POTENTIAL HEALTH RISK OF HEAVY METALS VIA FISHES FROM DIFFERENT WATER SOURCES IN SOME PARTS OF PATUAKHALI DISTRIC OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled **"*POTENTIAL HEALTH RISK OF HEAVY METALS VIA FISHES FROM DIFFERENT WATER SOURCES IN SOME PARTS OF PATUAKHALI DISTRICT OF BANGLADESH*"** submitted to the Faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE IN AGRICULTURAL CHEMISTRY*** embodies the result of a piece of bona fide research work carried out by ***MORIOM BEGUM MOURI*, Registration No.18-09183** under my supervision and guidance. No part of the thesis has been submitted for any other degree

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated: December, 2020
Dhaka, Bangladesh.

Professor Dr. Md. Sirajul Islam Khan
Department of Agricultural Chemistry

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Dedicated to

My Beloved Parents

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SAU, Dhaka ***The Author***

POTENTIAL HEALTH RISK OF HEAVY METALS VIA FISHES FROM DIFFERENT WATER SOURCES IN SOME PARTS OF PATUAKHALI DISTRICT OF BANGLADESH

Abstract

Irrigation by water with industrial effluents, wholesale use of pesticides and resources and impromptu urbanization result in heavy metal contamination in agricultural produces and thereby human health complicacies .Biomonitoring fish and their potential health risks involved are of prime importance for the time being. Concentration of heavy metals in nine different fish species (Spotted snakehead, Indian catfish, Tyangra, Yellow tail mullet, Panna croaker, Gora chela, Bombay duck, Gangetic sillago, Chinese silvery pomfret) collected from sea, river and pond at Patuakhali District, were measured by using the Atomic Absorption Spectrophotometer (AAS).Variations in concentrations of heavy metals were recorded among studied fish species. In all the sites, the pattern of metals exhibited the following trend: Cr > Ni > Pb> Cd. The concentration of all metals was found to be higher in the sea fishes as compared to river and pond fishes. Sea fish Chinese silvery pomfret (Rupchanda) and Gangetic sillago (Tulardandi) had the highest single factor pollution index (Pi) of most the heavy metals. . The sum of pollution index was also the highest in Chinese silvery pomfret (5.18), followed by Gangetic sillago (5.03) and Bombay duck(5.94).These a fishes had also the highest metal pollution index (0.0003 in Chinese silvery pomfret, 0.0003 in Gangetic sillago 0.0003 in Bombay duck). The hazard quotients were different for different people groups. Among the fishes, sea fishes had higher hazard quotients for Pb, Cr and Ni. The hazard quotient of Cr analyzed in fishes for male, female and children was higher than Pb, Cd and Ni. The highest hazard index was estimated for children (0.143),indicates alarming condition for the people group. The female group had the second highest hazard index, shows vulnerability to the heavy metals, if exposed for long-term. Continuous monitoring of heavy metal status in fishes should been forced all over Bangladesh for health concern of peoples of all ages.

CONTENTS

ABBRIVIATION

e.g. =Example

etc = etcetra

WHO = World Health Organization

FAO = Food and Agricultural Organization

USEPA = United States Environmental Protection Agency

AAS = Atomic Absorption Spectrophotometer

BARI = Bangladesh Agricultural Research Institute

SPI = Single Factor Pollution Index

MPI = Metal Pollution Index

mg = Milligram

kg = Kilogram

Cmetal = Concentration of metal

BO = Body weight

HQ = Hazard Quotients

HI = Hazard index

Chapter I

INTRODUCTION

The consumption of fish worldwide has increased speedily in recent years particularly with the awareness of its nutritional and therapeutic benefits. In addition to being important source of protein, fish are enriched with essential minerals, vitamins, and unsaturated fatty acids([El-Moshley, 2000). The American Heart Association recommended consumption of fish at least twice per week in order to reach the daily intake of omega-3 fatty acids (Kris-Etherton *et al.*, 2002). The presence of toxic heavy metals in fish can invalidate their beneficial effects. Several unfavorable effects of heavy metals to human health have been known for long time (Castro-González *et al*., 2008). This includes serious threats like renal failure, liver damage, cardiovascular diseases, and even death (Al-Busaidi *et al*., 2011). Thus, many local and international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al*., 2010). Fish constitute a major source of heavy metals in food (Sivaperumal *et al*., 2007). High level of metals in environment may lead to an excessive accumulation which cause problem to human, animal and plants (Al-Khateeb and Leilah, 2005). Fish has been found to be excellent indicator for the heavy metal contamination level in aquatic system because it occupies different food chain levels (Karadede-Akin and Unlu, 2007). A major concern and attention about the health effects people due to consumption of foodstuff contaminated with metals were paid (Iwegbue *et al.*, 2008). Heavy metals occur in the environment both as a result of natural processes as well as contaminants from human beings activities (Franc *et al*., 2005). Some heavy metals are known as potentially toxic include arsenic, lead, aluminium and cadmium, others are essential such as nickel, zinc and chromium (Abduljaleel and Shuhaimi-Othman, 2011).

Heavy metals are an important group of chemical pollutants, whereby food is the main route for entry into our body; some heavy metals irreversibly are bound to human body tissues, e.g., cadmium to kidneys and lead to bones (Kaplan *et al*., 2011). According to the literatures, metal bioaccumulation by fish and subsequent distribution in organs is greatly interspecific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming pattern, feeding behavior, and geographical location (Zhao *et al* ., 2011). However, fish normally accumulate heavy metals from food, water, and sediments (Yilmaz *et al* ., 2007) and this is a good indicator of heavy metals contamination in water (Voegborlo*etal* .,2012). The accumulation of heavy metals in fish has been extensively studied and well documented. However, the research has been generally focused on the muscle tissue, while the distribution pattern among other tissues, such as liver and gills have been mainly neglected (Jaric *et al.,* 2011). Furthermore, the contaminants also concentrate in some of the organs of fish and can cause lethal and a range of sub lethal effects (Ozmen*et al.,* 2008). It was known that active metabolite body parts such as liver, gill and kidney concentrate more on an amount of heavy metal than other parts like muscle (Dural *et al.,* 2007). Fishes are often seen at the top of the aquatic food chain and may accumulate large amount of heavy metals from its environment (Mansour and Sidky, 2002). In addition, fishes are one of the most indicative aspects in fresh water ambience, for the evaluation of heavy metals pollution and health risk potential of human consumption (Papagiannis *et al.,* 2004).

Patuakhali is recognized as a fisheries zone of Bangladesh. Many varieties of fish species are available in the fish landing centers of this district. Both inland (freshwater and brackish) and marine water fish species are found in the landing centers of Patuakhali district. This region supply one of the major amount of fish all over the country. Over exploitation of fisheries resources, river bank erosion and human activities are gradually damaging the aquatic life including fishes of pond, river, and sea in this region. This region also have a potential of fisheries production and the local peoples in this region consumed fishes mostly caught from different rivers, ponds and sea and they depend on this river in many ways. Bangladesh, as a developing country, is at a high risk of aquatic pollution. The river systems may be contaminated with heavy metals released from domestic, industrial, mining and agricultural effluents. The overuse of fertilizers and pesticides in the surrounding cultivable lands that washed out through surface runoff degrade the quality of the water and ultimately affecting the aquatic biota.

Heavy metal content in the tissues and organs of fishes indicates their accumulation in food chains. Since fishes are an integral component of the human diet, they need to carefully screen to ensure that unnecessary high level of heavy metals are not being transferred to human population via consumption of fish. Therefore, it is important to observe the level of heavy metals in consumed fishes to get some ideas about the safety of fish protein supplied to the consumers and to understand its harmful effects on individuals, population or ecosystem (Ahsan *et al*., 2018). Moreover, for the existence and conservation of aquatic resources, it is essential to investigate the fish quality of this region. In the present study, nine available fish species from different sources such as ponds, rivers and sea were collected. The studied fishes were Indian catfish (*Callichrus pabda*), Spotted snakehead (*Channa punctata),* Yellow tail mullet (*Sicamugli cascasia*),Gora chela (*Securicula gora*), Tyangra (Mystus cavasius), Panna croaker (*Panna microdon*), Bombay duck (*Harpadon nehereus*), Chinese silvery pomfret (*Pampus chinensis*), Gangetic sillago (*Sillaginopsis panijus*).These fish samples are mostly available and consumed by the consumers promptly in this region that's why I choose these fishes. Therefore, the present research work is undertaken with the following objectives:

Overall objective of the study was carried out to monitor the levels of heavy metals (Pb, Cd, Cr and Ni) in some selected fishes Patuakhali district.

1. To assess the highest and lowest concentration of heavy metals (Pb, Cd, Cr and Ni) in different type of fishes for comparing with safe value recommended by WHO/FAO/USEPA

2. To quantify the levels of heavy metals

3. To assess the health risk due to intake heavy metals through consumption of fishes from ponds, rivers and seas.

Chapter II

REVIEW OF LITERATURE

2.1 Heavy Metals in water

Trace elements of natural origin are transported by rivers and transferred to the coastal marine system through estuaries. There, trace elements are distributed between the dissolved and particulate phase, while their fate and bioavailability depend on the particle chemistry and competition between surface and dissolved forms in terms of complexation processes. Hence, the estuaries constitute a natural reactor in which heterogeneous processes at the interface between dissolved phase and suspended particulate matter and constitute an important part of the trace elements geochemical cycles. The distributions of trace metals and their rates of reactivity vary greatly between estuaries, depending on environmental factors, such as hydrodynamic residence times, mixing patterns and transport processes. Therefore, there is no universal pattern of trace metal behaviour in estuaries.

