HEAVY METAL CONTAMINATION IN COMMERCIAL FISH FEED AND CULTURED FISH

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Fisheries

Submitted by

Examination Roll: 4203

Registration No.: Ha-2381

Session: 2009-2010

Department of Fisheries

University of Dhaka

January, 2016

DEDICATED TO

MY

BELOVED PARENTS

Certificate

This is to certify that **Mohammad Alauddin** is a student of M.S. Program of 2013-2014 Session under the Department of Fisheries, University of Dhaka bearing Examination Roll No.: Curzon-4203, Registration no. HA -2381 (2009-2010). As part of his curriculum he has carried out a thesis under my supervision, the title of which is **"Heavy metal contamination in commercial fish feed and cultured fish"**

This is further to certify that it is an original work and suitable for partial fulfillment for the degree of Master of Science in Fisheries, University of Dhaka.

Dr. Md. Monirul Islam

Associate Professor
Department of Fisheries
University of Dhaka

Abstract

Fish is one of the major protein sources for Bangladeshis. It is a promising sector. One of the measures taken to encourage fish farming is the production of fish feeds. Fish feeds are usually in the form of granules or pellets which provide the nutrition in a stable and concentrated form, enabling the fish to feed efficiently and grow to their full potential. The feeds are combined with other ingredients such as vegetable, proteins, cereal grains, vitamins and minerals. Considering the importance of fish, the contamination of its feeds will greatly affect both the fish and the vulnerable population that depends on it as sources of proteins and as a food. In many ways these sources can be associated with anthropogenic heavy metal pollution. It is better to monitor any probable propagation of heavy metals into the food chain through the final feeds rather than the various raw materials for feeds. Three different commercial fish feeds Quality, Mega, Ruposhi and fish (*Oreochromisniloticus*) were collected from nine aquaculture farms ofMuktagachaUpazilla of Mymensingh district. Heavy metal (Cu, Fe, Pb, Cd, Na, Cr, Ni) concentration were determined by Atomic Absorption Spectrometer (AAS) in edible muscle from fish and in feed diets. The results show higher concentrations of Cd, Fe, Na and Pb in both fish feeds and fish muscles all of which exceeded the World Health Organization's or other standard limits for food safety. Target hazard quotient (THQ) and target cancer risk (TR) were calculated to estimate the non-carcinogenic and carcinogenic health risks from fish muscles, respectively. The highest THQ value was estimated for Na (1.51) followed by Cd (1.45). This indicates that excessive consumption of Na and Cd over a long time period might cause non-carcinogenic effect as their THQ values were higher than the acceptable guideline value of 1 (USEPA 2011). In case of TR, although consumption of tilapia at current accumulation level is safe but continuous and excess consumption for a life time of more than 70 years has probability of TR.Precocious steps must be taken to avoid use of such health hazardous concentrations of heavy meals in fish feed and fish.

Table of Contents

Chapters	Title	Page
	Abstract…………………………………………………………..	iii
	Table of Contents……………………………………………..	iv
	List of Figures………………………………………………...	vii
	List of Tables………………………………………………….	x
	List of Acronyms and Abbreviations……………………………	xi
1.	**Introduction**	1
	1.1 Background…………………………………………………	1
	1.2 Heavy metals…………………………………………….....	5
	1.2.1 Definition………………………………………………...	5
	1.2.2 Effect of metals……………………………………….	5
	1.2.3 Heavy metal contamination of aquatic system…….....	5
	1.2.4 Fate of heavy metals in aquatic system ………………	5
	1.3 Risk assessment of heavy metals in human…………………..	6
	1.4 Related works done in the region and around the world…….	6
	1.5 Selection of elements and their toxicity………………………	8
	1.5.1 Basis of selection of elements…………………………	8
	1.5.2 Selected elements and their toxicity………………….	8
	1.6 Aim and Objectives…………………………………………..	14
2.	**Materials and Methods**	15
	2.1 Collection of samples…………………………………………	15
	2.2 Laboratory facilities…………………………………………..	15
	2.3 Preparation of the samples……………………………………	16
	2.4 Analysis of heavy metal concentrations………………………	17
	2.4.1 Preparation of reagents………………………………..	17
	2.4.2 Atomic absorption spectrometer………………………	18
	2.4.3 Microwave digestion…………………………………..	21
	2.4.4 Blank preparation……………………………………..	21
	2.5 Sample analyses………………………………………………	24
	2.6 Analytical technique………………………………………….	24
	2.7 Calibration of instrument…………………………………….	26

Chapters	Title	Page
	2.8 Precautions followed to prevent contamination…………….	27
	2.9 Health risk estimation………………………………………..	27
	2.9.1 Target hazard quotient………………………………..	27
	2.9.2 Hazard index………………………………………….	28
	2.9.3 Target cancer risk……………………………………..	28
	2.10 Statistical analysis…………………………………………….	29
3.	**Results**	30
	3.1 Concentration of heavy metals in fish feed and tissue……….	30
	3.1.1 Copper (Cu)…………………………………………..	31
	3.1.2 Iron (Fe)……………………………………………….	32
	3.1.3 Lead (Pb)……………………………………………...	33
	3.1.4 Cadmium (Cd)……………………………………….	35
	3.1.5 Sodium (Na)…………………………………………	36
	3.2 Health risk estimation………………………………………	37
4.	**Discussion**	40
5.	**Conclusions and Recommendations**	45
	5.1 Conclusions…………………………………………………...	45
	5.2 Recommendations…………………………………………….	46
	References	47
	Appendix- Data analysis for samples	58

List of Figures

Figure	Title	Page
Figure 1	Sample collection from different fish farms............................	16
Figure 2	Collected different feeds………………………………………	16
Figure 3	Reagents used for sample preparation…………………………	17
Figure 4	Fish muscle separation………………………………………	17
Figure 5	AAS used for metal analysis (Shimadzu AA-7000)…………..	19
Figure 6	Different hollow cathode tube ………………………………	19
Figure 7	Flame in AAS ……………………………………..............	19
Figure 8	Schematic diagram of AAS equipment ………………………	20
Figure 9	Measurement of sample …………………………………….	22
Figure 10	Acid mixing ………………………………………………...	22
Figure 11	Sample tube set up……………………………...................	22
Figure 12	Set up for microwave digestion ………………………………	22
Figure 13	Ethos One microwave digester………………………………..	23
Figure 14	Sample after digestion ………………………………………..	23
Figure 15	Prepared samples………………………………………………	25
Figure 16	Sample set up in AAS ………………………………………..	25
Figure 17	Standard curve from standard solution.……………………...	25
Figure 18	Concentrations (mg/kg) of total copper in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh………………………………………………….	31
Figure 19	Concentrations (mg/kg) of total copper inedible tissue of cultured tilapia (*Oreochromis niloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh…………………………………..	31
Figure 20	Concentrations (mg/kg) of total iron in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.	32
Figure 21	Concentrations (mg/kg) of total iron inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different	32

	commercial fish feed collected from three farms in Mymensingh, Bangladesh…………………………………….	
Figure 22	Concentrations (mg/kg) of total lead in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.	33
Figure 23	Concentrations (mg/kg) of total lead inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh…………………………………..	34
Figure 24	Concentrations (mg/kg) of total cadmium in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh………………………………………………….	35
Figure 25	Concentrations (mg/kg) of total cadmium inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh………………………………….	36
Figure 26	Concentrations (mg/kg) of total sodium in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh…………………………………………………….	36
Figure 27	Concentrations (mg/kg) of total sodium inedible tissue of cultured tilapia (*Oreochromis niloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh…………………………………….	37
Figure 28	Composition of hazard index (HI) for different heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh………………………..	38
Figure 29	Target cancer risk (TR) of heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh…………………………………………………….	39

List of Tables

Table	Title	Page
1.	AAS operating conditions……………………………………………	27
2.	Heavy metals concentrations (mg/kg) in commercial fish feed and in edible tissue of cultured tilapia……………………………………….	30
3.	Target hazard quotient (THQ) for different heavy metals and their hazard index (HI) ……………………………………………………	38
4.	Target cancer risk (TR) of heavy metals from consumption of three brands feed fed tilapia……………………………………………….	39

List of Acronyms and Abbreviations

AAS	:	Atomic Absorption Spectrometer
BBS	:	Bangladesh Bureau of Statistics
BCSIR	:	Bangladesh Council of Scientific and Industrial Research
Cd	:	Cadmium
Cu	:	Copper
Cr	:	Chromium
DoF	:	Department of Fisheries
et al.	:	*et* alia(L), and others
EU	:	European Union
FAO	:	Food and Agriculture Organization
Fe	:	Iron
FRSS	:	Fisheries Resources Survey System
GDP	:	Gross Domestic Product
GNP	:	Gross National Product
GOB	:	Government of the People's Republic of Bangladesh
Hg	:	Mercury
HI	:	Hazard index
kg	:	Kilogram
Pb	:	Lead
mg/Kg	:	Milligram per kilogram
Na	:	Sodium
Ni	:	Nickel
ppm	:	Parts per million
SD	:	Standard Deviation
SPSS	:	Scientific Package for Social Sciences
TF	:	Transfer Factor
THQ	:	Target hazard quotient
TR	:	Target cancer risk
USEPA	:	US Environmental Protection Agency
WHO	:	World Health Organization
%	:	Percentage

Chapter 1 – Introduction

1.1 Background

The growing human population has increased the need for food supply. Because they are good protein sources, the demand for fish and shellfish products has increased. Worldwide, people obtain about 25% of their animal protein from fish and shellfish (Bahnasawy*et al*., 2009). In 2004, about 75% (105.6 million ton) of estimated world fish production was used for direct human consumption (FAO, 2007). It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 (Retnam*et al*., 2010).The real importance of fish in human diet is not only in its content of high-quality protein, but also to the two kinds of omega-3 polyunsaturated fatty acids: eicosapentenoic acid (EPA) and docosahexenoic acid (DHA). Omega-3 (n-3) fatty acids are very important for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Burger *et al*., 2005, Al bader*et al*., 2008). Fish also contain vitamins and minerals which play essential role in human health. But consumption of various chemical contaminated fish has become a global concern. Heavy metals contamination comprises significant portion of the problem as these metals known for their bioaccumulation and bio-magnification which cause various health hazards to human. Since diet is the main route of exposure to heavy metals, and fish represent a part of human diet, it is not surprising that polluted fish could be a dangerous dietary source of certain toxic heavy metals (Bogut*et al*., 1997).In the last two decades there has been a growing interest in assessing the levels of heavy metals in food including fish (mainly concerned with commercial species). Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazardous effect on human health.

As a result of increasing demand for food fish aquaculture has emerged as one of the most promising industries in the world with a considerable growth potential to improve human dietary standards by providing protein rich food and diversifying rural production and aquaculture potential (Dhawan*et al.*, 1998).World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2014). In the last five decades global fish production has grown steadily. In 2012, total world fish production was 158 million tons of which inland capture and culture productions were 11.6 and 41.9 million tons respectively (FAO, 2014).

In Bangladesh, The Fisheries and aquaculture sector is one of the major components of agricultural activities and plays a crucial role in economic emancipation through food security, poverty alleviation, improving the socio-economic status, income generation, the fight against malnutrition, foreign exchange earnings and creating employment opportunities and providing more than 60% of animal source food, representing a vital source of micro-nutrients, and possessing an extremely strong cultural attachment. The Fisheries sector is a source of cheap and nutritious food, also stimulates the growth of a number of subsidiary industries.

Bangladesh is one of the top ten countries in aquaculture (FAO, 2013). The country has the third largest aquatic fish biodiversity in Asia, after China and India, with about 800 species in fresh, brackish and marine waters (Hussain and Mazid, 2001). Numerous small rivers and a large number of big rivers including the Meghna, the Brahmaputra, the Surma, the Karnaphuli and the Padma flow through the country. The fisheries sector includes open water bodies such as rivers, canals, lakes, etc. and closed water bodies such as ponds and flood-control polders. There are rivers, tributaries, beels(permanent and seasonal lakes and wetlands), baors(oxbow lakes), haors(large deeply flooded depressions), and floodplains. Total area of inland water body is 46,99,345 hectors. The country is rich in her fish fauna supporting at least 265 freshwater fin fish species (Rahman, 2005). The fish production was 29,52,730 Metric Tons in 2014 (DoF, 2015).

In spite of steps to reduce poverty over the last three decades, Bangladesh remains one of the world's poorest and least developed countries. Within the overall agro-based economy of the country, fish production is crucial for livelihoods, income, animal protein, employment opportunities, nutritional security and food supply. About 11% of the population directly and indirectly depends on fisheries for their livelihood (DoF, 2015). Around 400,000 ha of fish ponds/ditches and more than 900,000 households are involved in aquaculture (ADB, 2010).

In Bangladesh, around 46% of children between the ages 6 to 7 years are stunted and 70% are wasted due to malnutrition (Ahmed *et al.*, 2007). The greater emphasis should be given to meet the animal protein deficiency among the people as well as to boost up fish production in this country through proper management of open water fishery and aquaculture. But fish production from open water bodies is decreasing day by day (DoF, 2015). Once upon a time, these unique ecosystems supported huge and diverse biodiversity. At present, most of the water bodies are contaminated by agricultural, industrial and municipal waste as a pollutant and those are accumulated by runoff into these resources. Aquatic organisms are silent victims of chlorine sub-lethal toxicity resulting from different types of pollutants (Bernet*et al*., 1997). Fish production is also decreasing due to natural causes like flood, drought, etc. (Chakraborty, 2009). But it is important that closed water or pond fishery production is increasing day by day due to necessitate and demand of the people (Ahmed, 2010). In addition more return also come from the fish production (DoF, 2015).

To fulfill the need of time, dependency on culture fisheries and pond aquaculture is increasing to a great extent, of which pond aquaculture is of greatest importance. With the increasing demand for fish and the decline in capture fish production, tilapia farming in Bangladesh is becoming more intensive (Ahmed *et al*., 2007). With increasing popularity among consumers, tilapia have become the world's second most important cultured fish after carps. Tilapias are considered as 'wonder fish' by some and as 'aquatic chicken' by others as opined by Gupta *et al*. (1992). Tsadik and Bart (2007) reported that the Nile tilapia, *O. niloticus*is a widely cultured fish because it can grow and reproduce in a wide range of environmental conditions and tolerate stress induced by handling. Production of Nile tilapia from ponds of Bangladesh was 8,221 MT which was only 1.52% of total pond fish production in 2001-2002. This production has increased to 98,758 MT which is 8.10% of total pond fish production in 2010-11 (FRSS, 2012). Obviously, the existing production will be much higher.

With the increasing demand for food fish and the decline in capture fisheries production, aquaculture in Bangladesh is heading towards intensification (Mahmud *et al*., 2012). Farmers shift gradually from no feed, through the use of farm-made feeds, to factory-made feeds (Mahmud *et al*., 2012). The success of intensive and semi-intensive fish culture depends on a large extent to the application of suitable feeds. This demonstrates a real possibility of increasing production and reveals the potential importance of aqua feeds in Bangladesh. Therefore, aquaculture feeds have been considered a major sub-sector of the feed milling industry.

Demand of fish feed is increasing day by day and number of fish feed companies are also increasing. The well-known industries are Ruposhi Feed, Mega feed, Saudi-Bangla Feed, Paragon Feed, Quality Feed, ACI Feed, C.P Feed, Kayer Feed, AIT Feed, New Hope Feed etc. (Hasan, 2010). These industries not only manufacture fish feed but also they produce livestock feed such as poultry, cattle, pet animal etc. On the other hand, hundreds of small-scale non-commercial and on-farm feed industries produce fish feed throughout the country.

Nowadays, it has been noted that some of the commercial feed producers failed to meet up with standards for the requirement of fish and in many ways, the source of raw material for the production of the feeds tends to be contaminated with heavy metals (Indrajit*et al.,* 2011). There is no or a little information evaluate the heavy metals of fish feed produced by different feed industries specially the local one. The farmers have to depend only on the existing information about the feed composition is given by the industry. The government of Bangladesh has a legal legislation but control over the feed components and feed quality is lacking. Also there is no guideline for establishment of a new industry. Although contamination of animal feeds by toxic metals cannot be entirely avoided given the prevalence of these pollutants in the environment, there is need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health (SCAN 2003). Some mineral elements such iron, manganese, copper and zinc are essential dietary nutrients for fish. However, all mineral elements can have an adverse effect upon human and fishes at excessively high or low concentration if included in the diet (Okoye*et al*., 2011).

1.2 Heavy metals

1.2.1 Definition

The term heavy metals is a general collective term which applies to group of metals and metalloids with atomic density greater than $4g/cm^3$ or 5 times or greater than water (Duruibe*et al*., 2007), they are also known as trace elements because they occur in minute concentrations in biological systems.

1.2.2 Effect of metals

Depending upon their concentration they may exert beneficial or harmful effects on plant, animal and human life (Forstner*et al*., 1981). Some of these metals are toxic to living organisms even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. When ingested in excess amounts heavy metals combine with body's biomolecules, like proteins and enzymes to form stable biotoxic compounds, thereby mutilating their structures and hindering them from the bioreactions of their functions(Duruibe*et al*., 2007).

