	**Weight (g)**	**Length (cm)**
L1	85.03	25
L2	93.46	22.5
L3	65.23	23
Sum	243.72	70.5
Mean	81.24	23.5
Standard Deviation	14.49159	1.322876
Pampus chinensis	**Weight (g)**	**Length (cm)**
R1	327.65	22.8
R2	349.42	22
R3	421.5	25.5
Sum	1098.57	70.3
Mean	366.19	23.43333
Standard Deviation	49.12108	1.833939
Hilsa ilisha	**Weight (g)**	**Length (cm)**
H1	560	36.2
H2	540	35
H3	600	37
Sum	1700	108.2
Mean	566.6667	36.06667
Standard Deviation	30.5505	1.006645

(Here L1, L2, L3= Loitta fish samples; H1, H2, H3= Hilsa fish samples; R1, R2, R3= Rupchanda fish samples.)

Appendix E: Heavy Metal Concentration in Kidney Tissues of Fish Species

Chromium Concentration in Kidney of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
L1k	0.0018/36	0.00005
L2k	0.0001/14	0.000714
L3k	0.0034/88	0.000386
Pampus chinensis	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
R1k	0.0012/15	0.00008
R2k	0.0007/25	0.000028
R3k	0.0076/50	0.000152
Hilsa ilisha	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
H1k	0.0139/225	0.000617
H2k	0.0016/370	0.00004
H3k	0.0006/630	0.000009

Lead Concentration in Kidney Of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Pb concentration (ppbmg)**
L1k	1.0998/36	0.03055
L2k	0.9923/14	0.070878
L3k	1.5098/88	0.01716
Pampus chinensis	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
R1k	1.0445/15	0.069633
R2k	0.9768/25	0.039072
R3k	1.0077/5	0.20154
Hilsa ilisha	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
H1k	1.5/225	0.000296
H2k	1.0016/370	0.00270
H3k	2.0006/630	0.003176

Arsenic Concentration in Kidney of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**As concentration (ppb/mg)**
L1k	1.3909/36	0.038636
L2k	1.2998/14	0.09284
L3k	1.4004/88	0.025913
Pampus chinensis	**ppb/sample(mg)**	**As concentration (ppb/mg)**
R1k	1.0425/150	0.00695
R2k	1.101/25	0.04404
R3k	1.0996/50	0.0218
Hilsa ilisha	**ppb/sample(mg)**	**As concentration (ppb/mg)**
H1k	1.5209/225	0.00675
H2k	1.5056/370	0.00406
H3k	1.5998/630	0.00253

(Here L1, L2, L3= Loitta fish samples; H1, H2, H3= Hilsa fish samples; R1, R2, R3= Rupchanda fish samples; K= Fish kidney)

Appendix F: Heavy Metal Concentration in Gill Tissues of Fish Species.

Chromium Concentration in Gill of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
L1G	1.0001/180	0.0055561
L2G	0.0001/200	0.0000005
L3G	0.0019/220	0.000006
Pampus chinensis	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
R1G	1.002/194	0.0051649
R2G	-0.0009/420	0
R3G	0.001/245	0.000004
Hilsa ilisha	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
H1G	1.0031/750	0.00133
H2G	0	0
H3G	-0.0087/750	0

Lead Concentration in Gill of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
L1G	1.0095/180	0.0056083
L2G	1.0005/200	0.0050025
L3G	1.5234/220	0.00692455
Pampus chinensis	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
R1G	1.0021/194	0.0051654
R2G	1.0009/420	0.0023830
R3G	1.5678/245	
Hilsa ilisha	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
H1G	1.31/750	0.00639918
H2G	1.1009/750	0.00146786
H3G	3.0088/750	0.00401173

Arsenic Concentration in Gill of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**As concentration (ppb/mg)**
L1G	1.0676/180	0.00593
L2G	1.0334/200	0.005167
L3G	1.0997/220	0.00499863
Pampus chinensis	**ppb/sample(mg)**	**As concentration (ppb/mg)**
R1G	1.0445/194	0.0053840
R2G	1.0873/420	0.0025888
R3G	1.1009/245	0.0044934
Hilsa ilisha	**ppb/sample(mg)**	**As concentration (ppb/mg)**
H1G	2.0112/750	0.0026816
H2G	2.1667/750	0.0028889
H3G	2.1123/750	0.0028164

(Here L1, L2, L3= Loitta fish samples; H1, H2, H3= Hilsa fish samples; R1, R2, R3= Rupchanda fish samples; G= Fish gill)

