CHAPTER I

Introduction

I.1 Introduction

Fishes are important animal vertebrates present in the aquatic environment all over the World. They live on aquatic ecosystems and survive by using their various body parts like gills which are used as a breathing apparatus and fins which are used as a movement apparatus and also they are one of the major biodiversity components that are composed of the river ecosystem (Dalimunthe, 2020). The term biodiversity is essential for understanding the intrinsic worth of all species present on the earth and gives an overall view of the environmental quality which is used for the stabilization of ecosystem protection. Fish is considered as the animal which is related to characterize both intrinsic and extrinsic environmental quality of the world because some microorganisms are ubiquitous and found in the entire environment such as soil, water, air as well as in association with fish body parts like gastrointestinal, skin, lung, etc. Among these, fish is the easy source for studying the overall microflora of a particular environment. Therefore, the study of microbial diversity from fish with their functional capabilities in specific habitat plays an important role in understanding more about microbial ecology and evolution (Porchas and Albores, 2015). Now a day's overall microbial diversity of a particular environment can be easily determined by using the method Metagenomics (Handelsman et al., 1998; Tringe et al., 2005; Alves et al., 2018). Therefore, fish is considered one of the most important biodiversity components of the aquatic environment. So, it is very important to study the distribution of fishes in a particular biosphere to understand the ecological significance of the species. There are many factors that are established for determining the distribution of fishes in river or stream habitats such as altitude, habitat type, water temperature, food availability, predator, ecological barrier, etc. (Arunkumar et al., 2017). The fishes present in various aquatic environments like marine and freshwater environments change their physical characteristics to survive in the specific environment. Some fishes cannot survive in other environments due to which they lose their habitat nature. Due to the loss of specific habitat conditions of nature some fish species are slowly declining from the environment. Fishes are considered as one of

the major groups of vertebrates which have very many effects on human civilization from ancient times by influencing human life in various ways to link the ecological food chain (Mohit et al., 2012; Ubharhande and Sonawane, 2012). Therefore, it is important for balancing the ecosystem.

I.2. Abundance

Abundance study is the relative representation of a species in a particular area (Amber et al., 2015). The numbers of individuals found per sample are count and the ratio of abundance found in one species to another species is called relative species abundance. These are an indicator which is relevant to computing biodiversity. It includes quantity, variety, and distribution, ranging through genetics to species, populations, communities, and ecosystem levels (Gowda et al., 2015; Hashemi et al., 2015). It is noted to be one of the most significant features of the community organization and structure (Ahmed et al., 2018). It provides a brief idea about species diversity and species richness because species richness and relative species abundance describe the key elements of biodiversity (Yadav and Mishra, 2013). The most common natural biodiversity study is fish diversity within different locations because fishes are very important from the biodiversity point of view, enjoying different ecosystems, habitats, and niches of the aquatic environment (Parvathy, 2018). It is considered to be one of the most important aspects of community organization and structure (Ahmed et al., 2018). It provides a brief idea about species diversity and species richness because species richness and relative species abundance describe the key elements of biodiversity (Yadav and Mishra, 2013). The most common natural biodiversity study is fish diversity within different locations because fishes are very important from the biodiversity point of view, enjoying different ecosystems, habitats and niches of the aquatic environment (Parvathy, 2018). The study of fishes are not only important indicators of ecological health and abundance but it also maintains a balance in the food chain by consuming plankton and small animals to form food for many animals, and also fishes are commercially important species that can improve the livelihood of the people around the rivers (Thirumala and Kiran, 2017; Negi and Mamgain, 2013). Fishes are well-suited for studying the relationship between species diversity and size diversity as it is a key components of aquatic environment (Brucet et al., 2017). The relationship between species diversity and size diversity may help to integrate variation at both interspecific and intraspecific levels (Tongnunui et al., 2016). It was observed that the fish diversity is extremely sensitive due to climatic changes because breeding, feeding, migration and other biological activities are affected in the growth of fish diversity (Aziz et

al., 2021). Therefore, it is important to study fish diversity to safe our aquatic biodiversity. There are several methods that were established to determine fish diversity such as Shannon-Wiener diversity index, Margalef's Richness and Pielou's Evenness indices (Negi and Mamgain, 2013; Sweke et al., 2013; Ataguba et al., 2014; Hashemi et al., 2015; Tessema and Mohamed, 2016; Aziz et al., 2021).

I.3. Nutritional significance of the fish

Nutrition is the most important support for the good health of human being, which affords sustenance of body parts, and promotes growth (Srivastava and Srivastava, 2008). The nutrients present in the varieties of foods can be found in various quantities. Among these, fish being an important source of nutrients can play a great role in the supply of nutrition to the human health as well as animal diet (Godfray et al., 2010; Tacon and Metian, 2013). Fishes are the most important inhabitants of the aquatic ecosystem mainly marine and freshwater, which provides cheap and easily digestible proteins to the human population (Pathan, 2013). Fish is the most important aquatic fauna directly or indirectly related to human health and wealth. The nutrients found in the fish species play a significant role in maintaining human and animal health by stopping numerous diseases. It can be considered as 'rich food for poor people' because it contains high nutritional components as compared to the other meat products like beef, goat, lambs, buffalo, and chicken, and also it is available in all the aquatic environments (Uhe et al., 1992; Steffens, 2006; Sujatha et al., 2013; Mohanty, 2015; Mohanty et al., 2015;). The nutrients can be of two types and these are micronutrients and macronutrients. Macronutrients are proteins, carbohydrates and fats, and micronutrients are the minerals and vitamins, which are considered as indispensable dietary elements. These are needed in very small amounts for good functioning of human health. It cannot be synthesis by our body. So, it must be supplied from the outer source to the body (Mohanty, 2015). The deficiency of the micronutrients causes various diseases like anemia, goiter, osteoporosis, stunted growth, coronary heart disease, and genetic disorders (Hsieh et al., 2011; Bhandari and Banjara, 2015). Micronutrients such as mineral and vitamin deficiencies are also recognized as 'hidden hunger', as they determine the health status and quality of our life. Hidden hunger is the persistent lack of minerals and vitamins which affect one-third of people globally according to the United Nation Millennium Summit (2000) (Mohanty et al., 2019). The major nutritional problems that have suffered globally are iron, vitamin A and iodine deficiencies. Currently, the IDA (iron deficiency anemia) is one of the most common global micronutrient deficiencies. It is reported that more than two billion people are

suffering from IDA globally, among these women and children are mostly affected. Mohanty et al. (2019) also mentioned vitamin A deficiency disease such as blindness that affects more than 2.8 million of the children under 5 years of age and more than 740 million people are affected by the deficiency disorders of iodine. Mohanty et al. (2012) mentioned that about 2 billion populations of the third world countries are suffering from the deficiencies of micronutrients. The nutrients such as lipids, proteins, vitamins and minerals help in many ways in maintaining our body healthy and the most abundant and easiest sources of these are considered to be the fish species. Most of the common people are generally eating fish deliciously because of their taste but they do not know their nutritional benefits. To fulfil the nutrient deficiencies among the masses, the government of Nepal has recommended a 30 g fish diet in a day (Gurung, 2016), but people are still unaware of the nutritional benefits of fish consumption, people still prefer food items like mutton, chicken, etc. in place of fish as a diet of food. So, it is the urgent need of the hour to try to increase the awareness about the nutritional benefits of eating fish among the unaware people.