The ability of a water body to support aquatic life, as well as its suitability for other uses depends on many trace elements. Certain metals like Mn, Zn and Cu present in trace concentrations are important for the physiological functions of living tissue and regulate many biochemical processes. Water pollution by heavy metals resulting from anthropogenic impact has caused serious ecological problems in many parts of the world. This situation is provoked by the lack of natural elimination processes for metals. As a result, metals shift from one compartment within the aquatic environment to a great extent, including the biota, often with harmful effects.

The Department of Environment reported higher concentrations of heavy metals in the waters of the west coast of Peninsular Malaysia compared to other areas because of the extensive land use and industrialization. In 1990, Sg Perak, Sg Selangor, Sg Kelang, Sg Linggi and Sg Melaka recorded samples exceeding the standard values of0.02mg/l of lead. Rivers that recorded values exceeding the proposed value of

0.4 mg zinc/litre included Sg Sepang Sg Langat and Sg Kelang. Sg Bernam which located in the west coast of Peninsular Malaysia, recorded copper values higher than the proposed value of 0.012mg/litre. Concentrations of cadmium were negligible in most of the riversmonitored.

Approximately all the samples collected from the coastal waters of Malaysia contained values of lead, copper and cadmium above the proposed standards of 0.05mg/l lead, 0.01mg/l copper and 0.005mg/l cadmium. The coastal waters of Perak and Penang recorded high levels of cadmium, copper, lead, mercury and nickel. In 1990, around 50 percent of the 41 samples collected from Perak had value exceeding the proposed standard of 0.005mg mercury/litre. In 1989, more than 80 percent of the 42 samples collected from the coastal waters of Perak had values above the proposed standard of 0.01 mgnickel/litre.

A considerable amount of research has been conducted to decide concentrations of heavy metals in water. In northwestern part of Thailand Gulf. A geochemical survey was carried out in order to define concentrations and circulation patterns of selected heavy metals in the coastal system and estuarine area of the Mae Klong river. The results indicated the presence of two different sources of heavy metals in the studied environment and further identified a lithogenic component that might. significantly influence the composition of coastal waters and suspended particulate matter (Censi*et al*., 2006).

Perez et al., (1999) had studied on heavy metal concentrations in water and underneath sediments of a Mexican reservoir. The results showed that mercury, lead, chromium and iron were the main metal contamination problem. In the same study, spatial and temporal distributions of total metal levels had also been identified. No organized pattern was detected for any particular metal concentration. The chronological variations of metal concentrations showed evidence of the water self-cleaning capacity of the reservoir, despite high level metal pollution being determined (Perez *et al*., 1999).

In Port Jackson estuary, the concentrations of dissolved metals were gritty for summer and winter under low-flow conditions. Dissolved Ni and Mn behaved mostly conservatively, whereas Cd, Cu and Zn showed mid-estuarine maxima. Peaks in Cd, Cu and Zn concentrations were located in the upper estuary, independent of the salinity and suspended particulate matter loading, and were consistent with anthropogenic inputs of metals in the estuary. Concentrations of dissolved Cu were highest in summer, whereas concentrations of Cd, Ni and Mn were significantly lower in summer than winter. The increase in temperature and biological activity during summer explained the seasonal deviation (Hatje*et al*., 2003).

2.2 Heavy Metals in aquatic organisms

Fish and marine products contain many elements which are essential for human life at low concentration. Nevertheless, they can become toxic at high concentrations. However, certain heavy metals such as mercury, cadmium and lead do no show essential functions in life and are toxic even at low concentration when ingested over long period. These metals were present in the aquatic environment long before human being existed. The proportion between the natural background concentration of heavy metals and anthropogenic heavy metals in fish varies from element to element. In unpolluted areas, fish normally carry natural burden of heavy metal concentration. In heavily polluted areas, the heavy metal concentrations actually found are exceeding the natural concentration (Kalay*et al*.,1999).

Fish and other aquatic animals take up heavy metals from their food and water that passes through their gills. The uptake of metals is often dependent on the amount of food ingested and on the heavy metal content of the food. Accumulation takes a long time and may result in high concentrations in aged organisms. Some species, which are relatively long lived, are known for storing higher amounts of heavy metals in different organs. The main organs which are used in fish for storage and detoxification of heavy metals are liver, kidney and bones. These organs are normally not used for human consumption in Europe and America. In Asia, however, smaller fish are consumed wholely and other gut contents often are used in fermented sauce or salted. There is a vast literature on the content of heavy metals in fish, mollusca and crustaceans. Majority of these papers have reported high concentrations of heavy metals which are due to anthropogenic activities.

The parts that are mostly investigated for heavy metal concentration are organs and tissues which normally accumulate and store heavy metals. Less information is available about the heavy metal content in the edible part consumed by humans.

Abdullah *et al.*, (2007) found that mollusk has a potential to be used as bioindicator for the contamination of Cd and Zn in water and sediment of an estuarine environment, as indicated by its high concentration factors (BCFs) values. The species seemed to accumulate certain metals in

Study done by Hashmi *et al* (2002) on heavy metal concentrations in water and tiger prawn (*Penaeus monodon*) from Sabah, East Malaysia had shown that there was no general correlation between metal levels in water and in the prawn tissue. The information suggested in uptake and retention of metals in tiger prawns. The animal seemed to resist the build-up of certain metals whereas it allowed the entry of others to the extent of exceeding the proportion that occurred in the environment. Some of the controlling factors include the nature of the metals, environmental factors, the body's reaction, physiological tolerance, tissue thresholds and regulatory mechanisms (Hashmi *et al*.,2002).

Ahmad Ismail, *et al.*,(1995) had studied the level of heavy metals in ten species of marine prawns along the coastal areas of Peninsular Malaysia. It was found that the levels of trace metals in most of the samples studied were below the maximum permissible limit of the Food Regulations in Malaysia, with the exception of a few samples collected from the coasts of Melaka and Matang, which contained higher levels of Pb and Cd. However, there was no clear evidence on the relationships of heavy metals in sediments and prawns (Ahmad Ismail *et al.*,1995).

In the southern part of the Korean Peninsula, concentrations of trace elements in the tissue of Mytillus galloprov in caialis varied with size of mussels, season, depending on many factors like sexual development, and seawater temperature. The levels of some trace metals in seawater were correlated significantly with those in soft tissue and byssal threads. There were spatial variations in metal concentrations in the soft tissue and byssus attributed to different sources of trace elements located near the sampling sites. Significant relationships were found between heavy metals in mussel soft tissues and byassal threads and suspended matter.

2.2 Distribution pattern of heavy metals in tissues and organs

2.2.1. Lake/Pond Fish

The difference in the accumulation of trace metals in various organs of fishes may be attributed to the proximity to the tissues to the accessibility of the metals. The age, size and feeding habits of fish or aquatic animals as well as their maintenance in polluted waters may affect the heavy metals accumulation in these organisms. For fish, the gills, skin and digestion tract are potential sites of assimilation of water borne chemicals. There may be variations in the bioaccumulation of different metals in fish. Kidneys and liver have a tendency to accumulate maximum heavy metals concentrations due to their capability to accumulate trace metals brought by blood from other parts including gills and muscles. This will encourage the binding protein, metallothionein that is believed to play a crucial role against the trace metals by binding them. (Adhikari *et al.,* 2009).

Adhikari *et al*.(2009) found that muscle of various fish species from Kolleru Lake, India contained the lowest concentrations of trace metals among all the tissues investigated. Muscle does not approach into direct contact with the metals as it is totally covered externally by the skin that in many ways help the fish to ward off the penetration of the trace metals (Adhikari *et al.*, 2009). Since it is not an vigorous site for detoxification and therefore transport of trace metals from other tissues to muscle does not seem to arise.

Liver tissues in fish are more often recommended as environmental indicator organisms of water pollution compared to any other fish organs. This is possibly because liver tend to accumulate pollutants of various kinds at higher levels from their environment as reported by Galindo *et al*. (1986). The accumulation of the tested metals in liver could be based on the greater tendency of the elements to react with the oxygen carboxylate, amino group, nitrogen or sulphur of the mercapto group in the metal othionein protein (Kendrick *et al*.,1992).

Campbell (1994) conduct a research on Concentrations of heavy metals associated with urban runoff in fish living in storm water treatment ponds. He found that Redear sunfish from storm water ponds contained significantly higher ($p<0.005$) concentrations of cadmium, nickel, copper, lead, and zinc than redear from controls. Largemouth bass collected from stormwater ponds contained significantly higher ($p<0.005$) concentrations of cadmium and zinc than those from control sites. Bluegill from storm water ponds contained significant copper concentrations ($p<0.005$) as compared to bluegill from control sites.

