ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA PRESENT IN COMMERCIALLY IMPORTANT FISH, SHELLFISH, WATER AND SOIL SAMPLES OF KAPTAI LAKE

Susmita Chakma

Roll No. 0120/05

Registration No. 878

Session: 2020-2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in technology

Department of Fishing and Post-Harvest Technology

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

December 2022

Authorization

I hereby declare that I am the sole author of the thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Susmita Chakma

ISOLATION AND IDENTIFICATION OF PATHOGENIC BACTERIA PRESENT IN COMMERCIALLY IMPORTANT FISH, SHELLFISH, WATER AND SOIL SAMPLES OF KAPTAI LAKE

Susmita Chakma

Roll No. 0120/05

Registration No. 878

Session: 2020-2021

This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

---	---
Dr. Md. Faisal	**Prof. Dr. M Nurul Absar Khan**
Supervisor	**Co-Supervisor**

Chairman of the Examination Committee

Department of Fishing and Post-Harvest Technology
Faculty of Fisheries
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

ACKNOWLEDGEMENTS

First and foremost, all praises to Almighty God for giving me the health, ability and strength to accomplish this MS research work as well as the thesis on due time. Without His help, I would not be to finish this thesis work.

I would like to convey my earnest gratitude to my beloved parents and siblings for their ultimate understanding, inspirations, moral support, kindness and blessings, forbearance and endless love to complete this study.

I sincerely express my deepest sense of gratitude, sincere appreciation, profound regards and immense indebtedness to my honorable teacher and research supervisor **Dr. Md. Faisal**, Assistant Professor & Head, Department of Fishing and Post-Harvest Technology, Chattogram Veterinary and Animal Sciences University for his valuable suggestions, intellectual guidance, constructive and constant inspiration throughout the entire period of the study and in preparations of this manuscript.

I feel proud in expressing my immense gratitude to my respected teachers of Department of Fishing and Post-Harvest Technology, Chattogram Veterinary and Animal Sciences University, for their kind co-operation, intellectual guidance, valuable suggestions and constructive criticism throughout the research period and for the thesis work.

I wish to express my heartfelt gratitude to our honorable respective Dean **Prof. Dr. M Nurul Absar Khan** for their supportive administrative coordination to fulfill my MS research work.

I would like to express appreciation to the lab technician and lab attendant of Processing and Nutrition Laboratory, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University for providing laboratory facilities for this study.

At the end, I would like to expresses cordial thanks to my loving friends and well-wishers for their co-operation, cheerfulness and inspiration during the course of this study. Last but not least, to everyone who has supported me directly or indirectly in completing this thesis.

The Author

Table of Contents

Contents	Page No
Title page	i
Authorization	ii
Signature page	iii
Acknowledgement	iv–v
Table of content	vi–viii
List of figures	ix–x
List of tables	ix
List of abbreviations	xii
Abstract	01
Chapter-1: Introduction	02–03
Chapter-2: Review of Literature	
2.1 Kaptai Lake	04
2.2 Present status of fish biodiversity in Kaptai Lake	05
2.3 Fish microbiology	06–07
2.4 Microbiological relation with fish nutrition	08
2.5 Fish market and marketing channels	09
2.6 Water Microbiology	09–10
2.7 Bacteriological Characteristics of Kaptai Lake Water	10–11
2.8 Soil microbiology	12–13
Chapter-3: Materials and Methods	
3.1 Sampling Area	14–15
3.2 Collection of water and soil samples	15
3.3 Microbiological analysis	
3.3.1. Sterilization of materials	15
3.3.2. Sample preparation	15
3.3.3. Preparation Serial dilution	15
3.3.4. Procedure of Plate Count Agar (APC)	16
3.3.5. Inoculation of sample in agar plates	17
3.3.6. Counting of bacterial load	17
3.4. Identification of Pathogenic Bacteria	
3.4.1. Identification of *E. coli*	18
3.4.2. Identification of *V. cholera*	19
3.4.3. Identification of *V. vulnificus and V. parahaemolyticus*	
3.4.4. Identification of *Salmonella* and *Shigella* identification	20

Chapter-4: Results

4.1. Bacterial quantification, isolation and identification from fish samples 21–24

4.2. Bacterial quantification, isolation and identification from water and soil samples 25–32

Chapter-5: Discussion 33–35

Chapter-6: Conclusions 36

Chapter-7: Recommendations and Future Prospects 37

References 38–45

Appendices 46–48

List of Figures

Serial No.	Figure No	Description of the Figures	Page No
01	01	Sampling sites at Kaptai Lake	13
02	02	Collection of soil and water sample and sample preparation	14
03	03	Procedure of Plate Count Agar (PCA)	16
04	04	Inoculation of Sample in agar plate	17
05	05	Procedure of of *E. coli* Identification	18
06	06	*E. coli* in EMB Agar	18
07	07	Procedure of *Vibrio cholera* Identification	19
08	08	Procedure of *V. vulnificus* and *Vibrio parahaemolyticus* Identification	20
09	09	Identification of *Salmonella* and *Shigella*	21
10	10	Colony of bacteria found in fish sample	22
11	11	Colony of bacteria found in water samples	26
12	12	Colony of bacteria found in soil samples	29

List of Tables

Serial No.	Table No	Description of the Tables	Page No
01	01	The Species of fishes caught in different months in Kaptai Lake	5-6
02	02	Pathogenic bacteria present in fish samples where "R" represent the replication of fish sample	23-25
03	03	Pathogenic bacteria present in water samples where "R" represent the replication of fish sample	27-29
04	04	Pathogenic bacteria present in fish samples where "R" represent the replication of soil sample	30-32

List of Abbreviations

Words	Abbreviation
KL	Kaptai Lake
sp.	Species
Mm	Milimeter
µm	Micrometer
Ml	Mililiter
µg	Microgram
Vol	Volume
L	Liter
Mg	Miligram
Gm	Gram
CFU	Colony Forming Unit
^{o}C	Degree Celcius
BPW	Buffer Peptone Water
TSA	Trypticase Soy Agar
Mac	Macconkey Agar
XLD	Xylose Lysine Deoxycholate
TCBS	Thiosulfate-citrate-bile-salts-sucrose agar
EMB	Eosin Methylene Blue Agar
TSI	Triple Sugar Iron
GP	Gram Positive
+	Positive
-	Negative
Hr	Hour
%	Percentage
G	Gram
Lbs	Pound
Min	Minute
Cm	Centimeter
E.	*Escherichia*
R	Replication

Abstract

Organisms that cause human infections are transmitted from fish or the aquatic environment is quite common. To analyze the bacterial population (pathogenic) of fish, soil and water from Kaptai Lake, this study aims to isolate enteric pathogenic bacteria from fish, soil and water that might be transmitted to humans after handling and consumption of these. To determine the presence of *E. coli*, *Salmonella*, *Shigella*, *V. cholera*, *V. parahaemolyticus*, *V. vulnificus* from fish, soil and water and to isolate those bacteria, different kinds' agar like EMB agar, Xylose Lysine Deoxycholate (XLD) Agar and TSI agar were used in this study. To determine the bacterial load Total Plate Count method was used. Result showed that, among 20 fish species the highest and lowest amount of bacterial load was found in *Puntius ticto (*$8.53\pm0.21\times10^6$ CFU mL^{-1}) *and Microbrachium rosenbergii (*$2.45\pm0.25\times10^4$ CFU mL^{-1}). Moreover, in case of soil ($6.13\pm0.66\times10^6$ CFU mL^{-1}) and water ($3.90\pm0.20\times10^6$ CFUmL^{-1}) highest bacterial load was found in station-12. Due to the presence of E coli in most of the water and soil of most of the sampling sites, it can be concluded that, these sites may be contaminated by human manure. Besides this, as sampling sites at Baghaichhari area was highly contaminated with pathogenic enteric bacteria which suggest reducing human interference in this area.

Keywords: Pathogenic Bacteria, Isolation, Identification, Colony Count

Chapter-1: Introduction

One of the biggest man-made freshwater bodies in Southeast Asia, Kaptai Lake (KL), is situated in Bangladesh's Rangamati district. The people who live on the lake's islands use the lake water directly to drink and other domestic needs before even purifying it. It has a surface area of 68800 ha on average (DOF, 2019; Uddin et al., 2014). It is enhanced by a wide variety of fish species. A variety of 71 species of fish, including 5 foreign fish species and 2 prawn species, were estimated to yield 10152 MT annually (DOF, 2019 and 2018). As the fishing industry mimics one of Bangladesh's most innovative and forward-thinking industries. Fish makes nearly 60% of all animal protein consumed in Bangladesh, making it a crucial source for animal protein for the country's citizens (DoF, 2018). This industry is making a significant contribution to food security by offering safe and high-quality animal protein. The GDP contribution from the fishing industry is 3.61% and the agricultural GDP is 24.41%. (DoF, 2018). According to thorough ARG's (Aquatic Research Group) and Bangladesh Fisheries Development Corporation field studies, the landings of large carps actually fell in 1985 and represented the lowest level in the fishery's history at Kaptai Lake. It took place as a result of excessive carp harvesting. After the proportion of carps gradually decreased, the output of pelagic clupeids significantly rose. Two species—Chapila (*Gudusia chapra*) and Keski (*Corica soborna*), which currently account for more than 50% of the total catch—have taken the lead in this growth. A fish fauna inventory identified eight species as being of significant commercial importance: *Gibelion catla, Cirrhinus mrigala, Lebeo rohita, L. calbasu, L. goinus, Notopterus chitala, Wallago attu,* and *G. chapra.*

Depending on the season, a person's interaction with fish and related surroundings, their dietary choices, and their immune system health, human diseases brought on by pathogens transferred via fish and other aquatic environments are relatively frequent. They are frequently facultatively pathogenic bacteria that might be acquired from fish without showing any outward signs of illness. Fish kept for consumption or as a hobby could be the source of the infection (Acha and Szyfres, 2003). Numerous bacteria have been linked to human diseases and toxins; among them, *Listeria monocytogenes, Vibrio cholera, Vibrio parahaemolyticus, Salmonella spp., Escherichia coli*, and *Clostridium*

botulinum are the principal culprits (Weir et al., 2012). Additionally, the people residing in the lake's islands uses the lake water for drinking and other domestic needs without first undergoing any kind of purification treatment (Barua et al., 2016; Chakma, 2008). Water tainted with pathogenic bacteria has the ability to spread a number of contagious diseases to people (Sadeghi et al., 2007). The most prevalent gastrointestinal illnesses spread through water contaminated with patient feces are cholera, salmonellosis, and shigellosis (Cabral, 2010).

One of Bangladesh's main animal protein sources is the fish of Kaptai Lake. The most crucial factors in ensuring good health are contamination- and pathogenic-bacteria-free seafood. The bacterial population found in fish, water, and sediments will be categorized in this study. Additionally, draw attention towards the pathogenic bacterial colony that can harm everyone's health if they consume fish. People who live along the shore of Kaptai Lake and utilize the dirty lake water for household purposes may frequently be seriously ill. The harmful bacteria in sediments can potentially contaminate fish that live at the bottom of the food chain. Thousands of people get sick every year by eating pathogenic fish and drinking dirty water, yet these illnesses are not properly recorded because of a lack of proper diagnosis. In addition, contaminated fish frequently infects not just customers but also anyone involved in the catch, handling, shipping, and processing of fish. Consequently, this study was created to fulfill the following goals:

i. To determine the total microbial load in fish, shellfish, water and soil samples of Kaptai Lake.
ii. To identify the pathogenic bacteria in fish, shellfish, water and soil samples

Chapter-2: Review of literature

It is crucial to look at the existing research activity on connected themes before starting new research under a specific experimental approach. Below is a survey of literature that is pertinent to the current research-

2.1. Kaptai Lake

The East Pakistani government started building the hydroelectric plant's reservoir in 1956. As a result, the lake was formed by the submergence of 54,000 acres (220 km2) of cropland in the Rangamati District. The United States provided funding for the hydroelectric project. In 1962, the project was completed. The contract for building the dam was given to Utah International Inc. and International Engineering Company. The dam measures 54.7 meters high and 670.8 meters long. The dam has 16 gates along its 745-foot (227-meter) long spillway. Water may flow through the spillway at a rate of 5,250,000 cu ft/s (149,000 m3/s). 40% of the region's total arable land was submerged as just a result of the construction of the dam. Additionally, 234 square miles (610 km2) of additional forested area and 29 square miles (75 km2) of government-owned forest property went under water. Also displaced were roughly 18,000 hagu households, totaling over 100,000 people. The Chakmas king's palace was likewise flooded and is currently submerged (Sadeghi et al., 2007).

A sum of 54,000 acres, or 40% of the indigenous people's arable land, have been taken by this lake. The population boom and shrinking amount of arable land have put considerable strain on the forest. Native Americans traditionally engage with in "slash and burn" method of farming, or jhum. A hill should have a recovery period of 15-20 years after slash and burn farming to restore the flora that was burned. In the past, the land to human ratio was optimal, and the projected jhum cycle interval was upheld. However, due to overpopulation, this period has now only been 2-3 yrs, which is incredibly short to allow for the growth of flora and the restoration of the woods. The viperous cycle of jhum farming is one of the main causes of the destruction of native forests, which are necessary for the springs to form. Additionally, the disappearance of native forests is to blame for rising temperatures and, as a result, falling precipitation (Cabral, 2010).

