EFFECT OF HEAVY METALS AT DNA LEVEL OF *CLARIAS GARIEPINUS* BURCELL, 1822 (AFRICAN CATFISH) COLLECTED FROM TANNERY WASTE POLLUTED AREA AND LOCAL MARKETS

A DISSERTATION
SUBMITTED TO THE UNIVERSITY OF DHAKA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF M.S. IN FISHERIES

MASRUPA TASLIM
EXAM. ROLL NO. 709
SESSION: 2014-2015

29 DECEMBER, 2015

EFFECT OF HEAVY METALS AT DNA LEVEL OF *CLARIAS GARIEPINUS* BURCELL, 1822 (AFRICAN CATFISH) COLLECTED FROM TANNERY WASTE POLLUTED AREA AND LOCAL MARKETS

A DISSERTATION
SUBMITTED TO THE UNIVERSITY OF DHAKA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF M.S. IN FISHERIES

29 DECEMBER, 2015

EXAM. ROLL NO. 709
SESSION: 2014-2015
REG. NO. : 2010-013003

EFFECT OF HEAVY METALS AT DNA LEVEL OF *CLARIAS GARIEPINUS* BURCELL, 1822 (AFRICAN CATFISH) COLLECTED FROM TANNERY WASTE POLLUTED AREA AND LOCAL MARKETS

DEPARTMENT OF FISHERIES
UNIVERSITY OF DHAKA
DHAKA-1000
BANGLADESH

29 DECEMBER, 2015

MASRUPA TASLIM
EXAM. ROLL NO. 709
SESSION: 2014-2015

Dedicated
To
Prof. Dr. Sheikh Shamimul Alam
And
Members of Cytogenetics lab, DU

Dedicated
To
My Beloved Parents

Certificate

*This is to certify that this thesis entitled "**EFFECT OF HEAVY METALS AT DNA LEVEL OF CLARIAS GARIEPINUS BURCELL, 1822 (AFRICAN CATFISH) COLLECTED FROM TANNERY WASTE POLLUTED AREA AND LOCAL MARKETS"** submitted by Masrupa Taslim has been carried out under my supervision. This is further to certify that it is an original work and suitable in partial fulfillment for the degree of MS in Fisheries.*

Date: 29.12.2015

(Dr. Mohammad Shamsur Rahman)
Associate Professor
Department of Fisheries
University of Dhaka
Dhaka-1000, Bangladesh

ACKNOWLEDGEMENT

At first thanks to Almighty Allah for giving me patience, strength and courage to complete my work in due time.

I feel pleasure to express my immense and heartfelt gratitude to Dr. Sheikh Shamimul Alam, Professor, Department of Botany, University of Dhaka, under whose constant guidance and supervision the present investigation was carried out.

I am grateful to Dr. Mohammad Shamsur Rahman, Associate Professor, Department of Fisheries, University of Dhaka for his supervision and sincere guidance during the whole research work.

My sincere thanks to Md. Alamgir kabir, Assistant Professor, Department of Fisheries, University of Dhaka for his continuous supervision and inspiration.

I express my sincere and heartiest gratitude to Dr. Syeda Sharmeen Sultana, Assistant Professor, Department of Botany, University of Dhaka, for her help, invaluable suggestions and warm encouragement to complete the study.

I am grateful to Dr. Rokeya Begum, Professor, Department of Botany, University of Dhaka, for her generous co-operation, guiding and encouragement throughout the research period.

My heartful thanks and gratitude to the Chairperson Mrs. Wahida Haque, Department of Fisheries, University of Dhaka for providing the research facilities of the Department.

I am grateful to Chairman, Professor Dr. Abul Bashar, Department of Botany, University of Dhaka for providing the facilities of the Department.

Special thanks to Md. Moniruzzaman, Senior Scientific Officer, Soil and Environmental Section, Biological Division, BCSIR, Dhaka and Dr. Ahsan Habib, Senior Scientific Officer, Tanjina Banu, Senior Scientific Officer and Mousona Islam, Scientific Officer, Plant Tissue Culture , BCSIR, Dhaka for their logistic help and kind cooperation for conducting this research. I am really very grateful to them from the core of my heart.

Very special thanks to dear Riffat Apu, Ph. D student of Cytogenetics Lab, Department of Botany, University of Dhaka and Mahin apu, Lecturer, Department of Botany, University of Barisal for their kind support and hard work to complete my thesis work.

Heartiest thanks to Dr. Kazi Nahida Begum, Assistant Professor, Department of Botany, University of Jagannath and Asma Ahmed Warasi, Assistant Professor, Department of Botany, University of Jahangirnagar for their encouragement.

My cordial and heartiest thanks to Uzzal Vaia, Riad Vaia, Imran Vaia, Laila Apu, Mohona, Suma and all the other members of cytogenetics laboratory, Department of Botany, University of Dhaka for their kind cooperation.

Sincere thanks to Emon Vai, Mojammel Vai, and Jashim vai, lab attendant of Department of Fisheries, University of Dhaka for their co-operation.

Special thanks to Dilip Da and Sawkot Vai of Department of Botany, University of Dhaka for their help.

I express my sincere and heartiest gratitude to all of my family members including my beloved parents and my only younger brother and my friends, since this work would not be possible without their patience and continuous co-operation and encouragement.

Finally, this thesis is dedicated to my beloved parents.

– The Author

Contents

Contents

Contents

Contents

Abstract

This research was undertaken to determine the amount of heavy metals in soil, water and fish samples from tannery waste affected areas of Hazaribagh. In addition, to know whether these heavy metals have any impact at the DNA level of this fish. Besides, samples were collected from two local fish markets. As control, sample from a reputed farm was selected. The quality of water in the tannery waste polluted area was much below than the standard level. The DO is 0.3 mg/l which gradually decreasing in comparison to previous studies. The below standard physico-chemical features of tannery waste polluted water are becoming a threat to the aquatic organisms living in these water body. High concentration of heavy metals like Pb, Cr and Cd are found in the sample fishes. Unexpectedly Cr was present 26 times higher than the normal limit to the control fish. In the fish from tannery waste water, the amount of Cr was about 200 times more than the normal level. A number of polymorphism was observed in four samples after RAPD analysis. Presence and absence of some bands indicated deletion and newly originating DNA fragments. The alteration at DNA level was probably due to accumulation of high level heavy metals. The presence of high amount of heavy metals in fish muscles is becoming a threat not only for the fishes but human health also. Therefore, the people should avoid African catfish those are selling in the local market and even from farms.

List of tables

List of tables

List of figures

List of figures

List of figures

Chapter 1
Introduction

Most of the peoples of Bangladesh depend on fish as a source of protein. The annual per head consumption of fish in Bangladesh is 19 kg (DoF, 2014). About 57% of the protein consumption of Bangladesh's population comes from fish and presently Bangladesh stands in 5th position in producing sweet water fish (DoF, 2014). From 2004 till 2014, fish production of Bangladesh has increased by 53%. Bangladesh Bureau of Statistics (BBS)'s latest economic census says that in the 2013-14 fiscal year, the country produced approximately 3.46 million tons of fish, of which about 2 million tons were farmed.

The new varieties of fish bred by Bangladesh's fishery experts and the rapid expansion in this regard have given rise to this significant increase in fish production. There has been a virtual revolution in fish farming in the ponds at Mymensing, Bogra, Comilla and in the fish farms of the southwest regions. Among the farm fishes "Koi" and "Magur" are popular because of their high growth rate. African Magur (*Clarias gariepinus* Burchell, 1822) is an omnivorous fish which has high market demand in Bangladesh for the cheap price to the common people. Due to high environmental tolerance and wide food spectrum these fishes are being cultured in the industrially polluted water to minimize the cost.

Fish traders usually select polluted water body around tannery factories because the tannery waste is rich in organic matter. The tannery effluents are one of the major sources for direct and often continuous input of pollutants/toxicants into aquatic ecosystems with long-term implication (Odeighgah and Osanyipeju 1995, Chan *et al.* 2003, Lah *et al*. 2004, Smolders *et al.* 2004). As a result of continuous input of different pollutants, it lowers the quality of life in various aspects and affects health and life span (Grover and Kaur 1999). Unfortunately these industries do not follow biosafety guidelines. Even they do not have any recommended waste management system and thus discharging their effluents directly into different water bodies around the factories.

Many of the persistent compounds contained in these effluents are toxic and having a devastating effect on the environment. Industrial effluents have been found to increase heavy metal load in surrounding agricultural soils (Joardar *et al.* 2005). Among the

heavy metals, Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni), etc. are directly health hazardous (Huq *et al.* 2006). It has been reported that the heavy metals like As, Pb, Cr, Cd, Ni have carcinogenic effects on both plants and animals (Förstner and Wittmann 1981, Glanze 1996). In human, the increased level of Pb causes abdominal colic, aneamia, nervous system disorder, teratogenic and fetotoxic effects, while the diseases like skin rashes, allergic reaction, nose irritation, kidney and liver damage, alteration of genetic material, lung cancer, etc, are caused by high concentration of Cr (Crinnion 2009). The reason of bone demineralization, impaired lung function, lung cancer, pulmonary damage diseases are due to high accumulation of Cd (US National library of medicine, National institute of health 1999).

The African Magur is a popular fish to common people for its low cost and great taste. A number of people are having this fish without knowing the sources. Everyday huge amount of African Magur are coming from the various tannery waste affected areas to the local market and are being sold at cheap price. In this way, the toxic substances are entering into the human food chain and thus increasing the level after continuous consumption. Since the heavy metals have carcinogenic effects on human (DHHS, Hazardous Substances Data Bank 1993) may have great impact on DNA level. Therefore, it is an urgent need to know whether these effluents are causing any change at the molecular level of African Magur.

DNA finger printing by Random Amplified Polymorphic DNA (RAPD) is a novel method. The term DNA fingerprinting/profiling describes the combined use of several single locus detection systems. This method has been using as versatile tool for investigating various genomic aspects of organism. It includes characterization of genetic variability, genome fingerprinting, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, etc. Genetic analysis using molecular marker technologies can provide a powerful approach to understand the organization and distribution of genetic resources in natural and managed populations (Schierwater *et al*. 1994). RAPD markers generated by the polymerase chain reaction (PCR), has been widely using since late 80's of the last century to assess intra-specific genetic variation on a molecular level (Welsh and McClellland 1990, Williams *et al.* 1990). RAPD technique has been used for taxonomic and systematic analysis of various organisms and provided important applications in catfish (Bartish *et al*. 2000).

In this study, a tannery industry named Fatema Leather Industry, Hazaribagh, Dhaka has been selected as the source of heavy metals. This industry is discharging waste effluents directly to the nearby water bodies along with other industries. The heavy metals of soil, water and muscles of fish from the water body were measured to assess the rate of accumulation and uptake of these substances. Besides, two local fish markets *viz* Ananda Bazar and Hatirpool Bazar of Dhaka were selected where the African Magur are being sold for human consumption. A manually cultured African Magur from Suborna Agro-based Initiative Fish Farm, Sonapur, Noakhali were used as control.