Appendix G: Heavy Metal Concentration in Liver Tissues of Fish Species

Chromium Concentration in Liver of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
L1L	0.0019/16	0.00011875
L2L	0.0002/14	0.000014
L3L	0.0087/30	0.00029
Pampus chinensis	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
R1L	-0.0019/460	0
R2L	0.0013/670	0.0000016
R3L	0.0092/580	0.000015
Hilsa ilisha	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
H1L	-0.0009/500	0
H2L	0.0019/750	0.000002
H3L	0.0001/750	0.0000001

Lead Concentration in Liver of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
L1L	0.0098/16	0.0006125
L2L	1.0002/14	0.07144
L3L	0.0001/30	0.000033
Pampus chinensis	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
R1L	0.0093/460	0.00002
R2L	1.0007/670	0.0014935
R3L	0.0012/580	0.000026
Hilsa ilisha	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
H1L	1.0001/500	0.0020002
H2L	1.0017/750	0.0013356
H3L	1.0011/750	0.0013348

Arsenic Concentration in Liver of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**As concentration (ppb/mg)**
L1L	1.5906/16	0.0994125
L2L	1.2334/14	0.0881
L3L	1.499/30	0.0499666
Pampus chinensis	**ppb/sample(mg)**	**As concentration (ppb/mg)**
R1L	1.2304/460	0.0026747
R2L	1.2339/670	0.0018416
R3L	1.3789/580	0.002377
Hilsa ilisha	**ppb/sample(mg)**	**As concentration (ppb/mg)**
H1L	1.9909/500	0.0039818
H2L	1.6776/750	0.0022368
H3L	1.8778/750	0.0025037

(Here L1, L2, L3= Loitta fish samples; H1, H2, H3= Hilsa fish samples; R1, R2, R3= Rupchanda fish samples; L= Fish Liver)

Appendix: H: Heavy Metal Concentration in Muscle Tissues of Fish Species

Chromium Concentration in Muscle of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
L1M	0.0113/750	0.00015
L2M	0.0057/397	0.000143
L3M	0.0096/750	0.0000128
Pampus chinensis	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
R1M	0.0004/750	0.000005
R2M	0.0098/750	0.000013
R3M	-0.009/750	0
Hilsa ilisha	**ppb/sample(mg)**	**Cr concentration (ppb/mg)**
H1M	0.0213/750	0.0000284
H2M	0.0002/750	0.000026
H3M	-0.0087/750	0

Lead Concentration in Muscle of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
L1M	1.0334/750	0.00137786
L2M	1.899/397	0.00478337
L3M	1.5096/750	0.0020128
Pampus chinensis	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
R1M	1.011/750	0.001348
R2M	1.009/750	0.00134533
R3M	1.0087/750	0.0013449
Hilsa ilisha	**ppb/sample(mg)**	**Pb concentration (ppb/mg)**
H1M	1.9091/750	0.002545
H2M	1.9902/750	0.0026536
H3M	1.009/750	0.001345

Arsenic Concentration in Muscle of Fish Species

Herpodon nehereus	**ppb/sample(mg)**	**As concentration (ppb/mg)**
L1M	1.0111/750	0.0013481
L2M	1.0556/370	0.002852
L3M	1.1019/750	0.0014692
Pampus chinensis	**ppb/sample(mg)**	**As concentration (ppb/mg)**
R1M	1.5726/750	0.0020968
R2M	1.989/750	0.002652
R3M	1.0887/750	0.0014516
Hilsa ilisha	**ppb/sample(mg)**	**As concentration (ppb/mg)**
H1M	2.0111/750	0.00268146
H2M	2.1108/750	0.0028144
H3M	1.3998/750	0.0018664

(Here L1, L2, L3= Loitta fish samples; H1, H2, H3= Hilsa fish samples; R1, R2, R3= Rupchanda fish samples; M= Fish muscle)

Brief Bio-Data of the Author

Afifa Siddiqua completed B. Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh with CGPA 3.88 out of 4.00. Now, she is a candidate for the degree of MS in Food Chemistry and Quality Assurance under the department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). She has strong passionate in research of food science. Her research interests are food chemistry, food safety, processing, preservation, functional food product development and nutritional value analysis, quality control and quality assurance regarding food, characterization of physical and chemical properties of food materials, chemical and microbial analysis of food, new techniques to measure the food quality, taste and flavor and instrumental analysis with UV/Vis spectroscopy, Atomic Absorption Spectroscopy (AAS), Near-infrared Spectroscopy (NIRS), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Gas Chromatography-mass spectrometry (GC-MS), Liquid Chromatography-mass spectrometry (LC-MS).