I.3.1. Proximate composition

A proximate composition is an important tool for nutritional analyses of any food materials. It is a mixture of biochemical compositions such as moisture, ash, carbohydrate, protein, fats, etc. The proximate composition is variable from species to species as its results depend on the interactions between physical and biological features like temperature, seasonal changes, food complexity, body size, growth stage, spawning period, etc (Jeyasanta and Patterson, 2015; Mallipudi, 2020). Therefore, the studies on the biochemical composition of edible organisms are important from the nutritional point of view. It was observed that the different fish species showed different proximate composition values. So, the estimation of proximate profiles of a fish species is often necessary to make sure that they meet the requirements of food regulations and commercial specifications (Ahmed et al., 2017).

Moisture

Moisture content is the water content present in the food stuffs. Park (2016) stated that moisture content has been used interchangeably in the literature to designate the amount of water present in foodstuffs and other substances. The determination of moisture content is an important and most widely used analytical measurement i.e. the key quality measure in the processing and testing of food products (Park, 2016). The amount of moisture is a measure of quantity and yield of solid foods and is used normally as the stability, index of economic

value, and as the quality of food products. The moisture content varies from species to species. Generally, aquatic food contains a high percentage of moisture contents such as fish contains almost 70–80 % of moisture (EIShehawy et al., 2016; Park, 2016; Ahmed et al., 2017; Mallipudi, 2020).

Ash

Ash content is the residue that is formed after burning a sample at a very high temperature (Park, 2016; Mallipudi, 2020). In analytical chemistry, the determination of ash content is used for mineralization. Ash is one of the important components of proximate composition, which is used for the determination of mineral elements percentage of any food or biological materials. Unlike moisture, ash content also varies from species to species. It has been reported that fish species contain ash ranging from 1.10 to 8.00 % (EIShehawy et al., 2016). Fish meal is produced from whole fish or fish offal and thus contains also the skeleton and the bones. The skeleton and the bones are important sources of ash and minerals (e.g. calcium and phosphorus) and fish feeds with high fish meal levels have also high ash levels (Terpstra, 2015).

Crude protein

A protein is an important nutritional component, which regulates various body functions of our health. Crude protein is the amount of protein that is calculated from the determined nitrogen. Protein is the major organic compound of our health and consists of amino acids. It is an abundant macromolecule in the biological system. Proteins in the body tissues are built using about 23 amino acids. Of these, 10 are essential amino acids that must be supplied from the diet (Craig and Helfrich, 2009). The protein from outer sources i.e. obtained from diets is the source of essential amino acids and it provides nitrogen for the synthesis of non-essential amino acids. Proteins (or amino acids) are required for body maintenance, reproduction, growth, and the replacement of depleted tissues. In addition, certain amino acids are readily converted to glucose to provide an essential energy source for some critical body organs and tissues such as the brain and red blood cells (Cho, 1990). Therefore, a portion of the dietary protein is always used as an energy source in fish. Not all dietary proteins are identical in their nutritional values. To a large extent, the bio-availability of a protein source is a function of its digestibility and amino acid makeup. Some protein feedstuffs which contain a high level of crude protein (% of total nitrogen × 6.25) are low in amino-nitrogen and do not contribute toward the requirement of amino acids (Cho, 1990). A fish contains about 17–18

% protein and this percentage is rather constant for various types of fish and other animal species (Terpstra, 2015). Proteins are present in diverse forms such as structural elements, receptors, signalling molecules, enzymes, hormones, antibodies, and so forth, which are having specific biological functions. It provides essential amino acids which are related to the development and maintenance of muscle tissues (Cho, 1990; Mohanty et al., 2014). It is an important component of every cell in the body, mostly hair and nails are made up of protein. It is used to build and repair our tissues when damaged, as it is an important component of the building block of bones, muscles, cartilage, skin, and blood (Noguch and Djerassi, 2009). So, the finding of a high protein source is very important for human as well as animal health and nutrition. Fish can sometimes serve as a solution for health problems because it can regulate various body functions of our health. In many developing countries, fish is the main source of animal protein which is essential for providing micronutrients to vulnerable populations (Mohanty and Singh, 2018). The proteins obtained from fish make up to 37 % of the total protein intake and 76 % of the animal protein intake which contributes to the well-being of millions of people around the world (Vilain et al., 2016).

Crude fat

Fats or lipids are organic compounds that are important components of animals, plants, and microbes for many metabolic purposes (Coloso, 2014). Crude fat is the mixture of fat-soluble substance of a crude sample. It is the free lipid content or ether extract which was used to measure fat percentage in the food product, traditionally. The total fat content is determined on the basis of the solubility of the lipids in organic solvents (non-polar) like petroleum ether, hexane, etc., because, they are not soluble in water, but are soluble in organic solvents like ether and alcohol (Coloso, 2014). Lipid consumption in the diet acts as a source of energy mainly and essential fatty acids as well that cannot be made in the body of the animal, and they are also the important sources of fat-soluble vitamins. The lipid components provide essential fatty acids for the growth and dietary fuel of our body (Cho, 1990). It provides secondary storage of heat and energy that is 1 g of fat or lipid gives 9 kilocalories of energy (Coloso, 2014). Fish lipids are highly digestible by all species of animals and are excellent sources of the essential polyunsaturated fatty acids (PUFA) in both the ω-3 and ω-6 families of fatty acids (Miles and Chapman, 2006). The beneficial effects of lipids in fish diets are particularly evident in the structure and function of cell membranes and it helps to controls the passage of nutrients and other substances in and out of the cell interior

(Miles and Chapman, 2006). The unsaturated fatty acids present in the fish fats do not cause heart problems as they can help in reducing the blood triglycerides (Akpambang, 2015).