Therefore, the present research work was undertaken with the following aims-

i) To determine the amount of heavy metals in soil, water and fish samples collected from tannery affected area of Hazaribagh.
ii) To determine the rate of accumulation of these heavy metals in fish.
iii) To compare the amount of heavy metals present in control, tannery affected fish and fishes from two local markets.
iv) To determine the genetic alteration at molecular level using RAPD in these sample fishes.
v) To create a public awareness regarding the consumption of such fish.

Chapter 2
Materials and Methods

2.1. Materials

Clarias gariepinus Burcell, 1822 (African Catfish, African Magur) commonly growing in the industrial effluent affected areas was used as research material in this study. The samples were collected from three different areas of Dhaka city such as (i) Polluted, Hazaribagh, (ii) Ananda Bazar local market and (iii) Hatirpool local market. Culture of African Magur collected from Suborna Agro-based Itiitiative Fish Farm, Sonapur, Noakhali was used as control. All of these fish samples have been maintaining in the Aquatic Laboratory, Department of Fisheries, University of Dhaka.

2.1.1. Taxonomic Tree of African Magur

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Chordata

Subphylum: Vertebrata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: *Clarias*

Species: *Clarias gariepinus* (Burcell 1822)

2.1.2. Nature and morphological description of African Magur

Clarias gariepinus is an widely distributed and indigenous species in Africa. The species easily adapts to environments, where the water temperature is higher than 20° C. The Clariidae species display an anguilliform shape, having an elongated cylindrical body. The skin of African magur is scaleless, similar to that of other catfish species. It is generally dark at dorsal and lateral surface of body and grayish-white at ventral side. During stress, black spots develop on the body surface of African magur (Viveen *et al*. 1986). Around the mouth, 4 pairs of barbells can be distinguished (nasal, maxillary, the

longest and most mobile, outer mandibular and inner mandibular). Close to the nasal barbels, two olfactory organs are located. Magur recognizes its prey mainly by touch and smell.

African Magurs with their wide mouth are able to feed on a variety of food items ranging from minute zooplankton to large fish. They are able to suck benthos from the bottom, tear pieces of cadavers with the small teeth on its jaws and to swallow prey such as a whole fish. The mouth circumference is about 25% of its total length and it determines the maximum size of its prey. A Magur of 30 cm (approx. 200 g) has a mouth circumference of about 7.5 cm. During respiration, water is taken into the mouth, passed over the gills for gaseous exchange and is then expelled through the opercular opening. Gaseous exchange takes place via the arborescent organs in air chambers above the gills. Due to its ability to breath out of water, the African magur is capable of existing in mud during the dry season even for few weeks.

The unpaired fins of African magur consist of a dorsal, a caudal and an anal fin, while the paired fins consist of the pectoral and ventral fins. The pectoral fins have developed strong spines which have locomotory and protective functions. Micha (1973) considered African Magur as an omnivorous fish with a high tendency for predation. Four modes of feeding behavior have been observed in *C. gariepinus* (Bruton 1976) such as grasping, grazing, foraging and shovelling.

2.2. Methods

2.2.1. Collection of specimen

Catfish (*Clarias gariepinus*) samples were collected from three different areas of Dhaka city such as (i) Polluted water of Hazaribagh, (ii) Ananda Bazar local market and (iii) Hatirpool local market. As control, catfish specimens were collected from Suborna Agro-based Ititiative Fish Farm, Sonapur, Noakhali. During the transportation of all of the fishes, proper transportation measures were taken.

2.2.2. Rearing of fish

After collection, the fishes were immediately released into the plastic tanks in the Aquatic Laboratory, Department of Fisheries, University of Dhaka. The tanks were filled with pond water and the fishes were reared there for about 4-5 days in static condition.

Air compressor with air stones was used for oxygenation of water continuously. After acclimatization healthy fishes of were used for the research.

2.2.3. Sampling procedure

Pieces of dorsal fin of each individual live fish were collected carefully and were preserved in 95% ethanol contained in the micro centrifuge tube. Samples were brought to the Cytogenetics Laboratory, Department of Botany, University of Dhaka and stored at –20°C in freezer.

2.2.4. Research Station

The total part of the experiment was conducted both in the Aquatic Laboratory, Department of Fisheries, University of Dhaka, Cytogenetics Laboratory, Department of Botany, University of Dhaka, Plant Tissue Culture Laboratory, Bangladesh Council of Scientific and Industrial Research (BCSIR) and Soil and Environment Laboratory, BCSIR, Dhaka.

2.2.5. Physico-chemical, essential elements, heavy metal analysis of water sample

The high-density 1-liter PVC bottles were used for sample collection and preservation. One was preserved with toluene for regular water analysis and the other preserved with HNO_3 acid for the heavy metal analysis.

2.2.5.1. Temperature

The temperature of the samples was measured immediately in the field by a mercury thermometer of 0 °C to 50 °C range and with 0.2 °C least count (Gupta 2000).

2.2.5.2. Hydrogen ion concentration (pH)

Water pH was determined by glass electrode using pH meter and recorded (Model: Jenway instrument, pH meter 3305 Jackson 1967).

2.2.5.3. Electrical Conductivity (EC)

The EC of six water samples was determined by EC meter and recorded (Moled: HANNA HI-8633) in µs/cm Jackson 1967).

2.2.5.4. Total Dissolved Solids (TDS)

A rapid determination of total dissolved solids (TDS) of water samples was determined by TDS meter and recorded (Model: Mettler-Toledo Ag, DH-8603).

2.2.5.5. Dissolved Oxygen (DO)

Dissolved oxygen of water samples was determined in the field using DO meter (Model: HACH HQ 30d) (APHA, 1998).

2.2.5.6. Turbidity

Turbidity of water samples was determined using turbidity meter (Model: HACH 2100Q) followed by APHA 2130B (APHA, 1998).

2.2.5.7. Biochemical Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD5) of water samples was determined by Respirometric Method (APHA 5210D) using BOD Sensor Set (Model: HACH BOD TRACK II) (APHA,1998).

2.2.5.8. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) of water samples was determined by closed reflux, titrimetric method (APHA 5220C) using certified HACH COD vials and COD Reactor (Model: HACH COD Reactor) (APHA,1998).

2.2.5.9. Total Hardness

The hardness or total hardness (Ht), calcium plus magnesium or hardness as CaCO3, of the irrigation water sample was calculated by adding the millie quivalents of Ca and Mg per liter and multiplying the sum by 50 (Bouwer 1978).

2.2.6. Chemical Analysis

The chemical analysis was carried out with the advanced instrument of Soil and Environment Research Division, BCSIR Laboratories, Dhaka.

2.2.6.1. Sodium and Potassium

Sodium (Na) and potassium (K) of the water samples were determined by flame emission spectrophotometry method. The reading was taken in a flame photometer (Model no. Jencons, PEP7) at 589 nm and 769 nm of wavelength respectively (Jackson, 1967).

2.2.6.2. Chloride

The chloride of the water samples was determined by Mhor volumetric method (Jackson, 1967).

2.2.6.3. Sulphate

The sulphate content of water samples was determined by turbidimetric method (Matheny and Hunt, 1981). The intensity of the color was taken in a Spectrophotometer (Model no: Jenway 6100) at 420 nm of wavelength.

2.2.6.4. Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Iron (Fe), Manganese (Mn), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Copper (Cu) content of soil, water and from flesh of all the fishes

Lead (Pb), Cadmium(Cd),Chromium (Cr), Nickel(Ni), Iron(Fe), Manganese (Mn), Calcium (Ca), Magnesium(Mg), Zinc (Zn), Copper (Cu) of the samples were determined by atomic absorption spectrometer (Model: AA-7000, Shimadzu) (APHA 3111) following calibration of the equipment for every 10ml sample was include a certified reference material (CRMs) to insure QA/QC (APHA 1998).

2.2.6.5. Nitrogen content

Total Nitrogen, ammonium-N (NH4-N) and Nitrate-N (NO3-N) were determined by micro kjeldahl's distillation method (Jackson 1967).

2.2.6.6. Protein content of fish flesh

Protein content of fish samples was determined by Kjeldahl method (Kjeldahl 1883).

2.2.7. RAPD analysis

2.2.7.1. Collection of fin sample

The primary objective of the isolation process is to recover the maximum yield of high molecular weight DNA devoid of protein and other restriction enzymes (Sambrook *et. al.,* 1989). In the present study genomic DNA was prepared from fin tissue following the method as described by Kabir *et al.* (2012) with some modifications. Approximately 30 mg of caudal fin tissue was cut into small pieces with the help of sterilized scissors and washed well initially in distilled water and then ethanol. These were dried on fresh tissue paper to remove any other sources of foreign DNA.

2.2.7.2. Preparation of different stock solutions and working solutions used for DNA isolation

For conducting the isolation procedures, the following stock solutions and working solutions were prepared:

2.2.7.3. 1M stock solution of Tris HCl pH 8.0 (100 ml)

12.14 g of Trizma base (MW=121.14) was dissolved in 75 ml of distilled water. The pH of this solution was adjusted to 8.0 by adding about 5 ml of concentrated HCl in a fume hood. The volume of the solution was adjusted to a total of 100 ml with de-ionized distilled water. Then it was sterilized by autoclaving and stored at 4 ºC.

2.2.7.4. Stock solution (0.5 M) of EDTA pH 8.0 (100 ml)

18.61 g of EDTA (EDTA. $2H_2O$, MW = 372.24) was added to 75 ml of distilled water and stirred thoroughly with a magnetic stirrer. Approximately 2 g of NaOH pellets was added to adjust the final pH to 8.0. It may mention that EDTA alone will not dissolve, unless NaOH is added. The final volume of the solution was adjusted to 100 ml by adding sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4 ºC.

2.2.7.5. Stock solution (5 M) of NaCl (100 ml)

29.22 g of sodium chloride (NaCl, MW = 58.44) was dissolved slowly (not at once) in 75 ml of distilled water. The total volume of the solution was adjusted to 100 ml with distilled water. The solution was then heated by oven for 15 seconds and stirred

thoroughly on a magnetic stirrer to dissolve NaCl. It was then sterilized by autoclaving and stored at 4 ºC.

2.2.7.6. Tris-HCl Saturated Phenol

The crystal phenol was melted in a water bath at 65º C for 30 minutes. 100 ml of melted phenol was added to same volume of Tris-HCl (pH 8.0). It was mixed initially for at least 10 minutes with a magnetic stirrer and then for 5 minutes kept in rest. At this stage, two distinct phases are visible-colorless upper phase and colorful lower phase. With the help of a dropper, the upper phase was removed as much as possible. The same procedure was repeated until the pH of the lower phase rose up to 7.8. Repetition for several times was needed. In this experiment six times repetitions were done which required about 3.5 hours obtaining the pH 7.75. After saturation, the phenol became the half of the initial volume.