1.2.3 Heavy metal contamination of aquatic system

In the last decades, contamination of aquatic systems by heavy metals has become a global problem. Heavy metals may enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz*et al.*, 2009).

1.2.4 Fate of heavy metals in aquatic system (Bioaccumulation)

In natural aquatic ecosystems, metals occur in low concentrations. As they cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals and thus, causing heavy metal pollution in water bodies (Abdel-Baki*et al*., 2011). Metals entering the aquatic ecosystem can be deposited in aquatic organisms through the effects of bio-concentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level (Huang *et al*., 2003). In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues (Mansour *et al*., 2002). Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in the blood stream to either a storage point (i.e. bone) or to the liver for transformation and/or storage (Obasohan*et al*., 2008).

1.3 Risk assessment of heavy metals in human

Like in other organisms, heavy metals are not destroyed by humans (Castro-Gonzeza*et al*., 2008). Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans. Therefore, the heavy metals are among most of the pollutants, which received attention in various countries and considered the most dangerous category of pollutants in the sea (Hassaan*et al*., 2007).

An early example of an environmental problem due to heavy metal occurred in 1952, in the vicinity of the Japanese fishing harbour of Minimata. This disease (Minimata disease) was a result of consuming fish and shrimps contaminated by methyl mercury and non- organic mercury from the wastewaters discharged by Chlor-alkali factories. Another example is the Ita-Ita Disease in Fugawa, Japan in 1955(Dural *et al*., 2007). It was the result of consuming rice, fish and bivalves that were Cd-contaminated from wastewaters discharged by nearby mining (Dural *et al*., 2007, Chen *et al*., 2001).

1.4 Related works done in the region and around the world

Due to their toxicity and accumulation in biota, determination the levels of heavy metals in commercial feed and fish species have received considerable attention in different countries in the region and around the world. Such interest aimed at ensuring the safety of 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:

Horst *et al*. (1998) also found OCs, specifically chlordane compounds, in farmed salmon as well as fish meal, oil and food products made from the farmed fish.

Easton et al. (2002) associated the levels of OCs, polybrominateddiphenyl ethers (PBDE; flame retardants) and metals in farmed salmonids with the elevated levels of contamination in commercial feeds.

Hilton *et al*. (1983) formulated five test feeds using fish meal from several sources, and all of the resulting feeds had detectable concentrations of dieldrin, heptachlor and chlordane.

Shearer *et al*., (1994) analyzed eight feeds from a Norwegian feed manufacturer for select metals. Their results found Cu, 1.3–29.2 ppm; Fe, 68.7–353 ppm; Mg, 1860–2100 ppm; Mn, 5–120 ppm; Zn, 170–380 ppm

Shamshad*et al*. (2009) found the range of elemental concentrations (mg/kg diet, dry wt.) of fish feed were: As 0.14-0.91, Cd <0.1-2.1, Pb<0.1-8.57 and Hg was found to be below the detection limit (<0.03).

Mac. *et al*. (1979) found polychlorinatedbiphenyls (PCBs) and dichlorodiphenyl-trichloroethane (DDT), metabolites of namely dichlorodiphenyltrichloroethylene (p,p-DDE), in fish feeds.

Anhwange*et al*. (2012) determined the concentrations of some heavy metals in two synthetic fish feeds (Multi feed and Coppen). The results of the analysis indicated Multi feed contained Cd (30.00 μg/kg), Cu (157.00 μg/kg), Fe (2196.00 μg/kg), Ni (92.00 μg/kg), Pb (348.00 μg/kg) and Zn (1209.00 μg/kg); while Coppen feed contained Cd (20.00 μg/kg), Cu (204.00 μg/kg), Fe (2435.00 μg/kg), Ni (8.00 μg/kg), Pb (375.00

μg/kg) and Zn (3324.00 μg/kg). The results showed that the feeds contained some of the heavy metals analysed in varying proportions.

Abdel-Baki*et al*. (2011) determined the concentrations of some heavy metals (Pb, Cd, Hg, Cu and Cr) in water, sediment and tissues of tilapia fish collected from WadiHanifah during summer season. The concentrations of the heavy metal in water were within the international permissible level. Cu had the highest accumulating level in fish whilst Hg had the lowest. The transfer factors of all metals in fish from water were greater than those from sediments. This led to the conclusion that fish bioaccumulation with these metals was from water. Heavy metals under study in the edible parts of tilapia were within the safety permissible level for human use.

Saeed and Shaker (2008) presented a report about concentrations of Fe, Zn, Cu, Mn, Cd and Pb in *O. niloticus*(Tilapia) fish tissues, water and sediments in northern Delta Lakes. They found that the edible part of *O. niloticus*from Lake Edku and Manzala contained the highest levels of Cd while fish from Manzala Lake contained the highest level of Pb. They reported that Nile tilapia caught from these two Lakes may pose health hazards for consumers.

1.5 Selection of elements and their toxicity

1.5.1 Basis of selection of elements

Elements as Cd, Cr and Pb are nonessential metals and their toxic effect on human health is well known, while metals as, Cu, Na, Ni and Fe are essential metals, the toxic effect of them on human health begins when they are present in high levels. These elements may be added on the ecosystem through human activities or from natural sources as it was explained previously. Many studies assessed concentration of these heavy metals in fish species all over the world.

1.5.2 Selected elements and their toxicity

1.5.2.1 Lead (Pb)

Lead has a density of 11.3g/cm3 atomic number 82 and is obtained from its sulphide mineral galena, carbonate cerussite, and sulphate anglesite. The ores are frequently found in combination with other recoverable metals such as Cu, Zn and Cd. Lead exists in various oxidation states (O, I, II and IV), which are of environmental importance with oxidation +2, the form in which most Pb is bio-accumulated by aquatic organisms (Akan *et al*., 2009).Lead was placed position 2 on the Agency for Toxic Substances and Disease Registry's (ATSDR)top 20 list of most dangerous heavy metals and it accounts for most of the cases of paediatric heavy metal poisoning (ATSDR, 2002).Lead has been used in pipe making, drains and soldering materials as well as battery manufacture, plumbing, ammunition, fuel additives, paint pigments and pesticides (ATSDR, 2005).

Lead has been of particular concern due to its toxicity and ability to bioaccumulation aquatic ecosystems, as well as persistence in the natural environment (Miller *et al*., 2002; Anim*et al*., 2010). Lead is known to accumulate in fish tissues such as bones, gills, liver, kidneys and scales, while gaseous exchange across the gills to the blood stream is reported to be the major uptake mechanism (Oguzie, 2003;Tawari-Fufeyin and Ekaye, 2007). Some effects of Pb poisoning include deficiency in cognitive function due to destruction of the central nervous system, abdominal pain and discomfort, formation of weak bones as Pb replaces calcium and causes anaemia due to reduction of enzymes concerned with synthesis of red blood cells (Lars, 2003).

Lead also leads to decreased fertility, causes cancer and other minor effects like vomiting, nausea, and headache (Lars, 2003; WHO, 2008). Exposure to high Pb levels can severely damage the brain and kidneys, cause miscarriage in pregnant women, damage the organs responsible for sperm production in men and it may ultimately cause death (ATSDR, 2002). Since fish have ability to bioaccumulate metals for a long time, the level of metal ions at a particular time may not give accurate information on concentration at that particular time.

1.5.2.2 Nickel (Ni)

Nickel with density of 8.9g/cm3 readily forms alloys with iron, aluminium, zinc, molybdenum, and copper and can be dissolved in dilute acids (Reilly, 2002). The most common oxidation state of Ni is +2, but compounds of Ni+ and Ni3+ are well known, and Ni4+ has been demonstrated(Housecroft and Sharpe, 2008).Nickel (II) compounds are known with all common anions, that is, the sulphide, sulphate, carbonate, hydroxide, carboxylates, and halides. In its compounds, Ni has a number of chemical manufacturing uses, such as a catalyst for hydrogenation, as a cathode in many rechargeable batteries, including nickel-cadmium, nickel-iron, nickel-hydrogen, and nickel-metal hydride, and used by certain manufacturers in Li-ion batteries (Davis, 2000). About 60% of world production is used in nickel-steels (particularly stainless steel) (Obasohan, 2008). Nickel toxicity is generally low, but elevated levels have been reported to cause sub-lethal effects (Nussey*et al.*, 2000). Among the known health-related effects of Ni are skin allergies, lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation. Nickel sulphide fume and dust is believed to be carcinogenic, and various other Ni compounds may be as well (Kasprzak*et al.,* 2003). The toxicity of Ni carbonyls is a function of both the toxicity of the metal as well as the carbonyl's ability to give off highly toxic carbon monoxide gas, being explosive in air (Nussey*et al.*, 2000).

Fish are known to accumulate Ni in different tissues when exposed to elevated levels in their environment (Nussey*et al.*, 2000; Obasohan and Oronsaye, 2004). Studies of heavy metal concentration in fish from the Dhaleswari and Buriganga Rivers Bangladesh recorded mean concentrations that ranged from 9.55 to 13.35 mg/kg and from 0.09 to 0.48 mg/kg, respectively (Ahmad *et al*., 2009; Ahmed *et al*., 2009b). Other findings from Ikpoba River recorded 0.02 mg/kg mean concentration of Ni in the gills of chrysichthysnigrodigitatus in the rainy season which, did not constitute immediate hazards because they fell below the 0.4 mg/kg levels recommended in fish and fishery products by the Food and Agricultural Organization of the United Nations(FAO/WHO, 1984; Oguzie and Izevbigie, (2009). The sediments of Buriganga River, Bangladesh showed spatial and temporal variation of Ni that ranged from 147.06 to 258.17mg/kg. It was suggested that the Buriganga River was partly a heavy metal-polluted river and the sediments were not fully safe for human health and ecosystem (Ahmad *et al*., 2009). With the gradual development of industry, intensive use of pesticides and discharge of untreated domestic sewage may further exacerbate the situation in coming years which should otherwise be minimized (Ahmad *et al*., 2009). The typical concentrations of Ni in unpolluted surface water are given as 5.0 x10-4 mg/l and 0.015 to 0.020 mg/l (Salnikow and Denkhaus, 2002; Awofolu*et al*., 2005).Studies in river water have indicated various levels of Ni concentrations compared to the recommended limit of 0.07 mg/l Ni in drinking water by WHO (WHO, 2003; Awofolu*et al*., 2005; Wachira, 2007).

1.5.2.3 Cadmium (Cd)

Cadmium has oxidation state of +2 and forms a number of inorganic compounds such as sulphates, chlorides and acetates most of which are water soluble. Cd is a by-product of mining and smelting of Pb and Zn and is used in nickel-cadmium batteries and paint pigments. Cd can be found in soils under agriculture from insecticides, fungicides, sludge and commercial fertilizers. Ingestion of Cd can rapidly cause feelings of nausea, vomiting, abdominal cramp and headache, as well as diarrhoea and shock. Itai-itai disease in Japan was identified among people living in Cadmium-polluted areas where rice was irrigated. Target organs include liver, placenta, kidneys, lungs, brain and bones (Reilly, 2002).

1.5.2.4 Copper (Cu)

Copper belongs to group I-B of the periodic table, it has an atomic weight of 63.55 with a specific gravity of 8.96 with oxidation states of +2, +1. The important ores of Cu are Chalcocite (CuFeS2), Cuprite (Cu2O) and Malachite [CuCO3. Cu(OH)2]. Copper is widely used for wire production and in the electrical industry. Its main alloys are brass (with zinc) and bronze (with tin). Other applications are kitchenware, water delivery systems, and copper fertilizers (Bradi, 2005).Copper is considered as an essential constituent of metalloenzymes of living organisms and is required in hemoglobin synthesis and in catalysis of metabolic reactions. It plays a crucial role in many biological enzyme systems that catalyze oxidation reduction reactions. However, if present at relatively high concentrations in the environment, toxicity to aquatic organisms may occur. Copper under ionic forms Cu^{2+}, Cu_2OH^+ and $CuOH^+$ is toxic to fish (Moore, 1997). High copper levels lead to an increase in the rate of free radical formation teratogenicity (Stouthart*et al.,* 1996) and chromosomal aberrations (Bhunya, 1987; Fahmy, 2000).

1.5.2.5 Iron (Fe)

Iron is a heavy silver-gray metal that is lustrous, ductile, and malleable. Iron is known to exist in four crystalline forms. It rusts in damp air but not in very dry air. Iron dissolves readily in dilute acids and is chemically active. This heavy metal forms two major series of chemical compounds known as bivalent iron (II), or ferrous compounds, and the trivalent iron (III), or ferric compounds. Of all the heavy metals, iron is the most used, more than all of other metal tonnage produced worldwide. This is because iron is both low cost and has high strength. Iron has many applications from food containers to family cars, from screwdrivers to washing machines, from cargo ships to paper staples. The best known alloy of iron is steel, and other forms of iron are pig iron, cast iron, carbon steel, wrought iron, alloy steels, and iron oxides.

Iron is known to be the 10th most abundant element in the universe. Iron is also most abundant by mass, 34.6% in the Earth's crust. In the different layers of the Earth, iron ranges from a high of 60.4% at the inner core to about 5% in the outer crust. Most iron is found as different forms of iron oxides like hematite, magnetite, and taconite. The core of the earth is made up of a metallic iron-nickel alloy.

Iron (III)-o-arsenite, pentahydrate also known as ferric arsenite may be hazardous to the environment, and special attention is needed for plants, air, and water. Iron is like other heavy metals, accumulation of a high concentration of iron (III)-oarsenite can become toxic to human health. According to Lenntech, this chemical is persistent in the environment once it enters. Therefore, it should not be allowed to enter into the environment in the first place.

Iron is found in many food sources like meat and vegetables. The human body absorbs iron from animal products faster than iron in plant products. Iron is a necessary part of hemoglobin that transports oxygen through our bodies. Hemoglobin is a protein that is made of two parts, the globin and heme groups that contain iron. The globin group is formed from four polypeptide chains, and each of these chains is associated with a heme group. The iron is the center of each heme group that binds the oxygen.

In humans, high concentration of iron causes conjunctivitis and choroiditis. Chronic inhalation of excessive concentrations of iron oxide fumes or dusts may result in the development of benign pneumoconiosis, referred to as siderosis. No physical impairment of lung function has been associated with siderosis. However, it may enhance the risk of lung cancer development, especially in workers also exposed to pulmonary carcinogens.

1.5.2.6 Chromium (Cr)

Chromium has density of 7.2g/cm3 and is the 21st most abundant element in Earth's crust with an average concentration of 100 ppm (Emsley, 2001). Chromium compounds are found in the environment, due to erosion of Cr -containing rocks, animals, plants, soil and can be a liquid, solid or gas. Cr can exist in valences of +3and +6 with oxidation state in Cr (III) being stable and give series of chromic compounds, like oxides (Cr_2O_3), chlorides ($CrCl_3$) and sulphates ($Cr_2(SO_4)_3$) (Emsley, 2001; Gonzalez *et al*., 2005). Cr is used in metal alloys such as stainless steel, protective coatings of metal (electroplating), magnetic tapes, and pigments for paints, cement, paper, rubber and its soluble form is used in wood preservatives as well as additive in water to prevent corrosion in industrial and other cooling system (Hingston, 2001; WHO, 2003). Hexavalent Cr is very toxic and mutagenic when inhaled and is a known human carcinogen. Breathing high levels of the element in this form can cause irritation to the lining of the nose and breathing problems such as asthma, cough, shortness of breath, or wheezing where long term exposure can cause damage to liver, kidney circulatory and nerve tissues, as well as skin irritation(Dayan and Paine,2001).

Higher Cr mean levels than the recommended limit of 0.15 mg/kg have been recorded in fish from various rivers (FAO/WHO 2003; Obasohan, 2008;Abdel-Baki *et al*., 2011).

1.6 Aim and Objectives

The aim of the present study was to evaluate the presence of heavy metals in commercial fish feed and their accumulation in edible fish tissues in order to assess the carcinogenic and non-carcinogenic risk associated to consumption of fish. The specific objectives of the study were to-

i. investigate the presence of heavy metals (Cu, Fe, Pb, Cd, Na, Cr, Ni) in commercial fish feed and edible tissues of tilapia (*Oreochromisniloticus*);
ii. assess potential bioaccumulation of heavy metals in fish through consumption of commercial feeds; and
iii. assess the potential human health risk associated with the consumption of these fish species.

Chapter 2 - Materials and Methods

2.1 Collection of samples

For this study both fish feed and fish samples were collected from MuktagachaUpazilla of Mymensingh district. There are lots of fish farms in that area. The farmers cultured different species of fish. Tilapia (*Oreochromisniloticus*) is cultured widely in that area. Culture technique and facilities of culture this fish are also wide. Tilapia culture is more profitable than other fish farming to the farmers.