The dietary lipids are associated with cholesterol, triglycerides, and high-density lipoproteins (HDL) that are important constituents of the lipid fraction of the human body for various intermediate purposes (Cox and Palmieri, 1990). Cholesterol is the main sterol synthesized by animals, which is present in every cell of the body and maintains the normal function of all animal cells. It is the fundamental element of cell membranes and is also a major precursor of various critical hormones viz. adrenal and gonadal steroid hormones and bile acids and vitamin D (Cox and Palmieri, 1990; Hanukoglu, 1992; Sarma, 2015). Triglycerides are fatty acid esters of glycerol and represent the main lipid components of dietary fat and fat depots of animals. They store unused calories and provide our body with energy. Both cholesterol and triglycerides are non-polar lipid substances (insoluble in water) and do not travel freely through the bloodstream inside the body (Cox and Palmieri, 1990). Lipoproteins are complex particles, which are composed of multiple proteins. Their main purpose is to transport fat molecules in water, as in blood plasma or other extracellular fluids and they are typically composed of 80-100 proteins or particles (Sarma, 2015). Three types of lipoprotein generally occur and can be categorized based upon how much protein with the amount of cholesterol is present and these are Low-density lipoproteins (LDL), High-density lipoproteins (HDL), and Very low-density lipoproteins (VLDL). LDL generally is called “bad” cholesterol because it carries cholesterol to tissues including the arteries and that remains most of the cholesterol in the blood is the LDL form. The higher levels of LDL can increase the risk for heart disease (Colpo, 2005). It is universally known as a major risk factor for cardiovascular disease and type 2-diabetes, whereas LDL particle is more valuable as a risk indicator of LDL-attributable atherosclerosis (Otvos et al., 2011; Chen et al., 2017). Further, the small dense LDL subfraction shows a positive association with coronary artery disease and is thought to be anatherogenic lipoprotein (Zeljkovic et al., 2010; Diffenderfer and Schaefer, 2014). HDLs are sometimes called “good” cholesterols or reverse cholesterols transport as these carry cholesterol from the other parts of body and then back to the liver. A low level of HDL can increase the risk for heart disease and atheromatous disease in the arteries (Daniels et al., 2009). VLDL, sometimes also called “bad” cholesterol because, like LDL, it too contributes to the build-up of plaque in the arteries. But VLDL and LDL are not the same. VLDL mainly carries triglyceride and LDL mainly carries cholesterol. Therefore, the triglyceride, cholesterol with lipid components maintains some level of limits for proper maintaining of a body function (Brown, 2007).

Carbohydrate

Carbohydrates are the important nutrient components which are the main sources of energy in most of the animal diets and synthesized by plant via photosynthesis (Gatlin, 2010). These are classified based on the composition, structure, constituent sugars, degree of polymerization, and glycosidic linkage. All carbohydrates are not directly indigestible to humans. Carbohydrates like cellulose and other fibrous components are indigestible to humans and animals like fish (Gatlin, 2010). The nutritional value of carbohydrates varies among fishes i.e. warm-water fish can use much greater amounts of dietary carbohydrate than cold-water and marine fish (Washington, 1993). Carbohydrates are inexpensive and easily available sources in formulated feed which are efficiently utilized in several fish species and they may serve as precursors for the dispensable amino acids and nucleic acids, which are necessary for various metabolic intermediates for growth (Washington, 1993). Carbohydrate provides energy for various body metabolisms and some dietary carbohydrate is converted to lipid and deposited in the body, which gives energy for us (Gatlin, 2010). Animal tissues such as the liver and muscle contain small concentrations of soluble carbohydrates in the form of glycogen, which is structurally similar to starch. This glycogen reserve can be rapidly mobilized when the body needs glucose (Gatlin, 2010).

I.3.2. Minerals

Minerals are the chemical components that act as essential nutrients for the functioning of our body (Soetan et al., 2010). These cannot be biochemically produced by the living organisms. Therefore, in the human body, most of the minerals come from the outer sources through consumption of plants, animal foods and from the drinking water (Mohanty, 2015). The five major mineral elements found in our body are Ca, P, K, Na, and Mg, and all the other remaining elements are called trace elements *viz.* S, Fe, Co, Cu, Cl, Cr, Zn, Mb, Mn, I, Se, etc. (Oteng et al., 2020). These elements have some specific biochemical functions in our body. The elements carbon (C), hydrogen (H), and oxygen (O) are the most abundant element. The element Ca makes up about 1.5 % of weight of adult body with 99 % of it containing in the teeth and bones (Awuchi et al., 2020). The other major mineral elements like Na, Cl, K, S, and Mg make up only about 0.85 % of body weight. These eleven chemical elements C, H, O, N, Ca, K, P, Na, Cl, S, and Mg make up 99.85 % of the body (Nandi et al., 2021).

Calcium (Ca)

Calcium is one of the most plentiful (abundant) minerals found in the human body. It is distributed throughout the body and about 99 % of the calcium is found in the bone and 1 % in the blood circulation with the plasma level of the body (Phiraphinyo et al., 2006). Calcium levels in the blood and fluids must be needed to be maintained for normal physiological functioning for survival. It plays a vital role in many body functioning. Therefore, lack of calcium in the body suffers various deficiency diseases like osteoporosis, heart disease, and hemorrhage and it is required for growth of fetal, milk production, and growth in young children (Larsen et al., 2000; Phiraphinyo et al., 2006). Various calcium sources are there and among these fish can play major mineral-rich sources including Ca (Larsen et al., 2000; Mohanty et al., 2016; Balami et al., 2019; Kiczorowska et al., 2019). The whole part of small fish species are an extremely Ca-rich food source (Balami et al., 2019). Fish-bones can be used as a food supplement to enhance the calcium content in our body (Phiraphinyo et al., 2006). Japanese people consume large amounts of small fishes and these are serving as an significant source of dietary Ca because fish and its bones are rich sources of Ca (Shimosaka et al., 1996). The daily recommended intake of Ca is 400 to 500 mg/day for adults as per WHO/FAO (Tilami and Sampels, 2017).

Potassium (K)

Potassium is an essential mineral for the proper functioning of all cells, tissues, and organs in the human body and is the major cation of the intracellular fluid (Pohl et al., 2013). It plays a key role in normal digestive and muscular function. It maintains internal fluids and electrolyte balance alongside sodium. It is important for renal regulation of acid-base balance. It is also important for heart function and in the nerve cells, it generates sodium-potassium flux which helps to generate muscle contractions and regulates the heartbeat (Pohl et al., 2013). Adequate dietary K intake is important for bone and heart issues, and it decreases the risk of coronary heart disease and stroke, and low K intake along with high intake of salt raises blood pressure and cardiovascular disease risk increases (He and MacGregor, 2008; Weaver, 2013). Pohl et al. (2013) reported that normal plasma levels for potassium in adults range from 3.5 to 5.0 mEq/L and this balance is usually maintained in adults on an average dietary intake of 80 to 200 mEq per day. WHO (World Health Organisation) recommends K intake from food sources to reduce risk of stroke, blood pressure, cardiovascular disease, and heart disease. WHO suggests K intake of at least 90 mmol in a day (3510 mg/day) for adults (WHO, 2012). Therefore, it is important to find out the potassium sources available in nature.