2.2. Present status of fish biodiversity in Kaptai Lake

Inland fisheries account for 80.59 percent of Bangladesh's overall fish production. One of the significant inland water bodies, Kaptai Lake has 68,800 species and 9,000 MT of fish productivity. Although there is currently a 120 kg production per acre, the overall fish yield of commercially significant fishes is decreasing. Major carps from India made up 4% of all fish produced in 2007 compared to 81% in the early 1960s. 78 fish species, 2 shrimp species, 1 dolphin species, and 2 turtle species have all been identified as existing in the lake thus far (Table 2.1). Six species had vanished by 2007 (Claucas and Ward, 1996). The majority of the fishing equipment utilized throughout the Kaptai reservoir is conventional. Fishing enthusiasts at Kaptai Lake have grown accustomed to using the brush shelter, a fish aggregation technique, since the early 1990s. Locally, this is referred to as juk fishing. As per Ahmed and Hambrey's (1999) study, the average harvest per brush was 242 kg, with major carps making up 17% of the catch, followed by catfish (24%), clupeid (13%), featherback (9%), and tilapia (6%).

Table 01. The Species of fishes caught in different months in Kaptai Lake

Fish group	Local name	Common name	Scientific name
Carps	Mrigal	Mrigal	*Cirrhinus mrigala*
	Carpio	Common carp	*Cyprinus carpio var specularis*
	Kalibaus	Black rohu	*Labeo calbasu*
	Rui	Indian major carp	*Labeo rohita*
	Bata	Minor carp	*Labeo bata*
	Silver carp	Silver carp	*Hypophthalmichthys molitrix*
	Catla	Indian major carp	*Catla catla*
Barbs and Minnows	Chapila	Indian river shad	*Gudusia chapra*
	Mola	Barb	*Amblypharyngodon mola*
	Dhela	Barb	*Rohtee cotio*
	Jat puti	Spot fin swamp barb	*Puntius sophore*
	Kachki	Ganga river sprat	*Corica soborna*
	Darkina	Top minnow	*Esomus danricus*
Snakehead	Shol	Snakehead murrel	*Channa striatus*
	Taki	Spotted snakehead	*Channa punctatus*
	Chang/raga	Asiatic snakehead	*Channa orientalis*
	Gajar	Giant snakehead	*Channa marulius*
Catfish	Gulsha Tengra	Long whiskered	*Mystus gulio*
	Kajoli	Gangeti cailia	*Ailia coila*
	Tengra	Striped dwarf catfish	*Mystus vittatus*
	Pabda	Pabdah catfish	*Ompok pabda*

	Batashi	River catfish	*Pseudeutropius atherinoides*
	Air	Long whiskered catfish	*Sperata aor*
	Boal	Fresh water shark	*Wallago attu*
Eels	Guchi baim	Striped spiny eel	*Mastacembelus spancalus*
	Tara baim	One striped spiny eel	*Macrognathus aculeatus*
Perch	Kholisha	Striped gourami	*Colisa fasciatus*
	Kata chanda	Round glass perchlet	*Chanda baculis*
	Lal chanda	Indian glass perch	*Chanda ranga*
	Foli	Feather back	*Notopterus Notopterus*
	Gutum	Guntea loach	*Lepidocephalus guntea*
	Bailla	Bar-eyed goby	*Glossogobious giuris*
	Chitol	Humped feather back	*Notopterus chitala*
	Gura chingri	River prawn	*Macro brachium lamarrei*
	Koi	Climbing perch	*Anabas testudineus*
	Veda	Gangetic Leaffish	*Nandus nandus*
	Shing	Catfish	*Heteropneustes fossilis*

2.3. Fish microbiology

Fish is an essential component of human nutrition and provides around 60% of the protein consumed worldwide. Fish provides 30% of the annual protein needs for almost 60% of the developing world (Abisoye et al., 2011). It is the primary source of excellent protein for humans, accounting for roughly 16% of a animal proteins consumed globally (FAO, 1997). Fish is one of the most lowest sources of proteins in Africa and provides 17% of the protein consumed there (Claucas and Ward, 1996). Because of its great nutritional value and simple digestion, fish is advantageous as food. As in small-holder farming sector, fish should be seen as a ready source of money as well as nourishment (Smith and Yoshida, 2000). The reclaimed fields can be brought back to life and made fruitful through the production of fish in large dams or ponds. Additionally, small-scale fish farming enhances communal residents' quality of life and decreases the amount of people who are perpetually reliant upon the government for financial support. However, a variety of bacterial pathogens can infect fish, and the majority of these pathogens are thought by others to be opportunistic pathogens in origins (Lipp and Ross, 1997). Fresh fish muscle's microbial richness is influenced by the surrounding environment and fishing areas (Cahill, 1990). It has been proposed that a fish's habitat determines the type of microorganisms that are present in association with it (Claucas and Ward, 1996). Indigenous and non-indigenous bacterial diseases linked to fish have been distinguished

(Kvenberg, 1991). The non-native species, that included *Escherichia coli*, *Clostridium botulinum*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Listeria monocytogens*, and *Salmonella* contaminate the fish or the habitat in one way or another. The fish's habitat contains naturally occurring native bacterial diseases such *Vibrio* species and *Aeromonas* species (Rodricks, 1991). Only when fish are physiologically out of balance, nutritionally malnourished, or under other stressors like poor water quality and overcrowding that promote opportunistic bacterial infections do fish microorganisms turn into pathogens (Austin, 2011).

Salmonella spp., *Mycobacteium* spp., *Streptococcus* spp., *Vibrio* spp., *Aeromonas* spp., and other pathogenic and potentially harmful bacteria are linked to fish and shellfish (Lipp and Ross, 1997). Fish farming and livestock production are combined in integrated fish farming. A fish pond receives direct animal excrement discharge as fertilizer, which fosters the development of photosynthesis-dependent species. Supplemental feeding has a direct impact on fish growth, whereas fertilization stimulates growth by supplying planktonic natural food. In pond aquaculture, plankton serves as a fish meal as well as a gross provider of dissolved oxygen, which is necessary for fish growth, and the most significant sink of ammonia-nitrogen, which fish excrete (Green and Teichert-Coddington, 1993). The fishing industry using various types of livestock manure could result in a rise in dangerous microorganisms, endangering the health of the country town (Musaiger and D Souza, 2008). It has been noted that eating fish can be a significant way for people to become exposed to human pathogenic microbes and other food borne illnesses (Christopher et al., 2009). Fish-borne pathogens can transferred to people via both active and inactive contact and can result in food-borne illnesses such cholera, salmonellosis, typhoid, fever, and dysentery. In light of the potential health dangers, livestock-fish farming must be viewed in perspective (FAO, 2003). Possible disease transmission between animals and people is one of the concerns associated with livestock integrated fish aquaculture. Different types of livestock manure are contaminated with dangerous bacteria, including *Salmonella*, *Shigella*, *Pseudomonas*, *Vibrio*, *Streptococcus*, and *E. coli* species, according to prior study (Abdelhamid et al., 2006).

These diseases can spread to people through contaminated fish handling or badly cooked food. The immune-compromised, young children, and the elderly are particularly

vulnerable to food-borne illnesses including dysentery and diarrhea caused by the ingestion of contaminated seafood, which have been linked to significant economic losses. Fish's quality and safety for human consumption are compromised by microbial contamination; this is especially important when the microorganisms are opportunist or virulent in type (Mhango et al., 2010). The inhabitants of the nearby communities may run the danger of contracting food-borne illnesses if they ingest the fish as from earth dams.

2.4. Microbiological relation with fish nutrition

Fish must be free of chemicals, germs, and other possibly harmful substances as well as potentially hazardous substances. Microbes can contaminate fish through the soil, the air, the dust, medical devices (used in production or dispensing), people, or animal fluids (World Health Day, 2015). Because of the pathogens that can grow and become abundant inside of the storage untill they release a toxin, fishes may be poisonous (Eze et al., 2011). According to a National Standard created mostly by Indonesian National Agency of Drug and Food Control in 2007 with the code SNI 01-2332.52006, all fish products, as well as imported and exported seafood, must be free of pathogenic microorganisms. Kvenberg (1991) and Rodeick separate fish pathogenic microorganisms among non-native and indigenous pathogens (1991). There are pathogens nearby or in the fish habitat that are not local to the area. These pathogens include *Escherichia coli, Listeria monocytogenes, Clostridium botulinum, Salmonella* species and *Listeria* species (Bensley et al, 2011; Eze et al., 2011; and Dwiyitno 2010). Fish typically include hazardous natural bacteria like *Vibrio* and *Aeromonas* species. Clucas and Ward (1996) found that some microorganisms, including *S. aureus, Salmonella*, and *Vibrio parahaemolyticus*, are more likely to infect specific seafood.

2.5. Fish market and marketing channels

The current state of fish market place in Rangamati has been the subject of several prior investigations (Kabir et al., 2020; add others). Fish spoils quickly if it is not dispose of in a timely manner due to its high perishability. This not only lowers prices but also creates health risks. Because of this, it needs to be handled carefully and packaged carefully before being sold to consumers. Rodeick (1991). Despite the fact that fish is regarded as

an industry in many nations throughout the world, fishers in our nation do not interact directly with consumers. Fish market chains are thus in action, running from either the suppliers to the final consumers. The local fish merchant, beparies, aratdar, entire sellers, and retailers are only a few of the middlemen in this system. The total number of fish landings in the landing facility were discovered to vary sometimes. The number of fish landed in the winter were greater compared to other seasons. More fish were landed in the months of January, February, and March than in the months of April, May, and June. Rahman (2003) noted that a multitude of middlemen, including regional fish traders, agents, entire sellers, and retailers, were involved in the market chain in Gazipur from farmers to consumers. In the Mymensingh district, Quddus (1991) also discovered a comparable market chain. The observations mentioned above support our findings. Rahman et al. identified three different kinds of supply chain operations for the sale of fish through supermarkets (2017).

2.6. Water Microbiology

Freshwater resources are a vital part of the biosphere and a necessity for ensuring the survival of living things, although only making up 3% of the world's total water supply (Wilson and Carpenter, 1999). (Jackson et al., 2001). The exponential increase in human population, widespread urbanization, fast industrialization, and intensive agricultural methods make it impossible for freshwater supplies to replenish themselves (Fischer and Heilig, 1997; Douglas et al., 2002; Dale et al., 2005; Grimm et al., 2008). The physical, chemical, and biological features of water, or its "quality," indicate whether or not the water is contaminated in regards of consuming, the suitability of human touch, as well as the stability of ecosystem (Schleiger, 2000). Around 80% of public health issues have been brought on by polluted water (Marshall et al., 2006; Jones et al., 2007; O'Reilly et al., 2007; Peace and Mazumder, 2007; Jayana et al., 2009). Water-borne illnesses are to blame for developing and recurring infectious diseases globally. The possible carrier for transmitting a number of infectious illnesses to humans is water contaminated with harmful microorganisms (Sadeghi et al., 2007).

The three gastrointestinal illnesses that are most frequently contracted by drinking water contaminated with patient feces are cholera, salmonellosis, and shigellosis (Cabral, 2010). Water used for drinking should be devoid of turbidity, color, odor, and any

organic or inorganic contaminants that might have a negative physiological impact (Bhatt et al., 1999). The population of Bangladesh depends on tubewells, ponds, lakes, etc. for access to safe drinking water, particularly in remote areas. Until a significant geogenic arsenic pollution is revealed, tube-wells that use subsurface water are marketed as more than just a better source of drinking water. However, the greatest barrier to using surface water for drinkable purposes is the potential for exposure to harmful germs (Rahman et al., 2003; 2011a; 2011b; 2013). The biggest man-made lake in Southeast Asia is Kaptai Lake (KL), which is situated in the Rangamati neighborhood and 68.5 kilometers from Chittagong Metropolitan City. The lake was made when a dam was built on the Karnaphuli River, which flows in the southeast of Bangladesh, to provide hydroelectric power (Fernando, 1980; Karmakar et al., 2011). A sizable hilly region was drowned as a result of the dam building and reservoir development, causing profound changes in the environment (Newman, 1974). The native people was also compelled to relocate to areas that were more mountainous or to the little islands that created inside the lake. All those who reside nearby the lake become reliant on the water for drinking, domestic use, and other uses, and they all consume it directly without any intermediary filtration (Ahmed et al., 2001; Chakma, 2008).

2.7. Bacteriological Characteristics of Kaptai Lake

Water is necessary for life, yet many people lack access to clean, safe drinking water, and many of them pass away from bacterial diseases contracted from water. In a prior study (Barua et al., 2016), samples of lake water were examined with HPC and MPN indexing to evaluate the microbial traits that are a key indication of water quality. The previous studies of the viable cells in drinkable water is 500 CFU mL-1, but the HPC count in all of the samples was within 250 CFU mL-1 (US EPA, 2003). The HPC represents both the overall number of bacteria and the microbial activity in freshwater (Aksu and Vural, 2004). In a research on Ghana's Ahor Lake, which is utilized for recreation, a decreased level of HPC in lake was also noted (Amfo-Out et al., 2011). Water pH, temperature, remaining chlorine, and incorporable organic matter levels are known to have an impact on the HPC count of drinking water, which can range from 1 to >104 CFU mL-1 (LeChevallier et al., 1980). A high HPC density in drinking water, however, does not always signify a serious health danger (Allen et al., 2004).

A water body's TCC is employed as a possible indication of bacterial, virus, or parasite infestation. Since all of the KL samples collected were positive for coliform, they may be unsafe for human consumption. Some of the nearby residents just use KL as a place to dump animal and human waste, contaminating the water. Naturally, each gram of fresh human and animal feces contains 102–104 times more hermos-tolerant coliform species (Gleeson and Gray, 1997). Children under the age of five, seniors, and other persons with weak immune systems may become ill from drinking water that has been polluted with these feces.