There are many commercial feeds which are used by the farmers for production of Tilapia (*Oreochromisniloticus*). Among the large number of commercial feed some of the feeds used in the present study were collected from different fish farms by the author himself. Three most widely used feeds, namely Quality, Mega, and Ruposhi, were sampled. Three kinds of fish feeds (nursery, starter, grower) were sampled from 9 Tilapia farms.

Adult freshwater tilapia (*Oreochromisniloticus*) ranged between 100-150 g in weight were collected from 9 Tilapia farms (5 fish from each farm) all of which used above mentioned feeds. After collection, the samples were cleaned with deionized-distilled water, stored in pre-cleaned plastic bags, and kept frozen in an ice box. Samples were double bagged in separate new plastic bags, sealed and labeled accordingly and transported to the BCSIR laboratory.

2.2 Laboratory facilities

Heavy metal concentrations were tested in the Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.

2.3 Preparation of the samples

The samples were taken from the refrigerator and kept in room temperature for few hours. Then the required amount of samples were finely ground by a mortar and kept in airtight container for subsequent chemical analysis.

Figure 4: Sample collection from different fish farms

Figure 5: Collected different feeds

2.4 Analysis of heavy metal concentrations

2.4.1 Preparation of reagents

All reagents used in the present study were of analytical grade and consisted of nitric acid (65% v/v) and hydrogen peroxide (30% v/v) (Merck, Darmstadt, Germany). Standard stock solutions of Cd, Cu, Ni, Fe, Na, Cr and Pb with concentrations of 1,000 mg/L were prepared by diluting 1 mL of each single element stock in the combination list to 100 mL with deionized distilled water that contains 1% (v/v) nitric acid. Prior to the experimental process, the apparatus was sterilized by soaking it for approximately 24 h in diluted nitric acid (10% v/v); afterwards, the apparatus was rinsed with deionized water.

Figure 6: Reagents used for sample preparation

Figure 7: Fish muscle separation

2.4.2 Atomic absorption spectroscopy

Flame atomic absorption spectrophotometer is very common technique for detecting metals and metalloids in environmental samples. It is very reliable and simple to use. The technique is based on the principle of ground state metals absorbing light at specific wave length. Metal ions in a solution are converted to atomic state by means of a flame. Light of the appropriate wave length is supplied and the amount of light absorbed can be measured against a standard curve. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires a standard with known analyte content to establish the relation between the measured and the analyte concentrations and relies on Beer Lambert's law (Skoog*et al*., 2005; Christian, 2005).

The sample is converted into atomic vapours by a process known as atomization. The precision and accuracy of this method depends on the atomization step and therefore a good choice of the atomization method is required. The two types of atomizers are continuous and discreet atomizers. In continuous atomizers the sample is fed into the atomizer continuously at a constant rate giving a spectral signal which is constant with time. Atomization methods that are of continuous type are flame, inductively coupled argon plasma and direct current argon plasma. With the discrete atomizers, a measured quantity of a sample is introduced as a plug of liquid or solid. The spectral signal in this case rises to a maximum and then decreases to zero. An electro thermal atomizer is one of the discrete types. The atoms then absorb radiations of characteristic

Wavelengths from an external source. The atoms of lead, nickel, copper, iron, cadmium and chromium, absorb radiations of wavelengths of 217.0 nm, 232.0 nm, 324.8nm, 248.3 nm 228.8 nm and 357.9 nm, respectively from an external source which is usually a hollow cathode lamp (Kilic*et al*., 2004). This technique has been widely employed for elemental analysis in a number of matrices such as soils, water, nuts wine and wine products (Narin*et al*., 2000).

Figure 8: AAS used for metal analysis (Shimadzu AA-7000)

Figure 9: Different hollow cathode tube

Figure 10: Flame in AAS

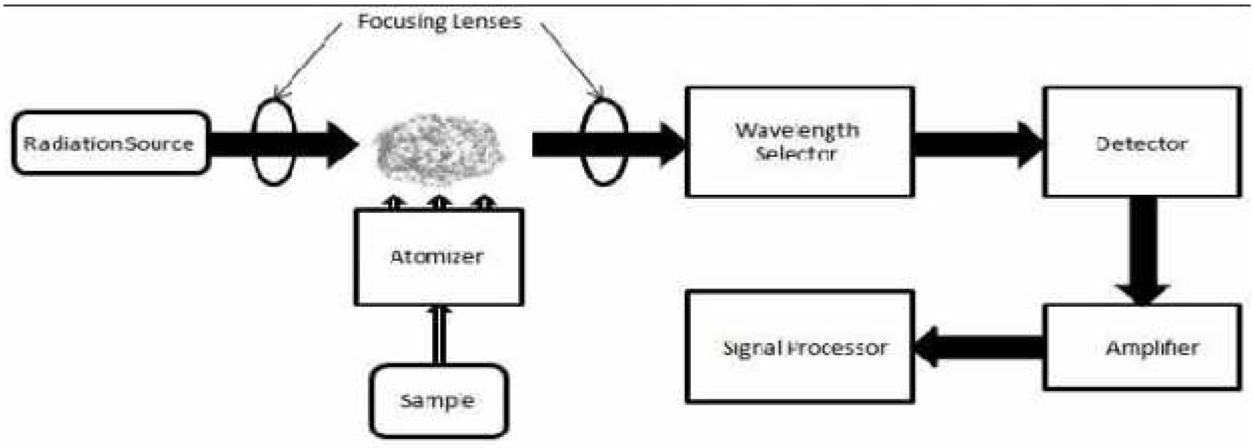

Figure 11: Schematic diagram of AAS equipment

Figure 8, shows a schematic diagram for the components of AAS. The two sources of radiation are continuous source which makes use of deuterium and mercury lamps and a hollow lamp which consists of an anode made of either tungsten wire or wink and a hollow cathode made of either the element of interest or its own salt. Flame atomization method consists mainly of a fuel and oxidant. Their temperatures are determined by flow rate and ratio of oxidant and fuel while the electro thermal atomizer is basically made of carbon rods. The free atoms are vaporized from the carbon atomizer into the optical light path to a monochromator which presents a monochromatic radiation to the detector. The radiations from the monochromators are received by detectors which converts them to electrical signals. Some commonly used detectors are photocells and photo multiplier tubes.

a. Radiation source (Hollow cathode lamp)

This is the source of analytical light line for the element of interest and gives a constant and intense beam of that analytical line.

b. Atomiser (Flame)

The atomiser will destroy any analyte ions and break complexes to create atoms of the element of interest.

c. Wavelength selector (Monochromator)

A wavelength selector isolates analytical line photons passing through the flame and remove scattered light of the other wavelength from the flame. This only impinges a narrow line on the photomultiplier tube.

d. Detector (Photomultiplier tube (PMT))

It determines the intensity of the analytical line exiting the monochromator. The PMT is the most commonly used detector for AAS.

2.4.3 Microwave digestion

The fish samples were dissected; the muscle were separated and dried in an oven at 80°C until constant weight was obtained. Afterwards, homogenized samples (between 0.5 and 0.4 g) and placed in a Teflon digestion vessel with 7 ml of ultra-pure HNO3 and 1 ml of Hydrogen Peroxide. Sealed containers were placed in a microwave oven and heated according to the digestion program (program : Power 1600W (100%), Ramp time 15 mints, Temperature 2000C, Hold time 15mints and cooling time 10mints). After digestion, sample solutions were cooled to room temperature then transferred quantitatively in to acid cleaned 100ml standard volumetric flasks and made up to 100 ml with double distilled deionized water and prepared under the same conditions as the calibration standards. After digestion, sample solutions were cooled to room temperature then transferred quantitatively in to acid cleaned 100ml standard volumetric flasks and made up to 100 ml with double distilled deionized water and prepared under the same conditions as the calibration standards. Microwave digestion is used instead of classical methods because of its shorter time, less acid consumption, and ability to retain volatile compounds in the solutions (Bashir *et al*., 2013).

2.4.4 Blank preparation

At each step of the digestion processes of the samples acid blanks (laboratory blank) were done using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contain the same digestion reagents as the real samples with the same acid ratios but without fish sample. After digestion, acid blanks were treated as samples and diluted with the same factor. They were analyzed by atomic absorption spectrophotometry before real samples and their values were subtracted to check the equipment to read only the exact values of heavy metals in real samples. Each set of digested samples had its own acid blank and was corrected by using the it's blank sample.

Figure 12: Measurement of sample

Figure 13: Acid mixing

Figure 14: sample tube set up

Figure 15: Set up for microwave digestion

Figure 16: Ethos One microwave digester

Figure 17: sample after digestion

2.5 Sample analyses

Analysis of the heavy metal content of the samples was performed with a flame atomic absorption spectrophotometer (Model Shimadzu AA-7000) using acetylene gas as fuel and air as an oxidizer. Digested samples were aspirated into the fuel-rich air acetylene flame and the metal concentrations were determined from the calibration curves obtained from standard solutions. Each determination was based on the average values of three replicate samples.

2.6 Analytical technique

Trace elements relate to the very small amounts of the analyte found in the sample which requires special instrumental techniques to be determined. Not long ago, trace levels were around µg/g levels, nowadays concentration levels are ranging from µg/g to ng/g or lower. On the other hand, one element at a high concentration in a sample can be considered as a trace in another. The analytical technique used to determine heavy metal levels in all samples was thermoelement Solar S4 Atomic Absorption Spectroscopy (International Equipment Trading Ltd, USA). It is a standard laboratory analytical tool for metal analysis and is based on the absorption of electromagnetic radiation by atoms. The absorption wavelengths and detections limits for the heavy metals were 217.0 nm and 0.001ppmfor Pb, 228.8 nm and 0.002 ppm for Cd , 324.7nm and 0.02 ppm for Cu and 232.0 nm and 0.01 ppm for Ni.

The key feature is the production of free, ground state atoms from the sample, whichpass through the light beam from the hallow cathode lamb. For many conditions the absorption of radiation follows Beer’s law:

$$A = abc$$

Where, A is the absorbance, a is the absorptivity, b is the bath-length of absorption and c is the concentration of the absorbing species.

Beer's law shows a relation between absorption and concentration of analyte, so calibration of the instrument is needed.

Figure 18: Prepared samples.

Figure 19: Sample set up in AAS

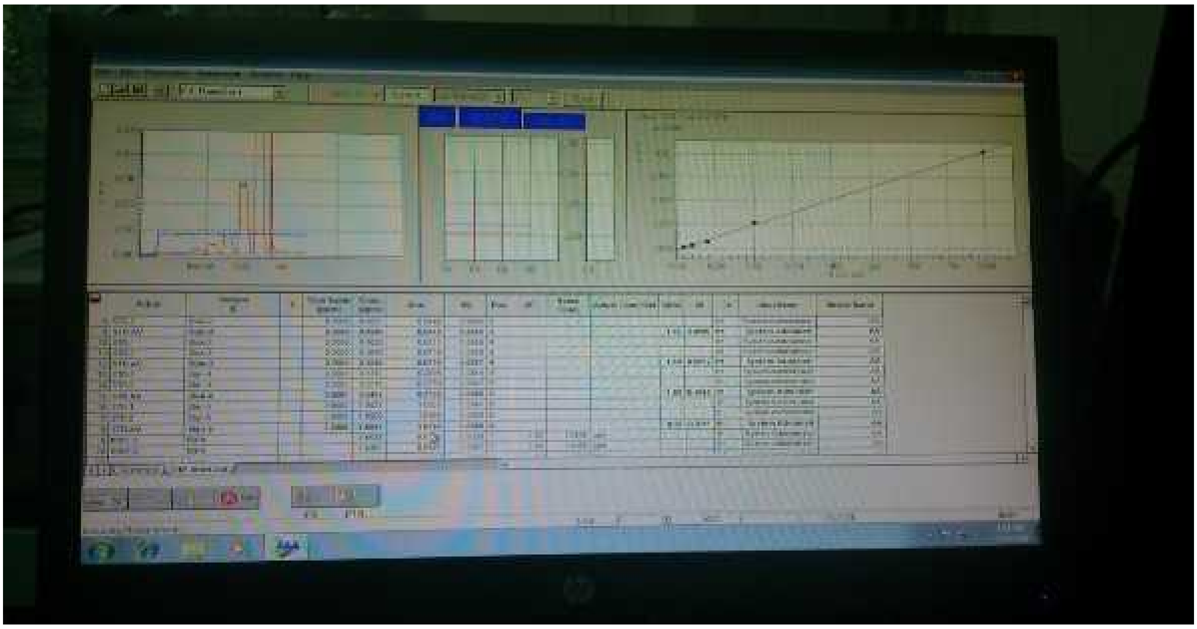

Figure 20: Standard curve from standard solution.

2.7 Calibration of instrument

Calibration requires the establishment of a relationship between signal response and known set of standards. The standards in atomic absorption spectrometry refer to the production of a series of aqueous solutions of varying concentrations (working standards) of the analyte of interest. By measuring the signals for a series of working solutions of known concentrations it is possible to construct a suitable graph. Then, by presenting a solution of unknown concentration to the instrument, a signal is obtained which can be interpreted from the graph, thereby determining the concentrations of the element in the unknown.

The actual concentration of each metal was calculated using the formula:

Actual concentration of metal in sample = (mg/kg)R × dilution factor

Where:

(mg/kg)R = AAS Reading of digest

Dilution Factor = Volume of digest used ⁄ Weight of digested sample

Table 1: The AAS operating conditions

Operating parameters	Pb	Cd	Ni	Cr	Fe	Cu	Na
Wavelen th (nm)	283.2	228.9	232.2	357.9	248.3	324.8	589.6
Flame type	Air Acetylene						
Oxidant flow rate (l/min)	1.5						
Sensitivity (ppm)	0.11	0.011	0.066	0.055	0.055	0.011	0.015
Detection limit (ppm)	0.02	0.0006	0.008	0.005	0.05	0.008	.0053
Lamp current (mA)	6	3	5	5	3	3	

2.8 Precautions followed to prevent contamination

One of the main problems in the sample preparation is the contamination of the sample during sample pre- treatment (weighting, cutting and digestion). Therefore, several precautions should be taken in order to prevent contamination, such as using acidic solution 10% (v/v) and deionized water to clean all bottles and glasswares prior using. Water samples were acidified at time of collection in order to reduce adsorption of the analytes onto the walls of containers, to prevent the precipitation of metals and to avoid microbial activity.

Fish samples were washed by deionized water prior cutting to remove adsorbed metals on skin. Contamination may also occur from acid mixture used for digestion.

2.9 Health risk estimation

2.9.1 Target hazard quotient

The target hazard quotient (THQ) is an estimate of the risk level (non-carcinogenic) due to pollutant exposure. To estimate the human health risk from consuming metal-contaminated fish, the target hazard quotient (THQ) was calculated as per USEPA Region III Risk-Based Concentration Table (USEPA 2011). The equation used for estimating THQ was as follows:

$$\square\square = \frac{\square l \times \square l \times \square\square \times \square l \times \square l}{\square\square \times \square\square \times \square\square} \times 10^{-3}$$

Where THQ is the target hazard quotient, EF is the exposure frequency (365 days/year), ED is the exposure duration (30 years for non-cancer risk as used by USEPA 2011), FIR is the fish ingestion rate (49.5 g/person/day; BBS 2011), Cf is the conversion factor (00.208) to convert fresh weight (Fw) to dry weight(Dw) considering 79 % of moisture content in fish, CM is the heavy metal concentration in fish (mg/kg d.w.), WAB is the average body weight (bw) (70 kg), ATn is the average exposure time for non-carcinogens (EF×ED) (365 days/year for 30 years(i.e., ATn=10,950 days) as used in characterizing non-cancer risk(USEPA 2011), and RfD is the reference dose of the metal (anestimate of the daily exposure to which the human population may be continuously exposed over a lifetime without an appreciable risk of deleterious effects.)

2.9.2 Hazard index

The hazard index (HI) from THQs is expressed as the sum of the hazard quotients (USEPA 2011).

HI = THQ(Fe) + THQ(Cr) +THQ(Cd) +THQ(Na)+ THQ(Cu) +THQ(Ni)+ THQ(Pb)

Where HI is the hazard index, THQ (Fe) is the target hazard quotient for Fe intake, and so on.

2.9.3 Target cancer risk

Target cancer risk (TR) was used to indicate carcinogenic risks. The method to estimate TR is also provided in USEPA Region III Risk-Based Concentration Table (USEPA 2011). The model for estimating TR was shown as follows:

$$\square = \frac{\square \times \square \times \square\square \times \square \times \square \times \square\square\square}{\square\square \times \square\square} \times 10^{-3}$$

Where TR is the target cancer risk, CM is the metal concentration in fish (μg/g), FIR is the fish ingestion rate (g/day), CPSo is the carcinogenic potency slope, oral (mg/kg bw/day), and ATc is the averaging time, carcinogens (365 days/year for 70 year as used by USEPA 2011). Since CPSo values were known for Ni, Cd, and Pb, so, TR values were calculated for intake of these metals.

2.10 Statistical analysis

The data were statistically analyzed using the statistical package, SPSS 16.0 (SPSS, USA). The means and standard deviations of the metal concentrations in fish species were calculated. Multivariate post hoc Tukey tests were employed to examine the statistical significance of the differences among mean concentrations of trace metals among different fish species for each metal.