Papagiannis *et al* (2004) have evaluated the level of contamination of two heavy metals appearing at high concentrations in lake water in four fish species (Cyprinuscarpio, Silurusaristotelis, Rutilusylikiensis, and Carassiusgibelio) caught in the lake. The metal concentrations were determined in three different tissues, namely muscle, liver and gonad in order to assess the metal contamination in these tissues. The study showed that Cyprinus carpioand Rutilusy likiens is were contaminated with the highest metal content. Tissue analysis revealed that liver and gonads have accumulated the highest levels of Cu and Zn. Metal concentrations in the edible part of the examined fish such as muscle were at the safety permissible levels for human consumption (Papagiannis *et al*., 2004)

Concentrations of cadmium and lead in five species of fish from River Neretva, Croatia were determined. The results showed that concentrations of cadmium were very high in kidneys of carp and mullet. Kidneys tend to accumulate cadmium, whereas the concentration of cadmium in muscle was lower. The lead concentration in all fish species is similar except carp. The carp had shown to accumulate this heavy metal in all tissue aparts from gonads (Has-Schon*et al*., 2006).

Öztürk et al.,(2009) conducted an experiment on determination of heavy metals in fish, water and sediments of Avsar dam lake in Turkey. 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. In the fish samples, cadmium, chromium, nickel and lead concentrations exceeded the tolerable values provided by international institutions.

Al-Yousuf *et al.,* (2000) had determined the influence of sex and body length on metal accumulation in fish. The results showed that the average metal concentration in liver, skin and muscle of the female fish were found to be higher than those found in the male fish. The accumulation of zinc, copper, manganese and cadmium in the liver tissues of *Lithrinus lentjan* was found high compared to skin and muscle tissues.

Bawuro *et al.,* (2018) studied on bioaccumulation of heavy metals (Zn, Pb, Cd, and Cu) was determined in the liver, gills, and flesh from benthic and pelagic fish species collected from Lake Geriyo covering two seasons. Fish species showed interspecific variation of metals. These differences were discussed for the involvement of potential factors that affected metals uptake like age, geographical distribution, and species-specific factors. The concentration of metals in fish flesh was established by the international legislation limits for Cu, Zn, and Cd; however, Pb transcend in *Clarias and Tilapia* during wet season and *Heterotis* in both seasons, hence unsafe for human consumption and a threat to public health. These levels might be due to anthropogenic inputs as there is no industrial activity around the lake.

2.2.2 River Fish

Ahsan *et al*., (2018) conducted a research on six available fish species (*Channa punctatus, Mastacembelusarmatus, Mystusvittatus, Puntius puntio, Amblycepsmangois and Metapenaeusspinulatus*) of the Dhaleshwari river. They found that the concentration of heavy metals in fishes of the study area ranged as Pb 0.086-0.288 mgkg-1, Cd 0.002-0.019 mgkg-1, Cr 0.006-0.159 mgkg-1, Hg 0.001-0.016 mgkg-1, As <0.005-0.128mgkg-1. Hg and As in fishes show negative correlations among themselves and positive correlations with Pb. The level of these heavy metals in fishes of the river were found below the acceptable limit. Thus, the study revealed that the consumption of the available fish species of the river is not harmful to human beings.

Mensoor and Said (2018) investigated in Determination of Heavy Metals in Freshwater Fishes of the Tigris River in Baghdad**.** The levels of heavy metals were determined by using an atomic absorption spectrophotometer (AAS). The results showed that concentrations of heavy metals in the sampled fishes exceeded the acceptable levels for food sources for human consumption. The results of this study showed high levels of cadmium and chromium levels in the tissues of the selected fish sample. Cd and Cr were among the highest concentrations and both exceeded the World Health Organization and Food and Agriculture Organization of the United Nations acceptable levels for heavy metals in fishes**.**

Ahmed *et al*., (2016) studied on Human health risks from heavy metals in fish of Buriganga river, Bangladesh. Whole body of five fish species (*Puntius ticto, Puntius sophore, Puntius chola, Labeorohita*and *Glossogobiusgiuris*) were analyzed which contained various concentrations of Cd, As, Pb, Cr, Ni, Zn, Se, Cu, Mo, Mn, Sb, Ba, V and Ag. Concentrations of Mn, Zn, Se and Pb in all fish species were above the Food Safety Guideline (FSG) by WHO/FAO. Measurement of noncarcinogenic health hazard by target hazard quotient (THQ) indicated no concern from consumption of these fish except for Mn. However, all metals together may affect human health as revealed by hazard index (HI). The target cancer risk (TR) values optional carcinogenic risk from Ni and As. Taken together it can be completed that there is potential human health risk in consuming fish from river Buriganga.

2.2.3 Sea Fish

Makedonski *et al.,(*2015) conducted a research on Determination of heavy metals in selected black sea fish species. The metal concentration of analyzed elements was highest in the gill for all fish species. The maximum metal concentration was measured for Cu (1.40 mg kg^{-1}w.w), Zn (11 mg kg^{-1}w.w) and Pb (0.08 mg kg^{-1}w.w) in muscle tissues of shad and sprat. The edible part of horse mackerel has the maximum value for Hg (0.12 mg kg^{-1}w.w) while Atlantic bonito predominantly accumulates As (1.10 mg kg^{-1}w.w). The analytical results obtained from this study were compared within acceptable limits for human consumption set by various health institutions.

Turkmen *et al.*, (2005) researched on Heavy metals in three commercially valuable fish species from İskenderun Bay, Northern East Mediterranean Sea, Turkey**.** They found that Concentrations of the heavy metals in examined fish species ranged as follows: Cd 0.01–4.16; Fe 0.82–27.35; Pb 0.09–6.95; Zn 0.60–11.57; Cu 0.04–5.43; Mn 0.05–4.64; Ni 0.11–12.88; Cr 0.07–6.46; Co 0.03–5.61; Al 0.02–5.41 mg kg^{-1} dry weight, respectively. The concentration of metals was significantly affected by the sampling site and fish species. Heavy metals in the edible parts of the investigated fish were in the permissible safety levels for human uses.

The concentrations of heavy metals (Cu, Zn, Pb, Cd, Fe and Mn) were measured in the liver, gills and muscles of fourteen benthic and pelagic fish species collected from three main landing areas (Shalateen, Hurghada and Suez) in the Egyptian Red Sea. The levels of heavy metals varied significantly among fish species and organs. Different species of fish showed inter-specific variation of metals, as well as variations between fish from the same species. These differences were discussed for the giving of potential factors that affected metals' uptake, like age, geographical distribution and species' specific factors. The concentration of metals in the here fish muscles were accepted by the international legislation limits and are safe for human consumption (El-Moshley *et al*.,2014).

Chapter III

MATERIALS AND METHODS

A study was conducted during July, 2019 to March, 2020 to determine the status of heavy metals in selected nine fishes collected from Patuakhali District. The details of materials used and methods applied for collection and analysis of fish samples presented in this chapter.

3.1. Sampling Sites

Fish samples were collected from randomly selected sites in Patuakhali district, Bangladesh. The geographical positions of the sites are shown in Table 3.1. Two sites at Patuakhali district (Kalapara and Galachipa) were randomly selected giving priority to the fish sources situated near Kuakata sea beach. The fishes collected were Spotted snakehead, Indian catfish, Tyangra, Yellow tail mullet, Panna croaker, Gora chela, Bombay duck, Gangetic sillago, Chinese silvery pomfret. Details are given in Figure 3.1, Table 3.1-3.2. Fishes samples were collected in clean polyethylene bags and brought to the laboratory for analysis.