Several bacterial pathogens, including Enterococcus spp., Salmonella spp., Pseudomonas spp., and Vibrio spp., were found utilizing microscopic, cultural, and biochemical testing to validate the possible contamination by human diseases. All KL specimens were discovered to be tainted with the aforementioned diseases. These bacteria are the primary etiological agents of several waterborne outbreaks as well as the cause of gastrointestinal disorders with symptoms such as diarrhea, vomiting, nausea, fever, and abdominal discomfort (Craun et al., 2006). Probable sources of contamination for the identified pathogens include human waste, animal excrement, fishing operations, persons bathing with skin sores, etc. It has been established that these bacteria are also to blame for the lake water pollution caused by rainstorms and stream flow incidents (Jamieson et al., 2005). The nature and frequency of diseases among residents in the vicinity of KL, however, were not documented. The information showed that the water in KL is tainted with dangerous microorganisms, making it unfit for drinking and domestic usage.

2.8. Soil microbiology

According to Wang et al. (2012), sediments are unique basin ecosystem components with a far larger variety of organisms and microbes than river systems (Zinger et al., 2011). Sediment, which makes up the majority of aquatic systems, is essential as a source, pool, and distributor of nutrients (Huang et al., 2015). There are several sorts of sediment in a typical river network region, including reservoirs, wetlands, and estuaries. Understanding the microbial communities in various basin regions is essential since sediment microorganisms from these places have an impact on overall freshwater environments (Shimeta & Cook, 2011; Shtarkman et al., 2013).

The primary elements of a simple river network are lakes, lake marshes, and estuaries, and deposits in these areas serve as the channels for matter change and movement. A lakeside wetlands is a landform in the lake region that serves as the lake outflow's filtering system. In lake wetlands, bacterial communities are common and play important roles in ecological processes, such as the cycling of biologically active substances (Newton et al., 2011; Van der Gucht et al., 2007; Woese et al., 1990). According to Ding et al. (2015) and Iasur-Kruh et al. (2010), wetland ecosystems near lake basins are the most ecologically diverse. They also provide habitat for biota (Wu et al., 2015). Estuaries are significant elements of lake basins and serve as buffer zones between rivers and lakes or seas. Runoff particles and suspended sediments are carried, stored, and altered in estuaries (Arzayus & Canuel, 2005; Sun et al., 2011). Microbial communities in sediments contribute significantly in narcotic exports, rejuvenation, and biochemical cycling in estuaries, where sediment typically stores inorganic and organic resources before releasing them back into the water column (Pinckney et al., 2001; Yokokawa & Nagata, 2010).

In reality, a significant majority of such nanoparticles portion of humus is made up of live and dead microbial cells, or their decomposing byproducts, making the microbiota of a soil an integral component of soil organic matter. No examination of the function of soil organisms in plant life would be complete without taking into consideration the type of the bacteria and subsequent biochemical transformations since the amount and qualities of organic matter in any particular soil rely on these factors (Craun et al., 2006). Other than viruses, four types of organisms make up the soil's microflora population. These include fungus, algae, bacteria, and actinomycetes. Each of these categories has several genera and numerous species. Besides of viruses that reside in the tissues of other creatures, bacteria are the most prevalent type of microorganism in soil and the smallest living thing. Actinomycetes are second only to genuine bacteria in terms of abundance in soils, where they can be found in concentrations as high as 200 million per gram. A very diverse range of organisms make up the microbial population of soils. Several hundred to several million algae per gram can be found in almost all cultivated soils. Numbers are meaningless for fungi and actinomycetes because the morphology and growth rate of algae vary so widely (Zinger et al., 2011).

Chapter-3: Materials and Methods

3.1. Sampling Area of Fish

The study was carried out at Kaptai Lake, Rangamati. The Kaptai Lake is situated in the Southeastern part of Bangladesh, occupying an average surface area of 58300 hactor with a water reserve of 525×10^6 m^3 (Uddin et al., 2014). It is under a humid tropical environment. Rainfall is bi-modally distributed with peaks around July and September of each year. Total twenty (20) species of fish and shrimp samples were collected in this study from the Fishery Ghat (Landing Centre). Maximum five (5) species with replications were collected in zip-locked bag for analysis in each sampling lot.

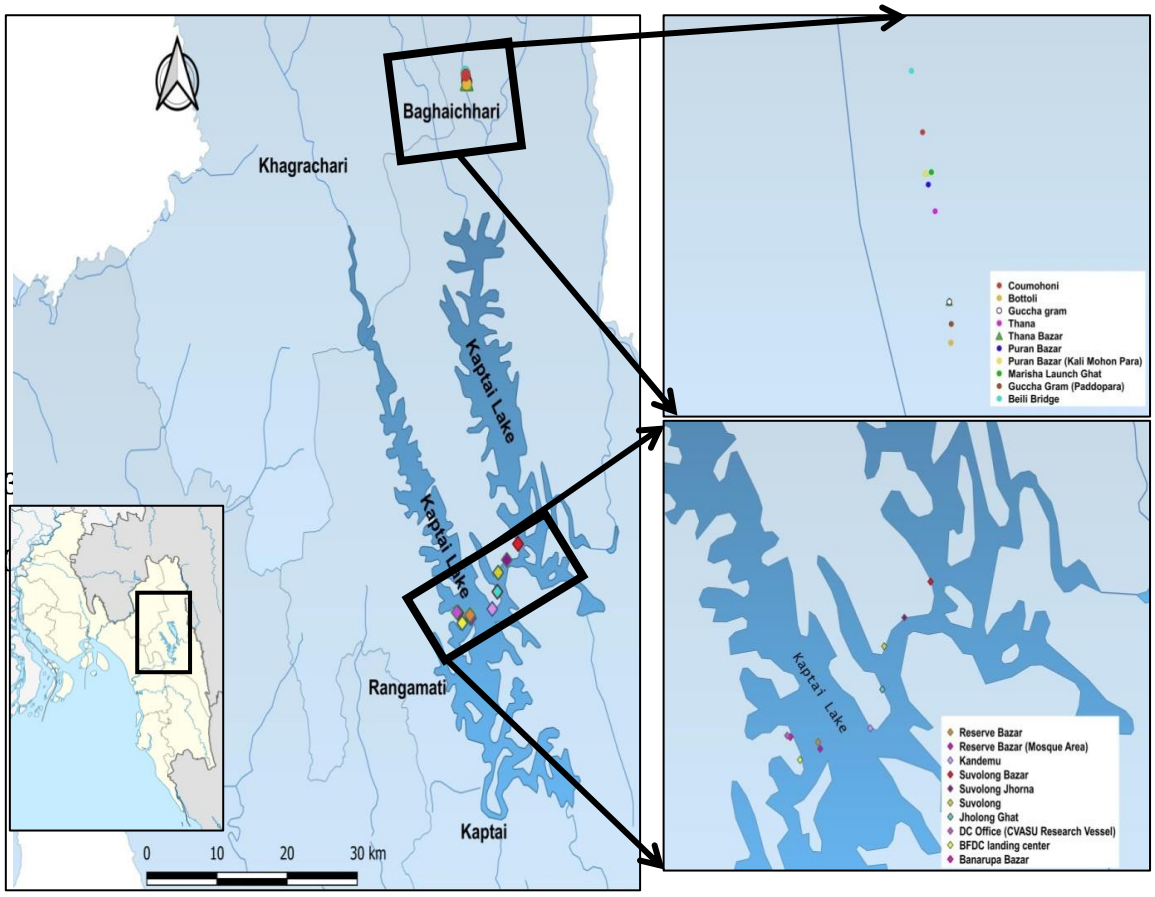


Figure 01: Sampling sites at Kaptai Lake

Samples were purchased from different sellers at Fishery Ghat during the early morning hours of the day (between 7:00 and 8:00 hour local time). Samples were placed in the ice

boxes to preserve the bacteriological properties and then transported to the Nutrition and Processing laboratory, CVASU for further analysis.

3.2. Collection of water and soil samples

The water and soil samples were collected in March 2021 at the end of winter season. Soil samples were collected from five different spots [Fishery Ghat, Reserve Bazar, Samata Ghat-1, Samata Ghat-2 and DC office Ghat] with replications and the spots were selected based on the inhabitants of the lake (Figure 01). Soil samples were also collected from different sampling stations and packed in zip lock poly bags. From the same sampling stations, water samples were also collected for microbiological analysis in sterile falcon tubes (50ml), placed in sampling buckets. Then all the collected water and soil samples were transported to the Disease and Microbiology laboratory of Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram. Analysis were performed immediately reaching the laboratory.

Figure 02: Collection of soil and water sample and sample preparation

3.3. Microbiological analysis

3.3.1. Sterilization of materials

All the glass-wares used were washed, dried and sterilized in hot air oven at a temperature of 150 °C for 1 h according to the method described by Adibe and Eze (2004) one day earlier before collection of samples. Culture media's were freshly prepared and sterilized in an autoclave at a temperature of 121°C for 15 min. The wire loop was also sterilized using spirit lamp.

3.3.2. Sample preparation

Sample preparation was made using the method described by Obi and Krakowiaka (1983). About 10 g of the fish sample was cut from the head, middle and tail regions with a sterile knife. The cut samples were crushed into small pieces in a sterile mortar. Then the crushed 10 g sample were transferred in a sterile beaker containing 90 ml of peptone water giving a 1:10 dilution. This was done for the 5 fish samples. Similar procedure was followed to prepare soil and water samples.

3.3.3. Preparation of serial dilution

Nine milliliters of sterile peptone water was poured aseptically into six tubes each and 1 ml from the first dilution of the original crushed fish sample was added to the first test tube and mixed thoroughly. Another 1 ml was taken from the first tube and added to the second test tube and mixed very well. From the second test tube, another 1 ml was taken and introduced into the third test tube and mixed very well. This procedure continued until the sixth test tube. The crushed sample was therefore diluted from 10^{-1} to 10^{-6} for each fish sample. Serial dilution was done for each fish, soil and water sample.

3.3.4. Preparation of Plate Count Agar (APC)

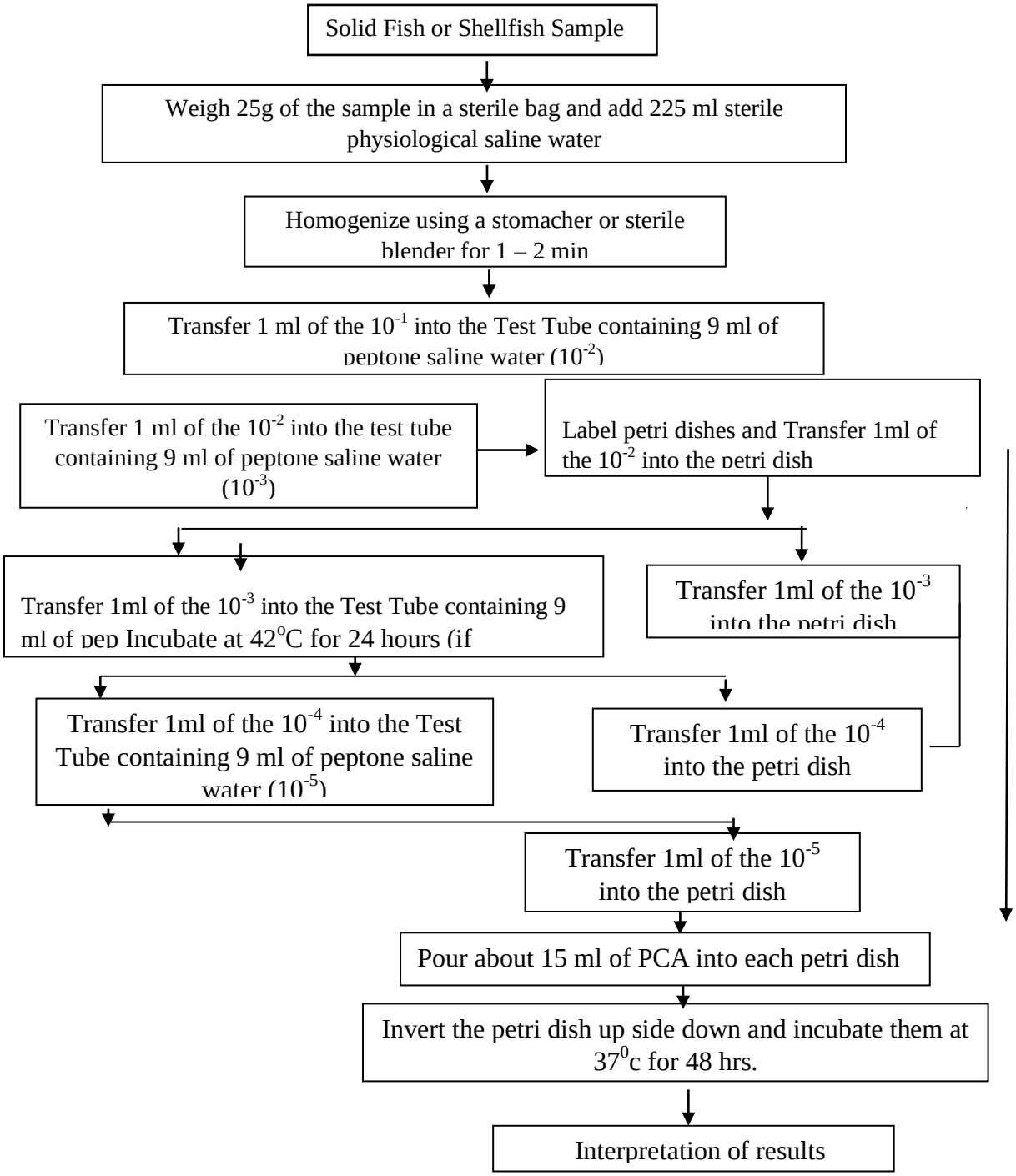


Figure 03**:** Procedure of Plate Count Agar (PCA)

3.3.5. Inoculation of sample in agar plate

Duplicate plates of nutrient agar were inoculated with 0.1 ml of the diluted solution (10^{-2} to 10^{-6}) using glass spreader technique. All plates were incubated at a temperature of 37°C for 24 h before colony enumeration and isolation. The temperature was chosen to differentiate the mesophiles which constitute most medically important pathogenic bacteria (Baker and Silverton, 1985).