Chapter 3 - Results

3.1 Concentration of heavy metals in fish feed and tissue

Mean concentrations and standard deviation of heavy metals in different fish feed and edible tissue of tilapia fed with those feed are presented in table 2. Cu, Fe, Pb, Cd and Na were found in all feed and fish samples, whereas, no Cr and Ni were found within the detection limit (0.0001 mg/Kg). There was no significant ($P < 0.05$) difference in metal concentrations between feeds. Concentration of all metals in fish fed with different feed were also not significantly ($P < 0.05$) different.

Table 2: Mean (±SD) heavy metals concentrations (mg/kg) in commercial fish feed and in edible tissue of cultured tilapia (*O. niloticus*) fed with respective feed in three farms in Mymensingh, Bangladesh

Name of heavy metal	Concentration of heavy metals (mg/Kg)					
	Quality		Mega		Ruposhi	
	Feed	Fish	Feed	Fish	Feed	Fish
Cu	26.75±6.23	21.78±2.43	35.08±3.34	23.76±1.41	32.2±92.23	21.13±1.44
Fe	607.67±146.20	193.98±7.17	709.55±108.53	151.49±24.27	831.16±351.06	159.21±27.40
Pb	9.34±1.78	9.92±3.51	10.12±1.83	14.83±1.36	9.25±0.49	11.78±0.88
Cd	8.20±0.12	9.84±0.55	9.21±0.52	9.43±0.37	8.64±0.01	9.79±0.35
Na	930.21±34.18	821.33±42.13	1005.38±76.01	852.42±81.52	1029.00±62.37	885.13±22.45

3.1.1 Copper (Cu)

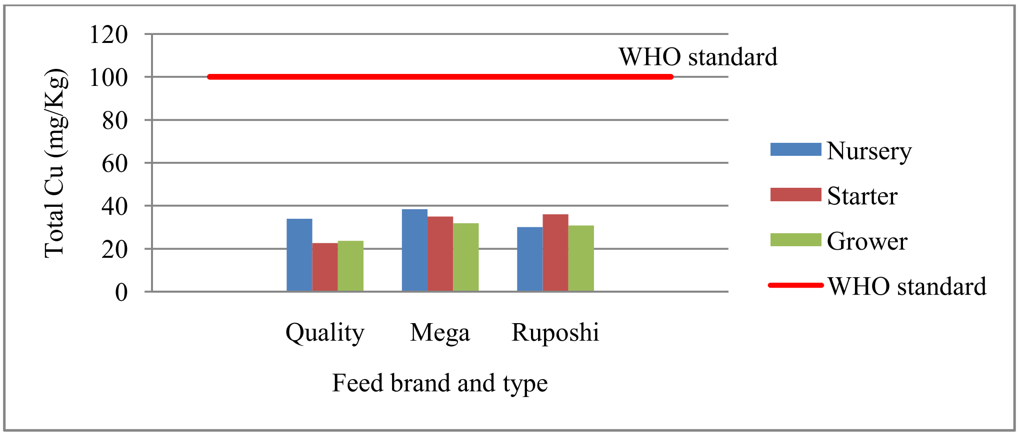

Figure 21: Concentrations (mg/kg) of total copper in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.

Copper concentrations were in a range of 22.618 to 38.480 mg/kg as shown in figure 18.The highest mean concentration of Cu (38.48 mg/Kg) was found in Mega (nursery), while the Quality (starter) had the lowest (22.61 mg/Kg) (Figure 18). Comparing with the maximum acceptable concentration of 100 mg/kg for Cu in feed as stipulated by WHO, all the samples were far below the limit. The Cu concentrations found in the present study is lower than 12.3–65.8 mg/kg obtained by Mahesar et al., (2010).

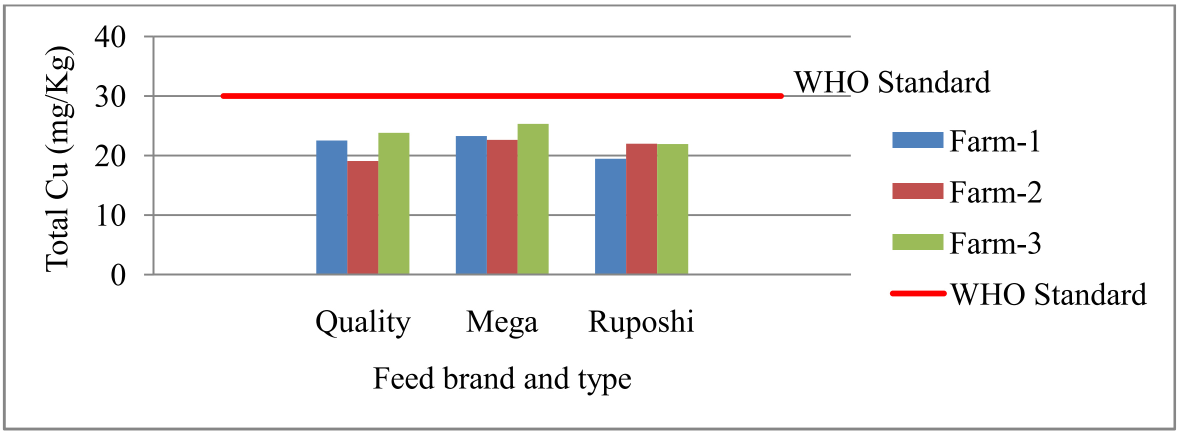

Figure 22: Concentrations (mg/kg) of total copper inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh

All fish samples contained Cu in muscle ranging from 19.073 to 25.343 mg/kg, with the highest content found in tilapia fed with Mega feed (Figure 19). The permissible limit of Cu proposed by WHO and FAO, was 30 mg/kg fresh weight (FAO, 1984).

3.1.2 Iron (Fe)

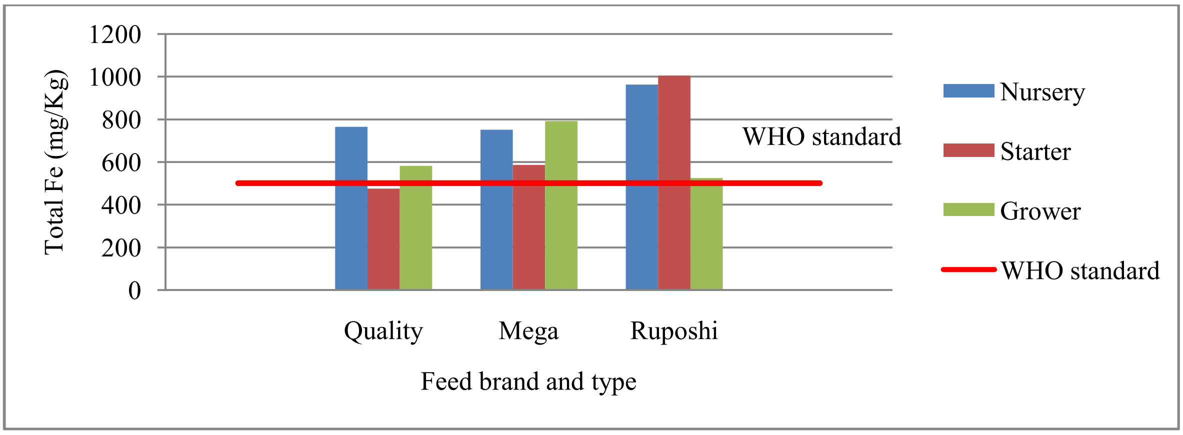

Figure 23: Concentrations (mg/kg) of total iron in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.

As shown in Figure 20, the mean concentrations of iron were in a range of 475.878 to 10004.855 mg/kg. The Ruposhi- Starter had the highest mean concentration of 10004.855 mg/kg while the Quality starter had the lowest concentration. However the Ruposhi brand had the highest mean concentration amongst all the brands.

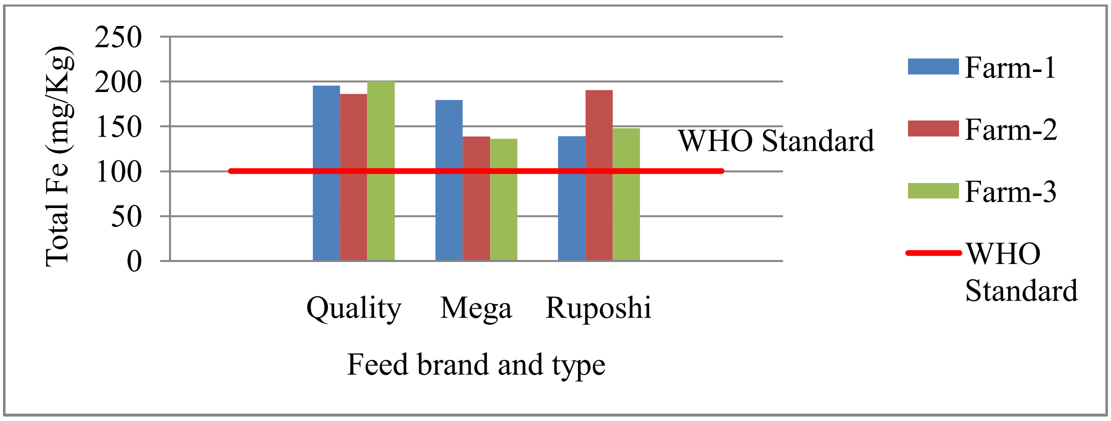

Figure 24: Concentrations (mg/kg) of total iron inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh

Iron was detected in all examined fish samples and its concentration ranged from 136.241 to 200.258 mg/kg, with the highest content found in Quality feed fed tilapia. The permissible limit of Fe proposed by WHO and FAO, was 100 mg/kg fresh weight (FAO, 1984).

3.1.3 Lead (Pb)

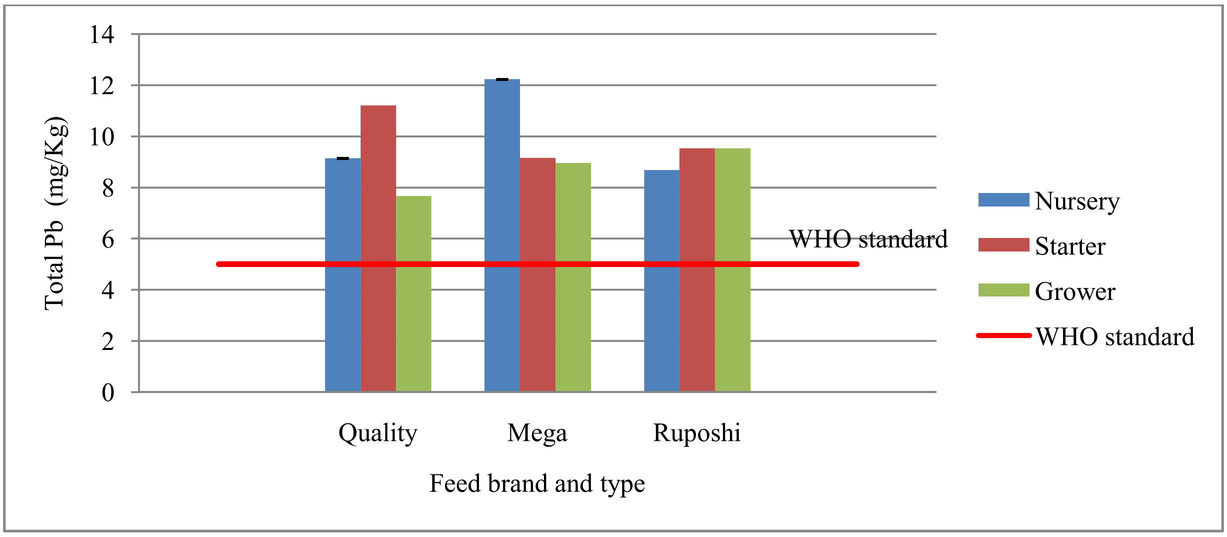

Figure 25: Concentrations (mg/kg) of total lead in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.

Lead is a non-essential element and it is well documented that Pb can cause neurotoxicity, nephrotoxicity, and many others adverse health effects (Garcia-Leston, Mendez, Pasaro, &Laffon, 2010). As shown in Table 2, the mean concentrations of lead in the different brands of feeds were in the range of 7.671 to 12.232 mg/kg. The highest mean concentration (12.232 mg/kg) was obtained in the Mega-nursery brand. The Quality brand had lowest concentrations for the grower. Comparing the values obtained with the maximum acceptable limit of 5mg/kg for lead in feed as stipulated by European Union (2003), all of the feed samples exceeded the limit. However the values obtained in this study were lower than 23.2–32.6 mg/kg obtained by Mahesar et al., (2010) in analysis of poultry feed.

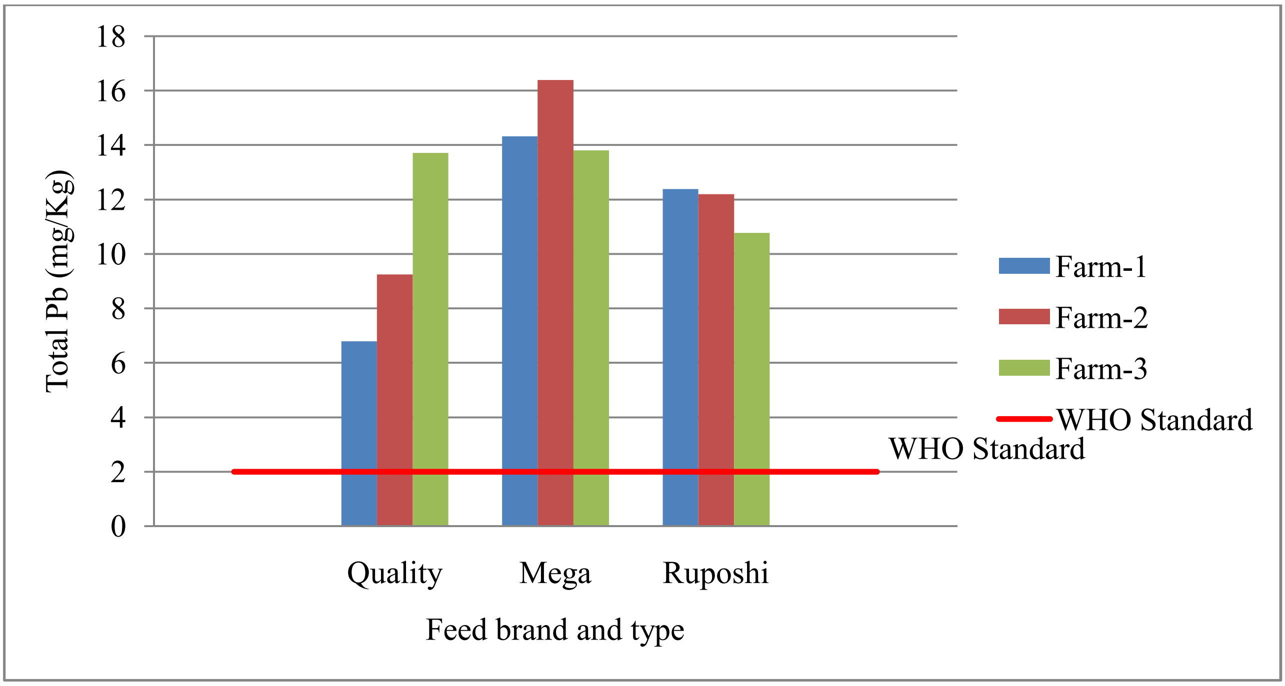

Figure 26: Concentrations (mg/kg) of total lead inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh

Lead was detected in all examined fish samples and its concentration ranged from 6.787 to 16.386 mg/kg, with the highest content found in Mega feed fed tilapia. The permissible limit of Pb proposed by WHO and FAO, was 2 mg/kg fresh weight (FAO, 1984). The maximum permitted concentration of Pb proposed by Australian National Health and Medical Research Council (ANHMRC) is 2.0 mg/kg as wet weight basis (Bebbington et al., 1977; Plaskett& Potter, 1979), According to UK Lead (Pb) in Food Regulations, Pb concentration in fish should not exceed 2 mg/kg as fresh weight basis (Cronin et al., 1998). There is also legislation in other countries regulating the maximum concentration of metals. For instance, Spanish legislation also limits the levels for Pb at 2 mg/kg (Demirak, Yilmaz, Tuna, &Ozdemir, 2006). The present observation showed that level of Pb in all fish were beyond the proposed acceptable limit for human consumption.