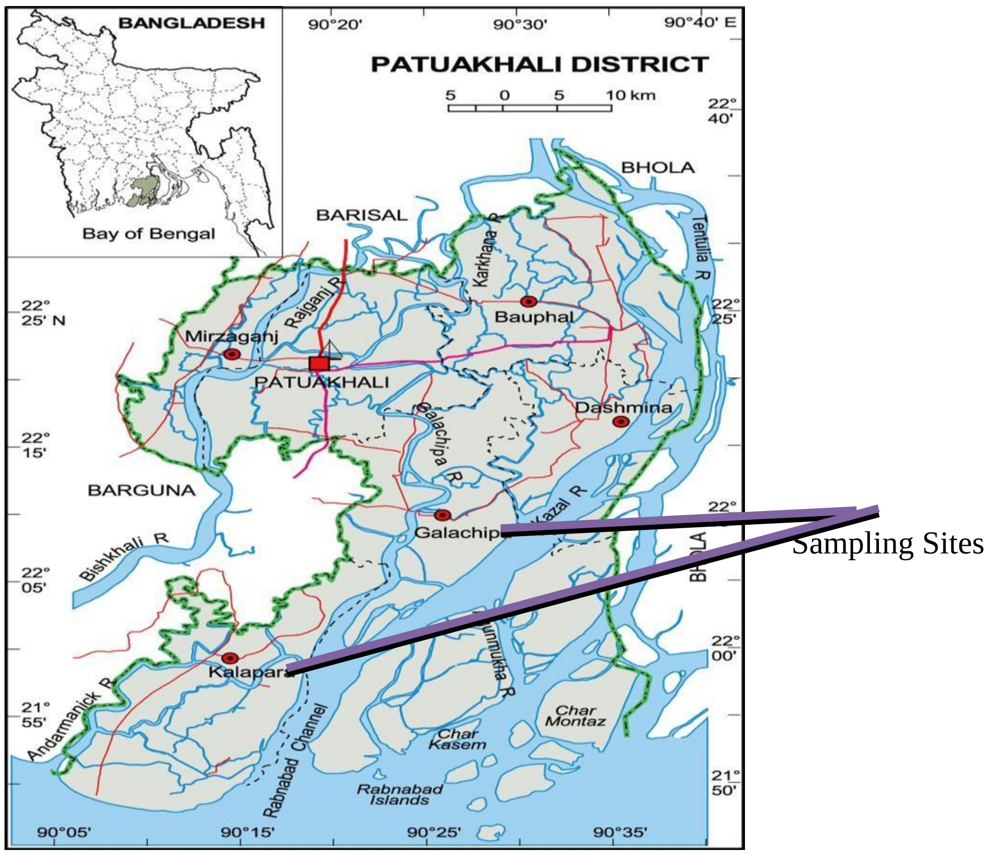


Figure 3.1: Map of Patuakhali district showing different sampling sites

Table 3.1: Location of the sampling sites at Patuakhali, Bangladesh

Sl. No.	Market	Geographical positions (Coordinates)
1	Galachipa	21°48' and 22°21'N 90°15' and 90°37'E
2	Kalapara	21°48' and 22°05'N 90°05' and 90°20'E

3.2 Collection of fish samples

Fish samples showed in Table 3.2 were collected at least from five fisherman. The organ (gills, liver, kidney, muscles, integument) were removed. Then sun dried for seven days and oven dried at 60^0C.The dried tissues were then reduced into powder in a pastle and mortar and sieved using a plastic sieve (0.2mm mesh size). The fish samples were put into the individual polythene bag with distinct marking and tagging and brought to the laboratory of Soil Science Division, Bangladesh Agricultural Research Institute(BARI),Gazipur

3.3 : Types of fishes:

The following fish samples were collected and they are listed below:

Table 3.2 List of fishes collected for the study

Sl. No.	Local name	English Name	Type	Scientific Name	Family
1	Taki	Spotted Snakehead	Pond fish	*Channa punctata*	Channidae
2	Pabda	Indian Catfish	Pond fish	*Callichrus pabda*	Siluridae
3	Tengra	Tyangra	Pond fish	*Mystus cavasius*	Bagridae
4	Kachki	Yellow Tail Mullet	River fish	*Sicamugili cascasia*	Mugilidae
5	Poa	Panna Croaker	River fish	*Panna microdon*	Sciaenidae
6	Chela	Gora Chela	River fish	*Securicula gora*	Cyprinidae
7	Loitta	Bombay Duck	Sea fish	*Harpadon nehereus*	Synodontidae
8	Tular Dandi	Gangetic Sillago	Sea fish	*Sillaginopsis panjus*	Sillaginidae
9	Rupchada	Chinese Silvery Pomfret	Sea fish	*Pampus chinensis*	Stromateoidae

3.3 Fish analysis

The collected fish samples were only analyzed to determine the Pb, Cd, Cr and Ni concentrations in fishes.

3.5 Digestion of fish samples

A sub-sample weighing 0.5 g was transferred into a dry clean digestion vessel. Then 5 ml of nitric acid (HNO_3) was added to the sample and was allowed for standing overnight under fume hood. On the following day, the vessels were placed on a heating block and heated at a temperature slowly raised to 120°C for two hours. After cooling, 2 ml of hydrogen per oxide (H_2O_2) was added into it and kept for few minutes. Again, the vessel was heated at 120°C. Heating was momentarily stopped when the dense white fumes occurred, after which the volume was reduced to 3 - 4 ml. The digest was cooled, diluted to 50 ml with deionized water and filtered through Whatman No. 42 filter paper into plastic bottle. The fish samples were then digested at digestion laboratory, Soil Science Division, Bangladesh Agricultural Research Institute, Gazipur.

3.6 Determination of Pb, Cd, Cr and Ni

Concentrations of Pb, Cd, Cr and Ni were determined at the laboratory of Soil Science Division, Bangladesh Agricultural Research Institute, Gazipur by using the Atomic Absorbtion Spectrophotometer (AAS) at 217, 193.7, 224.8 and 232 nm, respectively (Welsh *et al.*, 1990).

3.7 Statistic analysis

Mean concentrations of heavy metals in fishes were analyzed using excel computer package. One way analysis of variance (ANOVA) was used to determine significant difference ($p<0.05$) between groups using Statistix 10.

3.8 Data analysis

Content of heavy metals in fish samples was estimated. Apart from content, following parameters were assessed to estimate risk associated with uptake of metals:

3.8.1 Single Factor Pollution Index(SPI)

The pollution index (PI) is the ratio of metal concentration in a biotic or abiotic medium to that of the regulatory Standard of International bodies such as World Health Organisation (WHO), United States Environmental Protection Agency (USEPA) .

Mathematically, PI is expressed as:

$$Pi = C_{fish}/C_{USEPA\text{-}standard} \quad \ldots\ldots (1)$$

Where PI is the individual pollution index of study metal, C_{fish} is the concentration of the metal in fish. C_{USEPA}-standard is the value of the regulatory limit of the heavy metal by USEPA.

3.8.2 Sum of pollution index

Sum of Pollution index (SPI) previously described by Qingjie et al. (2008) was used for the present application.

$$SPI = Pi\mathit{Cd} + Pi\mathit{Cr} + Pi\mathit{Pb} + Pi\mathit{Ni} \quad \ldots\ldots (2)$$

Where Pi = single factor pollution index of heavy metals

3.8.3 Metal Pollution Index (MPI)

To examine the overall heavy metal concentrations of fishes , the metal pollution index (MPI) was computed (Usero et al., 1997). This index was obtained by calculating the geometrical mean of concentrations of all the metals in the fishes.

$$MPI\ (mgkg^{-1}) = (C_1 \times C_2 \times \ldots\ldots \times C_n)^{1/n} \ldots\ldots (3)$$

Where, C_n = concentration of metal n in the sample

Table 3.3 Showing reference oral dose for metals

Sl no.	Metal	Oral dose (mg/Kg/Day)	References
1	Lead (Pb)	0.004	USEPA 2015
2	Cadmium (Cd)	0.001	USEPA 2015
3	Chromium (Cr)	0.003	USEPA 2015
4	Nickel (Ni)	0.02	USEPA 2015

3.8.4 Hazard Quotient

The screening level risk associated with consumption of contaminated food can be assessed using hazard quotient. Hazard quotient for adults (male and female) and children (below 3 years) associated with the intake of metals along with fishes from experimental sites was assessed using the following formula:

$$HQ = \frac{(D)\times(Cmetal)}{RfD \times BO} \qquad (4)$$

where, D = daily intake of food (kg/day), C_{metal} = concentration of metal (mg/kg),
RfD = reference oral dose of metal (mg/kg of body weight/day) and BO = Body weight (kg). Daily intake of fishes was taken as 0.08kg for adults, as this is the minimum fish requirement for a balanced diet, it is prescribed that for a balanced diet, one must have a minimum serving of 80 g (National Institute of Nutrition, 2011).

Daily intake of fishes for children below 3 years was taken as 0.05 kg (National Institute of Nutrition, 2011). Average body weight for adult male was taken as 55 kg and average body weight of female as 45 kg and of children (below 3 years) is 12 kg. Reference oral dose (mg/kg/day) for metals is given in Table 3.3.

3.9 Hazard Index

Hazard index is used to evaluate the potential risk to human health when more than one heavy metal is involved. Hazard index was calculated as the sum of hazard quotients (HQs). Since different pollutants can cause similar adverse health effects, it is often appropriate to combine HQs associated with different substances.

$HI=\Sigma THQ(THQ_1+THQ_2+THQ_3\ldots\ldots\ldots THQ_n)$ ....(5)

3.10 Standards

Standard solutions of the heavy metals namely Lead (Pb), Cadmium (Cd), Nickel (Ni) and Cromium (Cr) was provided by Merck (Darmstadt, Germany). The standards were prepared from the individual 1000 mg/L standards (Merck) supplied in 0.1 N HNO_3.

3.11 Quality Assurance

Appropriate quality assurance procedures and precautions were taken to ensure the reliability of the results. Samples were carefully handled to avoid cross contamination. Glassware was properly cleaned, and reagents used were of analytical grades. Deionized water was used throughout the study. Reagent blank determinations were used to apply corrections to the instrument readings.