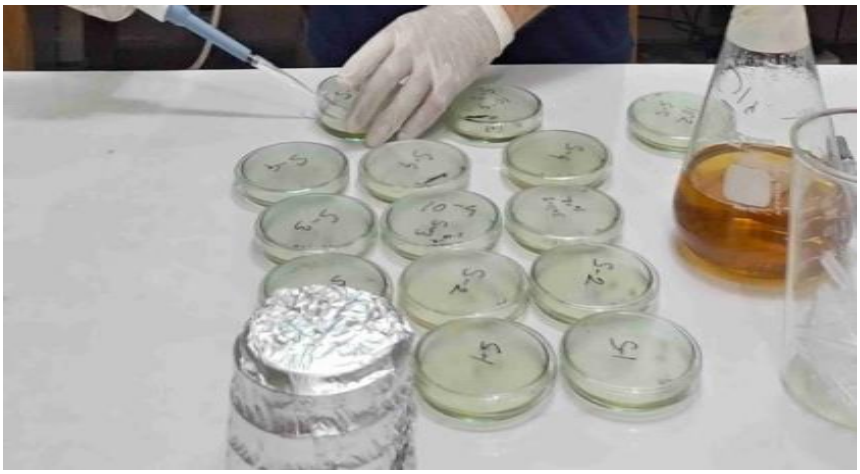


Figure 04: Inoculation of Sample in agar plate

3.3.6. Counting of bacterial load

The method described by Collins et al. (1989) for estimating bacteria counts was used to enumerate the total viable counts of the isolates. Countable plates showing 1 to 32 colonies were selected and counted. The mean colony count on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per gram (CFU mL^{-1}).

3.4. Identification of Pathogenic Bacteria

This study confirmed several pathogenic bacteria such as *Escherichia coli, Vibrio cholera, Salmonella, Shigella* which create food borne illness in human body**.**

3.4.1. Identification of *E. coli*

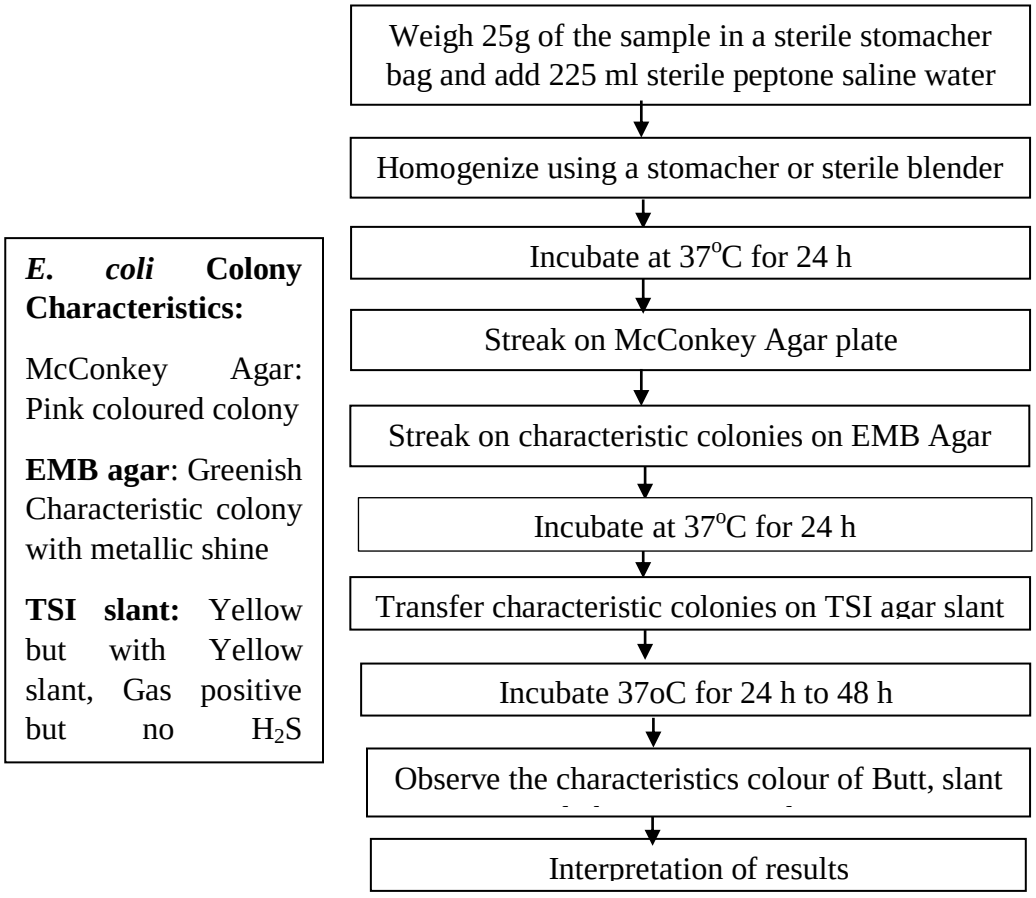


Figure 05**:** Procedure of of *E. coli* Identification

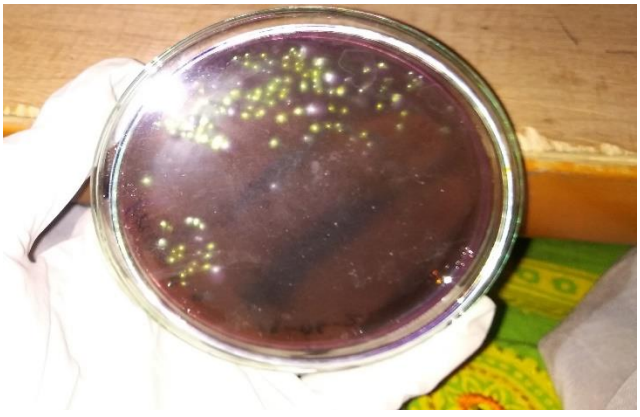


Figure 06: *E. coli* in EMB Agar

3.4.2. Identification of *Vibrio cholera*

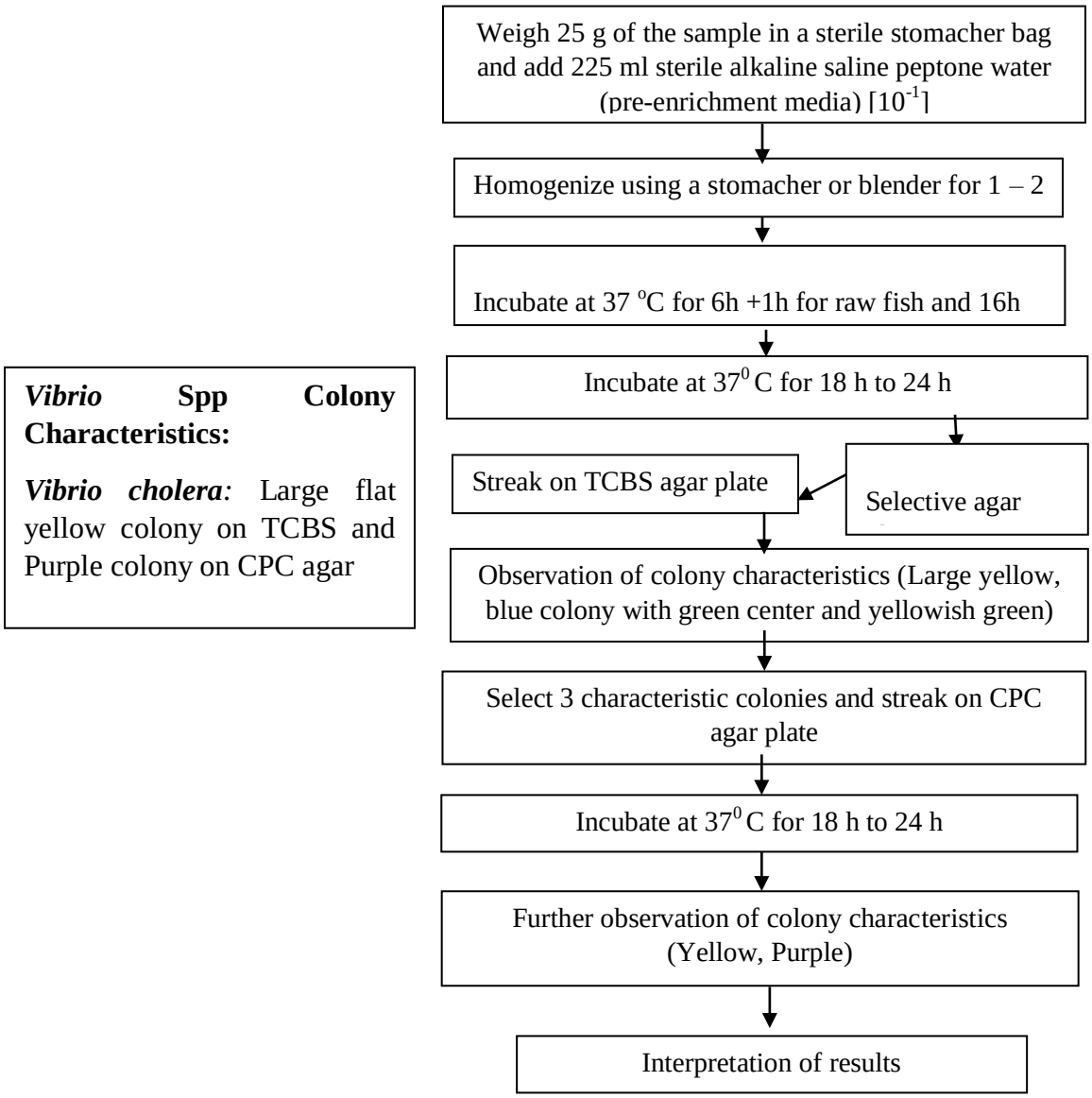


Figure 07: Procedure of *Vibrio cholera* Identification

3.4.3. Identification of *V. vulnificus* and *V. parahaemolyticus*

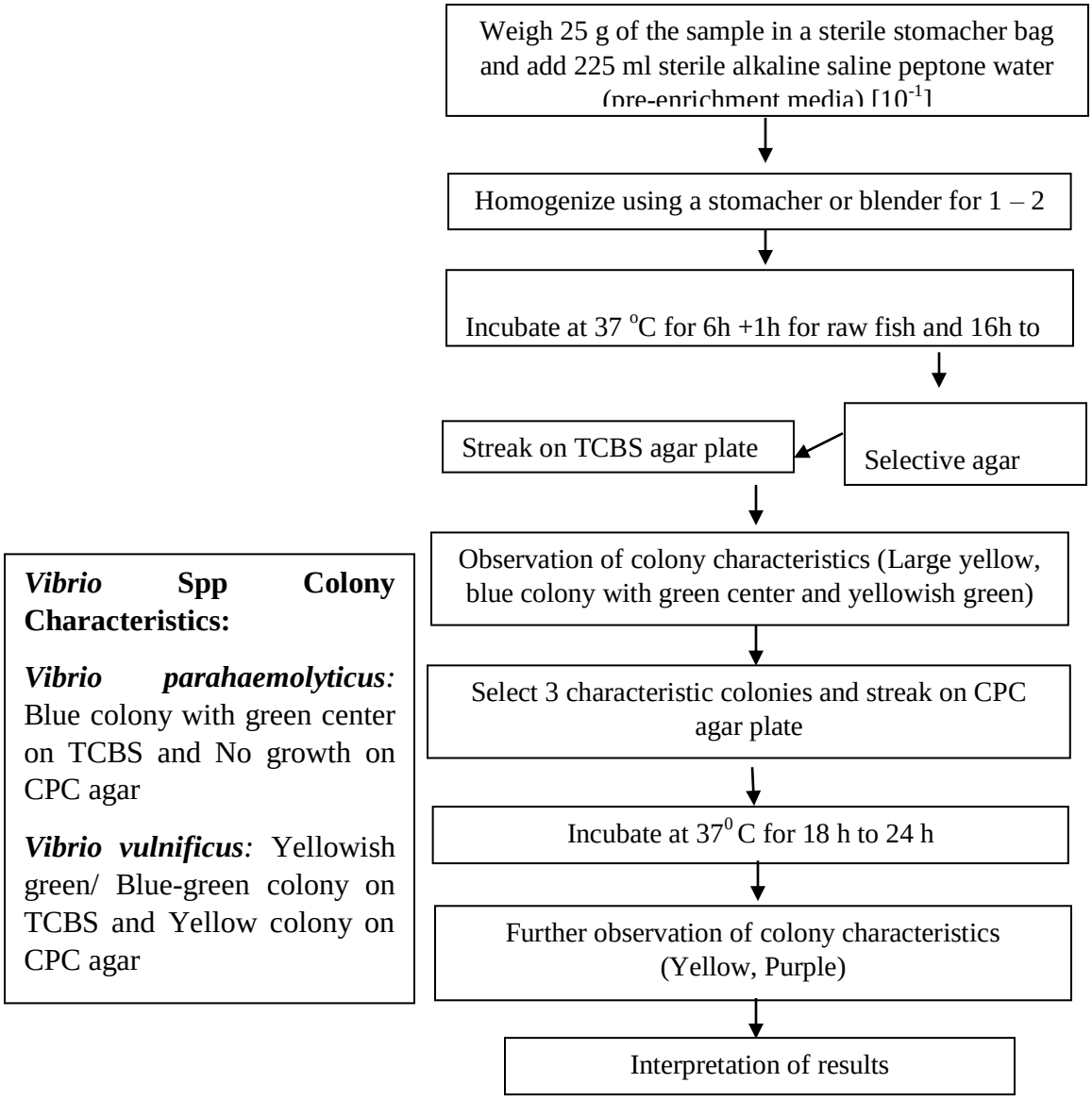


Figure 08: Procedure of *V. vulnificus* and *Vibrio parahaemolyticus* Identification