3.1.4 Cadmium (Cd)

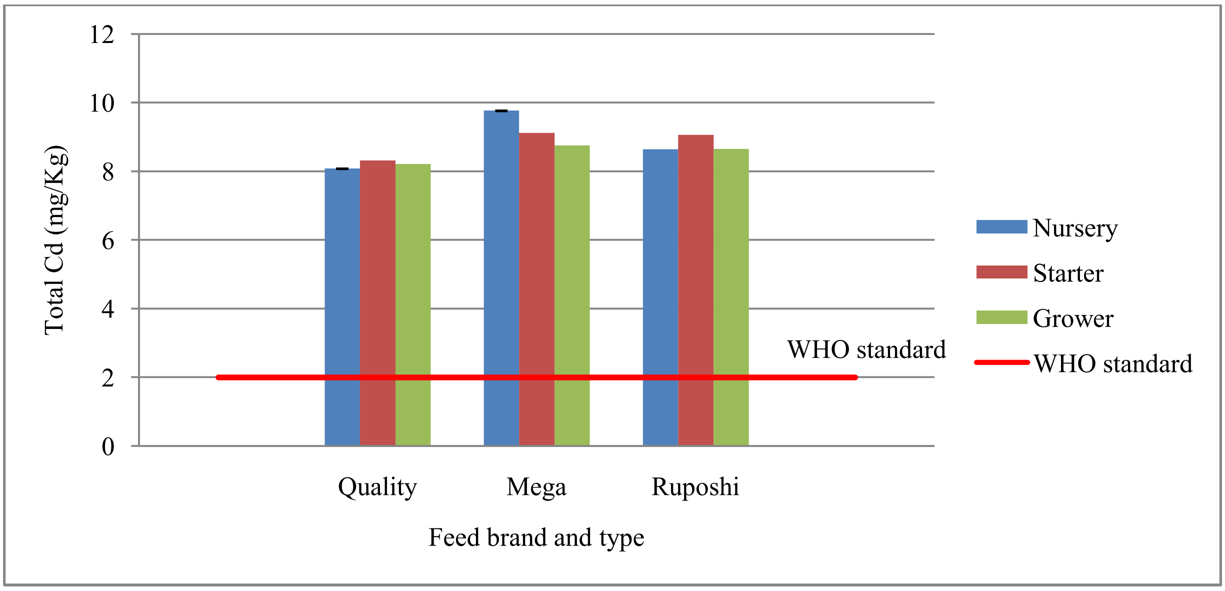

Figure 27: Concentrations (mg/kg) of total cadmium in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.

As shown in Table 7, the values of cadmium obtained were in a range of 8.082 to 9.771 mg/kg. The Mega- Nursery had a comparatively higher mean concentration compared to the other feed types while the Quality-Nursery had the lowest. However all feed had higher mean concentration of cadmium. Comparing with the maximum acceptable limit of 2 mg/kg cadmium in feed as stipulated by European Union (2003). However the values obtained in this study were lower than 3.8–33.6 mg/kg obtained by Mahesar et al., (2010).

Cadmium is a toxic element that could be present in fish organism at high concentrations (Türkmen et al., 2009). Cadmium was detected in all examined fish samples and its concentration ranged from 9.083 to 10.453 mg/kg (figure 24), with the highest content found in Quality feed fed tilapia. The permissible limit of Cd proposed by WHO and FAO, was 1 mg/kg fresh weight (FAO, 1984).

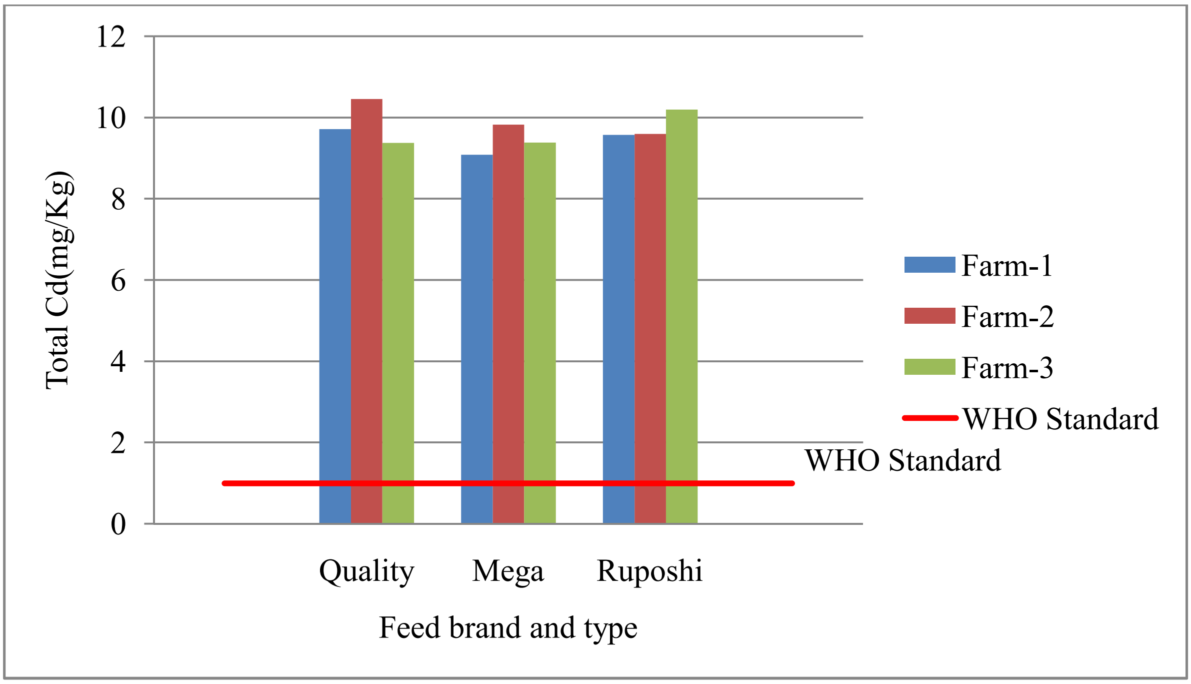

Figure 28: Concentrations (mg/kg) of total cadmium inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh

3.1.5 Sodium (Na)

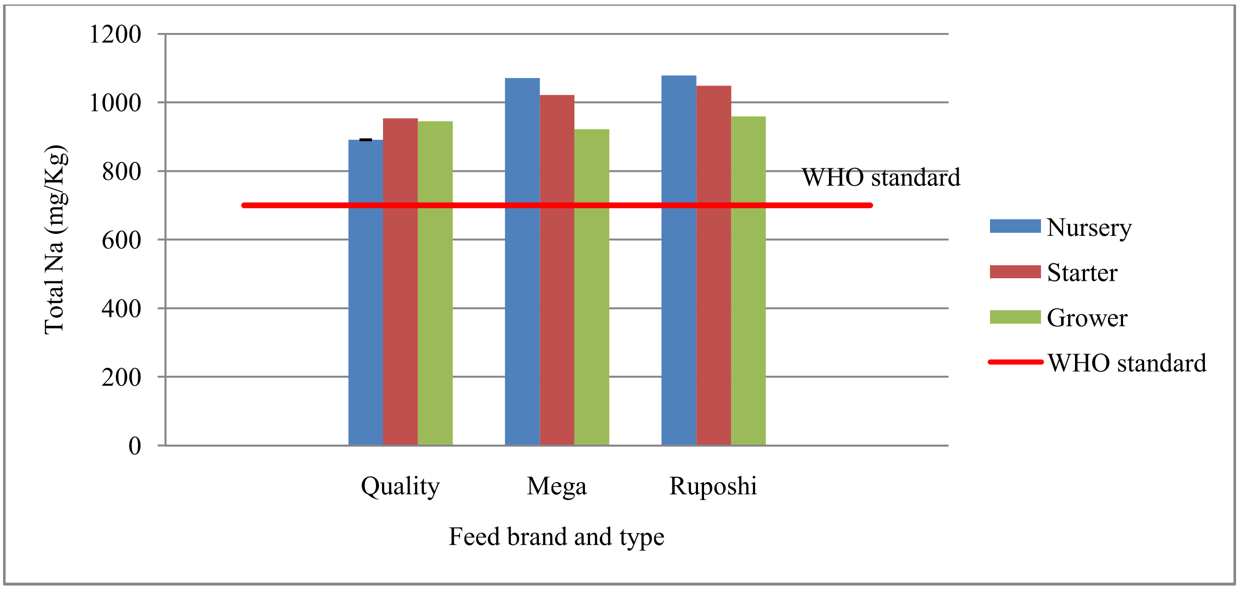

Figure 29: Concentrations (mg/kg) of total sodium in three commercial fish feeds collected from three farms in Mymensingh, Bangladesh.

As shown in Figure 26, the mean concentrations of sodium were in a range of 891.046 to 1079.131 mg/kg. The Ruposhi- Starter had the highest mean concentration of 1079.131 mg/kg while the Quality-nursery had the lowest concentration. However the Ruposhi brand had the highest mean concentration amongst all the brands.

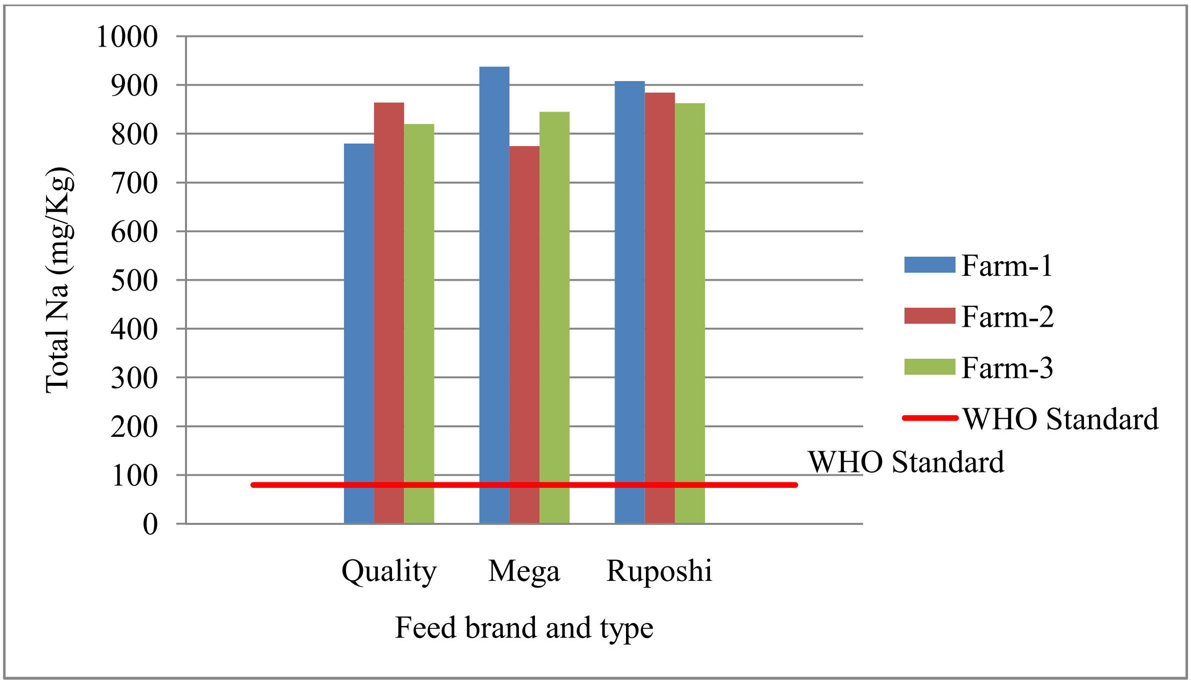

Figure 30: Concentrations (mg/kg) of total sodium inedible tissue of cultured tilapia (*Oreochromisniloticus*) fed with three different commercial fish feed collected from three farms in Mymensingh, Bangladesh

Sodium is a mineral element that could be present in fish organism at high concentrations (Türkmen et al., 2009). Sodium was detected in all examined fish samples and its concentration ranged from 821.33to 885.13mg/kg (figure 27), with the highest content found in Mega feed fed tilapia.

3.2 Health risk estimation

The health risk assessments are based on assumptions. The risk associated with the carcinogenic effects of target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years. The target hazard quotient (THQ) estimated for individual heavy metals through consumption of different fish species is presented in Table 3.

Table 3: Target hazard quotient (THQ) for different heavy metals and their hazard index (HI) from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh.

Heavy metals	RfD (mg/kg)	Target hazard quotient (THQ)		
		Quality	Mega	Ruposhi
Fe	0.7	4.08E-02	3.18E-02	3.35E-02
Na	0.08	1.51E+00	1.57E+00	1.63E+00
Cu	0.005	6.41E-01	6.99E-01	6.22E-01
Cd	0.001	1.45E+00	1.39E+00	1.44E+00
Pb	0.2	7.30E-03	1.09E-02	8.66E-03
HI	0.986	3.65E+00	3.70E+00	3.73E+00

The acceptable guideline value for THQ is 1 (USEPA 2011). THQ values were less than 1 for all individual heavy metal in all three brands feed fed tilapia indicating no non-carcinogenic health risk from ingestion of a single heavy metal through consumption of these fishes.

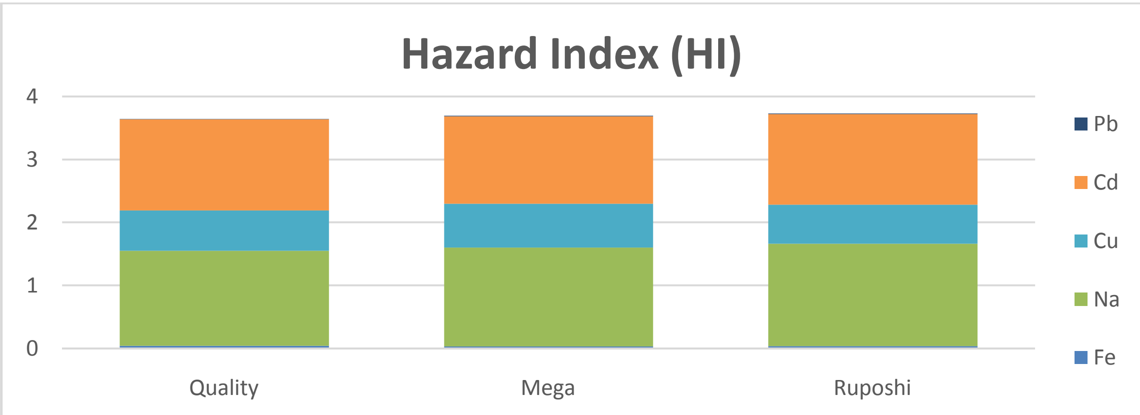

Figure 31: Composition of hazard index (HI) for different heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh.

The highest THQ value was estimated for Na (1.51) followed by Cd (1.45) contaminated in all farms. This indicates that excessive consumption over a long time period might cause non-carcinogenic effect as the THQ values were higher than the acceptable guideline value of 1 (USEPA 2011). Cd, Cu, Na and Pb contributed the most in the HI in all farms (Fig. 28).

Table 4: Target cancer risk (TR) of heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh.

Heavy metals	Target cancer risk (TR)		
	Quality	Mega	Ruposhi
Cd	9.12E-03	8.74E-03	9.07E-03
Pb	1.24E-05	1.85E-05	1.47E-05

The TR values were estimated for the metals reported with known carcinogenic effects. The TR values for Cd and Pb ranged from 9.12E-03 and 1.24E-05in Quality8.74E-03and1.85E-05in Mega, and 9.07E-03and1.47E-05 in Ruposhi, respectively (Table 4).

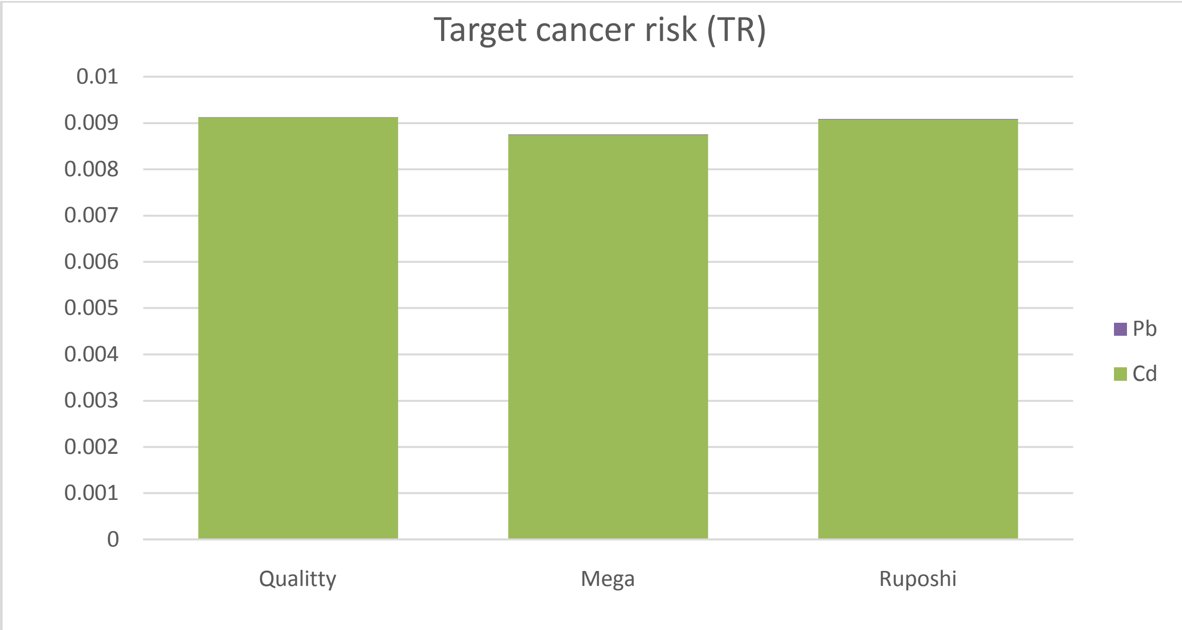

Figure 32: Target cancer risk (TR) of heavy metals from consumption of three brands feed fed tilapia collected from the Mymensingh, Bangladesh.