In that regard, various scientists reported that fish is also a common source of potassium. Pal et al. (2018) reported that fish is a good source of almost all the minerals present in seawater. Mogobe et al. (2015) reported that fish is commonly found in natural water bodies and well known for its superior nutritional quality with a very good supply of essential minerals. Steffens (2006), Mohanty et al. (2016), Teame et al. (2016), Paul et al. (2018), and Kiczorowska et al. (2019) reported that the fish species are important sources of K.

Sodium (Na)

Sodium ions are the major cation of extracellular fluid (Pohl et al., 2013). It is an important electrolyte involved in osmoregulation and the acid-base balance in the body (Gatlin, 2010). Sodium is an essential chemical element that is required for the normal maintenance of the human body that participates in several biochemical reactions like sodium along with potassium and play a key role in the transmission of nerve impulses and keeping electrolyte in balanced (Mogobe et al., 2015). Pohl et al. (2013) mentioned that normal plasma levels for sodium in adults range from 136 to 146 mEq/L and this is normally maintained by an average dietary intake of 90 to 250 mEq per day. Minerals like sodium are commonly found in fish flesh (Ahmed et al., 2017). Fish has a big potential for the strategy to provide a variety of nutrients including essential elements to the body (Mogobe et al., 2015; Mohanty et al., 2016; Paul et al., 2018).

Magnesium (Mg)

Magnesium is an important mineral in our body. It is found in bony tissue as well as complexed or protein-bound in all tissues. The highest concentration of magnesium is found in hard tissue of the dermal, skeletal bones, and scales (Bijvelds et al., 1998). It has several functions in our body. It is the most abundant cations of the cell. It acts as a cofactor for more than 300 enzymes that regulate several functions such as neuromuscular conduction, muscle contraction, bone and scale mineralization, myocardial contraction, glycemic control, osmoregulation, respiratory adaptation, energy metabolism, potential antiarrhythmic agent and protein synthesis, and blood pressure (Reigh et al., 1991; Kotecha, 2016; Alawi et al., 2018). It also regulates membrane permeability, $Na^+/K^+/Cl^-$ and K^+/Cl^- symport activity, insertion of proteins into membranes and cation channels (Bijvelds et al., 1998). Mg^{2+} is a second messenger for hormone action (Bijvelds et al., 1998). Magnesium supplements can modestly reduce the frequency of migraines (Edelstein and Mauskop, 2009). The main determinant of Mg balance is the level of serum magnesium (Ghosh and Joshi, 2008).

Magnesium is an important mineral constituent of fish muscle (Mogobe et al., 2015; Pal et al., 2018). Alawi et al. (2018) mentioned that according to the United States Food and Nutrition Board, the recommended daily allowance for magnesium is 420 mg for adult males and 320 mg for adult females.

Iron (Fe)

Iron is an essential mineral component of our health that takes part in various body metabolism of our system. It is especially important for hemoglobin which can transports oxygen throughout the body (Mohanty, 2015). It is occurred in physiological concentration and can regulate various biochemical processes in both humans and animals (Luczynska et al., 2009). A person with a deficiency of this element suffers various deficiency diseases. One of the most common worldwide iron deficiency diseases is anemia (Renzo et al., 2015). Anemia affects more than 3.5 billion people globally and the majority of people living with this condition are women and children in the developing world (Armstrong et al., 2017). Armstrong et al. (2017) mentioned that according to WHO, 66.4 % of pregnant and 57.3 % of non-pregnant women of reproductive age suffer from the anemia primarily due to the deficiency of Fe. It can also associate with maintaining a healthy immune system (Mohanty, 2015). Ahmed et al. (2017) mentioned that iron is a commonly found mineral in fish. Mohanty (2015) reported that fish is a useful source of iron. Pal et al. (2018) reported that fish contain the mineral iron with high bioavailability. Mogobe et al. (2015) mentioned that iron is a commonly found mineral in fish flesh.

Copper (Cu)

Cu is an important trace element that is needed for various body metabolisms in the living organisms (Woody and Neal, 2012). It is required in minute quantities for body function. The high amount of Cu is toxic and creates deleterious effects for creatures (Astani et al., 2018). Cu is toxic to aquatic species when it is just above the limit and causes harm to the species (Woody and Neal, 2012). Trace element Cu is essential to maintain its level for the normal metabolism of the body. Therefore, it is essential to aware of the knowledge about the appropriate level of it because a little amount of increased level leads to the serious health issue. Woody and Neal (2012) mentioned that humans need approximately 1–2.5 mg Cu daily. Minute quantities of Cu can take part in various functions like nervous system function, which forms part of many enzymes. Carpeni et al. (1999) mentioned that Cu is found in cuproenzymes such as tryptophan oxygenase, cytochrome-c oxidase, superoxide dismutase

and monoamine oxidase. Overdoses of Cu leads to several diseases like gastrointestinal distress, nausea, vomiting, abnormal bone development, headache, aortic aneurisms, and extreme higher doses can cause coma and even death (Woody and Neal, 2012). The increased level of Cu pollutant in the water bodies may be due to various reasons like anthropogenic activity, urbanization and disposal of industrial effluents into freshwater bodies and the biomagnification of these trace metals in the ecosystem is a major threat to human life (Uysal, 2011; Ejike and Liman, 2017; Lakshmanan et al., 2019). Cu is extremely lethal to freshwater fish species at the concentration range of 10–20 ppb (Woody and Neal, 2012).