2.2.7.7. Phenol: Chloroform: Isoamyl Alcohol (25: 24: 1) (100 ml)

50 ml of Phenol, 48 ml of Chloroform and 2 ml of Isoamyl alcohol were added and mixed properly using by vortex mixture under a fume hood. The solution was then stored at 4 $^{\circ}$C. The solution was shaken well before each use.

2.2.7.8. 70% Ethanol (100 ml)

30 ml double distilled water (ddH_2O) was added in 70 ml absolute ethanol.

2.2.7.9. Stock Solution of TE (Tris-HCl EDTA) Buffer pH 8.0 (100 ml)

1 ml of 1 M Tris-HCl was added to 0.2 ml (200 µl) of 0.5 M EDTA. The final volume was adjusted to 100 ml with sterile de-ionized distilled water. The solution was sterilized by autoclaving and stored at 4 $^{\circ}$C.

2.2.7.10. Sodium acetate (3 M) pH 5.2 (100 ml)

40.824 g of sodium acetate was mixed with 70 ml of ddH_2O and adjust the final volume to 100 ml with ddH_2O and sterilized by autoclaving.

2.2.7.11. Extraction Buffer

1. Extraction buffer: TEN buffer +1% SDS

2. TEN buffer (For 400 ml):
   Tris-HCl (100 mM): 4.844 g
   EDTA (Ethylenediaminetetraacetic acid) (10 mM):1.488 g
   NaCl (250 mM): 5.844 g
   PH =8.0 (Adjusted with HCl)

3. 1% SDS (Sodium Dodecyl Sulfate)
   20 g SDS (20%) were dissolved in 2000 ml of distilled water

4. Proteinase K:
   It was dissolved in sterile distilled water at a concentration of 20 mg/ml

2.2.8. Protocol Used for Genomic DNA Isolation

DNA was isolated using the mini preparation CTAB method (Doyle and Doyle, 1987) with minor modification. The protocol is described as follows:

2.2.8.1. Require Materials

1. Fin Tissue (Caudal fin tissue in this experiment)
2. Autoclave machine
3. Digital balance
4. Beaker
5. Centrifuge Machine with 10,000-14,000 rpm
6. Conical flasks
7. Eppendorf tubes
8. Gloves
9. Ice machine
10. Water bath capable of maintaining 65^0 C
11. Micropipettes and nuclease free micropipette tips
12. Mortar and pestle
13. pH meter
14. Refrigerator
15. Water de-ionizer

16. Water distillation plant
17. Fume hood

2.2.8.2. Requires Chemicals

1. Liquid Nitrogen
2. 100% Ethanol
3. Tris base
4. EDTA (ethylenediaminetetraacetic acid)
5. NaCl (Sodium chloride)
6. 1% SDS
7. Sodium acetate
8. Phenol
9. Chloroform
10. Isoamyl alchohol
11. Isopropanol
12. 70% ethanol
13. Proteinase K

2.2.8.3. Procedure

1. Freshly collected fin tissue 30mg was grinded in tissue homogenizer to homogenize. The fin tissue was taken in 15 ml Falkon centrifuge tube. 10 ml extraction buffer was added into 15 ml Falkon centrifuge tubes and vortexed for 5-10 second to mix the contents well. The tubes were put into 65º C preheated water bath and invert every 5-10 min to allow mixing. The samples were then cooled down to room temperature.
2. The samples were centrifuged at 13,000 rpm for 10 min at room temperature to remove non-soluble debris. The supernatant were transferred to fresh tubes.
3. Extract twice or thrice with an equal volume of Phenol: Chloroform: Isoamyl alcohol solution (25: 24: 1) was added and mixed well by inverting the tubes and centrifuged at 13,000 rpm for 5 minutes.
4. The supernatant was taken into another fresh tubes and add 0.1 vol. of 3 M sodium acetate (pH 5.2) followed by 0.6 vol. of 100% chilled Isopropanol and shaken slowly. In this step DNA became visible as whitish fibrous in the solution.

5. The solution was centrifuged for 10 min at 13,000 rpm at room temperature. The upper layer of the solution was discarded carefully by using adjustable micropipette.

6. The pellet was washed with 70% ice-cold ethanol. This washing step was repeated at least 2-3 times to thrice. Residual CTAB was removed by this step. The liquid was poured out and the tubes put on a paper towel for about 1 h at inverted position. Pellets should neither contain residual ethanol, nor allow for too dry. In both the cases, redissolving may be difficult.
7. The dried dna was dissolved in 500 µl of te buffer for 30 min at 37 ºC and store at -20 ºC.

2.2.8.4. Qualification and quantification of isolated DNA

Before PCR amplification it is important to know the concentration of genomic DNA because different DNA extraction methods produced DNA of widely different purity. It is necessary to optimize the amount of DNA achieve reproducibility and strong signal in PCR assay. Excessive genomic DNA may result smears lack of clearly defined bands in the gel. On the other hand, too little DNA will give non-reproducible patterns (Williams *et al*. 1993). Measurement of isolated DNA concentration can be done by comparing DNA with the standard DNA on agarose gel electrophoresis or by estimating the absorbance of DNA by spectrophotometer at 260 nm wave lengths. Both the methods were carried out in this experiment.

2.2.8.5. Measurement of DNA concentration and quality by agarose gel electrophoresis

The following equipments and chemicals were used to conduct agarose gel electrophoresis:

1. A horizontal electrophoresis chamber and power supply
2. Gel casting tray and combs
3. Gel Documentation System (Biosens SC Series-645)
4. Gloves
5. Pipette and tips
6. 1 kb DNA ladder
7. Electrophoresis buffer (TAE)

8. 6× sample loading buffer
9. Agarose
10. DNA stain (Ethidium bromide)

2.2.8.6. Preparation of stock solutions used for gel electrophoresis

For conducting the gel electrophoresis, the following stock solutions and other solutions were prepared:

2.2.8.7. TAE Buffer (50X) (pH 8.3)(1 litre)

242 g Trizma base (MW=121.14) was dissolved into 900 ml of sterile de-ionized distilled water. Then 57 ml glacial acetic acid was added to the solution. Finally, 100 ml 0.5 EDTA (pH 8.0) was added in it. These were mixed well. The pH of the solution was adjusted by mixing concentrated HCl to pH 8.3. The final volume of the solution was adjusted to 1000 ml.

2.2.8.8. Loading Dye (10 X)

For making 10 ml of 10× loading dye, 40 mg of Bromophenol blue (i.e., the final concentration was 0.4%), 40 mg of Xylene cyanol FF (i.e., the final concentration was 0.4%) and 5 ml of 98% glycerol (i.e., the final concentration was 50%) were added to 4 ml of sterile de-ionized distilled water and were mixed properly. The final volume was adjusted to 10 ml with sterile de-ionized distilled water and stored at 4 ºC.

2.2.8.9. Ethidium Bromide solution

For preparing 1ml solution, Ethidium Bromide (10 mg) was added to 1 ml of sterile de-ionized distilled water. It was then mixed by hand shaking. The solution was then transferred to a dark bottle and stored at room temperature. Stock solution of 10 mg/ml can be purchased directly from companies.

2.2.8.10. Preparation of 1% agarose gel

For agarose gel electrophoresis, 125 ml of 1% agarose gel was prepared (125 ml was required for 15 ×15 cm tray with 0.5 cm thickness).

2.2.8.11. The following steps were followed during casting the gel-

1.25 g agarose powder were measured and put in a 250 ml conical flask. 125 ml of of 1× TAE buffer was added to the flask. The agarose was melted in a microwave oven for several short intervals until the solution became clear. The solution did not allow boiling for long periods. When the agarose solution was cooled to about 50 ºC (the flask was cooled enough to hold comfortably with bare hand), 10 µl (from 10mg/ml solution) of ethidium bromide was added so that in the gel the concentration of ethidium bromide maintained 0.8 µg/ml (the final concentration of ethidium bromide in the melted agarose solution ranged from 0.5-1.0 µg/ml) and mixed well by gentle shaking.

2.2.8.12. Comb Set-up

The ends of the gel casting tray was sealed or fitted with casting dams and the combs placed in the gel casting tray. The melted agarose solution was poured into the casting tray and let it to cool until solid. The comb was removed carefully from the solidified gel. The casting dams or gates were removed from the edges of the gel tray carefully so that the gel does not slide off the tray.

2.2.8.13. Preparation of DNA sample for electrophoresis

At first 2 µl 6× loading dye was placed on a piece of parafilm by a micropipette. 5 µl DNA sample (after thawing from frozen stock) was added to it and mixed 2-4 times with the adjustable micropipette.

2.2.8.14. Electrophoresis

The gel was placed in the electrophoresis chamber in such a way that the sample wells remained near to the cathode (negative end generally marked as black). DNA sample would migrate towards the anode (positive end generally marked as red) during electrophoresis. The gel was covered by sufficient amount of 1× TAE buffer. The volume of the electrophoresis buffer should not above the maximum buffer mark on electrophoresis system. The prepared samples were loaded slowly and allowed to sink to the bottom of the wells. 7 µl of 1 kb DNA ladder (marker) was also loaded at least in one well (generally the first one). Electrophoresis was carried out at 50 volts and 100 mA for 1.5 hours. The separation process was monitored by the migration of the dye on the gel. When the first dye (bromophenol blue) had reached about three-fourths of the gel length, the electrophoresis was completed and stopped.

2.2.8.15. Documentation of the DNA sample

After electrophoresis, the gel was taken out carefully from the electrophoresis chamber and placed in Gel Documentation System (Biosens SC Series-645) for observing the DNA bands. The DNA was observed as band and photographed using Gel Documentation system.

2.2.8.16. Quantification and qualification of DNA by spectrophotometer

For more confirmation, DNA was also quantified through spectrophotometer. Spectrophotometer is commonly used in laboratories for the measurement of DNA concentration and purity. The DNA concentration was obtained by multiplying the absorbance at 260 nm wave length by a constant. The DNA purity was measured by dividing the absorbance at 260 nm wave length by the absorbance at 280 nm wave length. Good quality DNA should give the ratio (A260/A280) ranging from 1.8–2.0.The A260/A280 ratio higher than 2.0 lower than 1.8 generally indicates RNA and protein contamination respectively, during extraction process. Following Sambrook *et al*. (1989), the DNA concentration was calculated (1 O. D. at 260 nm corresponds to 50 µl/ml of double standard DNA.

2.2.8.17. Set-up the spectrophotometer

To estimate genomic DNA concentration, absorbance readings of the DNA samples were recorded at 260 nm using a spectrophotometer (Analylikjena, Specord 50, Germany).

At first, the spectrophotometer UV-lamp was turned on. After warming up the wave length was set at 260 nm. After washing the cuvette (the 'zero' or 'blank' cuvette) was filled with 2 ml sterile distilled water and placed on cuvette chamber. The absorbance reading was adjusted to zero for standardization.