Chapter IV

RESULTS AND DISCUSSION

4.1 Lead concentration in fishes

The lead (Pb) concentration in fishes studied is given in figure 4.1. The concentrations of Pb in fishes are quite variable, ranging from 0.0012-0.0023 μg/g. The lowest value was analysed in Panna croaker (Poa), while the highest in Chinese silvery pomfret (Rupchanda). However, the order of the fishes in containing Pb was Chinese silvery pomfret (Rupchanda)>Gangetic sillago

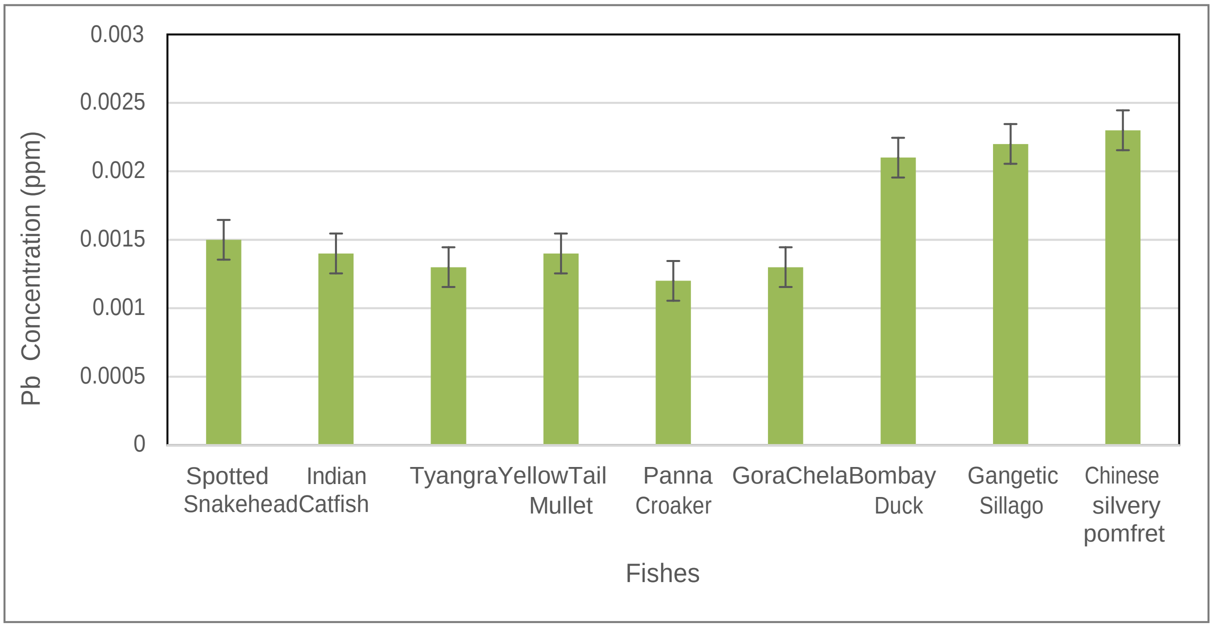


Figure 4.1 Lead concentration in fish collected from Patuakhali district. The bar on each bar graph represents standard error (±) calculated from sample variations (replicates).

(Tular dandi)>Bombayduck(Loitta)>Spotted snakehead(Taki)>Indian catfish(Pabda),Yellow tail mullet(Kachki)>Tyangra(Tengra),Gora chela(Chela)>Panna croaker(Poa). Safe value for lead in fishes recommended by the WHO/FAO is 0.05 μg/g (FAO,1983). Comparatively Chinese silvery pomfret (Rupchanda) had highest value

(0.0023) which was sea fish and Panna Croaker (Poa) had lowest value (0.0012) which was river fish. Based on the maximum permissible limit, the studied fishes had Pb lower than the maximum permissible limit. E.Rahimi *et al* (2010) conducted a research on Analysis and determination of mercury, cadmium and lead in canned tuna fish marketed in Iran. They founded Lead concentration varied from 0.021 to 0.301 (average of 0.096) which is well below the standards of FAO/WHO levels of these toxic metals.

4.2 Cadmium concentrations in fishes

The heavy metal Cd concentration varied among fishes (Figure 4.2). The Cd metal concentration in fishes ranged from 0.0010 µg/g to 0.0022 µg/g. The highest concentration (0.0022 µg/g) in Chinese silver pomfret (Rupchanda) was followed by Panna croaker(0.0013 µg/g) and Yellow tail mullet(0.0012 µg/g). The maximum allowable limit of Cd in fishes as determined by FAO/WHO is 0.2 µg/g (FAO,1983).Sea fish Chinese silver pomfret (Rupchanda) had maximum Cd value (0.0022).On the other hand Gora Chela had the lowest Cd value (0.0010) which was river fish. All of the fishes studied in the present research had Cd below the allowable limit according to FAO/WHO.

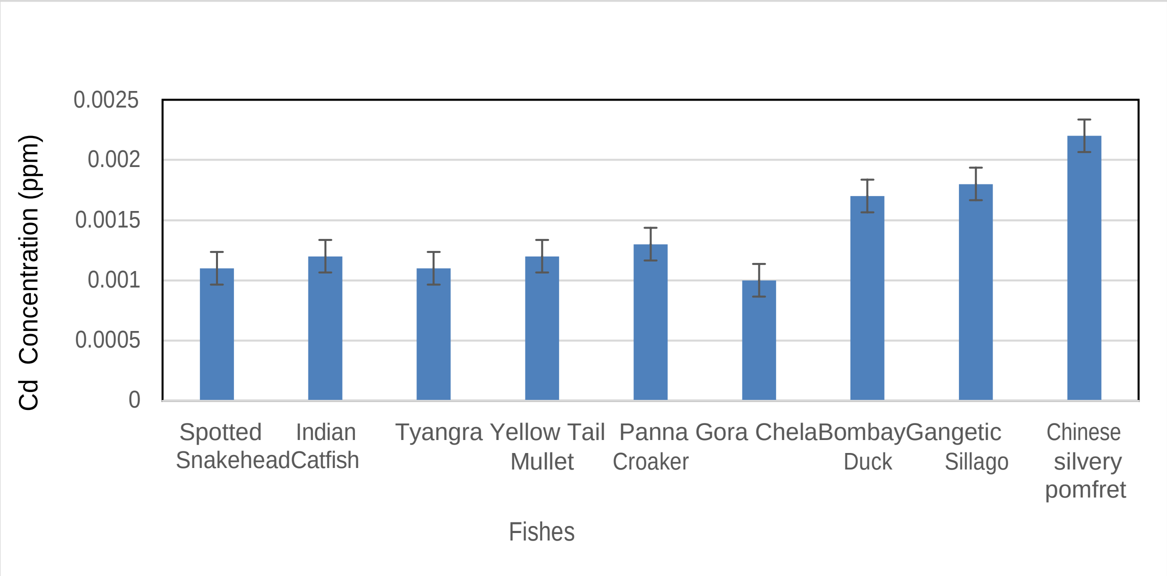


Figure 4.2 Cadmium concentration in fishes collected from Patuakhali district. The bar on each bar graph represents standard error (±) calculated from sample variations (replicates).

FM Kashkahi *et al* (2010) conducted a research on canned Tuna fish in Iran .They founded Cd concentration from 0.008 to 0.150 (average of 0.050) which well below the standards of FAO/WHO levels of these toxic metals.

4.3 Chromium concentrations in fishes

The heavy metal Cr concentrations in fishes are (Figure 4.3), ranging from 0.002µg/g in Yellow tail mullet (Kachki) to 0.008 µg/g in Gangetic sillago(Tulardandi). The order fishes containing heavy metals was Gangetic sillago>Gora chela>Chinese silvery pomfret, Panna croaker Indian catfish>Spotted snakehead, Tyangra, Bombay duck>Yellow tail mullet. Fishes collected from Patuakhali district had Cr concentrations lower than 2 µg/g level (Figure 4.3 The maximum allowable limit for Chromium(Cr) in fish is 2 µg/g (FAO,1983). Sea fish Gangetic sillago had the highest Cr value and river fish Yellow tail mullet had the lowest Cr value (Fig4.3)

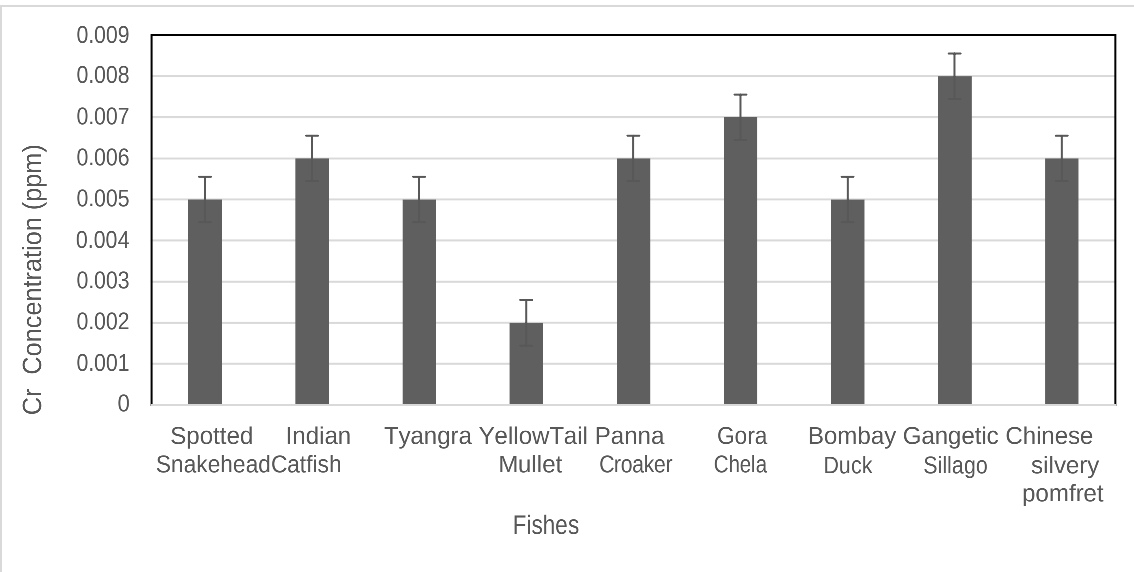


Figure 4.3 Chromium concentration in fishes collected from Patuakhali district. The bar on each bar graph represents standard error (±) calculated from sample variations (replicates)