3.4.4. Identification of *Salmonella* and *Shigella*

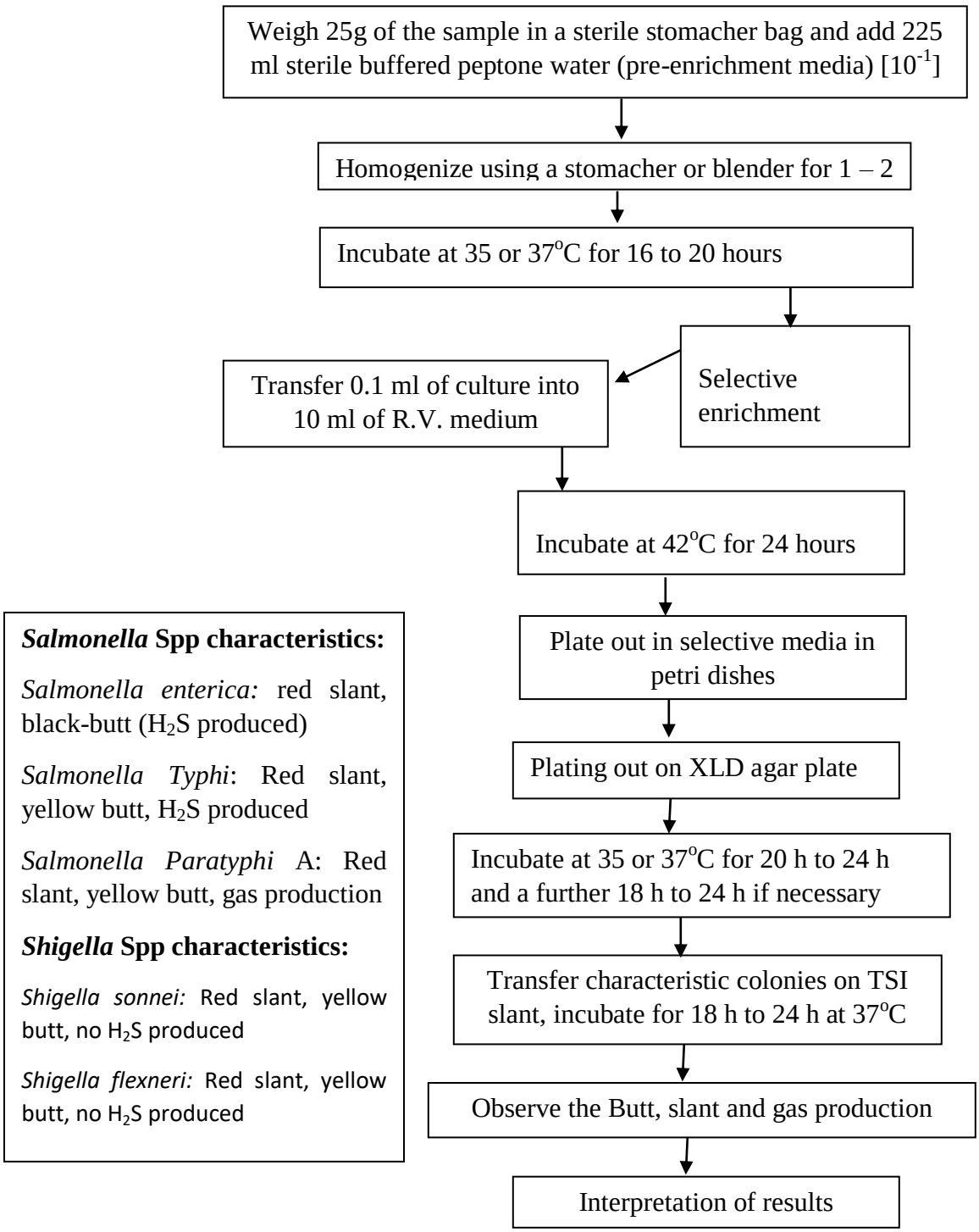


Figure 09**:** Identification of *Salmonella* and *Shigella*

Chapter-4: Results

4.1. Bacteria Isolated and Identified from Fish

Twenty fish samples from previously selected sampling areas collected. Among the fish samples, highest bacterial colony was found in Fish-4 (10^{-2}) and in Fish-3 (10^{-4}). No bacterial colony was observed in 10^{-4} dilution plates.

Vibrio vulnificus was found in all the fish samples whereas *Vibrio cholera* was only found in Fish-1, 2 and 5. *Salmonella* sp. was found in all the fish samples except Fish-5. *E. coli* was present in Fish-4 and Fish-5 (minute). *Vibrio vulnificus* and *Vibrio cholera* both were present in all the water samples.

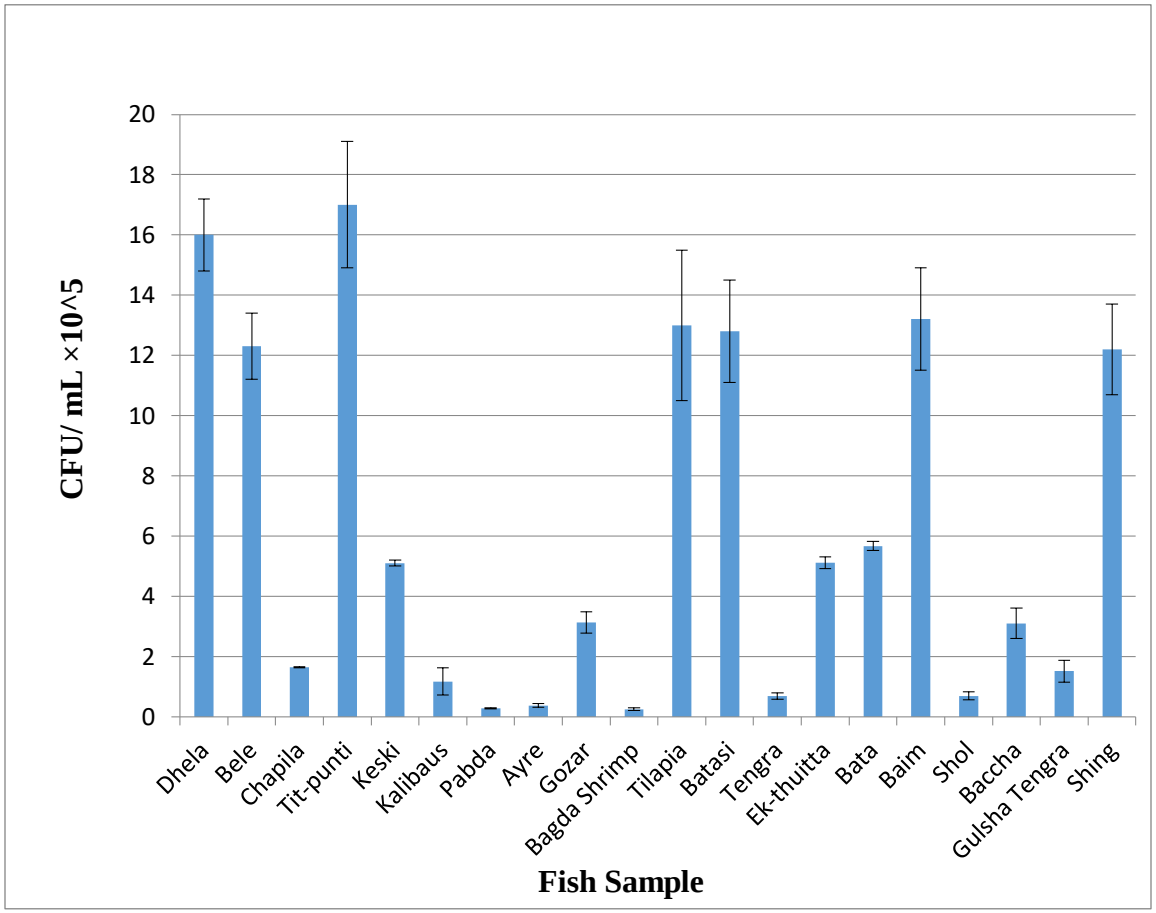


Figure 10**:** Colony of bacteria found in fish sample

Table 02: Pathogenic bacteria present in fish samples where "R" represent the replication of fish sample

Sample	**R**	***E. coli***	***V. cholera***	***V. vulnificus***	***V. parahaemolyticus***	***Salmonella***	***Shigella***
Fish 1: Dhela	R-1	+	+	-	-	-	-
	R-2	+	-	+	-	-	-
	R-3	+	+	-	-	-	-
Fish 2: Bele	R-1	-	+	+	-	+	-
	R-2	-	-	-	-	+	-
	R-3	-	+	-	-	+	-
Fish 3: Chapila	R-1	-	+	-	-	-	-
	R-2	+	+	-	+	-	-
	R-3	-	-	-	+	-	+
Fish 4: Tit-punti	R-1	-	-	+	+	-	-
	R-2	+	+	-	+	+	+
	R-3	-	-	+	+	-	+
Fish 5: Keski	R-1	+	-	-	-	+	-
	R-2	+	-	+	-	+	+
	R-3	+	-	-	-	-	-
Fish 6: Kalibaus	R-1	-	+	-	-	+	-
	R-2	-	+	+	-	+	+
	R-3	-	-	+	+	-	+
Fish 7: Pabda	R-1	-	+	-	-	+	-
	R-2	+	+	-	-	+	-
	R-3	-	-	+	-	-	+
Fish 8: Ayre	R-1	-	+	-	-	+	-
	R-2	-	-	-	+	-	+
	R-3	-	+	+	-	+	-

Fish 9: Gozar	R-1	-	-	-	-	+	-
	R-2	-	-	+	-	-	-
	R-3	-	-	+	+	+	+
Fish 10: Bagda Shrimp	R-1	+	+	-	-	-	-
	R-2	-	-	-	-	-	-
	R-3	+	+	-	+	-	+
Fish 11: Tilapia	R-1	+	+	+	+	+	-
	R-2	+	+	+	-	+	-
	R-3	+	+	-	-	-	+
Fish 12: Batasi	R-1	+	+	-	-	+	+
	R-2	-	-	+	+	+	+
	R-3	+	+	+	-	+	-
Fish 13: Tengra	R-1	-	+	+	-	+	+
	R-2	-	-	+	+	+	-
	R-3	+	+	+	-	+	+
Fish 14: Ek-thuitta	R-1	-	+	-	-	-	-
	R-2	-	-	-	-	-	-
	R-3	-	+	+	-	-	+
Fish 15: Bata	R-1	+	+	-	-	-	-
	R-2	+	+	-	+	+	-
	R-3	+	-	+	+	+	-
Fish 16: Baim	R-1	+	+	-	-	+	-
	R-2	+	-	+	+	+	-
	R-3	-	-	-	-	-	+
Fish 17: Shol	R-1	+	+	-	-	+	-
	R-2	-	+	-	+	+	-
	R-3	+	-	-	+	+	-
Fish 18:	R-1	-	-	+	-	-	-

Baccha	R-2	-	-	+	-	-	+
	R-3	-	+	-	+	-	-
Fish 19: Gulsha Tengra	R-1	-	+	-	-	-	-
	R-2	-	+	+	-	-	-
	R-3	-	-	+	+	-	-
Fish 20: Shing	R-1	+	+	-	-	-	+
	R-2	+	-	-	-	+	-
	R-3	+	+	-	-	+	-

4.2. Bacterial quantification, isolation and identification from water and soil samples

Water and soil samples were collected from previously selected 11 sampling areas (Figure 01). Number of bacterial colony found in water and soil samples are shown in Table 3 and Table 4 respectively. Highest bacterial load was found in water samples collected from Baghaichari (Water 11- 3.90×10^6 CFU/g). Whereas, the lowest number of bacterial colony was found in samples collected from Kandemu (Water 7- 1.75×10^4 CFU/g). After quantifying the number of bacterial colony in soil samples, it was found that both the highest and lowest number of colony was present in the samples collected from Baghaichari (Soil 11 and Soil 14).

Bacteria isolated and identified from different water samples are shown in Table 3. *E. coli* and *V. cholera* was found in all the water samples. *V. vulnificus* was also present in most of the water samples except Sample Water- 4, 7 and 8 (Jalojan Ghat, Kandemu and Shuvolong jhorna). *V. parahaemolyticus* was found in most of the water samples except Water- 7, 16 and 18. *Salmonella* and *Shigella* both were absent in the water sample-5, 16 and 18. *Salmonella* sp. was also absent in the sample- 4, 6, 7, 17.