2.2.8.18. Preparation of the DNA sample for spectrophotometry

The test samples were prepared by taking 2µl of each DNA sample in the cuvette containing 1,998 µl sterile distilled water. The samples were mixed well by using an adjustable micropipette. The reading was taken by viewing the monitor of spectrophotometer at 260 nm and 280 nm wave length. After recording the absorbance readings, the cuvette was rinsed out with sterile distilled water, tamped out on a paper and wiped. The absorbance reading for each sample was recorded in the same way. The ratios of spectrophotometric absorption readings at 260 nm of different samples for quality determination of DNA are shown in the (Table 1).

2.2.8.19. Calculation for the concentration of DNA

Before PCR, DNA concentrations were determined according to the following formula:

$$\text{DNA concentration} = A260 \times \text{Dilution factor} \times \text{Conversion factor}$$

$$= A260 \text{ X } \frac{\text{Volume of distilled water (µl)}}{\text{Amount of the DNA sample (µl)}} \text{ X } 50$$

$$= (\text{ng/µl})$$

$$= (\text{µg/ml}) \text{ [since } 1\text{µg} = 10^{-3}\text{ng i.e., µg/ml = ng/µl]}$$

A260 = Spectrophotometric absorbance reading at 260 nm of DNA sample.

Dilution factor = the ratio of distilled water (µl) to amount of DNA sample (µl).

Conversion factor 50 = the 50µg/ml of DNA contained in a solution which gives the spectrophotometric absorbance reading at 260 nm equal to 1.

Note: The calculated DNA concentration expressed in µg/ml gave a fraction. To avoid fraction it was converted into ng/µl ($1\mu g = 10^{-3}$ ng) and therefore, multiplied with 1000.

Table 1. Spectrophotometric absorbance readings at 260 nm wave length and concentration of DNA of three climbing perch forms.

Fish Sample collection area	Absorbance Reading at 260 nm	Concentration of DNA (ng/µl)
Control (Suborna Agro-based Itiitiative Fish Farm, Sonapur, Noakhali)	0.192	9600
Polluted water, Hazaribagh	0.162	8100
Ananda Bazar local market	0.124	6200
Hatirpool local market	0.189	9450

2.2.8.20. Amplification of DNA by Polymerase Chain Reaction (PCR) using RAPD markers

To perform the amplification of RAPD, a single oligonucleotide of arbitrary DNA sequence is mixed with genomic DNA in the presence of a thermostable DNA polymerase with suitable buffer and subjected to temperature cycling conditions typical for the polymerase chain reaction (PCR).

2.2.8.21. Preparation of working solution (25 ng/µl) of DNA samples for PCR reaction

Original stock solution concentration of each DNA sample was adjusted to a unique concentration (25 ng/µl) using the following formula:

$S_1 \times V_1 = S_2 \times V_2$

$V_1 = S_2 \times V_2 / S_1$

Where,

S_1= stock DNA concentration (ng/µl)

V_1= volume require (µl)

S_2= working DNA concentration (ng/µl)

V_2= working volume of DNA solution (µl)

Original stock DNA (2 µl) was taken in a 2 ml eppendorf tube and required amount of TE buffer calculated from the above formula added to it. Calculated required volume of TE buffer for each sample was shown in Table 2.

Table 2. Preparation of working DNA solution (25 ng/µl) for PCR reaction.

Climbing perch forms	Working Solution (25 ng/µl) for PCR	
	TE buffer/ de-ionized water (µl) required	DNA (µl) required
Control	149.2	2.0
Polluted	127.6	2.0
Local Market (Ananda Bazar)	98.0	2.0
Local Market (Hatirpool Bazar)	118.4	2.0

2.2.8.22. Primer test

Primarily 15 decamer primers were tested for RAPD amplification of which four primers exhibited good quality banding patterns and sufficient variability. Ten primers were selected for further analysis. The details of the seven primers were given in (Table 3).

Table 3. Parameters of the random primers used for DNA screening in this study

Serial No.	Primer codes	Nucleotide length	Sequence (5' to 3')	Annealing Temperature (°C)	(G+C)%
1	OPAO1	10-mer	CAGGCCCTTC	36.40	70.0
2	OPAO3	10-mer	AGTCAGCCAC	34.30	60.0
3	OPA04	10-mer	AATCGGGCTG	35.10	60.0
4	OPAO5	10-mer	AGGGGTCTTG	32.60	60.0
5	OPAO7	10-mer	GAAACGGGTG	47.40	60.0
6	OPAO8	10-mer	GTGACGTAGG	22.00	60.0
7	OPA10	10-mer	GTGATCGCAG	39.80	60.0
8	Primer 12	10-mer	GTATGGGGCT	32.70	60.0
9	Primer 21	10-mer	GGCACTGAGG	33.60	70.0
10	Primer 23	10-mer	GTCAGGGCAA	34.70	60.0

2.2.8.23. Preparation of Primers

The supplied primers were diluted to 100 µM. To make 100 µM of each primer, the following mathematical deduction was followed.

We know

n = c v (where n= number of mole)
c= molarity i.e. concentration in molarity
v= volume

Generally, 100 µM concentration of primer has to prepare as main stock solution. In this case, c=100 µM since 53.4 nanomole of primer was present in the vial used in the investigation, supplied from the company, n= 53.4 nanomole.
And v=? Required volume of TE buffer has to add in the supplied vial to make 100 µM main stocks.

Using the formula

$$n = c\,v$$

$$v = \frac{n}{c}$$

$$v = \frac{53.4\ \text{nmol}}{100\ \mu\text{M}}$$

$$v = \frac{53.4\times10^{-9}\ \text{mole}}{100\times10^{-6}\ \text{molar}}$$

$$v = \frac{53.4\times10^{-5}\ \text{mole}}{\text{molar}}$$

$$v = \frac{53.4\times10^{-5}\text{mole}}{\text{molL}^{-1}}$$

$$v = \frac{53.4\times10^{-5}}{\text{L}^{-1}}$$

$$v = 53.4\times10^{-5}\text{L}$$

$$v = 53.4\times10^{-5}\text{x}1000\ \text{ml}$$

$$v = 53.4\times10^{-2}\ \text{ml}$$

$$v = 53.4\times10^{-2}\times1000\ \mu\text{l}$$

$$v = 534\ \mu\text{l}$$

Therefore, 534 µl of TE buffer was added to the vial to make 100 µM main stock. Using the above deduction method, all primer pairs were diluted to 100 µM main stock. All primers were diluted to 50 times i.e., 2 µM to make working solution for use.

2.2.8.24. Preparation of PCR reaction mixture/ PCR cocktail

The following components were used to prepare PCR cocktail (Table 4). The total volume of PCR cocktail was 6.2 µl per sample.

Table 4. Component of PCR cocktail (for 15 reactions)

Sl. No.	Reagents	Amount per sample	Total
1	Sterile de-ionized distilled water	18.8 µl	282.0 µl
2	*Taq* Buffer A 10X (Tris with 15 mM $MgCl_2$)	2.5 µl	37.5 µl
3	Primer	1.0 µl	15.0 µl
4	dNTPs 2.5 mM	0.5 µl	7.5 µl
5	*Taq* DNA Polymerase 5U/µl	0.2 µl	3.0 µl
6	Template DNA 25 ng/µl	2.0 µl	30.0 µl
	Total	**25.0 µl**	**375.0 µl**

During the experiment, PCR buffer, dNTPs, primers and DNA sample solution were thawed from frozen stocks, mixed well by vortexing and kept on ice. Template DNA (25 ng/µl) were pipetted (2.0 µl) first into PCR tubes (0.5 ml) compatible with the thermocycler. For each DNA sample being tested, a pre-mix was prepared in the following order- buffer, dNTPs, DNA template and sterile distilled water. *Taq* DNA polymerase enzyme was added to the pre-mix. The pre-mix was then mixed well and aliquoted into the tubes containing primers. The tubes were then sealed and placed in a thermocycler and the cycling started immediately.

2.2.8.25. PCR amplification

PCR amplification was done in an oil-free thermal cycler (Biometra UNO II, Germany). Standardization of different PCR parameters are given in Table and the optimum amplification cycle was given in Table 6.

PCR amplification

PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany). The optimum amplification cycle was as follows:

Denaturation/ Annealing/ Extension		Temperature	Time (min.)
	Initial denaturation	94º C	5 minutes
45 cycles	Denaturation at	94º C	1 minute
	Annealing at	34-36º C	30 second
	Extension at	72º C	3 minutes
	Final extension at	72º C	5 minutes

After completion of cycling programme, the reactions were held at 4º C.

2.2.8.26. Electrophoresis of the amplified products and documentation

The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide 8 µl and 100 ml 1× TAE buffer. Agarose gel electrophoresis was conducted in 1× TAE buffer at 50 Volts and 100 mA for 1.5 hours. 1kb DNA ladder was electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a Gel Documentation system as before.

Chapter 3
Results

3.1. Physico-chemical paramaters

Different physico-chemical parameters of water and soil samples collected from the polluted water bodies near the tannery factory, Hazribagh, Dhaka were analyzed. Both soil and water of this area were alkaline with pH more than 9 (Tables 9, 10). The high salinity of soil and water was due to the deposition of salt from the tannery factory. The dissolved Oxygen (DO) was 0.3mg/l in the polluted water (Table 10). The water was not transparent rather dark gray and excessive turbid. The electrical conductivity (EC) is high 8.6µS/cm indicating the presence of bigger particles in the water (Table 10).

3.2. Trace element analysis

The amount of trace elements from fish muscles of different samples was carried out (Table 6, Fig. 2). The amount of sodium (ppm) is about 16 times greater in the muscles of fish collected from tannery polluted area whereas this amount is more or less similar in the other three samples (Table 6).

3.3. Protein content

The protein content of the control fish is highest among four samples (22.43%) and lowest in the sample collected from tannery waste polluted area (18.25%), Table 13). The percentage of protein in other two samples was little bit higher than the samples collected from tannery waste polluted area which was insignificant.

3.4. Heavy metal analysis

Lead (Pb) was found in only two samples *viz*. i) those collected from tannery waste polluted area and ii) those from Hatirpool fish market. The amount of Pb is more than 5 times in tannery samples than Hatirpool fish market samples (Table 12). Cadmium (Cd) was also found in these two samples. The samples collected from tannery waste polluted area had 0.8 ppm Cd whereas 0.03 ppm Cd was found in the sample collected from Hatirpool fish market (Table 12). On the other hand, Chromium (Cr) was found in the four samples where the tannery waste affected sample had about 9 times more Cr than the other 3 samples (Table 12). However, Nickel (Ni) and Cobalt (Co) were not found in any sample (Table 12). The

concentrations of above three heavy metals were much above to the permissible limits. In control, the concentration of Cr was 26 times more than the permissible limit (Table 12). Samples collected from Ananda Bazar fish market and Hatirpool fish market had 32 and 52 times more Cr concentration, respectively (Table 12). Eighty times higher level of Cd was present in the sample collected from tannery waste affected areas whereas it is 3 times in the sample collected from Hatirpool fish market (Table 12).