Zinc (Zn)

Zinc is an important micronutrient which can play key roles in living processes for both human and animals because it is involved in more than 300 metabolic functions (Carpeni et al., 1999; Bhowmik et al., 2010; Vilain et al., 2016). Zinc is mostly found as a cofactor in enzyme reactions (Mogobe et al., 2015). Zinc is an integral part of about 20 metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase (Watanabe et al., 1997; Lokuruka, 2012). It is connected with prostaglandin metabolism and also may have a structural role in nucleoproteins (Watanabe et al., 1997). Zinc is the second most prevalent vestigial element in the human body (Sousa et al., 2019). It plays a great role in the maintenance of physiological processes, metabolism, signaling, transduction, cell growth, and differentiation, and is required for the synthesis of DNA, normal growth, gene expression, gene regulation, cell division, growth, and immune competence (Watanabe et al., 1997; Vidyavati et al., 2016; Chasapis et al., 2020). Zinc is also critical to tissue growth, wound healing, taste acuity, connective tissue growth and maintenance, immune system function, prostaglandin production, bone mineralization, proper thyroid function, blood clotting, cognitive functions, fetal growth, and sperm production (Bhowmik et al., 2010). Zinc is abundantly present in the synaptic vesicles and plays an essential role in learning and memory (Chasapis et al., 2020). Zn can perform various roles in our body within the limits because excess amount of Zn in the body lead to toxic or poisoning and also below the level, Zn causes several deficiency diseases (Weiling et al., 1991; Uysal, 2011; Ejike and Liman, 2017; Astani et al., 2018). Children, pregnant women, and elderly people are more at risk of zinc deficiency (Berhe et al., 2019). Zinc deficiency is dangerous, and its expression depends on age (Vilain and Baran, 2016). The recommended dietary intake, expressed as elemental Zn, is 11 mg for adult males and 9 mg for adult females (Sousa et al., 2019). Therefore, it is very essential to search for available Zn sources for our healthy life. It has been reported that

zinc bioavailability is maximal in aquatic animals particularly fish and seafood (Watanabe et al., 1997; Mogobe et al., 2015; Mohanty, 2015).

Manganese (Mn)

Manganese is an essential constitutive heavy metal of our body, which is used in various ways to regulate our body. It can take part in various functions such as synthesis of a series of various essential enzymes, hormones, activation of certain metalloenzymes, immunological system function, nervous system function, and cofactors for brain function such as glutamine synthetase, superoxide dismutase (Watanabe et al., 1997; Arndt et al., 2014; Harford et al., 2015; Tuzuki et al., 2017; Agwu et al., 2018). Manganese plays an essential role in the regulation of cellular energy, bone and connective tissue growth, blood clotting, and also an important cofactor for a variety of enzymes, including those involved in neurotransmitter synthesis and metabolism (Sousa et al., 2019). Manganese is required for various physiological functions of our body but it may become very toxic to the organisms at concentrations above the optimal level (Noureen and Javed, 2017; Tuzuki et al., 2017). Therefore, various scientists reported an adequate level of Mn. Sousa et al. (2019) mentioned that the adequate intake of Mn for adult men is 2.3 mg/day, for women is 1.8 mg/day and the tolerable upper intake level for adults is 11 mg/day. Avila et al. (2013) mentioned that adequate intake of Mn for newborns (less than six months of age) is 3 µg/day, at seven to twelve months of age, its level 600 µg/day, in children one to three years of age, its level approximates 1.2 mg/day and in children four to eight years of age, its level increases to 1.5 mg/day. Agwu et al. (2018) reported that fish is an excellent indicator for heavy metal contamination in aquatic and marine environments because they occupy different levels of the food chain. Ekeanyanwu et al. (2010) reported that pollution of the aquatic environment with heavy metals has become a worldwide problem in recent times because they are indestructible and most of them have toxic effects on organisms. It was observed that higher levels of manganese in the aquatic ecosystems are mainly attributed to various anthropogenic activities, industrial and mining operations (Noureen and Javed, 2017; Tuzuki et al., 2017). Therefore, it is very necessary to find out the Mn concentration in aquatic species fish.

I.3.3. Fatty acids

The fatty acid is generally a long hydrocarbon chain having a terminal carboxylic group and it is found either in saturated or unsaturated form. Saturated fatty acid (SFA) is a simple form of fatty acid where it doesn’t have any double bonds, but unsaturated fatty acids (UFA)

have one or more double bonds in between them. Most naturally occurring fatty acids have 4 to 28 carbon numbers with unbranched hydrocarbon chains. These form of fatty acids play a very significant role in our body and play various body functions which are necessary for a healthy body. It can serve as a dietary source of fuel for humans as well as animals and also important for structural components of cells. People with deficiency or inappropriate concentrations of these components may suffer various fat-related diseases. Therefore, it must be supplied from outer dietary sources to the body. For this, we need to find out the easiest fatty acid sources which can help people to maintain their good health easily. There are various sources of fatty acids. Among these, fish can play a great role in the fatty acid sources because fish contains all the essential fatty acids (EFA). Fish is the easiest source of fatty acids as it is available in all the aquatic environments of the world (Steffens, 2006; Mohanty, 2015; Mohanty et al., 2019). The fatty acids found in fishes are mainly composed of ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA; C20:5), docosahexaenoic acid (DHA; C22:6) and α-linolenic acid (ALA; C18:3) (Watters et al., 2012; Cervera, 2020). These groups of fatty acids are very much essential for our body and known as essential fatty acids because our body cannot synthesize these PUFAs and these must be supplied to the human body through dietary sources such as fish species. These can regulate various body functions such as for the development of Babies' early nerve cells membranes of the cerebral cortex, can boost fetal growth, can improve mental health, can protect the heart and behavioral disorders, and can contribute positively to the brain functioning and memory of both adults and children (Swanson et al., 2012). The cardioprotective role of dietary fish intake includes prevention of coronary heart disease, reduced incidence of myocardial attack and protection against thrombosis and heart arrhythmia, reduced risk of sudden death (Albert et al., 2002). Fish consumption also has a positive impact on eyesight because the fatty acid found in fish i.e. DHA is integrated into the nerve cells of the retina during pregnancy (Swanson et al., 2012). The most effective function of fish lipid is mainly preventing the diseases like cardiovascular diseases (Nejad and Bikdeli, 2014). DHA together with EPA is used to prevent several diseases like dementia, atherosclerosis, Alzheimer's disease, rheumatoid arthritis, and age-related macular degeneration (Cebrian et al., 2015). Simopoulos (2002) suggested that for an adult it is needed to take the fatty acids (EPA and DHA) of at least 0.22 g each on a daily basis. Many countries have started to set up their recommendations for the daily intake of fatty acids such as EPA and DHA. Therefore it is important to find out the high percentage of fatty acid sources. It has been reported that freshwater fishes are high sources of fatty acids (Ugoala et

al., 2009; Robert et al., 2014; Vasconi et al., 2014). Therefore sufficient amount of fish as a source of fatty acids must be included in the dietary lipids.