4.4 Nickel concentration in fishes

The Ni concentration in fishes collected from Patuakhali is given in Figure 4.4. The concentrations of Ni in fishes are variable. The Ni concentration varied from 0.002-0.006 µg/g. The lowest value was analysed in Tyangra, while the highest in Bombay duck (Loitta). However, the order of the fishes in containing Ni was Bombay duck>Chinese silvery pomfret,Gora chela>Spotted snakehead,Panna croaker>Indian catfish>Yellow tail mullet,Gangeticsillago>Tyangra. The results showed that Ni content in sea fish like Bombay duck(Loitta) and Chinese silver pomfret (Rupchanda) was higher than pond and river fishes The maximum permissible limit of Ni in fishes is recommended to be 0.05 µg/g by the WHO/FAO (FAO,1983). Though the sea fish Bombay duck had Ni concentrations higher than river fishes, the fishes studied had Ni lower than the maximum permissible limit.

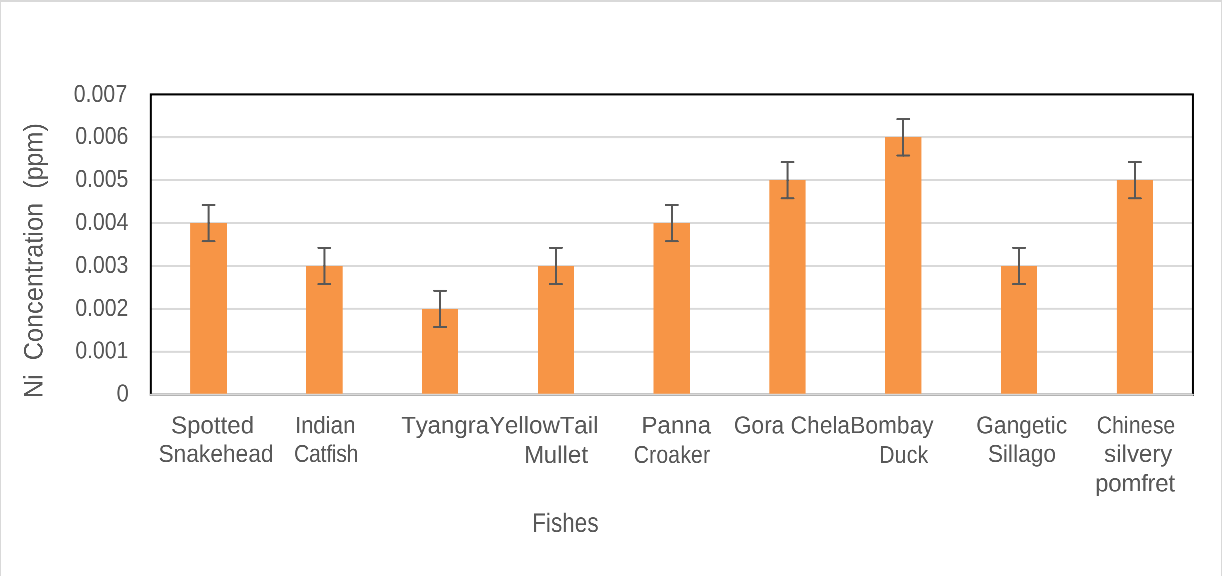


Figure 4.4 Nickel concentration in fishes collected Patuakhali district. The bar on each bar graph represents standard error (±) calculated from sample variations (replicates).

Table 4.1. Single factor pollution index, metal pollution index and sum of pollution index of fishes from different sources:

Fishes	Single factor pollution index (Pi)				Sum of pollution index	Metal pollution index
	Pb	Cd	Ni	Cr	SPI	(MPI)
Spotted snakehead	0.38	1.1	0.2	1.67	3.35	0.002
Indian catfish	0.35	1.2	0.15	2	3.7	0.002
Tyangra	0.33	1.1	0.10	1.67	3.2	0.001
Yellow tail mullet	0.35	1.2	0.15	0.67	2.4	0.001
Panna croaker	0.30	1.3	0.2	2	3.8	0.002
Gora chela	0.33	1.1	0.25	2.33	4.01	0.002
Bombay duck	0.53	1.7	0.3	1.67	4.2	0.003
Gangetic sillago	0.56	1.8	0.15	2.67	5.18	0.003
Chinese sivery pomfret	0.58	2.2	0.25	2	5.03	0.003

4.5 The single factor pollution index (Pi)

The single factor Pi of Pb varied among fish types (Table 4.1). The Pi of Pb recorded in sea fishes Gangetic sillago (Tulardandi) and Chinese silvery pomfret (Rupchanda) was higher than river fishes. The Pi of Pb in Bombay duck, Gangetic sillago, Chinese silvery pomfret were 0.53, 0.56,0.58 respectively. Pi of Pb were in the range of 0.3 to 0.35 in case of river fish. Pi of Pb were 0.35 and 0.38 in case of Spotted snakehead (Taki) and Indian catfish(Pabda) respectively, which were pond fish . The Pi of Cd also varied among fishes studied in the present study (Table 4.1). The Pi of Cd was the highest (2.2) for Chinese silvery pomfret (Rupcanda), followed by 1.8 for Gangetic sillago (Tulardandi). The lowest Pi of Cd was estimated in Spotted snakehead, Tyangra, Gora chela. Bombay duck had highest Ni value (0.3).Chinese silvery pomfret had the second highest Pi of Ni (0.25).Spotted snakehead and Indian catfish had 0.2 and 0.15 respectively, while the lowest Pi of Ni was estimated in Tyangra (0.1). Single factor pollution index of Cr was variable among fishes studied in the present research. Unlike the Pi of Cd, the Pi of Cr was higher than 1.0 in all the fishes except Yellow tail mullet (0.67). The Pi of Cr was the highest in Gangetic sillago(2.67), followed by Gora chela (2.33).The Pi of Cr was lowest in Yellow tail mullet (0.67). The single factor pollution index (PI) is the ratio of metal concentration in a biotic or abiotic medium to that of the regulatory Standard of International bodies such as World Health Organisation (WHO), United States Environmental Protection Agency (USEPA) .Values of Pi<1 value indicates that the plant material is not yet contaminated, whereas Pi >1 indicates pollution. On the other hand, Pi = 1 reveals a critical state.

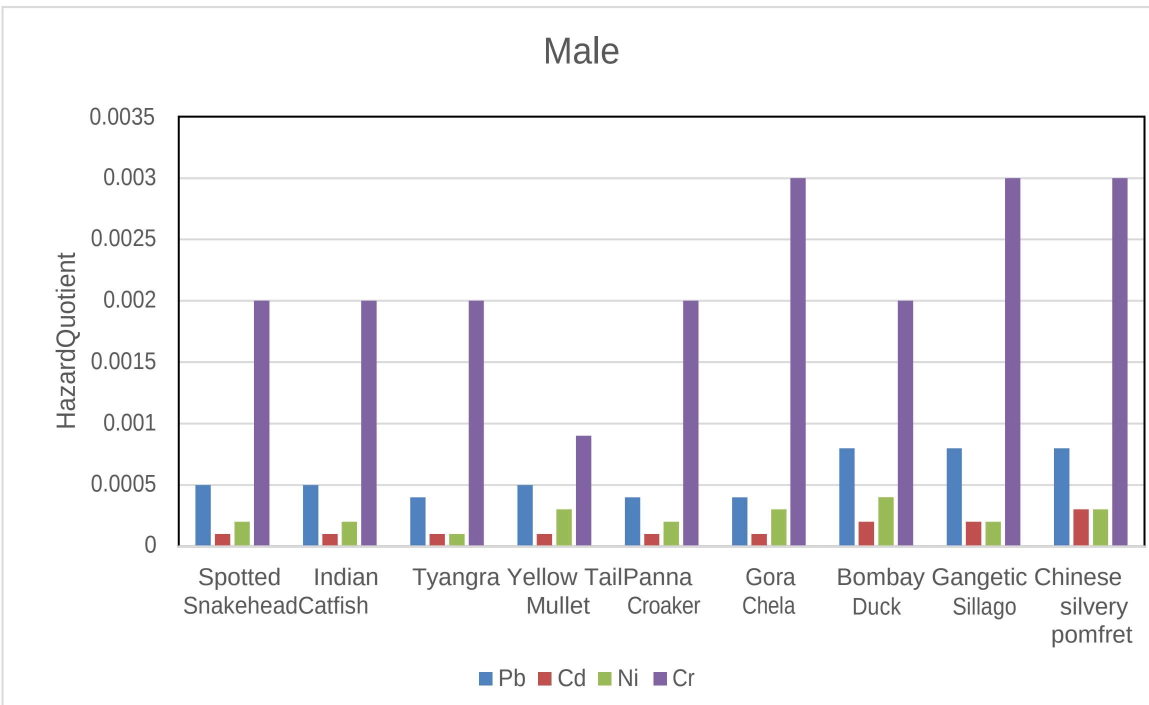
4.6 Sum of pollution index (SPI)