About 5 times Pb was present in the sample collected from Hatirpool market. In contrast, it was 20 times more than in the samples collected from tannery waste polluted water (Table 12). Different heavy metals are accumulated in the fish muscles from the polluted water of Hazaribagh at different rates. The percentage of accumulation of Cd in fish muscles was the highest (36.36%) whereas Pb was the lowest (9.39%) and Cr intermediate (16.42%, Table 12).

3.5. RAPD analysis

Initially 15 primers were tried for RAPD study of which 10 showed good and reproducible bands and thus selected. The ten primer combinations were OPA-1, OPA-3, OPA-4, OPA-5, OPA-7, OPA-8, OPA-10, Primer-12, primer-21 and primer-23. Each primer combination showed different banding pattern. The primer wise RAPD analysis of different samples of *Clarias gariepinus* is described below:

3.5.1. Primer OPA-1 (5'-CAG GCC CTT C-3')

In the control and tannery sample, no band was observed with this primer. In the sample collected from Ananda Bazar, a total of 7 bands was found of which 4 (3600, 3000, 2300 and 1400 bp) were bright and 3 (1900, 1700 and 1100 bp) light bands. In the sample collected from Hatirpool Bazar, a total of 8 bands was found of which 4 (3600, 3000, 2300 and 1400 bp) were bright and 4 (1900, 1700, 1100 and 900 bp) light bands. One unique band (900 bp) was found in the sample collected from Hatirpool Bazar (Fig.14, Table 14).

3.5.2. Primer OPA-3 (5'-AGT CAG CCA C-3')

In the control sample, a total of 6 bands 6 was found of which 1 (2400 bp) was bright and 5 (4000, 2500, 2000, 1500 and 1200 bp) light bands. A total of five bands 5 was found in tannery sample of which 1 (2400 bp) was bright and 4 (4000, 2000, 1500 and 1200 bp) light

bands. In the sample collected from Ananda Bazar, there were total 7 bands. Among the 7 bands, 1 (2400 bp) was bright and 5 (4000, 3200, 2900, 2000, 1500 and 1200 bp) light. In the sample collected from Hatirpool Bazar, two bright bands (4000, 2400 bp) and 7 light bands (7000, 5900, 3800, 3200, 2900, 2000 and 1800 bp) were observed. The band of 3800 bp was unique in the sample collected from Hatirpool Bazar (Fig.15, Table 15).

3.5.3. Primer OPA-4 (5'-AAA CGG GCT G-3')

Total of 5 bands were found in the control sample of which 1 (1600 bp) bright and 4 (4000, 3200, 2700 and 2200 bp) light bands. Tannery sample showed 1 bright (1600 bp) and 4 were light (4000, 3200, 2700 and 2200 bp) bands. The sample collected from Ananda Bazar, had 1 bright (1600 bp) and 3 light (4000, 3200 and 2000 bp) bands. Among these 5 bands 2000 bp was unique in this sample. Two bright (3200 and 1600 bp) and one light (4000 bp) band were observed in the Hatirpool Bazar sample. Three fragments (4000, 3200 and 1600 bp) were common in four samples (Fig. 16, Table 16).

3.5.4. Primer OPA-5 (5'-ACC GGT CTT G-3')

In the control and Hatirpool samples, only one light (500 bp) band was found whereas Ananda Bazar sample had two light (1100 and 800 bp) bands. These two bands were unique among four samples. No band was found in the tannery sample (Fig. 17, Table 17).

3.5.5. Primer OPA-7 (5'-GAA ACG GGT G-3')

In the control sample, a total of 5 bands was found of which 1 (2500 bp) bright and 4 (3400, 1800, 1500 and 1250 bp) light bands. The band of 2500 bp was unique in control sample. The tannery sample showed 2 (3400 and 1500 bp) bright and 5 (2700, 2400, 2000, 1800 and 1250 bp) light bands. Among these bands 2 (2400 and 2000 bp) were unique for this sample. In the sample collected from Hatirpool Bazar, a total of 4 light bands (3400, 2700, 1600 and 1400 bp) was found of which 1 (1400 bp) was unique. No band was found in the Ananda Bazar sample (Fig.18, Table 18).

3.5.6. Primer OPA-8 (5'-GTG ACG TAG G-3')

A light band (2400 bp) was observed in control, tannery and Hatirpool Bazar samples. On the other hand, three unique light bands (1900, 1500 and 1100 bp) were found in Ananda Bazar samples. (Fig. 19, Table 19).

3.5.7. Primer OPA-10 (GTG ATC GCA G-3')

A bright band (2400 bp) was observed in tannery and Hatirpool Bazar samples. In the control sample, 2 (2400 and 2000 bp) bright and 1 (1250 bp) light band were observed of which bands of 2000 bp and 1250 bp were unique. No band was found in the Ananda Bazar samples (Fig. 20, Table 20).

3.5.8. Primer -12 (5'-GTA TGG GGC T-3')

In tannery samples, two (3000 and 2500 bp) bright and 5 (5000, 4000, 2000, 1800 and 1600 bp) light bands were observed. Hatirpool samples had 2 (3000 and 2500 bp) bright and 6 (6000, 5900, 5000, 4000 3500 and 2000 bp) light bands. Three (2500, 1800 and 1600 bp) and four unique bands (6000, 5500, 3500 and 2500 bp) were found in the tannery and Hatirpool samples, respectively with this primer. No band was observed in the control and Ananda Bazar samples (Fig. 21, Table 21).

3.5.9. Primer 21 (5'-GGC ACT GAG G-3')

Two light (2400 and 2000 bp) and one bright band (1600 bp) were observed in the control samples. In tannery samples, two bright (2000 and 1600 bp) and one light band (2500 bp) were found. The Hatirpool samples had 2 (4000 and 3200 bp) light and 3 (2500, 2000 and 1600 bp) bright bands. One (2400 bp) and three unique bands (4000, 3200 and 2500 bp) were found in the control and Hatirpool samples, respectively. In contrast, no band was observed in Ananda Bazar samples (Fig. 22, Table 12).

3.5.10. Primer 23 (5'-GTC ACC GCA A-3')

A total of six bands were observed in the control samples of which 4 (4000, 3200, 2500 and 800 bp) light and 2 (1800 and 1400 bp) bright. Among these six bands, three fragments (4000, 2500 and 1800 bp) were unique in the control samples. In tannery samples, three light (2250, 800 and 600 bp) and one bright band (1400 bp) were found. Band of 2250 and 600 bp were unique. In Hatirpool samples, 4 light bands (4000 3200, 2250 and 1400 bp) were found of which 400 bp was unique. No band was observed in Ananda Bazar samples (Fig. 23, Table 23).

.

Table 5. Comparative heavy metal analysis from fish muscles of different samples of *Clarias gariepinus*

Collection Areas	Pb (ppm)	Cr (ppm)	Cd (ppm)	Ni (ppm)	Co (ppm)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	0.00	1.31	0.00	0.00	0.00
Tannery, Hazaribagh, Dhaka	1.01	9.72	0.80	0.00	0.00
Ananda Bazar, Nimtoli, Dhaka (Local Market)	0.00	1.63	0.00	0.00	0.00
Hatirpool Bazar, Dhaka (Local Market)	0.24	2.63	0.03	0.00	0.00
*Permissible Limit(mg/l)	0.05	0.05	0.01	0.05	0.05

* Source: Guide to the environmental conservation at 1995 and rules 1997.

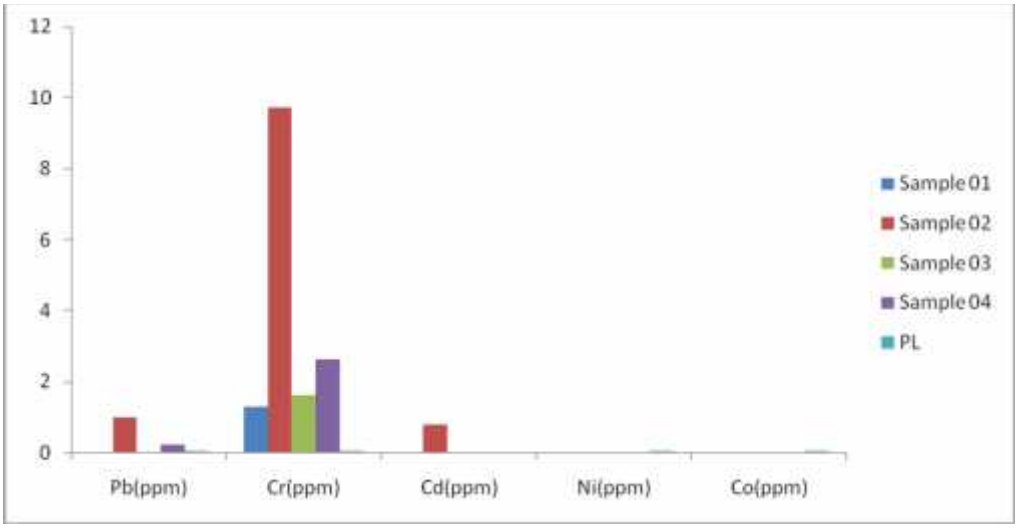

Fig. 1. Comparative heavy metal analysis from fish muscles of different samples of *Clarias gariepinus*.