Bacteria isolated and identified from different soil samples are represented in Table 4. After identifying the isolated bacterial colony, it was found that, *E. coli* was present in all the soil samples collected from different sampling areas. *V. cholera, V. vulnificus*, *V.*

parahaemolyticus, *Salmonella* and *Shigella* were not found in the sample 6 and 16 (collected from Jholong ghat and Baghaichari)

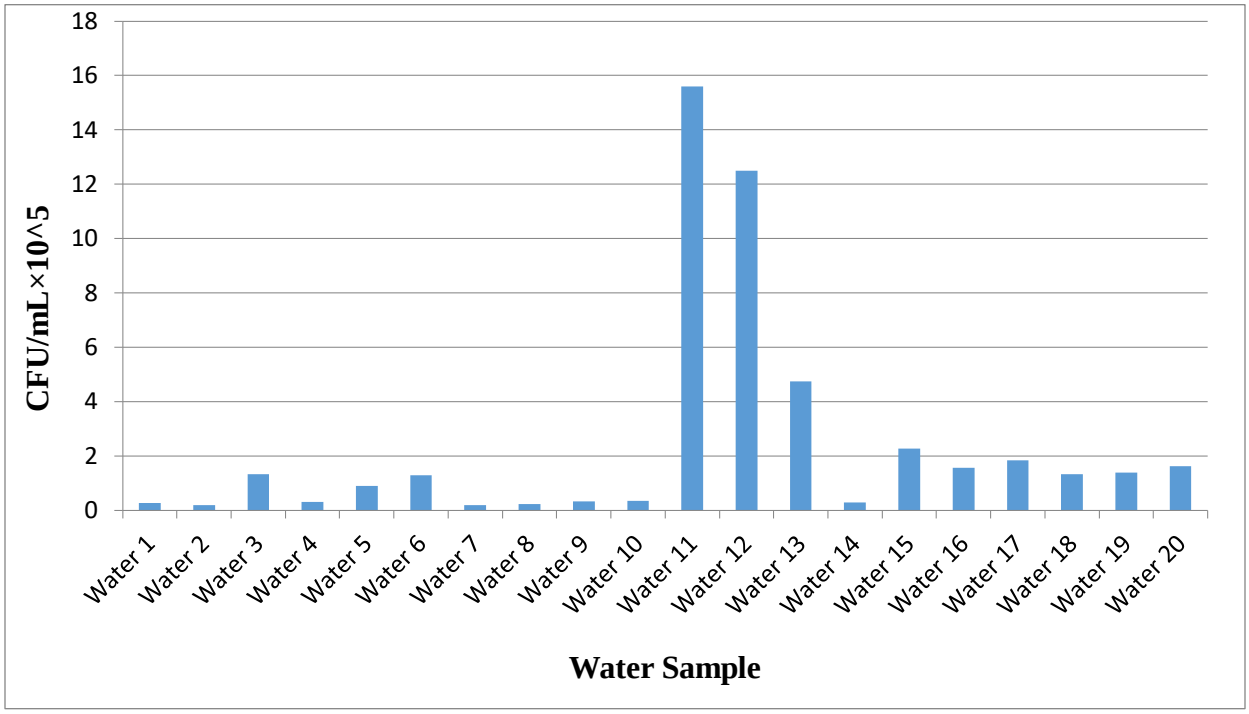


Figure 11: Colony of bacteria found in water samples

Table 03: Pathogenic bacteria present in water samples where "R" represent the replication of water sample

Sample	**R**	***E. coli***	***V. cholera***	***V. vulnificus***	***V. parahaemolyticus***	***Salmonella***	***Shigella***
Water 1	R-1	+	+	+	+	+	-
	R-2	+	+	-	+	+	+
	R-3	+	+	-	+	+	-
Water 2	R-1	+	+	+	+	+	+
	R-2	+	+	+	-	+	+
	R-3	+	+	-	-	+	-
Water 3	R-1	+	+	+	+	+	+
	R-2	+	+	+	+	+	-
	R-3	+	-	+	-	+	+
Water 4	R-1	-	+	-	-	-	-
	R-2	-	+	-	+	-	+
	R-3	-	-	-	+	-	-
Water 5	R-1	+	+	-	-	-	-
	R-2	+	+	+	-	-	-
	R-3	+	+	+	+	-	-
Water 6	R-1	-	-	-	-	-	-
	R-2	-	-	-	+	-	+
	R-3	-	-	+	-	-	+
Water 7	R-1	+	-	-	-	-	-
	R-2	+	+	-	-	-	+
	R-3	-	-	-	-	-	-
Water 8	R-1	+	-	-	-	+	+
	R-2	+	-	-	-	+	-
	R-3	+	+	-	-	+	+
Water 9	R-1	+	+	-	+	+	-

	R-2	+	+	+	-	+	+
	R-3	-	+	-	+	+	-
Water 10	R-1	+	-	-	-	-	-
	R-2	-	-	-	+	-	+
	R-3	-	-	-	-	-	-
Water 11	R-1	+	+	+	+	+	+
	R-2	+	+	-	-	+	+
	R-3	+	+	-	-	+	-
Water 12	R-1	+	+	+	+	+	+
	R-2	+	+	+	+	-	+
	R-3	+	-	-	-	+	-
Water 13	R-1	+	+	-	-	-	-
	R-2	+	+	+	+	-	-
	R-3	-	-	-	-	-	-
Water 14	R-1	+	+	-	-	+	-
	R-2	+	+	-	-	+	-
	R-3	+	+	+	-	+	+
Water 15	R-1	+	+	+	+	+	+
	R-2	+	+	-	-	+	+
	R-3	+	+	-	-	+	+
Water 16	R-1	-	+	-	-	-	-
	R-2	+	+	+	-	-	-
	R-3	-	+	-	-	-	-
Water 17	R-1	+	+	+	-	-	-
	R-2	+	+	+	+	-	-
	R-3	+	+	+	+	-	+
Water 18	R-1	+	+	-	-	-	-
	R-2	+	-	+	-	-	-
	R-3	+	+	+	-	-	-

Water 19	R-1	+	+	-	+	+	-
	R-2	+	+	+	+	+	+
	R-3	-	-	-	-	+	-
Water 20	R-1	+	+	-	-	+	-
	R-2	+	+	+	-	+	-
	R-3	+	+	-	+	+	+

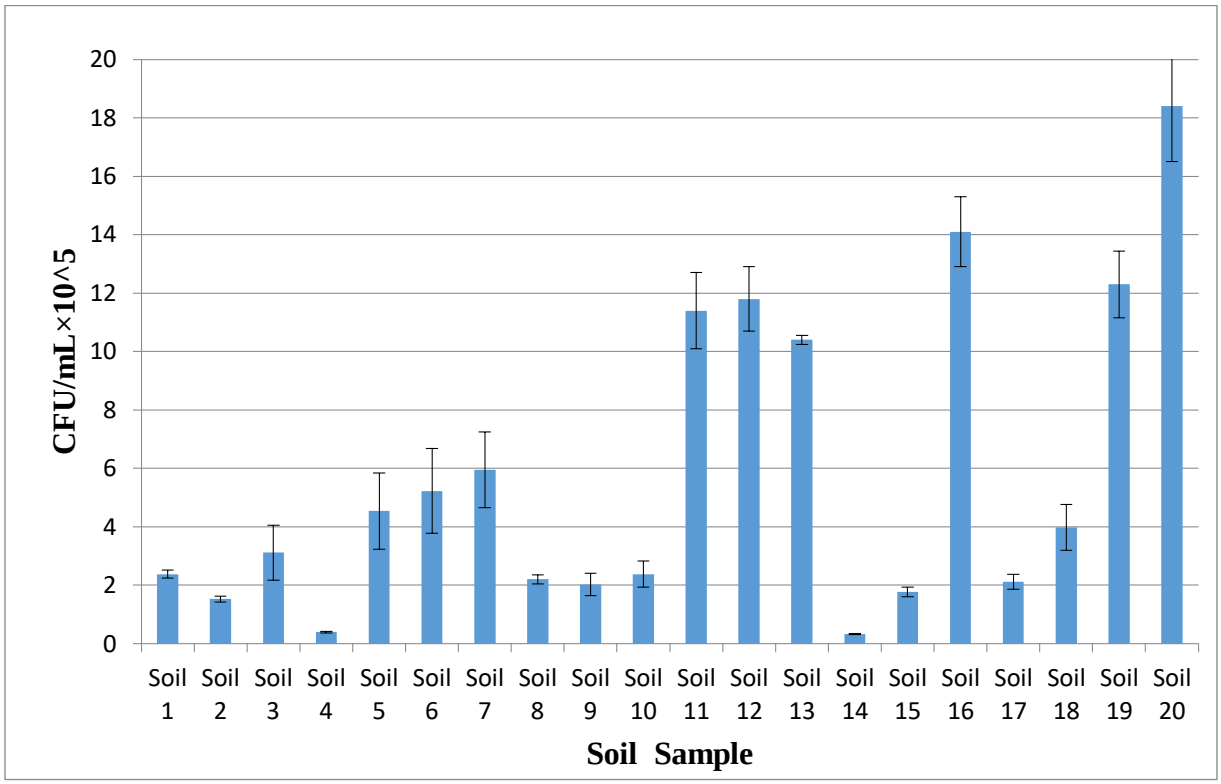


Figure 12: Colony of bacteria found in soil samples

Table 05: Pathogenic bacteria present in soil samples where "R" represent the replication of soil sample

Sample	**R**	***E. coli***	***V. cholera***	***V. vulnificus***	***V. parahaemolyticus***	***Salmonella***	***Shigella***
Soil 1	R-1	+	+	+	+	+	-
	R-2	+	+	-	+	+	+
	R-3	+	+	+	+	+	-
Soil 2	R-1	+	+	+	+	+	+
	R-2	-	+	-	-	+	+
	R-3	-	+	+	-	+	-
Soil 3	R-1	+	+	+	-	+	+
	R-2	+	+	+	-	+	+
	R-3	+	-	+	+	+	+
Soil 4	R-1	-	+	-	-	-	-
	R-2	-	-	+	+	-	-
	R-3	-	-	+	+	-	-
Soil 5	R-1	+	+	-	-	-	-
	R-2	+	+	+	-	-	-
	R-3	+	+	+	-	-	+
Soil 6	R-1	+	-	-	-	-	-
	R-2	-	-	-	-	-	-
	R-3	+	-	-	-	-	-
Soil 7	R-1	-	+	-	-	+	-
	R-2	-	+	+	-	+	+
	R-3	+	+	-	-	+	+
Soil 8	R-1	+	-	-	-	+	-
	R-2	+	-	-	-	-	+
	R-3	+	+	-	+	+	+
Soil 9	R-1	+	+	-	-	-	-

	R-2	+	+	-	+	-	+
	R-3	-	+	-	+	-	-
Soil 10	R-1	+	-	-	-	+	-
	R-2	+	+	-	-	+	+
	R-3	+	-	-	-	+	-
Soil 11	R-1	+	+	+	+	+	-
	R-2	-	-	-	+	+	-
	R-3	-	+	+	+	+	-
Soil 12	R-1	+	+	+	+	+	+
	R-2	+	+	+	-	+	+
	R-3	+	+	+	-	-	+
Soil 13	R-1	+	-	-	-	-	-
	R-2	+	+	-	+	-	-
	R-3	+	-	-	-	-	-
Soil 14	R-1	+	-	+	+	+	-
	R-2	+	-	+	+	+	+
	R-3	+	-	+	+	+	-
Soil 15	R-1	+	+	+	+	+	+
	R-2	+	+	+	-	+	+
	R-3	+	-	-	-	+	+
Soil 16	R-1	+	-	-	-	-	-
	R-2	+	-	-	-	-	-
	R-3	-	-	-	-	-	-
Soil 17	R-1	+	+	-	-	-	-
	R-2	+	+	-	-	+	+
	R-3	+	-	-	-	+	-
Soil 18	R-1	+	-	-	-	-	-
	R-2	-	-	-	-	-	-
	R-3	+	+	+	+	-	-

Soil 19	R-1	+	+	+	-	+	+
	R-2	+	+	-	+	+	+
	R-3	-	+	-	-	+	-
Soil 20	R-1	+	+	-	+	-	-
	R-2	+	-	+	+	-	-
	R-3	+	+	-	+	-	-

"+"the result is positive
"- the result is negative

Chapter-5: Discussion

Freshwater resources are a vital part of the biosphere and a necessity for ensuring the survival of living things, although only making up 3% of the world's total water supply (Wilson and Carpenter, 1999). (Jackson et al., 2001). Water tainted with harmful bacteria has the ability to spread a number of contagious illnesses to people (Sadeghi et al., 2007). The most prevalent gastrointestinal illnesses spread by water contaminated with patient feces are cholera, salmonellosis, and shigellosis (Cabral, 2010).

The most frequent pathogenic bacteria discovered in connection with fish, soil, and water from several sites of Kaptai Lake were *E. coli*, *Vibrio cholera*, and *Salmonella* sp. Their prevalence was ascribed to the fish ponds being contaminated by animal feces (Abdelhamid et al., 2006). *Salmonella* and *E. coli* were isolated from fish, soil, and water samples, which suggests that animal manure added to fish reservoirs as feed and agricultural fields as organic fertilizer has contaminated the lake. The findings imply that perhaps the microbial quality of the fish under investigation is within acceptable ranges and doesn't now constitute a possible concern to the public health, as per published microbiological criteria mentioned by Gilbert et al. (1996). This could be explained by the little amounts of manure (around 50 kg/ha/week) used. Due to the typically high demand for animal dung to fertilize croplands in the remote areas under study, little is left over for fish feeding in earth dams. As little to no antibiotics are given to the rural animals under investigation, the topic of maximum residue limits in the utilized manure was not raised in order to elicit any reaction that would be worth looking into. However, the variety of possible infections found in the fish samples is concerning, especially at a time when so many people in our communities have impaired immune systems as a consequence of various diseases.