Chapter 4 - Discussion

This study was undertaken to investigate heavy metal concentrations in commercial fish feeds and edible parts (muscles) of a commercially important fish species (*O. niloticus)* in Mymensingh, Bangladesh and to detect whether their levels are potentially harmful for human health if included in the diet. *O. niloticus*was selected because it is one of the most commonly consumed fish in Bangladesh. The levels of heavy metals were determined in the muscles because of its importance for human consumption.

It is well known that it is very difficult to compare the metal concentrations even between the same tissue in different fish because of the difference in many factors as, the aquatic environments, feed, concerning the type and the level of water pollution, etc. Romeo, *et al*. (1999), described that the ability of fish to accumulate heavy metals depends on ecological needs, metabolism, degree of pollution in sediment, water and food, as well as salinity and temperature of water. Kamaruzzaman, *et al*. (2010), indicated that there were a relation between metal concentration and several intrinsic factors of fish such as organism size, genetic composition and age of fish. This investigation showed that different feed contained different concentrations of a certain metal. Kalay*et al*. (1999), reported that different fish species accumulate metals in their tissue in significantly different values.

Moreover, Canli and Atli, (2003), reported that levels of heavy metals in fish vary in various species and different aquatic environments. On the other hand, Farkas*et al*. (2005), attributed the differences of concentrations of metals between fishes to feeding habits, the bio-concentration capacity of each species and to the biochemical characteristics of the metal.

The concentrations of Cu in the feed samples analyzed ranged from 26.75±6.23to 35.08±3.34mg/kg. However, the concentration of copper measured in feeds was far below the FAO guideline of 100 mg/kg. The values found from this study was also lower than 12.3–65.8 mg/kg obtained by Mahesar*et al*. 2010. The concentrations of Cu in the fish samples analyzed ranged from 21.13±1.44to 23.76 ±1.41mg/kg. The permissible limit of Cu proposed by WHO and FAO, was 30 mg/kg fresh weight. Thus, the concentrations of Cu in the fish and feed samples analyzed were all below the FAO recommended guideline (FAO 1983). Ahmed *et al*. (2015) investigated the Cu concentration 575.34±61.86 mg/kg in prawn from the Buriganga River, Bangladesh.

Cu is an essential element that is carefully regulated by physiological mechanisms in most organisms (Erdoĝrul and Ates2006).Copper is an essential part of several enzymes and is necessary for the synthesis of hemoglobin (Sivaperumal, Sankar, & Nair, 2007).However, studies have shown that Cu is highly toxic in aquatic environments and has effects on fish, invertebrates, and amphibians, with all three groups equally sensitive to chronic toxicity. Kamaruzzaman*et al*. (2010) observed that copper will bio-concentrate in many different organs in fish and mollusks. While mammals are not as sensitive to copper toxicity as aquatic organisms, bio-magnifications play critical role in their toxicity. Toxicity in mammals include a wide range of animals and effects such as liver cirrhosis, necrosis in kidneys and the brain, gastrointestinal distress, lesions, low blood pressure and fetal mortality (Ezeonyejiaku*et al.*, 2011).

The concentrations of Pb in the feed samples analyzed ranged from 9.25±0.49to 10.12±1.83mg/kg. Comparing the values obtained with the maximum acceptable limit of 5mg/kg for lead in feed as stipulated by European Union, (2003) all of the feed samples exceeded the limit. However the values obtained in this study were lower than 23.2–32.6 mg/kg obtained by Mahesar*et al*. (2010) in analysis of poultry feed.

The concentrations of Pb in the fish samples analyzed ranged from 9.92±3.51to 14.83±1.36mg/kg. The permissible limit of Pb proposed by FAO, was 2 mg/kg fresh weight. Thus, the concentrations of Pb in the fish and feed samples analyzed were all higher than the recommended guideline (FAO 1983, European Union 2003). Rahman*et al*. (2012) reported Pb in *C. soborna* (10.27 mg/kg)from the Bangshi River, Bangladesh. Ahmad *et al*. (2010) reported more Pb in chapila fish (13.52 mg/kg) from the Buriganga River. Dural *et al*. (2002) reported that the Pb contents were in the range of 0.40 to 2.44mg/kg in muscle and 1.41 to 3.95 mg/kg in liver tissues of fish of Tulza Lagoon.

The concentrations of Fe in the feed samples analyzed ranged from 607.67±146.20to 831.16±351.06mg/kg. However, the concentration of copper measured in feeds washigher than the European Union (2003) guideline of 500 mg/kg. The concentrations of Fe in the fish samples analyzed ranged from151.49±24.27 to 193.98±7.17mg/kg. The permissible limit of Fe proposed by WHO and FAO, was 80 mg/kg fresh weight (FAO, 1984).Thus, the concentrations of Fe in the fish and feed samples analyzed were all higher than the recommended guideline (FAO 1983, European Union 2003). Other authors reported Fe level ranges of 36.211mg/kg (Tuzen, 2009) and 6.570mg/kg (Mendil, Ünal, Tüzen, &Soylak, 2010) in fishes from Turkey, of 27.22mg/kg in fishes from Cambodian (Roos*et al*., 2007), and 8.819mg/kg in fishes from Italy (Minganti, Drava, Pellegrini, &Siccardi, 2010).

Iron deficiency is frequently associated with anemia and, thus, with reduced working capacity and impaired intellectual development. The RDA for children and adults (male and female) is 11 and 18 mg/day, respectively (Schümann, Ettle, Szegner, Elsenhans, &Solomons, 2007).Iron is a critical nutrient for the proper functioning of the body organs. It is a component of the respiratory pigments and enzymes concerned in tissue oxidation and it is essential for oxygen and electron transport within the body. When the body absorbs more iron than it can take, the excess iron cannot be discarded naturally and is stored in the body tissues of the liver, pancreas and heart. This situation is associated with general body weakness, fatigue, muscle impuissance, hair loss, abdomen pains (near the liver), enlarged liver and impotence may result.

The concentrations of Cd in the feed samples analyzed ranged from 8.20±0.12to 9.21±0.52mg/kg. Comparing with the maximum acceptable limit of 2 mg/kg cadmium in feed as stipulated by European Union (2003), all of the samples exceeded the limit. However the values obtained in this study were lower than 3.8–33.6 mg/kg obtained by Mahesar*et al*. (2010)

The concentrations of Cd in the fish samples analyzed ranged from 9.43±0.37 to 9.84±0.55mg/kg. The permissible limit of Cd proposed by FAO, was 1 mg/kg fresh weight. Thus, the concentrations of Cd in the fish and feed samples analyzed were all higher than the recommended guideline (FAO 1983, European Union 2003). Ashraf (2006) studied 57 samples of canned tuna fish and found the concentration of Cd ranged between 0.08 and 0.66 mg/kg which is much lower than the present findings. Ahmed *et al*. (2009) investigated the heavy metal concentration in fish from the Shitalakhya River, Bangladesh, and found seasonal variation of Cd, ranged from 1.09 to 1.21 mg/kg which is also lower than the present findings. However, the values reported in the literature are generally lower than the values from this study.

Cadmium is a serious contaminant, a highly toxic element, which is transported in the air. The level of contamination of fish with Cd is largely affected by environmental pollution. Cadmium occurs naturally in low levels in the environment and is also used in batteries, pigments, and metal coatings. Industrial processes such as smelting or electroplating and the addition of fertilizers can increase the concentration of Cd in the pond (environment). Long-term or high dose exposure to cadmium can cause kidney failure and softening of bones (Vannoort and Thomson 2006), and high levels of cadmium have been linked to prostate cancer (Gray *et al*., 2005).

The concentrations of Na in the feed samples analyzed ranged from 930.21±34.18to 1029.00±62.37mg/kg. The concentrations of Na in the fish samples analyzed ranged from 821.33±42.13to 885.13±22.45mg/kg.

Sodium is required for humans as being a component nutrient and probably other metallo enzymes (Chaney, 1992). It is relatively non-toxic to animals and toxic to plants at higher levels (Manahan, 1997). All the samples contained sodium very much exceeding the nutritional requirement. But as no standards were fixed for maximum permissible limit as contaminant, it cannot be shown whether the contents in the samples were harmful.

Generally, in present study, concentrations of non-essential elements (Cd, Cr and Pb) in fish muscles were lower than those of essential metals (Fe, Na, Ni and Cu) (Table).This result is consistent with what Huang et al. (2003) reported, that the accumulation levels of the essential metals in fish are generally higher and more homeostatic than the non-essential metals.

Among heavy metals iron concentration was the highest of all metals in all fish species (Table). This agrees with several studies performed in many countries. Huang (2003), found the order of concentrations of four heavy metals in common benthic fishes decreasing as: Fe> Cu > Cd = Pb, in addition Chen and Chen (2001), ordered the concentrations of some metal in fish as the following: Fe> Cu = Mn> Cd. Bahnasawy*et al*. (2009), summarized that the average concentrations of the metals in fish tissues from Lake Manzala, Egypt exhibited the following order: Fe>Cu>Pb>Cd. These results were in agreement with results from present study in which concentrations of the metals followed the order Na>Fe> Cu >Pb> Cd.

The acceptable guideline value for THQ is 1 (USEPA 2011). THQ values were less than 1 for all individual heavy metal in all three brands feed fed tilapia indicating no non-carcinogenic health risk from ingestion of a single heavy metal through consumption of these fishes. The highest THQ value was estimated for Na (1.51) followed by Cd (1.45) contaminated in all farms. This indicates that excessive consumption over a long time period might cause non-carcinogenic effect as the THQ values were higher than the acceptable guideline value of 1 (USEPA 2011). Cd, Cu, Na and Pb contributed the most in the HI in all farms (Fig. 1). This indicates that continuous and excessive intake of this fish could result in chronic non-carcinogenic effect.

The TR values were estimated for the metals reported with known carcinogenic effects. The TR values for Cd and Pb ranged from 9.12E-03 and 1.24E-05 in Quality 8.74E-03 and 1.85E-05in Mega, and 9.07E-03 and 1.47E-05 in Ruposhi respectively (Table 2). This indicates that excessive consumption over a long time period might cause carcinogenic effect as the TR values were higher than the acceptable guideline value of 10^{-6} (USEPA 2011). Although the fish species under the present study were found safe for human consumption, the probability of contracting cancer is present for continuous consumption for 70 years.

Chapter 5 - Conclusions and Recommendations

5.1 Conclusions:

This study was carried out as a first approach to understand trace metal accumulation from feed in farmed fish. The results of the study revealed that the fish feeds analyzed contained some of the heavy metals in varying proportions. As a whole, the average copper, chromium and nickel concentration in the feeds used in Mymensingh, was considerably below the maximum allowed limit, permitting a less frequent control of this element. The average cadmium and lead content in feeds were higher than the maximum allowed concentrations. This makes the feeds contaminated with cadmium and lead is not safe for fish consumption since heavy metals are bio-accumulative and have the tendency to be transferred to human after consumption. This signifies that practically, control for cadmium and lead amounts in commercial feed is necessary.

The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters in tissues. It is also evident from this study that the presence of heavy metals in the aquaculture environment is of global importance because they play an important role in the cultured fish bioaccumulation of transfer of heavy metals especially when they passed on to human being through the consumption of fish and other aquatic products. The present study concludes that tilapia collected from different farms accumulates various metals at concentrations more than the maximum permissible limits. Although, at current concentrations in fish no metal was found to pose potential non-carcinogenic health risk individually, but collectively, the metals were found enough to be considered as potential human health hazard. People who continuously consume tilapia contaminated with metals as found in the present study are under the target cancer risk in the long run.

To maintain the food chain safe from the entering of heavy metals and the subsequent consequences, it is necessary to have definite standards for heavy metals for all possible pathways towards food chain. Then any phenomenon allowing heavy metals to enter into food chain beyond those limits can automatically be regarded as unsafe and will be directed for mitigation strategies. Concordantly, most of the human aquaculture activities such as the use of chemicals like feed additives, antibiotics, soil and water treatment need to be addressed and thoroughly investigated from a toxicological point of view. Most importantly, each Government agencies should ensure that the standard set to ensure that the concentrations of heavy metals are not above than the permissible level set is strictly adhered to.

5.2 Recommendations:

Further studies should be done to cover more locations/divisions of the country. Also, other fish feeds available in the region should be screened for heavy metal contamination. Feed companies should periodically carry out heavy metal assessment of their feed products so as to keep them at a safe level.

It is recommended that further research should be done on the accumulation and concentration of heavy metals in fish in order to monitor and prevent them from exceeding permissible limits that make them toxic to human. Health screening should be undertaken on the inhabitants to check for symptoms of some of these heavy metals. .

References

ABDEL-BAKI, A.S., DKHIL, M.A. and AL-QURAISHY, S. 2013. Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of WadiHanifah, Saudi Arabia. *Afr. J. Biotechnol.* **10**(13): 2541-2547.

ADB. 2010. An evaluation of small-scale freshwater rural aquaculture development for poverty reduction. Asian Development Bank (ADB), Manila, Philippines.

AGATHA, A.N. 2010. Levels of Some Heavy Metals in Tissues of Bonga Fish, Ethmallosafimbriata from Forcados River. *J. Appl. Environ. Biol. Sci.* **1**: 44-47.

AHMAD, M.K., ISLAM, S., RAHMAN, M.S., HAQUE, M.R. and ISLAM, M.M. 2009. Heavy metals in water, sediment and some fishes of Buriganga River, Bangladesh. *Int. J. Environ. Res.* **4**(2): 321-332.

AHMAD, M.K., ISLAM, S., RAHMAN, M.S., HAQUE, M.R. and ISLAM, M.M. 2009. Heavy metals in water, sediment and some fishes of Buriganga River, Bangladesh. *Terres. Aqua. Environ. Toxicol.* **3**: 33-41.

AKAN, J.C., ABDULRAHMAN, F.I., SODIPO, O.A. and AKANDU, P.I. 2009. Bioaccumulation of some heavy metals of six fresh water fishes caught from lake chad in doronbuhari, maiduguri, borno state, nigeria. *J. Appl. Sci. Environ. Mgt.* **4**(2).

ALINNOR, I.J. and OBIJI, I.A. 2010. Assessment of trace metal composition in fish samples from Nworie River. *Pak. J. Nutr.***9**(1): 81-85.

AMIYA TIRKEY, P. SHRIVASTAVA and A. SAXENA. 2012.Bioaccumulation of Heavy Metals in Different Components of two Lakes Ecosystem Current World Environment, 2012, *Food Chem.* **7**(2):293-297

ANHWANGE, B. A., ASEMAVE, K., KIM, B. C. and NYIAATAGHER, D.T. 2012. Heavy Metals Contents of Some Synthetic Fish Feeds Found within Makurdi Metropolis.*Int. J. Food Nutri. Safety.***2**(2): 55-61

ANIM, A.K., AHIALEY, E.K., DUODU, G.O., ACKAH, M. and BENTIL, N.O. 2011. Accumulation profile of heavy metals in fish samples from Nsawam, along the Densu River, Ghana. *Res. J. Environ. Earth Sci.* **3(**1): 56-60.

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY (ATSDR), 2002 Toxicological profile for Manganese and Lead *(*Draft for Public Comment*)*.

Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

AWOFOLU, O.R., MBOLEKWA, Z., MTSHEMLA, V. and FATOKI, O.S. 2005. Levels of trace metals in water and sediment from Tyume River and its effects on an irrigated farmland. *Water SA*. **31**(1): 87-94.

BAHNASAWY, M., KHIDR, A.A. and DHEINA, N. 2009. Seasonal variations of heavy metals concentrations in Mullet, *Mugilcephalus* and *Liza ramada* (Mugilidae) from Lake Manzala, Egypt. *J. appl. sci. res.* **5**(7): 845-852.

BASHIR, F.H., OTHMAN, M.S., MAZLAN, A.G., RAHIM, S.M. and SIMON, K.D. 2013. Heavy metal concentration in fishes from the coastal waters of Kapar and Mersing, Malaysia. *Tur. J. Fish Aquat. Sci.* **13(**2).

BEBBINGTON, G.N., MACKAY, N.J., CHVOJKA, R., WILLIAMS, R.J., DUNN, A. and AUTY, E.H. 1977. Heavy metals, selenium and arsenic in nine species of Australian commercial fish. *Mar. Freshwater Res.* **28**(3): 277-286.

BERNET, D. H., SCHMIDT, W., MEIER, P., BURKHARDT, H. and WAHLI, T. 1997. Histopathology in fish, Proposal for a protocol to assess aquatic pollutions. *J. Fish Dts*. **22**(1): 25-34.