Sample 01- Control (Suborna Agro Based Fish Hatchery, Noakhali)

Sample 02- Tannery, Hazaribagh, Dhaka

Sample 03- Ananda Bazar, Nimtoli, Dhaka (Local Market)

Sample 04- Hatirpool Bazar, Dhaka (Local Market)

Sample 05- Permissible Limit (mg/l)

Table 6. Comparative trace elements analysis from fish muscles of different samples of *Clarias gariepinus*

Collection Areas	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	932.26	2750	222.66	953.05	68.42	1.075	3.89	3.380
Tannery Hazaribagh, Dhaka	**1354.84**	1750	112.16	339.9	44.03	0.56	1.80	2.987
Ananda Bazar, Nimtoli, Dhaka (Local Market)	774.19	2500	190.00	549.65	65.70	0.96	4.50	3.020
Hatirpool Bazar, Dhaka (Local Market)	806.45	2000	199.02	656.05	52.04	1.045	3.29	3.705

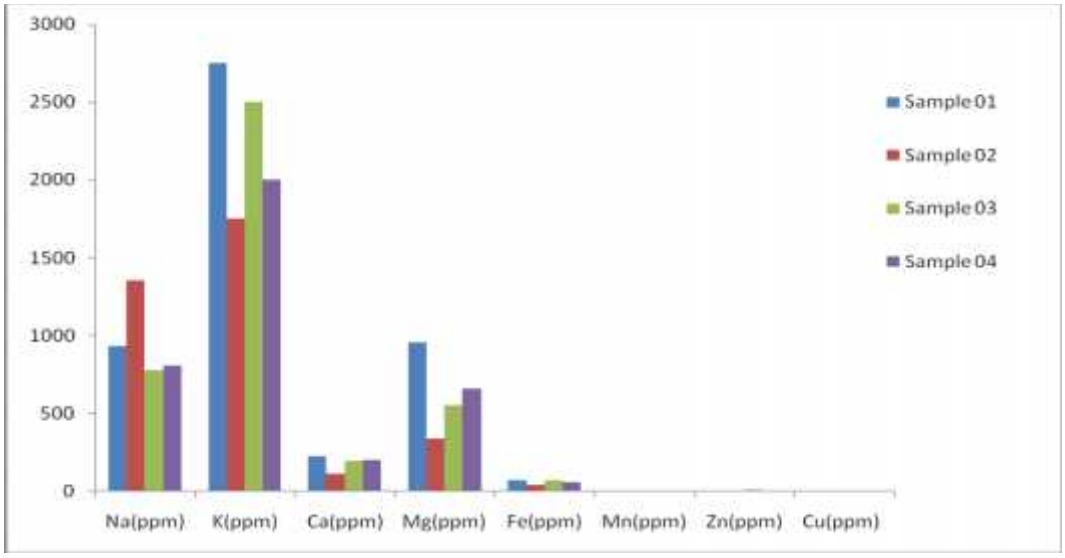

Fig. 2. Comparative trace elements analysis from fish muscles of different samples of *Clarias gariepinus*.

Sample 1- Control (Suborna Agro Based Fish Hatchery, Noakhali)

Sample 02- Tannery, Hazaribagh, Dhaka

Sample 03- Ananda Bazar, Nimtoli, Dhaka (Local Market)

Sample 04- Hatirpool Bazar, Dhaka (Local Market)

Table 7. Heavy metals present in soil, water and fish muscles collected from water body near to tannery factory of Hazaribagh, Dhaka

Samples	Cr (ppm)	Cd (ppm)	Pb (ppm)	Ni (ppm)
Soil	403.20	11.20	25.78	35.08
Water	59.20	2.20	10.75	13.28
Muscle	9.72	0.80	1.01	0.00

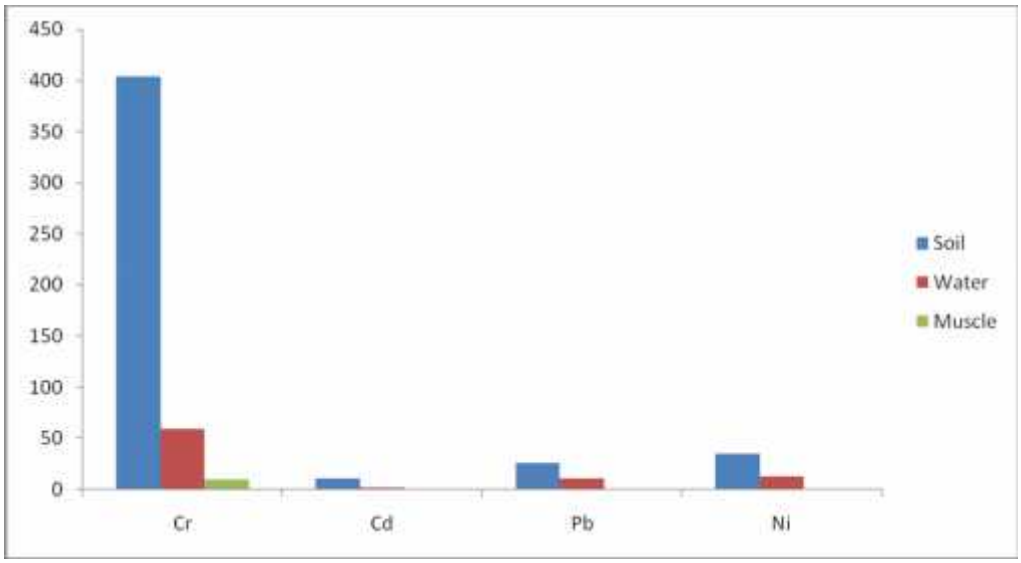

Fig. 3. Heavy metals present in soil, water and fish muscles collected from near tannery factory of Hazaribagh, Dhaka.

Table 8. Chemical parameters in water and soil sample collected from water body near to tannery factory of Hazaribagh, Dhaka

Parameters	Water (ppm)	Soil (ppm)
Sodium	**1475**	**1987**
Zinc	61.96	10.2
Copper	19.23	38.56
Fluoride	1.83	2.36
Chloride	7.57	45.15
Nitrite	7.17	10.25
Bromide	2.31	3.25
Nitrate	50.81	45.69
Sulfate	11.41	215.14

Table 9. Physico-chemical parameters in soil sample collected from water body near to tannery factory of Hazaribagh, Dhaka

Parameters	Polluted soil sample
Temperature	27.8°C
pH	**9.2**
Salinity	7.4ppt
Odor	**Disagreeable smell**
Color	**Dark gray**
Total Dissolved Solids(TDS)	8124mg/l
Total Suspended Solids(TSS)	745mg/l

Table 10. Physico-chemical parameters in water sample collected from water body near to tannery factory of Hazaribagh, Dhaka

Parameters	Polluted water sample
Temperature	27.8°C
pH	**9.6**
Salinity	**6.9 ppt**
Odour	Disagreeable smell
Colour	Dark Gray
DO	**0.3mg/l**
Turbidity	Excessive Turbid
Electrical Conductivity(EC)	8.6μS/cm
Total Hardness	6100mg/l
Total Dissolved Solids(TDS)	**7928mg/l**
Total Suspended Solids(TSS)	**647mg/l**
Biochemical Oxygen Demand(BOD)	**623 mg/L**
Chemical Oxygen demand(COD)	**858 mg/L**

Table 11. Accumulation (%) of heavy metals in fish muscles from polluted water

Samples	Cr (ppm)	Cd (ppm)	Pb (ppm)	Ni (ppm)
Water	59.2	2.2	10.75	13.28
Muscle	9.72	0.800	1.01	0
Accumulation (%)	16.42	36.36	9.39	0

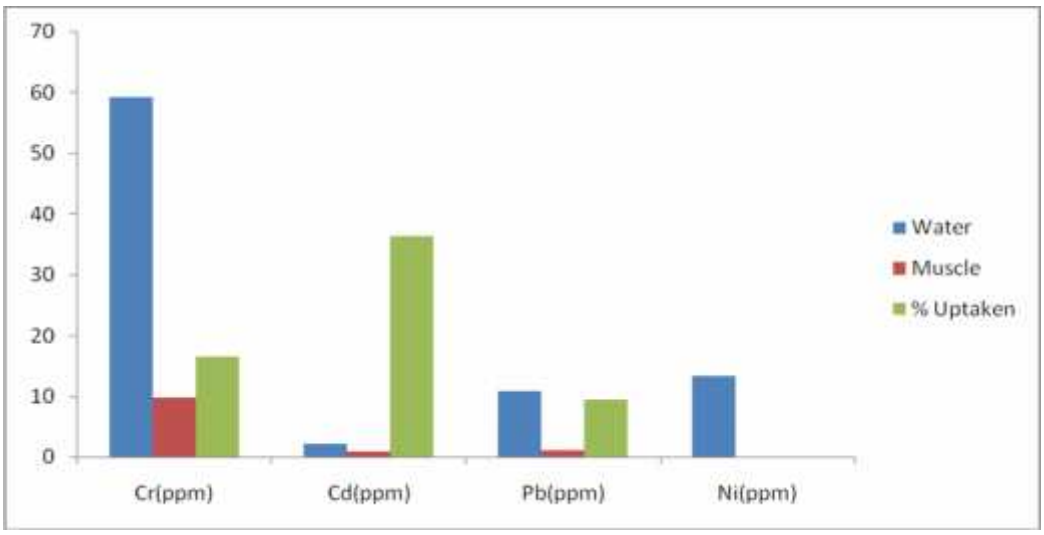

Fig. 4. Accumulation (%) of heavy metals in fish muscles from polluted water.

Table 12. Presence of heavy metals in sample tissues in comparison to the Permissible Limit

Collection Areas	Cr (ppm)	Times	Cd (ppm)	Times	Pb (ppm)	Times
Control (Suborna Agro Based Fish Hatchery, Noakhali)	1.31	**26.2**	0.00	0	0.00	0
Tannery, Hazaribagh	9.72	**194.4**	0.80	**80**	1.01	**20.2**
Ananda Bazar, (Local Market)	1.63	**32.6**	0.00	0	0.00	0
Hatirpool Bazar, (Local Market)	2.63	**52.6**	0.03	3	0.24	4.8
Permissible Limit(mg/l)	0.05		0.01		0.05	

Table 13. Protein content analysis from fish muscles of different samples of *Clarias gariepinus*

Collection Areas	% Protein Content
Control (Suborna Agro Based Fish Hatchery, Noakhali)	22.43
Tannery Hazaribagh, Dhaka	**18.25**
Ananda Bazar, Nimtoli, Dhaka (Local Market)	20.12
Hatirpool Bazar, Dhaka (Local Market)	19.06

Table 14. RAPD analysis with primer OPA-1 (5'-CAG GCC CTT C-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	--	--	--		--
Tannery, Hazaribagh, Dhaka	--				--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	7 (3600, 3000, 2300, 1900, 1700, 1400, 1100)	3 (1900, 1700, 1100)	4 (3600, 3000, 2300, 1400)	--	--
Hatirpool, Dhaka (Local Market)	8 (3600, 3000, 2300, 1900, 1700, 1400, 1100, 900)	4 (1900, 1700, 1100, 900)	4 (3600, 3000, 2300, 1400)		1(900)

Table 15. RAPD analysis with primer OPA-3 (5'-AGT CAG CCA C-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	6 (4000, 2500, 2400, 2000, 1500, 1200)	5 (4000, 2500, 2000, 1500, 1200)	1 (2400)		-
Tannery, Hazaribagh, Dhaka	5 (4000, 2400, 2000, 1500, 1200)	4 (4000, 2000, 1500, 1200)	1 (2400)		-
Ananda Bazar, Nimtoli, Dhaka (Local Market)	7 (4000, 3200, 2900, 2400, 2000, 1500, 1200)	5 (4000, 3200, 2900, 2000, 1500, 1200)	1 (2400)	2 (4000, 2000)	-
Hatirpool Bazar, Dhaka (Local Market)	9 (7000, 5900, 4000, 3800, 3200, 2900, 2400, 2000, 1800)	7 (7000, 5900, 3800, 3200, 2900, 2000, 1800)	2 (4000, 2400)		1 (3800)