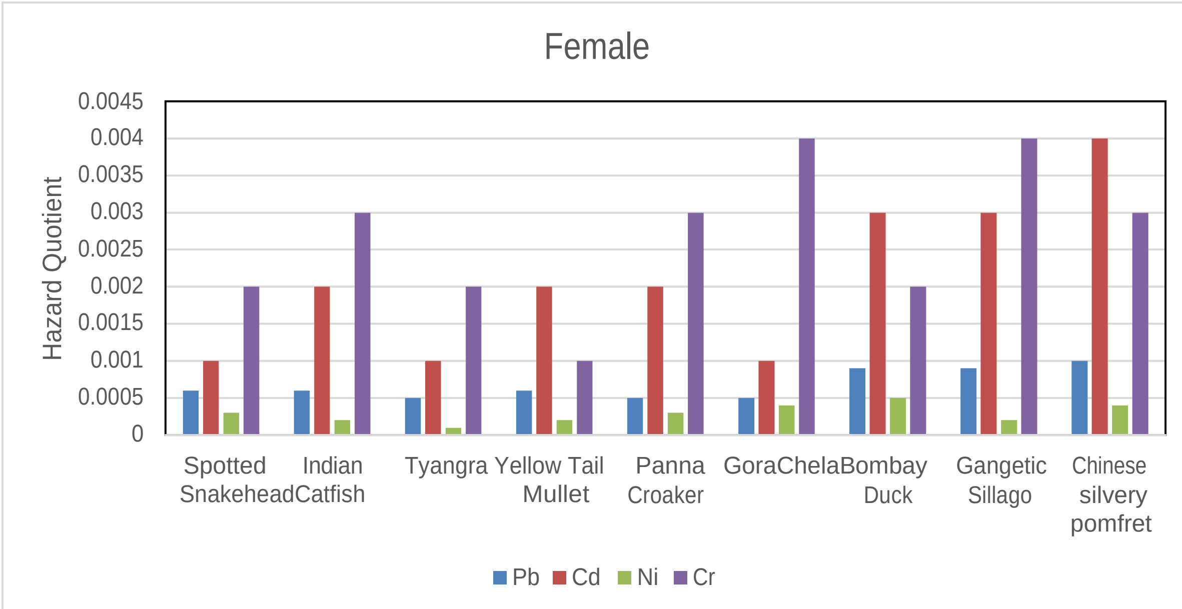
The sum of pollution index (SPI) of the four heavy metals in fishes in Patuakhali, Bangladesh was estimated and is presented in Table 4.1. The sum of pollution index showed wide range of disparity. The sum of pollution index 5.18 the highest was estimated in Gangetic sillago and 5.03 in Chinese silvery pomfret. Among theriver fishes, the highest SPI was estimated in Gora chela (4.01), followed by Panna croaker (3.8). The lowest SPI was estimated in Yellow tail mullet. The variation among the different vegetables could also be due to source locations.

4.7 Metal pollution index

Metal pollution in samples is reliably estimated using metal pollution index (MPI). Table 4.1 demonstrates metal pollution index of fish samples from Patuakhali area. It was found that metal pollution index varied among fish samples. All sea fishes had similar metal pollution index value. Among the river fishes Gora chela and Panna croaker had more MPI value than Tyangra and Yellow tail mullet.

4.8 Hazard Quotient





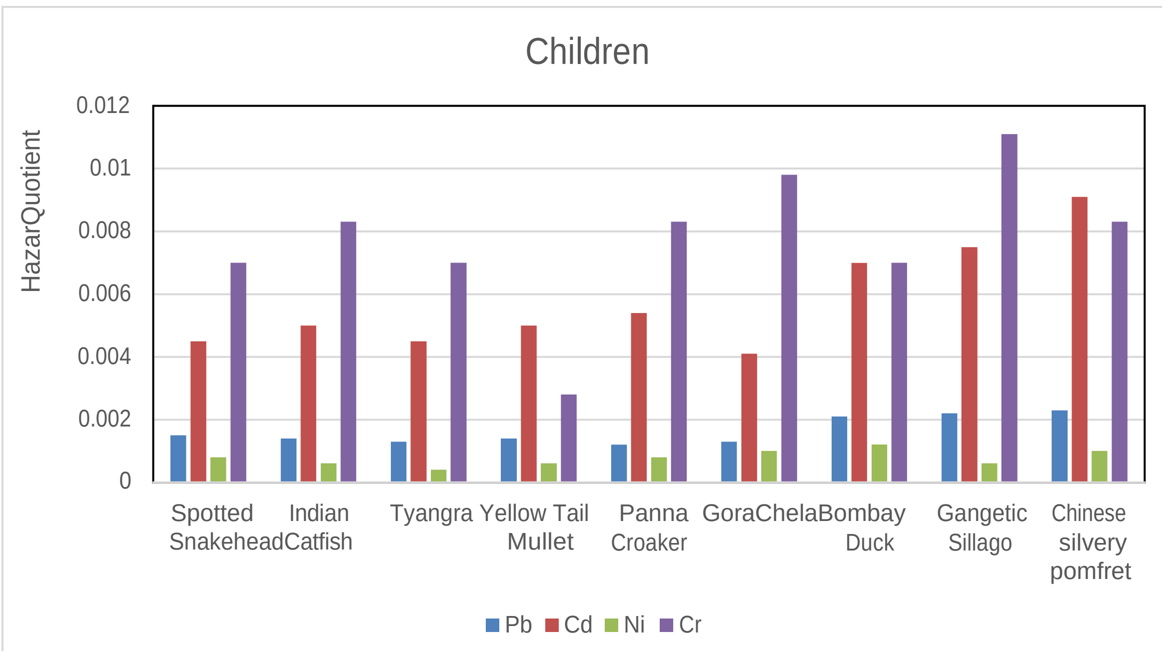


Figure 4.5 The estimates of hazard quotients of metals in the fishes collected from Patuakhali, Bangladesh

4.8 Hazard Quotient

The hazard quotient of all the metals estimated for the sea fishes, river fishes and pond fishes showed different results. The quotients were different for different people groups.

Among the fishes sea fishes Gangetic sillago, Chinese silvery pomfret, Bombay duck had higher hazard quotients for Pb, Cr and Ni. The hazard quotient associated with lead was below 1.0 in all samples for adults and children. Along with Chinese silvery pomfret, Gangetic sillago, Gora chela samples can be highly hazardous for children. Among the different people groups, children had the highest hazard quotients, followed by female. Health risk associated with any pollutant is dependent upon the level of exposure and amount of absorption by human body. Thus, hazard quotient is a valid tool to assess the level of risk associated with particular pollutant. If level of hazard quotient is less than 1.0, the risk associated with exposure of metal is negligible. However, if level of hazard quotient is higher than 1, the metal may pose serious health hazards. In the present study, the estimation of hazard quotient of metals in different fishes from se, river and pond demonstrated negligible health hazards, though children group showed highly under risk of health (Figure 4.5). Though the concentration of Cr was significantly higher in traditional fish samples but the health risk associated with other metals was also found to be negligible.

Lead is very toxic and has very chronic health implications even at low concentrations (Okoronkwo*et al*., 2005). Ingestion of lead could cause mental retardation in children, colic anaemia and renal diseases. The accumulation of Pb in fishes is more pronounced at locations close to the emission source of Pbvapour and fine particles (Abou-Arab *et al*., 2000). Exposure to its high levels can damage the brain, kidneys and ultimately cause death and long-term exposure result in decreased performance in some tests that measure the functions of the nervous system; weakness in fingers, wrists or ankles; small increase in blood pressure and anaemia (Lokeshappa et al., 2012).

Cadmium identified as potential carcinogen by USEPA (2010), was not found to be at hazardous level in fish samples. Apart from various other toxicity affects, USEPA (2015) identifies Cd to target kidney. Thus, this Cd can cause serious health effects at their target organs. Chromium (III) is an essential nutrient that helps the body use sugar, protein and fat but Chromium (VI) is carcinogenic . Adverse health effects may arise from excessive amount of chromium (III).

There is no upper tolerable intake level for chromium according to the Institute of Medicine but the AI of chromium for women and men 51 to 70 years old is 20 mg/day and 30 mg/day, respectively. Deficiency of Cr results in impaired growth and disturbances in glucose, lipid and protein metabolism. However, Cr (IV) is carcinogenic (USEPA, 2011).