The majority of the time, Salmonella sp. was discovered in soil, water, and fish samples. A significant health issue is the high prevalence of Salmonella in fish and water samples taken from earth dams. The presence of several enteric bacteria in fish, in particular of *Salmonella*, suggests contamination that might pose a risk to human health. It is strongly advised to implement strict rules and monitoring practices along with food safety education for suppliers (fishers and dealers) and, eventually, consumers on different

GHP, GMP, and HACCP topics. Since coliforms are not part of the typical commensal bacteria in fish and water, their presence in fish as well as other samples shows the extent of environmental pollution *E. col*i is a non-indigenous pathogen that contaminates fish or aquatic environment in some way among the microorganisms which were separated and identified (Kvenberg, 1991). Environmental pollution and faecal contamination are indicated by the identification of *Salmonella, Vibrio cholera*, and *E. coli* (Yagoub, 2009) in areas where there has been faecal infiltration from warm-blooded animals, coliforms like *E. coli* are frequently found (Chao et al., 2003). In tiny amounts, the bacterium *E. coli* is recognized as a trustworthy sign of fecal contamination, and in larger amounts, it is an indicator of mishandling (Eze et al., 2011). The only coliform species that is expelled in considerable amounts in feces and is found as a commensal in the human digestive tract and those of other warm-blooded animals is *E. coli* (Geldreich, 1983).

The detection of *Salmonella* and *E. coli* was linked to the faecal material supplied to the fish contaminating the fish sample. Being that the captured fish is handled roughly and kept until consumption, the elevated bacterial loads discovered in the fresh fish somewhere at source point are expected to have a compounding impact. Similar research found *Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae*, and *Escherichia coli* in the gills, intestines, muscles, and skin of *Megalaspis cordyla* and *Priacanthus hamrur* from Royapuram waters in India (Sujatha et al., 2011). This was ascribed to the substantial amount of sewage dumped into the oceans, which might serve as an environment favorable for the survival and proliferation of human infections. Known as opportunistic pathogens, members of the genera *Vibrio* are prevalent in soil and natural water sources. They are significant phytopathogens and causes of human infections (Sujatha et al., 2011).

The TCC is employed to detect possible pathogen, virus, or parasite contamination in a body of water. Using the usual MPN indexing method, the TCC in samples collected from Kaptai Lake was previously studied. It was discovered that the TCC varied between 4 and 140 per 100 mL. The WHO and Bangladesh EQS both recommend that the TCC be zero per 100 milliliters of drinking water. All of the water samples taken from Kaptai

Lake tested positive for coliform, making it potentially hazardous for human consumption. The Kaptai Lake is used as a disposal facility for animal and human wastes by certain locals, contaminating the water. Naturally, each gram of fresh human and animal feces contains 102–104 times more thermotolerant coliform organisms (Gleeson and Gray, 1997). Infants, small children, the elderly, and other individuals with significant immunological impairment may become ill by drinking water that has been polluted with these feces.

In our investigation, the presence of many bacterial pathogens, including *Salmonella* spp., *E. coli*, and *Vibrio* spp., was determined by culture and biochemical testing of fish, water, and soil samples from different sections of Kaptai Lake in order to validate the possible contamination by human diseases. We discovered that the aforementioned infections were present in all of the Kapta Lake samples. Only the fish, water, and soil samples taken from Sampling-1 station had *Vibrio vulnificus*. Other samples taken from other parts of Kaptai Lake were devoid of this bacterial species. All of those bacteria are the main causative agents of several waterborne outbreaks as well as the cause of gastrointestinal disorders with symptoms such as diarrhea, vomiting, nausea, fever, and abdominal discomfort (Craun et al., 2006). Virobacteria are mostly found in water. The distribution of species is influenced by water temperature and salt content. Septicemia and wound infections, which are frequently deadly, are frequently brought on by *V. vulnificus*. The total and fecal coliform bacteria in untreated water meant for consumption should be fewer than 10/100 mL, per recommended values for bacteriological parameters. Additionally, more than 75% of the samples should be in good condition (Busari, 1999).

The identified pathogens might have been contaminated by human waste, animal excretions, fishing operations, persons bathing with skin sores, etc. It has been established that these bacteria can contaminate lake water as a result of storms and stream flows (Jamieson et al., 2005). The forms and frequency of sickness among those who lived close to Kaptai Lake, however, were not documented. Our research shows that the water in Kaptai Lake is tainted with dangerous germs, making it unfit for drinking and domestic usage.

Chapter-6: Conclusions

The bacteria isolation and identification results revealed the presence of commensal bacterial load, such as- *E. coli, Vibrio cholera, Vibrio vulnificus* and *Salmonella* sp.The results revealed that among 20 fish species, *Puntius ticto* ($8.53\pm0.21\times10^{6}$ CFU mL^{-1}) and *Microbrachium rosenbergii* ($2.45\pm0.25\times10^{4}$ CFU mL^{-1}) had the greatest and lowest amounts of bacterial burden, respectively. Moreover, station 12 had the maximum bacterial load in both the soil ($6.13\pm0.66\times10^{6}$ CFU mL^{-1}) and the water ($3.90\pm0.20\times10^{6}$ $CFUmL^{-1}$).The microbial population was found to be higher than the approved safety standard by NAFDAC. Since these microorganisms could contaminate fish and therefore a source of food poisoning; harvesting, handling and cooking should be done properly done so as to reduce the bacterial load. Therefore, the purpose of this study is to offer fundamental knowledge about these bacteria that, when found in fish taken from Kaptai Lake, are likely to result in food-borne illness.

Chapter-7: Recommendations and Future perspectives

Bacteriological analysis is mostly based on the notion of fecal indicator microorganisms Based on the ecology and biology of the causative agents as well as the features and life cycles of the environment, this study provides a broad characterization of the three most significant waterborne bacterial diseases: *cholera*, typhoid fever, and bacillary dysentery. The role of infectious *Escherichia coli* strains as well as evolving microorganisms in drinking water-transmitted illnesses is also briefly highlighted. It introduces and discusses the most significant fecal indicator bacteria as well as the most common bacteria found in human and animal feces (with an emphasis on how these bacteria behave in their host and environmental contexts). You'll also get a quick overview of some of the major contributors to the bacterial feces contamination of natural waterways. In the last item it is discussed which markers of fecal contamination should be employed in contemporary drinking water microbiological examination. In order to ensure that everyone in the world has access to clean water, microbiological management of water supplies must become the standard worldwide.

- Assaying for the presence of various harmful bacteria using culture techniques should be part of a water system's standard operating procedure for basic microbiological examination.
- To complement fecal coliform results, enterococci quantification should be performed whenever feasible.
- Additional research is required to determine if ammonia may be used as a first-stage screening tool in the event of an emergency involving fecal contamination.
- Human and animal feces bacteria in environmental waters: a better knowledge of their ecology and behavior requires investment of time and money.

References

Abdelhamid AM. Gawish MM, Soryal KA. 2006. Comparative study between desert cultivated and natural fisheries of mullet fish in Egypt. II Microbiological concern. Journal of Agricultural Science. 31: 5681 5687.

Acha PN, Szyfres B. 2003. Zoonoses and communicable diseases common to man and animals. Vol. I. Bacterioses and mycoses. 3rd ed. Scientific and Technical Publication No. 580, Pan American Health Organization, Regional Office of the WHO, Washington, USA, ISBN 9275-31580 9. Pp. 384.

Adibe NC, Eze EI. 2004. General Laboratory Techniques for Tertiary Institutions, Mike Social Publishers, Enugu, Nigeria.

Ahmed KKU, Hambrey JB, Rahman S. 2001. Trends in inter-annual yield variation of reservoir fisheries in Bangladesh, with special reference to Indian major carps. Lakes & Reservoirs: Research & Management. 6: 85–94.

Ahmed KK, Hambrey JB. 1999. Brush shelter: a recently introduced fishing method the Kaptai Reservoir fisheries in Bangladesh. NAGA. 22:20-23

Aksu H, Vural A. 2004. Evaluation of microbiological risks in drinking water. Tesisat, 98: 120–131.

Allen MJ, Edberg SC, Reasoner DJ. 2004. Heterotrophic plate count bacteria-what is their significance in drinking water? International Journal of Food Microbiology. 92: 265–274.

Amfo-Out R, Wiafe ED, Kocke BB. 2011. Assessment of water quality in Ahor Lake – Ghana. African Journal of Environmental Science and Technology. 5: 1093–1099.

Baker FJ, Silverton RE. 1985. Introduction to Medical Laboratory Technology. 6th Edition Butterworths, Toronto.

Barua R., Barua S., Fatema-Tuz-Zohora R. M., Uddin M. S., Hasegawa H., Rahman I. M. 2016. Bacteriological and physicochemical characteristics of Kaptai Lake water in

terms of public health significance. International Journal of Scientific Research in Environmental Sciences. 4(2): 31-39.

Bhatt L, Lacoul P, Lekhak H, Jha P. 1999. Physicochemical characteristics and phytoplanktons of Taudaha Lake, Kathmandu. Pollution Research. 18: 353–358.

Cabral JP. 2010. Water microbiology. Bacterial pathogens and water. International Journal of Environmental Research and Public Health. 7: 3657–3703.

Chakma K. 2008. Proposal for Improving Water, Sanitation, Health and the Environment of the Kaptai Lake Chittagong Hill Tracts (CHT), Bangladesh CHT Indigenous Jumma Association Australia, Australia.

Chao KK, Chao CC, Chao WL. 2003. Suitability of the traditional microbial indicators and their enumerating methods in the assessment of faecal pollution of subtropical freshwater environments. Journal of Microbiological and Immunological Infection. 36: 288 293.

Collins CH, Lyne PM, Grange GM. 1989. Collins and Lyne Microbiological Methods, 6th Ed. Butterworth, London.

Craun MF, Craun GF, Calderon RL, Beach MJ. 2006. Waterborne outbreaks reported in the United States. Journal of Water and Health. 4: 19–30.

Dale V, Archer S, Chang M, Ojima D. 2005. Ecological impacts and mitigation strategies for rural land management. Ecological Applications. 15: 1879–1892.

DoF. 2018. Jatiya Matshya Saptah. Department of Fisheries, Government of the People's Republic of Bangladesh.

DoF. 2019. Jatiya Matshya Saptah. Department of Fisheries, Government of the People's Republic of Bangladesh.

Douglas I, Hodgson R, Lawson N. 2002. Industry, environment and health through 200 years in Manchester. Ecological Economics. 41: 235–255.

Eze El, Echezona BC, Uzodinma EC. 2011. Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (*Scomber scombrus*) in a humid tropical environment. African Journal of Agricultural Research. 6(7): 1918 1922.

Fernando C. 1980. The fishery potential of manmade lakes in Southeast Asia and some strategies for its optimization. In: Soerianegara, S (Ed.). Biological Resource Management for Economic Development in Southeast Asia. BIOTROP Special Publication, Indonesia. Pp. 25–38.

Fischer G, Heilig GK. 1997. Population momentum and the demand on land and water resources. In: Greenland, DJ, Gregory, PJ, Nye, PH (Eds.). Land Resources: On the Edge of the Malthusian Precipice? CAB International, Wallingford, England. Pp. 9–29.

Geldreich EE. 1983. Bacterial populations and indicator concepts in feces, sewage, storm water and solid wastes. In: Berg, G. (Ed.), Indicators of viruses in water and food. Ann. Arbor Science Publishers, Inc., Orlando, Fla. Pp. 51 97.

Gilbert RJ, de Louvois J, Donovan T. 1996. Microbiological guidelines for some ready to eat foods sampled at the point of sale. Microbiological Digest. 13: 41 43.

Gleeson C, Gray NF. 1997. The Coliform Index and Waterborne Disease. E & FN SPON, London; New York.

Grimm NB, Grove JM, Pickett STA, Redman CL. 2008. Integrated approaches to long-term studies of urban ecological systems. In: Marzluff, JM, Shulenberger, E, Endlicher, W, Alberti, M, Bradley, G, Ryan, C, Simon, U, ZumBrunnen, C (Eds.). Urban Ecology. Springer US. Pp. 123–141.

Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW. 2001. Water in a changing world. Ecological Applications. 11: 1027–1045.

Jamieson R, Joy DM, Lee H, Kostaschuk R, Gordon R. 2005. Transport and deposition of sediment associated Escherichia coli in natural streams. Water Research. 39: 2665–2675.

Jayana BL, Prasai T, Singh A, Yami KD. 2009. Assessment of drinking water quality of madhyapur-thimi and study of antibiotic sensitivity against bacterial isolates. Nepal Journal of Science and Technology. 10: 167– 172.

Jones AQ, Majowicz SE, Edge VL, Thomas MK, MacDougall L, Fyfe M, Atashband S, Kovacs SJ. 2007. Drinking water consumption patterns in British Columbia: An investigation of associations with demographic factors and acute gastrointestinal illness. Science of the Total Environment. 388: 54–65.

Karmakar S, Sirajul Haque SM, Mozaffar Hossain M, Shafiq M. 2011. Water quality of Kaptai reservoir in Chittagong Hill Tracts of Bangladesh. Journal of Forestry Research. 22: 87–92.

LeChevallier MW, Seidler RJ, Evans T. 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Applied and Environmental Microbiology. 40: 922–930.

Marshall JK, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM. 2006. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. Gastroenterology. 131: 445–450.

Kabir MTH, Rahman MA, Chowdhury P, Haque SM, Miah MI. 2020. Kaptai Lake and fisheries: Gears, species and marketing channel with management practices. Journal of Entomology and Zoology Studies. 8(1): 19-25.