Table 16. RAPD analysis with primer OPA-4 (5'-AAA CGG GCT G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	5 (4000, 3200, 2700, 2200, 1600)	4 (4000, 3200, 2700, 2200)	1 (1600)		--
Tannery, Hazaribagh, Dhaka	5 (4000, 3200, 2700, 2200, 1600)	4 (4000, 3200, 2700, 2200)	1 (1600)	3 (4000, 3200, 1600)	--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	4 (4000, 3200, 2000, 1600)	3 (4000, 3200, 2000)	1 (1600)		1 (2000)
Hatirpool, Dhaka (Local Market)	3 (4000, 3200, 1600)	1 (4000)	2 (3200, 1600)		--

Table 17. RAPD analysis with primer OPA-5 (5'-ACC GGT CTT G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	1 (500)	1 (500)	--	--	--
Tannery, Hazaribagh, Dhaka	--	--	--		--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	2 (1100, 800)	2 (1100, 800)	--		2 (1100, 800)
Hatirpool, Dhaka (Local Market)	1 (500)	1 (500)	--		--

Table 18. RAPD analysis with primer OPA-7 (5'-GAA ACG GGT G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	5 (3400, 2500, 1800, 1500, 1250)	4 (3400, 1800, 1500, 1250)	1 (2500)	--	1 (2500)
Tannery, Hazaribagh, Dhaka	7 (3400, 2700, 2400, 2000, 1800, 1500, 1250)	5 (2700, 2400, 2000, 1800, 1250)	2 (3400, 1500)		2 (2400, 2000)
Ananda Bazar, Nimtoli, Dhaka (Local Market)	--	--	--		--
Hatirpool, Dhaka (Local Market)	4 (3400, 2700, 1600, 1400)	4 (3400, 2700, 1600, 1400)	--		1 (1400)

Table 19. RAPD analysis with primer OPA-8 (5'-GTG ACG TAG G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	1 (2400)	1 (2400)	--	--	--
Tannery, Hazaribagh, Dhaka	1 (2400)	1 (2400)	--		--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	3 (1900, 1500, 1100)	3 (1900, 1500, 1100)	--		3 (1900, 1500, 1100)
Hatirpool, Dhaka (Local Market)	1 (2400)	1 (2400)	--		--

Table 20. RAPD analysis with primer OPA-10 (GTG ATC GCA G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	3 (2400, 2000, 1250)	1 (1250)	2 (2400, 200)	--	2 (2000, 1250)
Tannery, Hazaribagh, Dhaka	1 (2400)	--	1 (2400)		--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	--	--	--		--
Hatirpool, Dhaka (Local Market)	1 (2400)	--	1 (2400)		--

Table 21. RAPD analysis with primer-12 (5'-GTA TGG GGC T-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	--	--	--		--
Tannery, Hazaribagh, Dhaka	7 (5000, 4000, 3000, 2500, 2000, 1800, 1600)	5 (5000, 4000, 2000, 1800, 1600)	2 (3000, 2500)		3 (2500, 1800, 1600)
Ananda Bazar, Nimtoli, Dhaka (Local Market)	--	--	--	--	--
Hatirpool, Dhaka (Local Market)	8 (6000, 5900, 5000, 3000, 3500, 3000, 2500, 2000)	6 (6000, 5900, 5000, 3500, 3000, 2000)	2 (3000, 2500)		4 (6000, 5500, 3500, 2500)

Table 22. RAPD analysis with primer-21 (5'-GGC ACT GAG G-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	3 (2400, 2000, 1600)	2 (2400, 2000)	1 (1600)		1 (2400)
Tannery, Hazaribagh, Dhaka	3 (2500, 2000, 1600)	1 (2500)	2 (2000, 1600)		--
Ananda Bazar, Nimtoli, Dhaka (Local Market)	--	--	--	--	--
Hatirpool, Dhaka (Local Market)	5 (4000, 3200, 2500, 2000, 1600)	2 (4000, 3200)	3 (2500, 2000, 1600)		3 (4000, 3200, 2500)

Table 23. RAPD analysis with primer-23 (5'-GTC ACC GCA A-3') of different samples of *Clarias gariepinus* (Burchell, 1822)

Collection Area	Total no. of bands with molecular weight (bp)	No. of light bands with molecular weight (bp)	No. of bright bands with molecular weight (bp)	No. of common bands with molecular weight (bp)	No. of unique polymorphic bands with molecular weight (bp)
Control (Suborna Agro Based Fish Hatchery, Noakhali)	6 (4000, 3200, 2500, 1800, 1400, 800)	4 (4000, 3200, 2500, 800)	2 (1800, 1400)		3 (4000, 2500, 1800)
Tannery, Hazaribagh, Dhaka	4 (2250, 1400, 800, 600)	3 (2250, 800, 600)	1 (1400)		2 (2250, 600)
Ananda Bazar, Nimtoli, Dhaka (Local Market)	--	--	--	--	--
Hatirpool, Dhaka (Local Market)	4 (4000, 3200, 2250, 1400)	4 (4000, 3200, 2250, 1400)	--		1 (4000)

Chapter 4

Discussion

Due to wide adaptation and food habit range fish traders usually like to culture rapid growing fish like African Magur. Big size and cheap price attract common people to this fish without knowing the sources. The fish traders generally use tannery waste polluted water body to rare this fish because this type of water bodies are rich in organic matter by continuous deposition of tannery wastage. Tannery wastes contain different heavy metals and other toxic substances harmful to human health. In this study, an attempt has been taken to determine the concentration of heavy metals in fish muscles and to know whether there is any impact of these substances at DNA level.

4.1. Quality of soil and water

To test the quality of water in the tannery waste polluted water body, different physico-chemical tests have been carried out. In addition, the same tests have also been done with the polluted soil of the water body. The results showed that both soil and water of the tannery waste polluted area were highly alkaline with pH more than 9 (Tables 9, 10). The salinity of water was much higher than the normal. The increased salinity in that area was due to deposition of salt from the tannery factories (Tables 9, 10). The water was dark gray and turbid, not usual transparent (Tables 9, 10). In this study, the amount of DO was only 0.3mg/l indicating high anoxic nature (Table 10). The presence of organic matter will promote anaerobic action leading to the accumulation of toxic compounds in the water bodies. Islam *et al.* (2014) worked on tannery waste water from Sudan and obtained the similar results.

Moniruzzaman *et al.* (2009) studied on the water body near tannery factories of Hazaribagh and reported that the DO was within the range of 2.20-6.62mg/l. Later Nahar and Alam (2015) reported 0.88mg/l DO in the same area which was less than the former report. The present report showed that the DO (0.3mg/l, Table 10) was much less than that of Nahar and Alam (2015). The comparison of DO concentration was indeed alarming. The DO is

decreasing day by day lowering the quality of water around the tannery factories in Hazaribagh.

The TSS and TDS are other parameters indicating the quality of water. The recommended TDS and TSS according to WHO standard limit for good water quality are 500mg/l and 1000mg/l, respectively (Chapman 1996). In this study, the TDS was 7928mg/l (Table 10) and TSS was 647mg/l (Table 10) for the water collected from tannery waste polluted areas. The present value was much higher than the recommended value causing lower water quality in this area.

In this present study, 623mg/l BOD and 858mg/l COD were recorded (Table 10). The recommended value for BOD is 3-6mg/l and COD is 20mg/l (Chapman 1996). The present result was more than the recommended values. This level of BOD and COD are responsible for the disagreeable smell and dark gray color of the polluted water from Hazaribagh (Table 10).

High level of total suspended solids present in the tannery effluents could be attributed to their accumulation during the processing of finished leather. As a result the water quality is degrading and becoming a severe threat to the aquatic lives.

4.2. Trace element and protein content

The recommended range of sodium for freshwater fish muscle is 30-134mg/100g body weight (Murray and Burt 1969). High amount of Na was present in the muscle tissues of four samples. However, Na was about 16 times higher in the muscle of fishes collected from the Hazaribagh area than rest samples (Table 6). The excessive Na in muscle tissue of these fish is correlated to excessive salinity of water in that area (Table 10). Sodium ion is water soluble may easily enter into human body after consumption of this fish. High Na is dangerous for human health. It may cause hypertension, cardiac problem, imbalanced osmo-regulation, etc. Thus these kinds of fishes are threat to human health.

The recommended amount of protein in an ideal catfish is 20-26% in wet weight basis (Garcia *et al.* 2004). The percentage of protein (22.43%, Table 13) in the control fish was

within the recommended range. However, the percentage of protein in the rest three samples were lower of which the samples from tannery area was the lowest (18.25%, Table 13). Morphologically the fish from tannery area are bigger with more flesh than other samples (Figs. 5-8), thus people choose the tannery waste polluted fish and getting less amount of protein along with other toxic substances.

4.3. Heavy metal concentration

In the control specimen, 1.31 ppm of Chromium (Cr) was observed (Table 12) and the amount is about 26 times more than the normal limit (0.05ppm, WHO 1990). Chromium is considered to be carcinogenic because of its powerful oxidative potential and ability to cross cell membranes (WHO 1990, Eisler 2000, Lushchak *et al*. 2009). The toxic effects include alteration of histological, hematological and morphological features, reduction of growth and impaired immune functions (Ricketts *et al.* 2015, Vera-Candioti *et al.* 2011). It was expected that like other heavy metals, Cr should not present in the flesh of control fish. Why heavy metal like Cr was found in the flesh of control sample in high amount is not clear. However, there might be two possibilities- either (i) the fish sample inherently carrying Cr or (ii) the supplied fish food contained Cr. Therefore, the farm owner must take maximum care during collecting the fish fry for culture and application of fish food. Otherwise Cr will be continuously transmitting to human body.

The sample collected from tannery waste polluted water body had Pb, Cr and Cd in high amount (Table 7). Among the three heavy metals, the amount of Cr was maximum. Cr was about 200 times higher than the permissible limit (0.05mg/l) (WHO 1990, Eisler 2000, Lushchak *et al*. 2009). In addition, Pb and Cd were also much higher than the permissible limits (Table 12, Fig. 4). Moreover, the heavy metals were found in the samples collected from Ananda Bazar and Hatirpool Bazar fish market (Table 7, Fig. 3). This is indeed alarming. It means the African Magur those have been selling in the markets were collected from water that polluted with heavy metals. Table 11 and Fig. 4, clearly showed that the high rate of accumulation of heavy metals from polluted water to the fish flesh. If this rate is

allowing to continue, muscle tissues of such fishes will be enriched with heavy metals that would not only be a threat to fish but for human health also.