BURGER, J. and GOCHFELD, M. 2005. Heavy metals in commercial fish in New Jersey. *Environ. Res.* **99**(3): 403-412.

BOGUT, I. 1997. Water pollution by heavy metals and their impact on fish and human health. *HrvatskeVode*. **5**: 223-229.

CRAFFORD, D. and AVENANT-OLDEWAGE, A. 2011. Uptake of selected metals in tissues and organs of *Clariasgariepinus* (sharptooth catfish) from the Vaal River System–Chromium, copper, iron, manganese and zinc. *Water SA*. **37**(2).

CANL, M. AND ATLI, G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ. Poll.* **121**: 129-136

CASTRO-GONZÁLEZ, M.I. and MÉNDEZ-ARMENTA, M. 2008. Heavy metals: Implications associated to fish consumption. *Environ. Toxicol. Pharmacol.* **26**(3): 263-271.

CLEMENTS, W.H. and REES, D.E. 1997. Effects of heavy metals on prey abundance, feeding habits, and metal uptake of brown trout in the Arkansas River, Colorado. *Trans. Am. Fish Soc.* **126**(5): 774-785.

CRONIN, M., DAVIES, I.M., NEWTON, A., PIRIE, J.M., TOPPING, G. and SWAN, S. 1998. Trace metal concentrations in deep sea fish from the North Atlantic. *Marine Environ. Res.* **45**(3): 225-238.

DAYAN, A.D. and PAINE, A.J. 2001. Mechanisms of chromium toxicity, carcinogenicity and allergenicity: review of the literature from 1985 to 2000. *Hum. Exp. Toxicol.* **20**(9): 439-451.

DEMIRAK, A., YILMAZ, F., TUNA, A.L. and OZDEMIR, N. 2006. Heavy metals in water, sediment and tissues of *Leuciscuscephalus* from a stream in southwestern Turkey. *Chemosphere*. **63**(9): 1451-1458.

DENKHAUS, E. and SALNIKOW, K. 2002. Nickel essentiality, toxicity, and carcinogenicity. Critical reviews in oncology/hematology. **42**(1): 35-56.

DOF. 2013. National Fish Week 2013 compendium (in Bengali). Department of Fisheries (DoF), Ministry of Fisheries and Livestock, Bangladesh. **144** p.

DOF. 2011. Fishery Statistical Yearbook of Bangladesh. Fisheries Resources Survey System, Department of Fisheries, Dhaka, Bangladesh.

DURAL, M., GÖKSU, M.Z.L. and ÖZAK, A.A. 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem.* **102**(1): 415-421.

DURUIBE, J.O., OGWUEGBU, M.O.C. and EGWURUGWU, J.N. 2007. Heavy metal pollution and human biotoxic effects. *Int. J. Phys. Sci*. **2**(5): 112-118.

EASTON, M.D.L., LUSZNIAK, D., V and GEEST, E. 2002. Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. *Chemosphere*. **46**: 1053–1074.

ENEJI, I.S., SHA’ATO, R. and ANNUNE, P.A. 2011. Bioaccumulation of Heavy Metals in Fish (*Tilapia Zilli* and *ClariasGariepinus*) Organs from River Benue, North–Central Nigeria. *pak. J. Anal. Environ. Chem.***12**.

ENUNEKU, A., EZEMONYE, L.I. and ADIBELI, F. 2013. Heavy metal concentration in surface water and bioaccumulation in fish (*Clariasgariepinus*) of river Owan, Edo state, Nigeria. *Eur. J. Sci. Res.***2**(7): 31-39.

EUROPEAN COMMISSION (2003a). Opinion of the scientific committee on animal nutrition on the use of copper in feedstuffs. European Commission, Health and Consumer Protection Directorate, Brussels, Belgium.

EUROPEAN COMMISSION (2003b). Opinion of the scientific committee on animal nutrition on undesirable substances in feed. European Commission, Health and Consumer Protection Directorate, Brussels, Belgium.

EZEONYEJIAKU, C.D., OBIAKOR, M.O. and EZENWELU, C.O. 2011. Toxicity of copper sulphate and behavioral locomotor response of tilapia (*Oreochromisniloticus*) and catfish (*Clariasgariepinus*) species. *Online J. Anim. Feed Res*. **1**(4): 130-134.

FAO/WHO. (1984) List of maximum levels recommended for contaminants by the Joint FAO/WHO. *Codex Alimentarius Commission*. **3**:1-8.

FARKAS, A., SALANKI, J. AND VARANKA, I.2000. Heavy metal concentrations in fish of Lake Balaton. Research & Management. *J. Biol. Sci.* **10**: 157-161.

FERNANDES, C., FONTAÍNHAS-FERNANDES, A., CABRAL, D. and SALGADO, M.A. 2007. Heavy metals in water, sediment and tissues of *Liza saliens* from Esmoriz–Paramos lagoon, Portugal. *Environ. Monit. Assess.* **136**(1-3): 267-275.

FÖRSTNER, U. 1987. Sediment-associated contaminants—an overview of scientific bases for developing remedial options. *Hydrobiologia*. **149**(1): 221-246.

FRSS. 2012. Fisheries Statistical Yearbook of Bangladesh. Fisheries Resources Survey System(FRSS), Department of Fisheries, Bangladesh. **46**p.

GARCIA-VAZQUEZ, E., PEREZ, J., MARTINEZ, J.L., PARDINAS, A.F., LOPEZ, B., KARAISKOU, N., CASA, M.F., MACHADO-SCHIAFFINO, G. and TRIANTAFYLLIDIS, A. 2010. High level of mislabeling in Spanish and Greek hake markets suggests the fraudulent introduction of African species. *J. Agric. Food Chem.* **59**(2): 475-480.

GARCÍA-LESTÓN, J., MÉNDEZ, J., PÁSARO, E. and LAFFON, B. 2010. Genotoxic effects of lead: an updated review. *Environ. Int.* **36**(6): 623-636.

GONZALEZ, A.R., NDUNG'U, K. and FLEGAL, A.R. 2005. Natural occurrence of hexavalent chromium in the Aromas Red Sands Aquifer, California. *Environ. Sci. Technol.* **39**(15): 5505-5511.

GORELL, J.M., JOHNSON, C.C., RYBICKI, B.A., PETERSON, E.L., KORTSHA, G.X., BROWN, G.G. and RICHARDSON, R. J. 1997. Occupational exposures to metals as risk factors for Parkinson's disease. *Neurology*. **48**(3): 650-658.

GUPTA, M. V., AHMED, M., BIMBAO, M. P and LIGHTFOOT, C. 1992. Socio-economic impact and farmers assessment of Nile tilapia (*Oreochromisniloticus*) culture in Bangladesh. International Centre for Living Aquatic Resource Management (ICLARM), Tech Rep. **35**: 50p.

HASAN, M.N., RASHID, H., TANU, M.B., PARVEEN, R., SUKHAN, Z.P., SATTAR, M.A. and MAHMUD, Y.2011 Monitoring Pollution Level of the River Khiru at Mymensingh, Bangladesh. In Proceedings of the International Conference on Environmental Aspects of Bangladesh (ICEAB 2011). **1**: 4p.

HASSAAN, M.H., AL-KAHALI, M. and AL-EDRES, M. 2007. Heavy Metal Contamination in the White Muscles of Some Commercial Fish Species From Al-Hodeidah-Red Sea coast of Yemen. *Food Chem.* **78(**1): 15-22.

HILTON, J.W., HODSON, P.V., BRAUN, H.E., LEATHERLAND, J.L., SLINGER, S.J. 1983. Contaminant accumulation and physiological response in rainbow trout (*Salmogairdneri*) reared on naturally contaminated diets. *Can. J. Fish. Aquat. Sci.***40**: 1987–1994

HINGSTON, J.A., COLLINS, C.D., MURPHY, R.J. and LESTER, J.N. 2001. Leaching of chromated copper arsenate wood preservatives: a review. *Environ. Poll.* **111**(1): 53-66.

HORST, K., LEHMANN, I., OETJEN, K. 1998. Levels of chlordane compounds in fish muscle, meal, oil, and feed. *Chemosphere.* **36**: 2819–2832.

HUANG, W.B. 2003. Heavy metal concentrations in the common benthic fishes caught from the coastal waters of Eastern Taiwan. *J. Food Drug Anal.* **11**(4).

HUSSAIN, M. G. and KOHINOOR, A. H. M. 2003. Breeding, monosex male tilapia seed production and culture technologies of BFRI super tilapia. Extension Manual No. 25. Bangladesh Fisheries Research Institute, Mymensingh.

ISLAM, M.M., BANG, S., KIM, K.W., AHMED, M.K. and JANNAT, M. 2010. Heavy metals in frozen and canned marine fish of Korea. *J. Sci. Res*. **2**(3): 549.

JAVED, M. and USMANI, N. 2014. Assessment of heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) in rivulet water, their accumulations and alterations in hematology of fish *Channapunctatus*. *Afr. J. Biotechnol.* **13**(3): 492-501.

JÄRUP, L., 2003. Hazards of heavy metal contamination. *Br. Med. Bull*. **68**(1): 167-182.

KALAY, M., ALY, O. AND CANIL, M. 1999.Heavy metal concentrations in fish tissues from the Northeast Mediterranean sea. *Bull. Environ. Contam. Toxicol.* **63**: 673-681.

KAMARUZZAMAN, B. Y., ONG, M. C., RINA, S. Z., and JOSEPH, B. 2010. Levels of some heavy metals in fishes from pahang river estuary, Pahang, Malaysia. *J. Biol. Sci.* **10**: 157-161.

KARANJA, N.N., NJENGA, M., PRAIN, G., KANG'ETHE, E., KIRONCHI, G., GITHUKU, C., KINYARI, P. and MUTUA, G.K. 2009. Assessment of environmental and public health hazards in wastewater used for urban agriculture in Nairobi, Kenya. *J. Agric. Food Chem.* **12**(1): 85-97.

KILIC, E., DEMIROGLU, A., SARAYMEN, R. and OK, E. 2004. Comparative quantative analysis of zinc, magnesium, and copper content in the scalp hair of healthy people and breast cancer patients. *J. Trace Elem. Exp. Med*. **17**(3): 175-180.

KRISHNA, P.V., RAO, K.M., SWARUPARANI, V. and RAO, D.S. 2014. Heavy metals concentration in fish *Mugilcephalus* from Machilipatnam coast and possible health risks to fish consumers. *Br. biotechnol. J*. **4**(2): 126-135.

KUMAR, B., SAJWAN, K.S. and MUKHERJEE, D.P. 2012. Distribution of heavy metals in valuable coastal fishes from north east coast of India. *Turkish J. Fish Aquat. Sci.* **12**(1).

MAC, M.J., NICHOLSON, L.W., MCCAULEY, C.A. 1979. PCBs and DDE in commercial fish feeds. Prog. *Fish Cult.***4**: 210–211.

MANAHAN, S.E. 1997. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chem.* **78(**1): 15-22.

MENDIL, D., ÜNAL, Ö.F., TÜZEN, M. and SOYLAK, M. 2010. Determination of trace metals in different fish species and sediments from the River Yeşilırmak in Tokat, Turkey. *Food Chem. Toxicol.* **48**(5): 1383-1392.

MINGANTI, V., DRAVA, G., DE PELLEGRINI, R. and SICCARDI, C. 2010. Trace elements in farmed and wild gilthead seabream, *Sparusaurata*. *Mar. Pollut. Bull.* **60**(11): 2022-2025.

MOHAMED, F.A. 2008. Bioaccumulation of selected metals and histopathological alterations in tissues of *Oreochromisniloticus* and *Latesniloticus* from Lake Nasser, Egypt. *Global Veterinaria*. **2**(4): 205-218.

MOHAMED, H. and AMAAL, M. 2005. Studies of some heavy metals in water, sediment, fish and fish diets in some fish farms in El-Fayoum province, Egypt. *STUDIES*. **31**(2): 261-273.

MOHSIN AB, HAQUE SM, GALIB SM, FAHAD MF, CHAKI N, ISLAM MN, and RAHMAN MM. 2013. Seasonal Abundance of Fin Fishes in the Padma River at Rajshahi District, Bangladesh. *World J. Fish Mar. Sci.* **5**(6):680-5.

MOODY, F.O., AKINWANDE, A.A. and ADEWUNMI, A.A. 2013. Bioaccumulation of some heavy metals and the haematological indices in some selected freshwater fishes in river Manyara, Nigeria. *Int. J. Agr. Sci*. **3**(8): 602-607.

MORGANO, M.A., RABONATO, L.C., MILANI, R.F., MIYAGUSKU, L. and BALIAN, S.C. 2011. Assessment of trace elements in fishes of Japanese foods marketed in São Paulo (Brazil). *Food Control*. **22**(5): 778-785.

MWEGOHA, W.J.S. and KIHAMPA, C. 2010. Heavy metal contamination in agricultural soils and water in Dares Salaam city, Tanzania. *Afr. J. Environ. Sci. Technol.* **4**(11): 763-769.

NARIN, İ., SOYLAK, M., ELÇI, L. and DOĞAN, M. 2000. Determination of trace metal ions by AAS in natural water samples after preconcentration of pyrocatechol violet complexes on an activated carbon column. *Talanta*. **52**(6): 1041-1046.

NASREDDINE, L., NASHALIAN, O., NAJA, F., ITANI, L., PARENT-MASSIN, D., NABHANI-ZEIDAN, M. and HWALLA, N. 2010. Dietary exposure to essential and toxic trace elements from a total diet study in an adult Lebanese urban population. *Food Chem. Toxicol*. **48**(5): 1262-1269.

NASIM AL MAHMUD, M.D., HOSSAIN, M.B. and MINAR, M.H. 2012. Proximate Composition of Fish Feed Ingredients Available in Lakshmipur Region, Bangladesh.

NUSSEY, G., VAN VUREN, J.H.J. and DU PREEZ, H.H. 2000. Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeoumbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. *Water SA.* **26**(2): 269-284.

OBASOHAN, E.E. 2008. Bioaccumulation of chromium, copper, maganese, nickel and lead in a freshwater cichlid, *Hemichromisfasciatus* from Ogba River in Benin City, Nigeria. *Afr. J. Agric. Res*. **4**(3): 141-152.

OGUZIE, F.A. 2003. Heavy metals in water and sediment of the lower Ikpoba River, Benin City, Nigeria. *Pak. J. Sci. Ind. Res.* **46**(3): 156-160.

OKOYE, C.O.B., IBETO, C.N. and IHEDIOHA, J.N. 2011. Assessment of heavy metals in chicken feeds sold in south eastern, Nigeria. *Adv. Appl. Sci. Res.* **2**(3): 63-68.

OLMEDO, P., PLA, A., HERNÁNDEZ, A.F., BARBIER, F., AYOUNI, L. and GIL, F. 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environ. Int*. **59**: 63-72.

ONSANIT, S., KE, C., WANG, X., WANG, K.J. and WANG, W.X. 2010. Trace elements in two marine fish cultured in fish cages in Fujian province, China. *Environ. Poll.* **158**(5): 1334-1342.

ORONSAYE, J.A.O., WANGBOJE, O.M. and OGUZIE, F.A. 2010. Trace metals in some benthic fishes of the Ikpoba river dam, Benin City, Nigeria. *Afr. J. Biotechnol.* **9**(51): 8860-8864.

OSMAN, A.G. and KLOAS, W. 2010. Water quality and heavy metal monitoring in water, sediments, and tissues of the African catfish *Clariasgariepinus* (Burchell, 1822) from the river Nile, Egypt. *J. Envir. Protect. Sci.* **1**(04): 389.

ÖZTÜRK, M., ÖZÖZEN, G., MINARECI, O. and MINARECI, E. 2009. Determination of heavy metals in fish, water and sediments of Avsar Dam Lake in Turkey. *Iranian J. Environ. Health Sci. Eng.***6**(2). pp.: 73-80.

PLASKETT, D. and POTTER, I.C. 1979. Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. *Mar Freshwater Res.* **30**(5): 607-616.

REILLY, C. 2002. Metal contamination of food. Backwell Science Limited. USA. *J. Fish Biol.***81**-194.

ROOS, N., THORSENG, H., CHAMNAN, C., LARSEN, T., GONDOLF, U.H., BUKHAVE, K. and THILSTED, S.H., 2007. Iron content in common Cambodian fish species: Perspectives for dietary iron intake in poor, rural households. *Food Chem.* **104**(3): 1226-1235.

ROMEO, M., SIAUB, Y., SIDOUMOUB, Z., AND GNASSIA-BARELLIA, M. 1999.Heavy metal distribution in different fish species from the Mauritania coast. *Sci. Total. Environ.* **232(3)**: 169-175

RUDD, J.W. and TURNER, M.A. 1983. The English-Wabigoon river system: II. Suppression of mercury and selenium bioaccumulation by suspended and bottom sediments. *Can. J. Fish. Aquat. Sci.* **40**(12): 2218-2227.