Obi SKC, Krakowiaka A. 1983. Theory and Practice of Food Microbiology (Unpublished manual).

O'Reilly CE, Bowen AB, Perez NE, Sarisky JP, Shepherd CA, Miller MD, Hubbard BC, Herring M, Buchanan SD, Fitzgerald CC, Hill V, Arrowood MJ, Xiao LX, Hoekstra RM, Mintz ED, Lynch MF, Group OW. 2007. A waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio, 2004. Clinical Infectious Diseases. 44: 506–512.

Peace T, Mazumder A. 2007. Tracking patterns of enteric illnesses in populations and communities. Environmental Health Perspectives, 115: 58–64.

Rahman IMM, Begum ZA, Nakano M, Furusho Y, Maki T, Hasegawa H. 2011a. Selective separation of arsenic species from aqueous solutions with immobilized macrocyclic material containing solid phase extraction columns. Chemosphere. 82: 549–556.

Rahman IMM, Begum ZA, Sawai H, Maki T, Hasegawa H. 2013. Decontamination of spent iron-oxide coated sand from filters used in arsenic removal. Chemosphere. 92: 196–200.

Rahman IMM, Islam MM, Hossain MM, Hossain MS, Begum ZA, Chowdhury DA, Chakraborty MK, Rahman MA, Nazimuddin M, Hasegawa H. 2011b. Stagnant surface water bodies (SSWBs) as an alternative water resource for the Chittagong metropolitan area of Bangladesh: physicochemical characterization in terms of water quality indices. Environmental Monitoring and Assessment. 173: 669–684.

Rahman MA, Akter S, Haidar MI and Majumder MWR. Present status (Biodiversity and Conservation) of fish at Chalan Beel in Bangladesh. 2017. International Journal of Zoology Studies. 2(2):31-37.

Rahman MM. 2003. Status of Fish Marketing in Gazipur, Bangladesh, MS Thesis, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Barua R, Barua S, Zohora1 FT, Mutsuddi R, Uddin MS, Hasegawa H, Rahman IMM. 2016. Bacteriological and Physicochemical Characteristics of Kaptai Lake Water in Terms of Public Health Significance. International Journal of Scientific Research in Environmental Sciences. 4(2), pp. 0031-0039.

Sadeghi G, Mohammadian M, Nourani M, Peyda M, Eslami A. 2007. Microbiological quality assessment of rural drinking water supplies in Iran. Journal of Agriculture & Social Sciences. 3: 31–33.

Schleiger SL. 2000. Use of an index of biotic integrity to detect effects of land uses on stream fish communities in west-central Georgia. Transactions of the American Fisheries Society. 129: 1118–1133.

Sujatha K, Senthilkumar P, Sangeeta S, Gopalakrishnan MD. 2011. Isolation of human pathogenic bacteria in two edible fishes, *Priacanthus hamrur* and *Megalaspis cordyla* at Royapuram waters of Chennai, India. Indian Journal of Science and Technology. 4(5): 539 541.

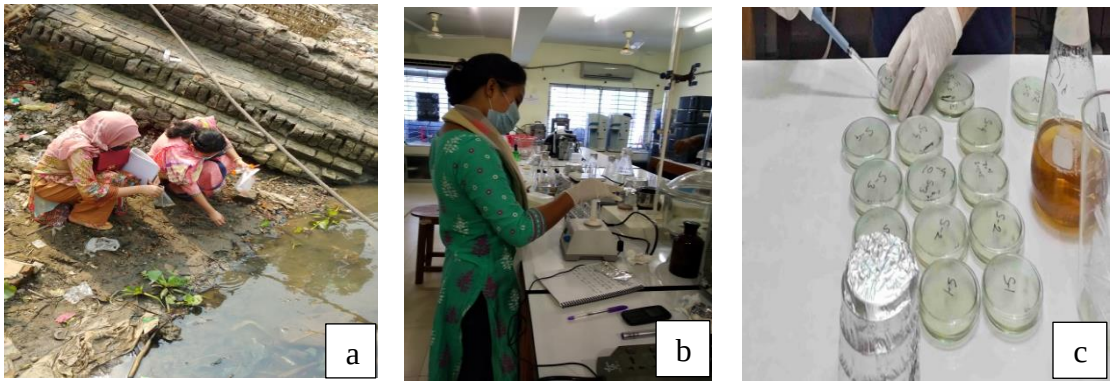
Uddin K, Islam A, Basak S, Rahman M, Bashar M, Mahmud Y. 2014. Species composition and abundance of fishes in the brush shelter fishery of kaptai Lake. Bangladesh Research Publications Journal. 10: 233–237.

US EPA. 2003. List of drinking water contaminants and maximum contaminant levels (EPA 816-F- 03-016). United States Environmental Protection Agency, Washington, DC.

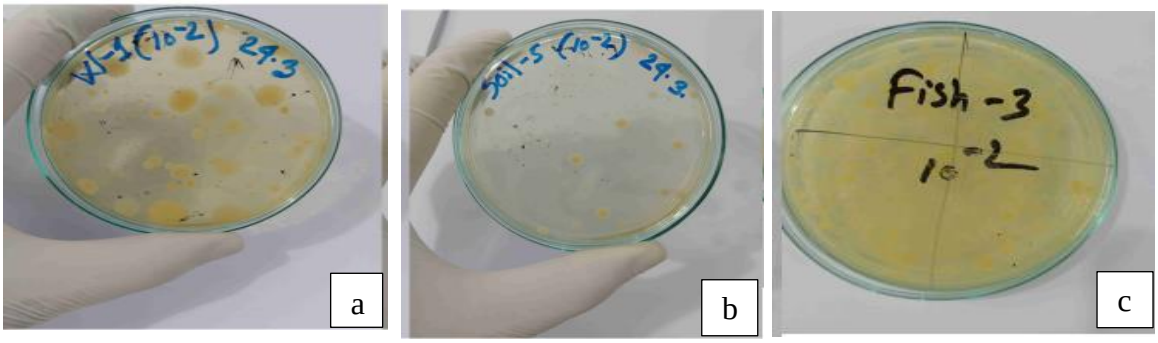
Weir M, Rajić A, Dutil L, Uhland C, Bruneau N. 2012. Zoonotic bacteria and antimicrobial resistance in aquaculture: opportunities for surveillance in Canada. The Canadian Veterinary Journal. 53(6), 619

Wilson MA, Carpenter SR. 1999. Economic valuation of freshwater ecosystem services in the United States: 1971–1997. Ecological Applications. 9: 772–783.

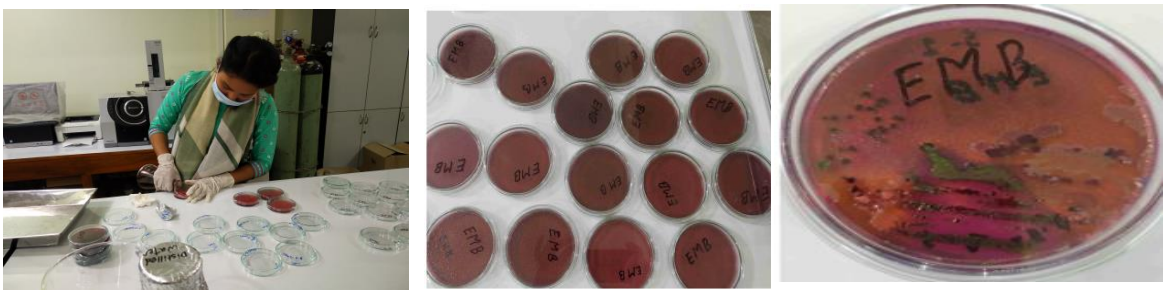
Yagoub SO. 2009. Isolation of *Enterobacteria* and *Pseudomonas* spp. from raw fish sold in fish market in Khartoum State. Journal of Bacteriological Research. 1(7): 85-88.



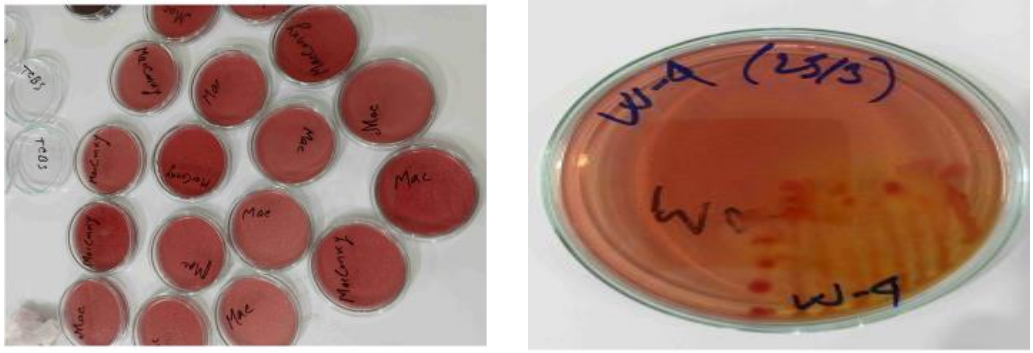
Appendix A: (a) Sample collection (b) Sample mixing (c) Sample Inoculation



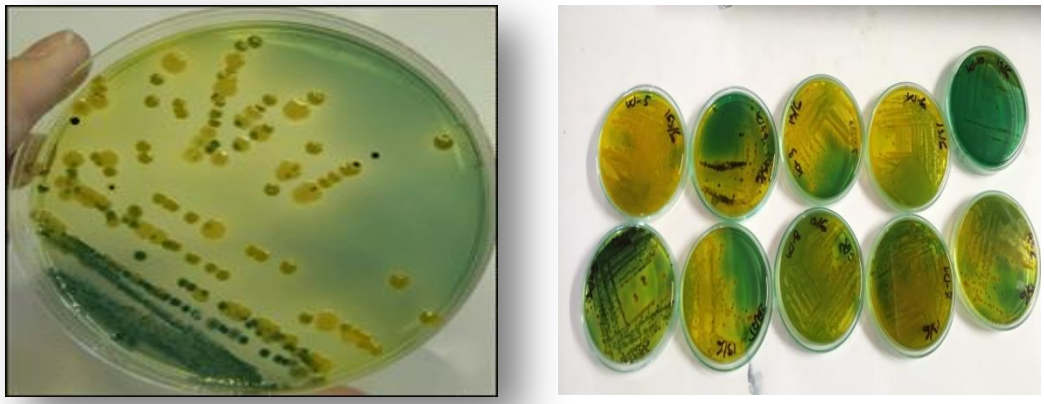
Appendix B: Bacterial colony in (a) Water sample (b) Soil sample (c) Fish sample



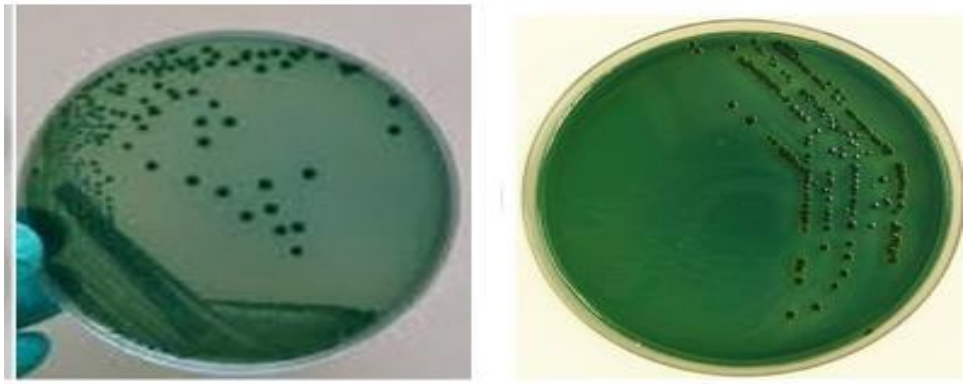
Appendix C: Identification of *E. coli* in EMB Agar



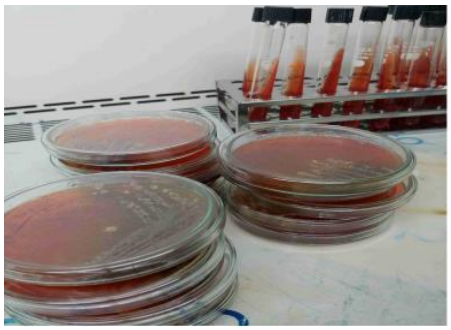
Appendix D: *E. coli* in Macconkey Agar

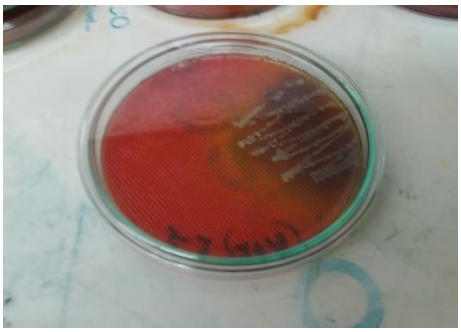


Appendix E: *Vibrio cholera* in TCBS Agar

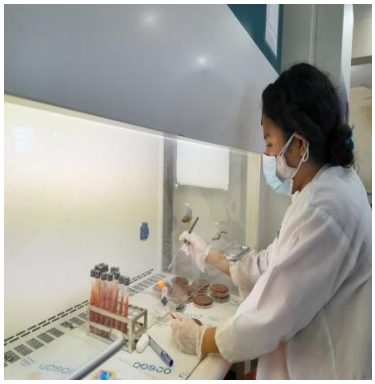


Appendix F: *Vibrio vulnificus* and *V. parahaemolyticus* in TCBS Agar





Appendix G: *Salmonella* in XLD Agar



Appendix H: *Salmonella* and *Shigella* in TSI Agar