I.3.4. Amino acids

Amino acids are organic compounds containing C, H, O, and N in the form of functional groups viz. amine (-NH_2) and carboxylic acid (-COOH) along with a side chain (alkyl group) specific to each amino acid (Azad, 2018). About 500 naturally occurring amino acids are known, but only 20 of them appear in the genetic code (Wagner and Musso, 1983). The amino acid residues (in the form of protein) are the second-largest component of human muscles and other body tissues (Azad, 2018). So, it is known to as the building blocks of proteins that play numerous critical roles in human body. They are needed for many vital processes like the synthesis of hormones and neurotransmitters (Dalangin et al., 2020). The human body needs 20 different amino acids to grow and function properly. There are only 9 amino acids which are classified as the essential amino acids (EAA) out of 20 amino acids, because these EAA can't be produced in the human body and must be obtained through the diet only (Ugoala et al., 2009; Wu, 2010; Bojarska et al., 2019). These 9 EAA are isoleucine, histidine, leucine, methionine, lysine, phenylalanine, tryptophan, threonine, valine, and their derivatives. Phenylalanine is the main precursor for neurotransmitters and plays an integral role in various structures and functions of proteins and enzymes (Humphries et al., 2008). Valine is the amino acid that consists of three branched-chains linkages. It helps to stimulate muscle growth, regeneration and also involved in energy production (Zaman and Lin, 2007). Threonine is a principal part of structural proteins viz. collagen and elastin, which are important components of the skin and connective tissue (Li and Wu, 2018). It plays an important role in fat metabolism and immune function (Qi et al., 2019). Tryptophan is important for maintaining proper nitrogen balance and is a precursor to serotonin, a neurotransmitter that regulates our body's appetite, sleep, and mood (Nayak et al., 2019). Methionine is a powerful antioxidant and a good source of sulfur, which prevents disorders of the skin, hair, and nails, also it assists the breakdown of fats which helps to prevent a build-up of fat in the liver and arteries (Nachimuthu et al., 2015). Leucine is a branched-chain amino acid that is critical for production of protein and muscle reparation. It also helps to regulate blood sugar levels, growth hormones and to stimulate wound healing (Sarojnalini and Hei, 2019). Isoleucine is also three branched-chain amino acids that are involved in the immune system, hemoglobin production, energy regulation, muscle metabolism, and muscle tissue (Bojarska et al., 2019). Lysine is used for the production of every immunological protein,

antibody, antigen, hormone, growth factor, and cytokine (protein) (Zaman and Lin, 2007). Histidine is used for histamine synthesis, which is a neurotransmitter and is important for digestion, immune response, and sexual function. It is critical for maintaining the myelin sheath protective barrier that surrounds our nerve cells (Bojarska et al., 2019; Sarojnalini and Hei, 2019). Wu (2016) reported that the dietary requirements of EAA for a healthy adults human based on FAO/ WHO/UNU (2007) for their daily allowances per 1 kg of body weight are isoleucine **(**20 mg/kg), histidine **(**10 mg/kg), tryptophan (4 mg/kg), lysine **(**30 mg/kg), threonine (15 mg/kg), leucine **(**39 mg/kg), valine (26 mg/kg), methionine with the non-essential amino acid (NEAA) cysteine **(**15 mg/kg), phenylalanine with the non-essential amino acid (NEAA) tyrosine **(**25 mg/kg). Thus, these essential amino acids perform various roles in our body such as energy production, tissue growth, nutrient absorption, and immune function (Rose, 2019). Therefore, we must need to know the high EAA sources which are required for our body. In this regard, various researchers reported that freshwater fishes are the most common and suitable sources of these essential amino acids (Bicudo and Cyrino, 2009; Osibona et al., 2009; Paul et al., 2018; Romharsha and Sarojnalini, 2018).

I.3.5. Vitamins

Vitamin is an essential micronutrient that is needed in small quantities for the proper functioning of body metabolism to sustain life. It cannot be synthesized in the organism either at all or not in sufficient quantities and therefore we must need it through different diets because different vitamins have different roles and are needed in different quantities. Vitamins based on their solubility can be classified into two main groups i.e. water-soluble and fat-soluble. Four fat-soluble vitamins are vitamin A, D, E and K. Nine water-soluble vitamins are eight B complex group vitamins and vitamin C. The water-soluble vitamins are easily dissolved in water and are readily excreted from the body but fat-soluble vitamins are stored in adipose tissues, which are not absorbed directly into the bloodstream, it transported through the lymphatic system and then released into the bloodstream (Thompson, 2021). Fat-soluble A and D vitamins are essential nutrients to maintain the normal body metabolism and functions and these are vital for the skeleton, animal growth, vision development, immune function, and reproduction (Qing-Song et al., 2007). Vitamin B plays various essential roles in cellular function (Kennedy, 2016). Vitamin C acts as a cofactor of many enzymes, which are involved in photosynthesis, hormone biosynthesis, regeneration of antioxidants, and vitamin E function as antioxidants (Mondo et al., 2020). Therefore, these vitamins play different specific and vital functions in health and can cause health problems when they are

either lacking or in excess intake (Uribe et al., 2017; Awuchi et al., 2020). The vitamin supply for a healthy life depends on the diet; but the foods that contain the necessary vitamins can have reduced vitamin contents after processing, storage, or cooking (Lee et al., 2018; Awuchi et al., 2020). Therefore, various scientists reported that fishes are good sources of some vitamins that are easily accessible (Fenwic, 1984; Rao and Raghuramulu, 1995; Lu et al., 2007; Thorgilsson et al., 2008; Lock et al., 2010; Youn et al., 2014; Lehmann et al., 2015; Merdzhanova et al., 2015; Balami et al., 2019; Tirakomonpong et al., 2019; Hasselberg et al., 2020; Nordhagen et al., 2020).

I.4. Metagenomics

The term metagenomics was first used by J. Handelsman in 1998 (Ghosh et al., 2018; Kimura, 2018; Chauhan, 2019). It is referred to as the assembly of genes-sequenced from the environments which give us better knowledge about the structure of the community and their physiological significance in the ecosystem (Ghosh et al., 2018; Chauhan, 2019). It is a culture-independent approach with the aim of access to the physiology and genetics of microorganisms growing in an ecosystem (Chauhan, 2019). It has been described as a direct extraction of DNA from an environment such as water, soil, gut microbiome, etc. (Chauhan, 2019). Therefore, metagenomic DNA represents the whole genetic information of available microorganisms that could be explored directly without culturing those (Mahapatra et al., 2020). The isolated metagenomic DNA from the environmental sample is partially digested and cloned into a suitable vector and then transformed into a suitable host organism (Garza and Dutilh, 2015; Kimura, 2018; Chauhan, 2019). This study has enriched our understanding of the uncultured microflora of the microbial world and helped in encountering novel genes (Handelsman, 2004). It provides unlimited resources for the development of natural products, bioactive compounds, enzymes, novel genes, and bioprocesses that may substantially impact biotechnological and industrial applications (Handelsman, 2004; Ghosh et al., 2018; Kimura, 2018; Chauhan, 2019).