SAEED, S.M. and SHAKER, I.M. 2008. Assessment of heavy metals pollution in water and sediments and their effect on *Oreochromisniloticus* in the northern delta lakes, Egypt. In 8th International Symposium on Tilapia in Aquaculture. *J. Fish Biol.***8**: 475-490.

SAEI-DEHKORDI, S.S. and FALLAH, A.A. 2011. Determination of copper, lead, cadmium and zinc content in commercially valuable fish species from the Persian Gulf using derivative potentiometric stripping analysis. *Microchem. J.* **98**(1): 156-162.

SHAMSHAD , B.Q., SHAHIDUR , R.K., AND TASRENA, R.C. 2009. Studies on toxic elements accumulation in shrimp from fish feed used in Bangladesh. *As. J. Food Ag-Ind.* **2**(4): 440-444

S.A. Mahesar, S.T.H. Sherazi, A. Niaz, M.I. Bhanger, S. Uddin and A. Rauf. 2010. *Food Chem. Toxicol.* **48**(8-9): 2357-2360

SCHÜMANN, K., ETTLE, T., SZEGNER, B., ELSENHANS, B. and SOLOMONS, N.W. 2007. On risks and benefits of iron supplementation recommendations for iron intake revisited. *J. Trace Elem. Med. Biol.* **21**(3): 147-168.

SHEARER, K.D., A ˚ SGA˚RD, T., ANDORSDO¨ TTIR, G., AAS, G.H. 1994. Whole body elemental and proximate composition of Atlantic salmon (*Salmosalar*) during the life cycle. *J. Fish Biol.***44**: 785–797.

SHINN, C., DAUBA, F., GRENOUILLET, G., GUENARD, G. and LEK, S. 2009. Temporal variation of heavy metal contamination in fish of the river lot in southern France. *Ecotoxicol. Environ. Safety*. **72**(7): 1957-1965.

SKOOG, D.A., WEST, D.M., HOLLER, F.J. and CROUCH, S.R. 2005. Fundamentals of Analytical Chemistry. Publisher, Chemistry: David Harries, 8th Ed, 720-723 p.

SIVAPERUMAL, P., SANKAR, T.V. and NAIR, P.V. 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India international standards. *Food Chem.* **102**(3): 612-620.

SRIVASTAV, A.K., RAI, R., SUZUKI, N., MISHRA, D. and SRIVASTAV, S.K., 2013. Effects of lead on the plasma electrolytes of a freshwater fish, *Heteropneustesfossilis*. *International Aquac. Res.* **5**(1): 1-7.

TABOURET, H., BAREILLE, G., MESTROT, A., CAILL-MILLY, N., BUDZINSKI, H., PELUHET, L., PROUZET, P. and DONARD, O.F.X. 2011. Heavy metals and organochlorinated compounds in the European eel (*Anguilla anguilla*) from the Adour estuary and associated wetlands (France). *J. Environ. Monit.* **13**(5): 1446-1456.

TAWARI-FUFEYIN, P. and EKAYE, S.A. 2007. Fish species diversity as indicator of pollution in Ikpoba river, Benin City, Nigeria. Reviews in Fish Biology and Fisheries. **17(**1): 21-30.

TSADIK, G.G. and BART, A.N. 2007. Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromisniloticus*. *Aquaculture*. **272**(1): 380-388.

TÓTH, T., ANDREJI, J., TÓTH, J., SLÁVIK, M., ÁRVAY, J. and STANOVIČ, R. 2012. Cadmium, lead and mercury contents in fishes—case study. *J MicrobiolBiotechnol Food Sci*, **1**: 837-847.

TÜRKMEN, M., TÜRKMEN, A., TEPE, Y., TÖRE, Y. and ATEŞ, A. 2009. Determination of metals in fish species from Aegean and Mediterranean seas. *Food Chem.* **113**(1): 233-237.

TUZEN, M. 2009. Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. *Food Chem. Toxicol.* **47**(8): 1785-1790.

WORLD HEALTH ORGANISATION (WHO) (2008) Guidelines for Drinking-water Quality; 3rd Edition **1**-459.

WORLD HEALTH ORGANIZATION WHO (2003) Chromium , zinc, lead, in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality,Geneva.

YILMAZ, F. 2009. The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugilcephalus* and *Oreochromisniloticus*) inhabiting Köycegiz Lake-Mugla (Turkey). *Turkish. J. Sci. Technol.***4**(1): 7-15.

YILMAZ, A.B., SANGÜN, M.K., YAĞLIOĞLU, D. and TURAN, C. 2010. Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay, Turkey. *Food Chem.* **123**(2): 410-415.

Appendix- Data analysis for samples

Table 1: Metal concentration of Cu in feed and fish

feed type	Cu concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Nursery		33.922	38.48	30.081
Starter		22.618	34.97	36.001
Grower		23.729	31.805	30.789
WHO standard	100	100	100	100

Table 2: Metal concentration of Cu in fish

Sample type	Cu concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Farm-1		22.504	23.293	19.468
Farm-2		19.073	22.649	22.006
Farm-3		23.791	25.343	21.924
WHO Standard	30	30	30	30

Table 3: Metal concentration of Cd in feed

feed type	Cd concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Nursery		8.082	9.771	8.639
Starter		8.313	9.116	9.067
Grower		8.214	8.754	8.651
WHO standard	2	2	2	2

Table 4: Metal concentration of Cd in fish

Sample type	Cd concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Farm-1		9.715	9.083	9.575
Farm-2		10.453	9.827	9.596
Farm-3		9.371	9.385	10.198
WHO Standard	1	1	1	1

Table 5: Metal concentration of Fe in feed

feed type	Fe concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Nursery		764.929	750.808	963.425
Starter		475.878	586.442	1004.86
Grower		582.203	791.413	525.199
WHO standard	500	500	500	500

Table 6: Metal concentration of Fe in Fish

Sample type	Fe concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Farm-1		195.524	179.489	139.13
Farm-2		186.167	138.762	190.428
Farm-3		200.258	136.241	148.083
WHO Standard	100	100	100	100

Table 7: Metal concentration of Pb in feed

feed type	Pb concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Nursery		9.145	12.232	8.684
Starter		11.209	9.163	9.532
Grower		7.671	8.962	9.538
WHO standard	5	5	5	5

Table 8: Metal concentration of Pb in fish

Sample type	Pb concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Farm-1		6.787	14.325	12.384
Farm-2		9.248	16.386	12.193
Farm-3		13.715	13.806	10.774
WHO Standard	2	2	2	2

Table 9: Metal concentration of Na in feed

feed type	Na concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Nursery		891.046	1071.63	1079.13
Starter		954.057	1022.28	1048.73
Grower		945.523	922.259	959.161
WHO standard	700	700	700	700

Table 10: Metal concentration of Na in fish

Sample type	Na concentration (mg/Kg)			
		Quality	Mega	Ruposhi
Farm-1		780.001	937.387	907.816
Farm-2		864.212	774.703	884.656
Farm-3		819.774	845.177	862.917
WHO Standard	80	80	80	80

Table 11: Post Hoc Tests for different feed

metal	Quality	Mega	Ruposhi	Q v M	Q v R	M v R
cu	33.922	38.48	30.081	0.13212	0.265338	0.356513
	22.618	34.97	36.001			
	23.729	31.805	30.789			
Fe	764.929	750.808	963.425	0.391591	0.406312	0.418668
	475.878	586.442	10004.9			
	582.203	791.413	525.199			
Pb	9.145	12.232	8.684	0.625837	0.939186	0.502191
	11.209	9.163	9.532			
	7.671	8.962	9.538			
Cd	8.082	9.771	8.639	0.07039	0.071445	0.196167
	8.313	9.116	9..067			
	8.214	8.754	8.651			

Na	891.046	1071.63	1079.13	0.223656	0.092521	0.699708
	954.057	1022.28	1048.73			
	945.523	922.259	959.161			

Table 12: Post Hoc Tests for fish farms

Metal	Quality	Mega	Ruposhi	Q v M	q v r	m v R
Farm-1	22.504	23.293	19.468	0.306938	0.713088	0.086709
Farm-2	19.073	22.649	22.006			
Farm-3	23.791	25.343	21.924			
Fe	195.524	179.489	139.13	0.083505	0.151878	0.733776
	186.167	138.762	190.428			
	200.258	136.241	148.083			
Pb	6.787	14.325	12.384	0.122579	0.456859	0.099095
	9.248	16.386	12.193			
	13.715	13.806	10.774			
Cd	9.715	9.083	9.575	0.350218	0.889559	0.295111
	10.453	9.827	9.596			
	9.371	9.385	10.198			
Na	780.001	937.387	907.816	0.59878	0.102087	0.564099
	864.212	774.703	884.656			
	819.774	845.177	862.917			

Table 13: Mean and SD calculation of heavy metals concentration (mg/kg) in feed

Cu	Quality	Mega	Ruposhi
Nursery	33.922	38.48	30.081
Starter	22.618	34.97	36.001
Grower	23.729	31.805	30.789
Mean	26.7563333	35.085	32.2903
SD	6.23046261	3.33899	3.23297
Fe	Quality	Mega	Ruposhi
Nursery	764.929	750.808	963.425
Starter	475.878	586.442	10004.9
Grower	582.203	791.413	525.199
Mean	607.67	709.554	3831.16
SD	146.198653	108.534	5351.06
Pb	Quality	Mega	Ruposhi
Nursery	9.145	12.232	8.684
Starter	11.209	9.163	9.532
Grower	7.671	8.962	9.538
Mean	9.34166667	10.119	9.25133
SD	1.77718016	1.83267	0.49133
Cd	Quality	Mega	Ruposhi
Nursery	8.082	9.771	8.639
Starter	8.313	9.116	9..067
Grower	8.214	8.754	8.651
Mean	8.203	9.21367	8.645
SD	0.11589219	0.51549	0.00849
Na	Quality	Mega	Ruposhi
Nursery	891.046	1071.63	1079.13
Starter	954.057	1022.28	1048.73
Grower	945.523	922.259	959.161
Mean	930.208667	1005.39	1029.01
SD	34.1832288	76.1031	62.3686

Table 14: Mean and SD calculation of heavy metals concentration (mg/kg) in fish

Cu	Quality	Mega	Ruposhi
Farm-1	22.504	23.293	19.468
Farm-2	19.073	22.649	22.006
Farm-3	23.791	25.343	21.924
Mean	21.7893333	23.7616667	21.1326667
SD	2.43884037	1.406821	1.44222652
Fe	Quality	Mega	Ruposhi
Farm-1	195.524	179.489	139.13
Farm-2	186.167	138.762	190.428
Farm-3	200.258	136.241	148.083
Mean	193.983	151.497333	159.213667
SD	7.17077966	24.2742438	27.4005476
Pb	Quality	Mega	Ruposhi
Farm-1	6.787	14.325	12.384
Farm-2	9.248	16.386	12.193
Farm-3	13.715	13.806	10.774
Mean	9.91666667	14.839	11.7836667
SD	3.51206952	1.36464171	0.87959669
Cd	Quality	Mega	Ruposhi
Farm-1	9.715	9.083	9.575
Farm-2	10.453	9.827	9.596
Farm-3	9.371	9.385	10.198
Mean	9.84633333	9.43166667	9.78966667
SD	0.55282668	0.3741889	0.35378289
Na	Quality	Mega	Ruposhi
Farm-1	780.001	937.387	907.816
Farm-2	864.212	774.703	884.656
Farm-3	819.774	845.177	862.917
Mean	821.329	852.422333	885.129667
SD	42.1270299	81.583651	22.4532474

Table 15: THQ estimation for Quality feed fed fish farms

Heavy metals	Concentration	Ingestion rate (g/d)	Exposure frequency (d/y)	Exposure duration (y)	Reference dose (mg/Kg)	Body weight of Adult (Kg)	Averaging time (d)	EDI	THQ
Fe	193.98	49.5	365	30	0.7	70	10950	0.02853	0.04076
Cr	0	49.5	365	30	0.003	70	10950	0	0
Na	821.33	49.5	365	30	0.08	70	10950	0.12081	1.510074
Ni	0	49.5	365	30	0.02	70	10950	0	0
Cu	21.78	49.5	365	30	0.005	70	10950	0.0032	0.640705
Cd	9.84	49.5	365	30	0.001	70	10950	0.00145	1.447323
Pb	9.92	49.5	365	30	0.2	70	10950	0.00146	0.007295

Table 16: THQ estimation for Mega feed fed fish farms

Heavy metals	Concentration	Ingestion rate (g/d)	Exposure frequency(d/y)	Exposure duration (y)	Reference dose (mg/Kg)	Body weight of Adult (Kg)	Averaging time (d)	EDI	THQ
Fe	151.49	49.5	365	30	0.7	70	10950	0.02228	0.031831
Cr	0	49.5	365	30	0.003	70	10950	0	0
Na	852.42	49.5	365	30	0.08	70	10950	0.12538	1.567235
Ni	0	49.5	365	30	0.02	70	10950	0	0
Cu	23.76	49.5	365	30	0.005	70	10950	0.00349	0.698951
Cd	9.43	49.5	365	30	0.001	70	10950	0.00139	1.387018
Pb	14.83	49.5	365	30	0.2	70	10950	0.00218	0.010906

Table 17: THQ estimation for Ruposhi feed fed fish farms

Heavy metals	Concentration	Ingestion rate (g/d)	Exposure frequency(d/y)	Exposure duration (y)	Reference dose (mg/Kg)	Body weight of Adult (Kg)	Averaging time (d)	EDI	THQ
Fe	159.21	49.5	365	30	0.7	70	10950	0.02342	0.033454
Cr	0	49.5	365	30	0.003	70	10950	0	0
Na	885.13	49.5	365	30	0.08	70	10950	0.13019	1.627375
Ni	0	49.5	365	30	0.02	70	10950	0	0
Cu	21.13	49.5	365	30	0.005	70	10950	0.00311	0.621584
Cd	9.79	49.5	365	30	0.001	70	10950	0.00144	1.439969
Pb	11.78	49.5	365	30	0.2	70	10950	0.00173	0.008663

Table 18: TR estimation of Quality feed fed fish

Quality fish											
Metal	EF	ED	FIR	Cf	CM	WAB	Atn	RfD	THQ	CPSo	TR
Fe	365	30	49.5	0.21	194	70	10950	0.7	0.04		0
Cr	365	30	49.5	0.21	0	70	10950	0.009	0		0
Na	365	30	49.5	0.21	821	70	10950	0.08	1.51		0
Ni	365	30	49.5	0.21	0	70	10950	0.14	0	1.7	0
Cu	365	30	49.5	0.21	21.8	70	10950	0.02	0.16		0
Cd	365	30	49.5	0.21	9.84	70	10950	0.005	0.29	6.3	0
Pb	365	30	49.5	0.21	9.92	70	10950	0.2	0.01	0	0

Table 19: TR estimation of Mega feed fed fish

Mega-fish											
Meta	EF	ED	FIR	Cf	CM	WAB	Atn	RfD	THQ	CPSo	TR
Fe	365	30	49.5	0.21	151	70	10950	0.7	0.03		0
Cr	365	30	49.5	0.21	0	70	10950	0.009	0		0
Na	365	30	49.5	0.21	852	70	10950	0.08	1.57		0
Ni	365	30	49.5	0.21	0	70	10950	0.14	0	1.7	0
Cu	365	30	49.5	0.21	23.8	70	10950	0.02	0.17		0

Cd	365	30	49.5	0.21	9.43	70	10950	0.005	0.28	6.3	0
Pb	365	30	49.5	0.21	14.8	70	10950	0.2	0.01	0	0

Table 20: TR estimation of Ruposhi feed fed fish

Ruposhi-feed											
Meta	EF	ED	FIR	Cf	CM	WAB	Atn	RfD	THQ	CPSo	TR
Fe	365	30	49.5	0.21	159	70	10950	0.7	0.03		0
Cr	365	30	49.5	0.21	0	70	10950	0.009	0		0
Na	365	30	49.5	0.21	885	70	10950	0.08	1.63		0
Ni	365	30	49.5	0.21	0	70	10950	0.14	0	1.7	0
Cu	365	30	49.5	0.21	21.1	70	10950	0.02	0.16		0
Cd	365	30	49.5	0.21	9.79	70	10950	0.005	0.29	6.3	0
Pb	365	30	49.5	0.21	11.8	70	10950	0.2	0.01	0	0

Table 21: HI Estimation of different fish farms

Heavy metals	*Quality fish*		*Mega fish*		*Ruposhi fish*	
	THQ	TR	THQ	TR	THQ	TR
Fe	0.04075955	-	0.03183145	-	0.033453595	-
Cr	0	-	0	-		-
Na	1.5100738	-	1.567235057	-	1.627374729	-
Ni	0		0			
Cu	0.6407053	-	0.698951314	-	0.621584229	-
Cd	1.4473234	0.009118	1.387018286	0.008738	1.439969143	0.009072
Pb	0.0072954	1.24E-05	0.010906406	1.85E-05	0.008663349	1.47E-05
HI	3.64615745		3.695942513	0.008757	3.731045045	0.0090867