4.4. RAPD fingerprinting

In total, 15 different primers were tried to find out proper RAPD profile. Among these 10 showed good banding profile and thus selected for this experiment (Tables 14-23). Common bands in all the four samples were found after using primer OPA-3 and OPA-4 (Tables 15, 16, Figs. 15, 16). The common bands revealed the sharing of similar sized fragments in different samples. It was quite expected that the different samples of a species should have similar DNA fragments. Since a number of common bands were observed with primer OPA-3 and OPA-4, these two primers are not suitable to determine the polymorphism among different samples of African Magur. This result may be used as reference for future RAPD work of African Magur. In contrast, no common band was found in the rest 8 primers. This result indicated the alteration of DNA fragments within the four samples of African Magur. Otherwise, at least few common bands should appear with these 8 primers as well.

In this research, unique bands were observed in different samples with different primers. The term unique band means a band appeared in a sample with certain primers absent in other samples with the same primers. A unique band was found in the control specimen with primer OPA-7 (Table 18, Fig. 18) and primer 21 (Table 22, Fig. 22), two with OPA-10 (Table 20, Fig. 20) and three with primer 23 (Table 23, Fig. 23). These bands were absent in other samples although supposed to be present. On the other hand, unique bands were found in the rest samples with different primers (Tables 14-17, 19, 21, Figs. 14-17, 19, 21) which absent in the control sample. Moreover, a number of variations regarding RAPD profiling were found in the four samples with different primers (Tables 14-23, Figs. 14-23).

The results showed that some bands were present in the control which absent in other samples indicating deletion of such fragments. In contrast, few new bands appeared in other samples those were absent in the control revealing originating of new fragments (Afroz *et al.* 2013, Sultana *et al.* 2012). Sultana (2014) reported that the presence and absence of RAPD

DNA fragments were associated with deletion and tandem duplication of CMA and DAPI bands from chromosomes of respective specimens. The present findings may correlate with deletion or duplication of DNA fragments. No RAPD fingerprinting report on African Magur was found in the available literature and internet sources. Thus it was not possible to compare the present data. Therefore, this study on RAPD fingerprinting is probably the first report on African Magur.

Chapter 5

Conclusions and Recommendations

From the forgoing discussion it becomes clear that water bodies nearby tannery factories of Hazaribagh, Dhaka are highly contaminated with tannery effluents containing heavy metals. The heavy metals from such water are entering into the fish muscles at very high rate. These toxic substances have negative impact at DNA level. There is every possibility to enter such heavy metals to human and raising the concentration after continuous consumption of these fishes. Therefore, the people should avoid African catfish those are selling in the local markets and even from farms.

Chapter 6

References

A.P.H.A. 1998**.** Standard methods for the examination of water and wastewater, 20th edition. *American Public Health Association*, Washington, D.C.

AFROZ, M., SULTANA, S.S. and ALAM, SK.S. 2013. Karyotype and RAPD analysis of three morphological forms of *Alocasia fornicata* (Roxb.) Schott. *Cytologia* **78**(3): 269–275.

B.B.S. 2014. Economic census 2013-2014 fiscal year.

BARTISH, I.V., GORKAVA, L.P., RUMPUNEN, K. and NYBOM, H. 2000. Phylogenetic relationship and differentiation among and within populations of Chaenomeles Lindl. (Rosaceae) estimated with RAPD's and isozymes. *Theor. Appl. Genet.* **101**: 554-563.

BOUWER, H. 1978. Mcgraw-Hill series in water resources and environmental engineering, *Ground Water Hydrology*, New York, USA 480 pp.

BRUTON, M.N. 1976. On the size reached by *Clarias gariepinus*. *J. Limnol. Soc. S. Africa.* **2**:57–58.

CHAN, Y.K., WONG, C.K., HSIEH, D.P.H., LAU, T.K. and WONG, P.K. 2003. Application of a toxicity identification evaluation for a sample of effluent discharged from a dyeing factory in Hong Kong. *Environ. Tox.* **18**: 312 – 316.

CHAPMAN, D. 1996. Water Quality Assessments -A Guide to Use of Biota, Sediments and Water in Environmental Monitoring -Second Edition, 651 pp.

CRINNION, W.J. 2009. Chlorinated pesticides, threats to health and importance of detection. *Altern. Med. Rev.* **14**: 347-359.

DoF. 2014. Fish week compendium, Department of Fisheries, Ministry of Fisheries and Livestock, Dhaka, Bangladesh.

DOYLE, J.J. and DOYLE, J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* **19**: 11-15.

EISLER, R. 2000. Handbook of chemical risk assessment: Health hazards to humans, plants, and animals. Boca Raton: CRC Press, Lewis publishers.

FORSTNER, U. and WITTMANN, G.T.W. 1981. Metal pollution in aquatic environment (2^{nd} eds.). Springer- Verlag, Berlin, Germany. 486 pp.

GARCIA-ARIAS, M.T., NAVARRO, M.P. and GARCIA-LINARES, M.C. 2004. Effects of different thermal treatments and storage on the proximate composition and protein quality in canned tuna. *Archivos Latinoamericanos de Nutrición* **54**: 112–117.

GLANZE 1996. Heavy metal toxicity. *International occupational safety and health information centre.*

GROVER, I.S. and KAUR, S. 1999. Genotoxicity of wastewater samples from sewage and industrial effluent detected by the *Allium* root anaphase aberration and micronucleus assays. *Mutat. Res*. **426**: 183 – 188.

GUPTA, P.K. 2000. Methods in environmental analysis: Water, Soil and Air. Agrobios, Jodhpur, India. pp. 443.

HUQ, I.S.M., HOSSAIN, A. and JOARDER, J.C. 2006. Comparison of arsenic accumulation by two ferns, *Pteris vittata* and *Nephrodium molle. Dhaka Univ. J. Biol. Sci.* **15** (2): 95-103.

ISLAM, B.I., MUSA, A.E., IBRAHIM, E.H., SHARAFA, S.A.A., ELFAKI, B.M. 2014. Evaluation and characterization of tannery wastewater. *J. Forest Prod. Ind.* **3**(3): 141-150.

JACKSON, M.L. 1967. Soil chemical analysis, Prentice Hall, Inc. Englewood Cliffs, N. J. USA. pp. 227-261.

JOARDER, J.C., RASHID, M.H. and HUQ, I.S.M. 2005. Adsorption of lead (Pb) by soil and their clay fraction. *J. Asiat. Soc. Bangladesh Sci.* **31**: 63-74.

KABIR, M.A., HABIB, M.A., HASAN, M. and ALAM, SK.S. 2012. Genetic diversity in three forms of *Anabas testudineus* Bloch. *Cytologia* **77**(2): 231–237.

*KJELDAHL, J. 1883. Neue methods zur bestimmung des stickstoffs in organischen korpern, *Z. Anal. Chem.* **22**:366-382.

LAH, B., GORJANE, G., NEKREP, F.V. and MARINSEK-LOGAR, R. 2004. Comet assay of wastewater genotoxicity using yeast cells. *Bull. Environ. Contam. Tox.* **72**: 607 – 616.

LUSHCHAK, O.V., KUBRAK, O.I., LOZINSKY, O.V., STOREY, J.M., STOREY, K.B. and LUSHCHAK, V.I. 2009. Chromium induces oxidative stress in goldfish liver and kidney. *Aquat. Toxicol* **93**: 45-52.

MATHENY, T.A. and HUNT, P.G. 1981. Effects of irrigation and sulphur application on soybeans grown on a Norfolk loamy sand. *Commun. Soil Sci. Plant Anal*. **12**: 147-159.

*MICHA, J.C. 1973. Eludedis populations piscicoles de I. vbangui et tentatives de selection et d' adaptators de quelques especes a l'etany de piscicultue-Nogent-san-Marine, *Centre Technique Forestier Tropical*. 110p.

MONIRUZZAMAN, M., ELAHI, S.F. and JAHANGIR, M.A.A. 2009. Study on temporal variation of physico-chemical parameters of Buriganga river water through GIS (Geographical Information System) Technology. *Bangladesh J. Sci. Ind. Res*. **44** (3): 327-334.

MURRAY, J. and BURT, J.R. 1969. The composition of fish. Torry Advis. Note, Aberdeen, **38**.

NAHAR, K.K. and ALAM, SK.S. 2015. Physical and chemical parameters and trace metal flux in water, soil and plant samples collected from industrial effluent affected areas in and around Dhaka city. *Bangladesh J. Bot.* **44** (2): 237-244.

ODEIGAH, C. and OSANYINPEJU 1995. Genotoxic effects of two industrial effluents and ethylmethane sulfonite in *Clarias lazera*. *Food Chem. Tox.* **33**: 501–505.

RICKETTS, C.D., BATES, W.R. and REID, S.D. 2015. The effects of acute waterborne exposure to sublethal concentrations of molybdenum on the stress response in rainbow trout, *Oncorhynchus mykiss*. *Plos One.* DOI:10.1371/journal.pone.0115334.

SAMBROOK, J., MANIATIS, T. and FRITSCH, E.F. 1989. Molecular Cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, USA.

SCHIERWATER, B., ENDER, A., SCHWENT, K., SPAAK, K., SPAAK, P. and STREIT, B. 1994. Molecular ecology evolution: approaches and applications. *Birkhauser Verleg*, Basel, Boston, 495-500.

SMOLDERS, R., BERVOETS, L. and BLUST, R. 2004. *In situ* and laboratory bioassays to evaluate the impact of effluent discharges on receiving aquatic ecosystems. *Environ. Pollut.* **132** (2): 231 – 243.

SULTANA, N., SULTANA, S.S. and ALAM, SK.S. 2013. Karyotype and RAPD analysis to elucidate taxonomic status in two morphological forms of *Egeria densa* Planch. and *Hydrilla verticillata* (L.f.) Royle. *Cytologia* **78**(3): 277–284.

SULTANA, S.S. 2014. Characterization of some cotton germplasms through molecular cytogenetics. Ph.D. Thesis, Department of Botany, University of Dhaka, Bangladesh.

U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) on Cadmium. National Center for Environmental Assessment, Office of Research and Development, National Institute of Health, Washington, DC. 1999.

VERA-CANDIOTI, J., SOLONESKI, S., LARRAMENDY, M.L. 2011. Acute toxicity of chromium on *Cnesterodon decemmaculatus* (Pisces: Poeciliidae). *Theoria* **20**: 81-88.

VIVEEN, W.J.A.R., RICHTER, C.J.J., Van Oordt, P.G.W.J., Janssen, J.A.L. and Huisman, E.A. 1986. Practical manual for the culture of the African Catfish (*Clarias gariepinus*). University of Wageningen, Netherlands. 121p

WELSH, J. and MCCLELLAND, M. 1990. Fingerprinting genomes using PCR with arbitary primers. *Nucleic Acids Res*. **18**: 7213-7218.

WHO (World Health Organization), 1990. Chromium, Nickel and welding. International agency for research on cancer. *IARC monographs on the evaluation of carcinogenic risks to humans,* France.

WILLIAMS, J.G.K., HANAFET, M.K., RAFALSKI, J.A. and TINGEY, S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods enzymol*. **218**: 705-740.

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. and TINGEY, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*. **18**: 6531-6535.

---

* = Original not seen.