Two major strategies have been used to identify novel natural compounds or genes related to biocatalysis from environmental samples (Kimura, 2018; Chauhan, 2019). These are

1. Function-based screening method for natural compounds and novel genes in metagenomics. It has been performed by detecting the enzymatic activity on the plates or in the liquid.

2. Sequence-based screening method which is done by using the PCR method with oligo primers targeting the conserved region of gene families. It is designed based on the sequence information in databases. Furthermore, the complete and random sequencing of metagenomic libraries presents useful information on novel genes. In addition to this large-scale shotgun sequencing of metagenomic DNA by a next-generation sequencer has provided genomic information on unculturable microbes in the environment.

I.5. 16S rRNA Gene

The prokaryotic 16S rRNA gene serves as the ideal molecular-marker for distinguishing the bacteria within a specified sample as it is present within all the bacterial genomes (Ellermann et al., 2017). The 16S rRNA gene sequence is about 1,550 bp long, which is composed of both variable and conserved regions (Clarridge, 2004). It contains 10 highly conserved regions and 9 variable regions (Ellermann et al., 2017). In clinical microbiology, it is used as a marker for bacterial identification or assignment of close relationships at the genus and species level (Clarridge, 2004). Because of its ubiquity across all domains, it provides valuable phylogenetic information for comparison to database collections (Bartram et al., 2011). Due to this unique structure, it is well-suited for classifying the bacteria using the molecular methods such as the High Throughput Sequencing of 16S rRNA (Ellermann et al., 2017). Kim and Chun (2014) mentioned that 16S rRNA gene sequences are used for the classification and identification of prokaryotes and are mainly dependent on a comparison against known sequences database. Clarridge (2004) mentioned that the 16S rRNA gene sequence is an excellent and extensively used choice for identifying an unknown organism. It was also reported that 16S rRNA gene sequence analysis is the backbone and most authoritative reference of the new edition of Bergey's Manual of Systematic Bacteriology (Clarridge, 2004). Janda and Abbott (2007) also mentioned that 16S rRNA gene sequences are used to study bacterial phylogeny and taxonomy. Therefore, for many years, Sanger sequencing is used to collect 16S rRNA genes sequence from environmental samples that have revealed sample sizes, coverage and compare microbial communities (Bartram et al., 2011). But now collections of thousands of sequences from multiple samples were possible using various platforms including 454-pyrosequencing (Hamady et al., 2008), Illumina Hiseq and Miseq (Bartram et al., 2011), and Ion Torrent (Whiteley et al., 2012). Computational techniques are applied to recognize bacterial taxa within each of the sample and to analyze microbial ecological measures like diversity, relative abundance, or microbial richness of a particular community (Ellermann et al., 2017).

I.6. North-East region of India

The country India is placed as the mega biodiversity country in the world because India supports about 1/6th of the world population, 1/50th of the world's land, and 1/25th of the world's water resources (Gangwar, 2013; Pathan, 2013). Khuntia et al. (2018) reported that fish constitutes half of the total number of vertebrates in the world. It has been reported that from 39,900 species of vertebrates, 21,723 are representing the living species of fish and out of these, 8,411 are freshwater species and 11,650 are marine species. They also mentioned that particularly in India, 2,500 species of fishes are present of which 930 live in freshwater and 1,570 are in the marine environment. Khodake et al. (2014) reported that fish exhibit the greatest biodiversity of the vertebrates with over 22,000 species. Out of these about 58 % are marine, 41 % are freshwater species and 1 % move back and forth between salt and freshwater. There are about 450 families of freshwater fishes globally and roughly 65 are represented in India.

The North-East (NE) region of India has consisted of eight states viz. Assam, Nagaland, Arunachal Pradesh, Manipur, Tripura, Mizoram, Meghalaya, and Sikkim with splits of international border with Bhutan, China, Myanmar, and Bangladesh, and contains more than one-third of the country's total biodiversity (Ramanujam et al., 2010; Hussain, 2012). Therefore, The NE region of India is considered as one of the hotspots of freshwater fish biodiversity in the world (Ramanujam et al., 2010). The Indian fish population represents 11.72 % of species, 23.96 % of genera, 57 % of families from 80 % of the global fishes. It is also reported that among 2200 species so far listed, 73 (3.32 %) belong to the cold freshwater regime, 143 (6.50 %) to the brackish waters, 544 (24.73 %) to the warm fresh waters domain, and 1440 (65.45 %) to the marine ecosystem (Shobhna et al., 2013). It is to be notes that the diadromous and freshwater fish species are highly endangered due to the factors like climate change, habitat loss, and flow regime modification (Moody et al., 2019). Globally, freshwater fishes are the most diverse group among all the vertebrate groups, but also they are the most highly threatened species through anthropogenic activities (Sarkar et al., 2013). The freshwater aquatic biodiversity is depleting unfortunately due to the introduction of exotic species, overexploitation, habitat loss, diseases, pollution, and other anthropogenic activities (Shobhna et al., 2013). In India, freshwater environments are undergoing serious threats to biodiversity. So, it is an urgent priority to promote fish biodiversity conservation and management search by other alternative techniques (Sarkar et al., 2013). It is highlighted that the diversity within the region of freshwater ecosystems of the Himalayas and Indo-Burma is

both highly diverse because of its great regional importance in terms of the livelihood and the economic importance of the people living around it (Goswami et al., 2012). Besides that, some small hill-streams are highly torrential with the huge altitudinal variation that showed great freshwater resources, which provide a variety of habitat for the subsistence of varied and large freshwater fish fauna (Singh and Agarwal, 2013).