The hazard quotient and metal concentration in food crops in markets in and around Dhaka may be associated with using pesticides and chemicals laden with heavy metals like Cd and Cr. Naser *et al*., (2009), Guerra *et al*. (2012), Orisakwee*et al*. 2012; Pedrero et al. (2010), Singh and Agrawal (2010) also found similar results but their study estimated higher hazard quotient which was more than 1. Various studies have reported increase in number of cancer cases, DNA damage and high frequency of micronuclei in buccal mucosa in people from a Village Mahal across the wastewater drain (Sambyal *et al.*, 2017). The hazard quotient of fishes tested in the present study varied among male, female and children groups (Figure 4.6). Among the groups, children were at highest hazard/risk of exposure to metals like Pb, Ni, Cd and Cr.

The uptake and bioaccumulation of heavy metals in fishes are influenced by many factors such as climate, atmosphere depositions, the concentrations of heavy metals in water, the nature of water. Air pollution may pose a threat to fishes during transportation and marketing causing elevated levels of heavy metals in. Elevated levels of heavy metals in fishes reported which such as long term uses of treated or untreated waste water. Other anthropogenic sources of heavy metals include the addition of manures, sewage sludge, fertilizers and pesticides which may affect the uptake of heavy metals.

4.9 Hazard Index

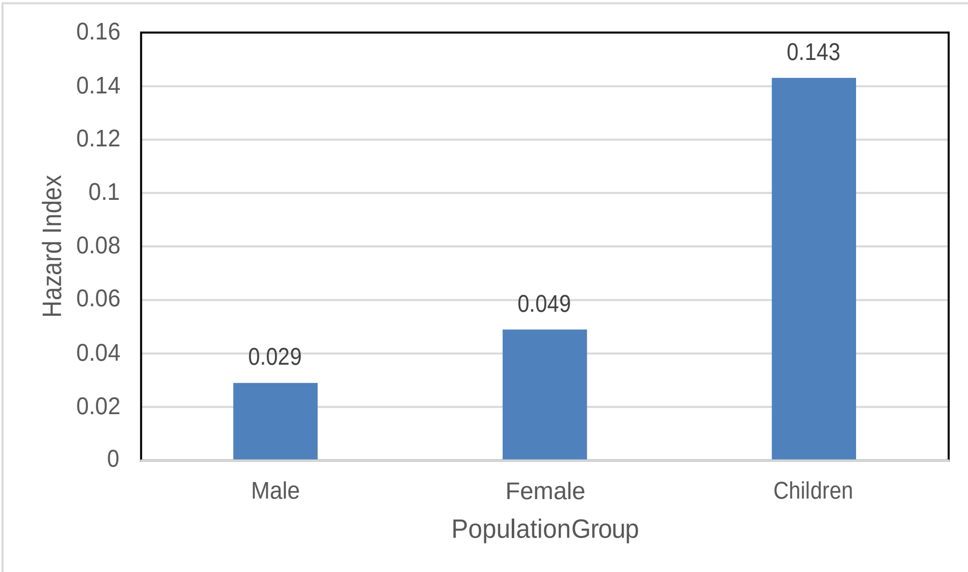


Figure 4.9 Hazard index of the fish estimated for male, female and children.

The hazard index was also estimated for male, female and children. The highest index was estimated for children (0.143), indicates alarming condition for the people group. The female group had the second highest hazard index, shows vulnerability to the heavy metals, if exposed for long-term (Figure 4.9). The estimation of hazard quotient of metals in different fishes from all the sites ponds, river, sea demonstrated negligible health hazards, though children group showed highly under risk of health (Figure 4.9). Though the concentration of Cr was significantly higher in all fish samples but the health risk associated with other metals was also found to be negligible.

Chapter V

SUMMARY AND CONCLUSION

A study was undertaken to determine the status of heavy metals in fishes collected from pond, river and sea of Patuakhali, Bangladesh. Concentration of heavy metals in nine different fish species (Spotted snakehead, Indian catfish, Tyangra, Yellow tail mullet, Panna croaker, gora chela, Bombay duck, Gangetic sillago, Chinese silvery pomfret) collected from Patuakhali District, measured by using the Atomic Absorption Spectrophotometer (AAS). Potential health risks of heavy metals to the local population via vegetables consumption were estimated.

The concentrations of Pb in fishes are quite variable, ranging from 0.0012 in Panna croaker – 0.0023 μg/g in Chinese silvery pomfret .The results showed that sea fishes like Bombay duck, Gangetic sillago, Chinese silvery pomfret contained higher Pb than river and pond fishes. All the fishes had Pb concentrations lower than the maximum permissible limit.

The Cd metal concentration in fishes ranged from 0.0010μg/g in Gora chela to 0.0022μg/g in Chinese silvery pomfret. The highest concentration (0.0022μg/g) in Chinese silvery pomfret was followed by Gangetic sillago(0.0018 μg/g) and Bombay duck(0.0017 μg/g).

The heavy metal Cr concentrations in fishes are variable, ranging from 0.002 μg/g in Yellow tail mullet to 0.008 μg/g in Gangetic sillago (Tulardandi).Fishes collected from Patuakhali had Cr concentrations lower than 0.05 μg/g level. However, the sea fishes contained Cr slightly higher than river fishes but all are below the permissible limit.

The concentrations of Ni in fishes are variable. The Ni concentration varied from 0.002 μg/g in Tyangra – 0.006 μg/g in Bombay duck. The results showed that Ni content in sea fishes like Bombay duck and Chinese silvery pomfret was higher than the river fishes. Though the sea fishes had Ni concentrations higher than river fishes, the fishes studied had Ni lower than the maximum permissible limit.

The estimates to relate to health hazards by taking fishes collected from the Patuakhali district have been reported and found varied among fish types. The Pi of Pb recorded in sea fishes (Gangetic sillago and Chinese silvery pomfret) was higher than river and pond fishes. Pi of Pb were in the range of 0.30 to 0.58. The Pi of Cd was the highest (2.2) for Chinese silvery pomfret, followed by 1.8 for Gangetic sillago. The lowest Pi of Cd was estimated in Spotted snakehead, Tyangra, Gora chela. The Pi of Cr was the highest in Gangetic sillago(2.67) , followed by Gora chela(2.33). Yellow tail mullet had Pi of Cr 0.67, which was lowest. All of the vegetables had Pi values of Ni lower than 1.0. However, Bombay duck had the highest Pi of Ni value (0.30). Gora chela and Chinese silvery pomfret had the second highest Pi of Ni (0.25).

Metal pollution in samples is reliably estimated using metal pollution index (MPI) which slightly varied among samples. The sea fishes Bombay duck, Gangetic sillago, Chinese silvery pomfret had the highest MPI than that of pond and river fishes. Metal pollution index of pond fishes like, Spotted snakehead, Indian catfish was 0.002. River fishes Tyangra and Yellow tail mullet found to be minimum. The general trend of MPI was sea fish>pond fish>river fish.

The hazard quotients were different for different people groups. Among the fishes, sea fishes had higher hazard quotients for Pb, Cr and Ni. The hazard quotient of Cr analyzed in fishes for male, female and children was higher than Pb, Cd and Ni. Along with Gangetic sillago and Chinese silvery pomfret, samples from pond and river can be hazardous for children. Among the different people groups, children had the highest hazard quotients, followed by female. The estimation of hazard quotient of metals in different fishes collected from pond, river and sea demonstrated negligible health hazards, though children group showed slightly under risk of health. Though the concentration of Cr was significantly higher in fish samples but the health risk associated with other metals was also found to be negligible. The hazard index was also estimated for male, female and children. The highest index was estimated for children (0.143), indicates alarming condition for the people group. The female group had the second highest hazard index, shows vulnerability to the heavy metals, if exposed for long-term.

The estimation of hazard quotient of metals in different fishes from pond, river and sea demonstrated negligible health hazards, though children group showed highly under risk of health. Though the concentration of Cr was significantly higher in fish samples but the health risk associated with other metals was also found to be negligible.

The heavy metal concentrations were variable in fishes collected from pond, river and sea at Patuakhali district, Bangladesh. The sea fishes had heavy metal concentrations higher than pond and river fishes. The results indicate that the sea fishes contained heavy metals slightly higher than pond and river fishes but below the maximum permissible limit Among the different people groups, children had the highest hazard quotients, followed by female. Among the fish samples, sea fishes Bombay duck, Gangetic sillago and Chinese silvery pomfret had the highest metal pollution index. Metal pollution index of river fishes like, Yellow tail mullet and Tyangra was found to be minimum than pond fishes. The general trend of metal pollution index from collected fish was Sea fish>Pond fish>River fish. The results, therefore, reported that the fish collected from Patuakhali district contained measured heavy metals within the safe limits prescribed by the WHO.

The present study produced an important result as human health is directly affected by ingestion of fishes; the biomonitoring of trace elements in fishes needs to be continued because fishes are one of the main sources of protein for humans in Bangladesh and around the world. Regulatory frameworks and guidelines for heavy metals in the environment and food stuffs should be developed and deployed in Bangladesh. Industrial effluents should be released after treating and making safe for environment and agricultural use.

Chapter VI

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APPENDICES

Figure 1: Oven Dried Fish Samples.

Figure 2: Oven Dried Fish Samples.

Figure 3: Taking weight of fish samples.

Figure 4:Taking 5ml of Nitric Perchloric acid.

Figure 5: Add 30ml distilled water and filtered with Whattman 42.

Figure 6:Take reading with Thermo AAS.