According to the report of Ramanujam et al. (2010), 930 species of fishes inhabit the freshwaters of India, and out of that, the NE India is represented by 267 species belonging to 114 genera under 38 families and 10 orders. Among all the fish families, *the Cyprinidae* family of fishes is widely scattered. He et al. (2008) reported that *Cyprinidae* is the largest freshwater fish family in the world which including over 200 genera and 2100 species. The *Hel* river, located in the Kokrajhar district in the state of Assam, India, is one of the rich freshwater evergreen biodiversity of Assam. The Kokrajhar district is in the western part of Assam and is situated on the North Bank of the Brahmaputra and lies between 89°46′ E to 90°38′ E Longitude and 26°19′ N to 26°54′ N Latitude (Narzary et al., 2013; Paron and Bharti, 2019). It is surrounded by the two districts viz. Bongaigaon on the East and Dhubri on the South, and the country Bhutan on the North, and an Indian State West Bengal on the West. The district covers a total of 3169.2 sq. km geographical areas of the state of Assam (Paron and Bharti, 2019). The district has a distinct topographical structure as the Bhutan hills form its Northern boundary and the Brahmaputra plain forms most of the district (Narzary et al., 2013). The district Kokrajhar also has several hill streams and rivers (Chakraborty et al., 2016). Among all the Bhutan hill rivulets, one of the important hill stream rivers is the *Hel* river, which is originated from Bhutan and passes through the Ramfalbil, Serfanguri, and Dotma and finally falls at Brahmaputra river. Bain (2011) reported that the freshwater fish family *Cyprinidae* has the highest diversity family in species. It is also observed the district Kokrajhar of Assam of NE India is considered as one of the important reservoirs of the *Cyprinidae* family fishes (Singha et al., 2017). The highest number of hill stream fishes found in Kokrajhar district streams belonged to several genera of the families *Cyprinidae* of order *Cypriniformes* (Baro et al., 2015). Some reports mentioned working on fishery resources from rivers and streams of Bhutan, Himalaya (Petr, 1999; Goswami et al., 2012; Acharjee and Barat, 2013; Gurung et al., 2013; Pathak et al., 2013; Baro et al., 2014; Thoni et al., 2016; Dorji and Gurung, 2017). However, the proper scientific investigation of nutritional and biochemical composition studies of the indigenous hill stream fish species of the *Cyprinidae* family are missing from the *Hel* river, Kokrajhar, Assam and not yet studied. The present study is therefore conducted to investigate the nutritional quality

and biochemical composition of the fish species of the *Cyprinidae* family found in the *Hel* river of Kokrajhar, Assam which is selected based on their high abundance. The study area selected for the present investigation is located within North: Dhanguri, South: Lungsung, East: Sialmari, and West: Moinaguri (Latitude – 26.56^{o}, Longitude – 90.13^{o}, Altitude – 57.9 m, Accuracy: 2.0 m, Bearing – 48.21^{o}) of Kokrajhar district.

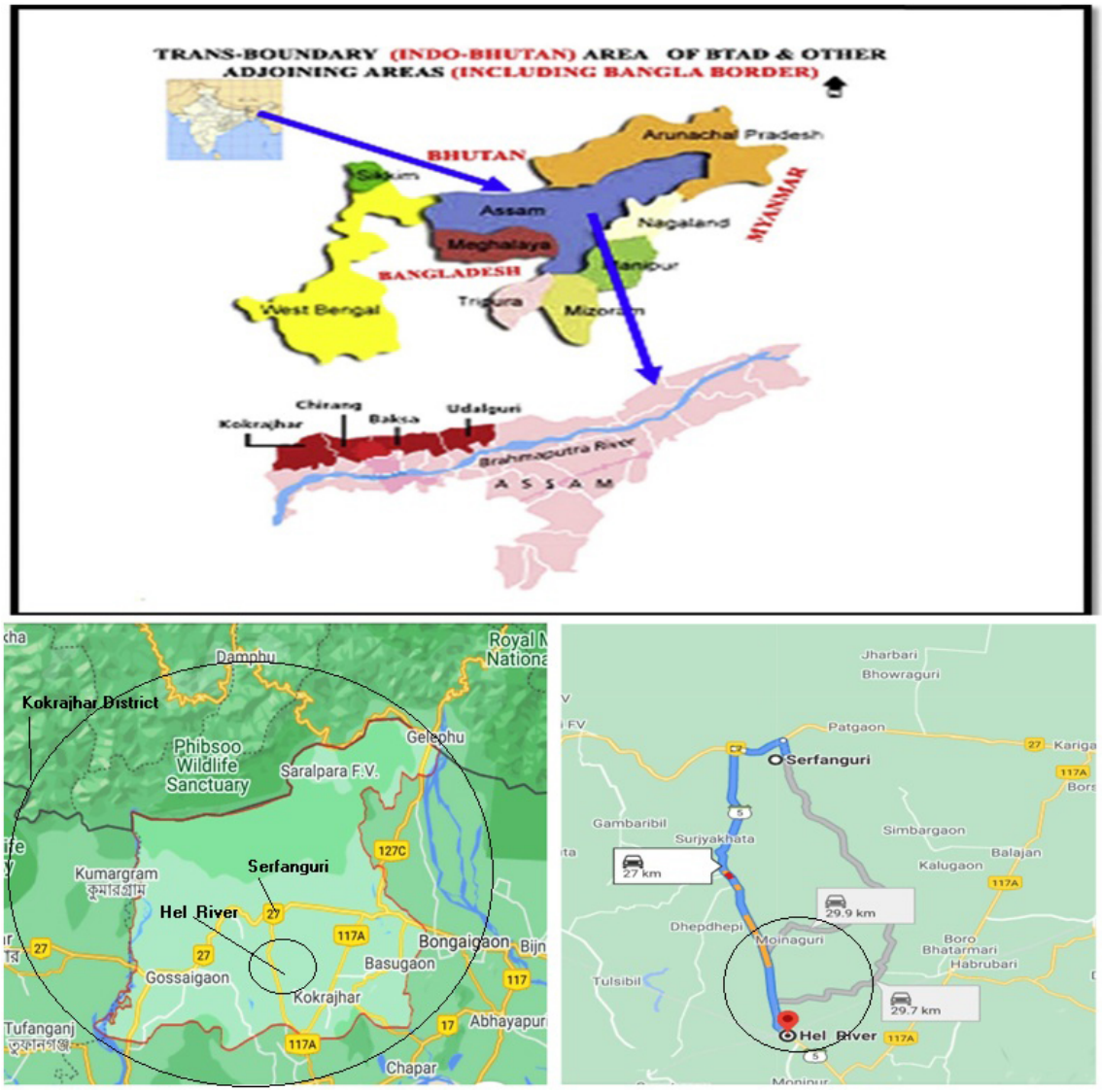

Fig.I.1. Map showing the study area.

Objectives of the present study

Considering the availability of indigenous fish species from the *Hel* river of Kokrajhar, Assam and their consumption by the local people, the focused objectives of the present work were:

1. To collect the fish species from the *Cyprinidae* family based on the abundance study.
2. To analyze the nutritional composition of candidate fish species.
3. To study the metagenomics of *Barilius bendelisis*.