INTRODUCTION
1. INTRODUCTION

Bangalore also called as Bengaluru lies in the southeast of the South Indian state of Karnataka (Plate - I). It is located at 12.97°N 77.56°E and covers an area of 741 km² (Finance budget, 2007-08). Bangalore, with annual rainfall of 900 mm (3.0 ft) with three different rainy seasons covering nine months of the year. June to October is the rainy season accounting for 64% of the total annual rainfall in the S-W monsoon period and 324 mm (1.1 ft) during (November – December) the N-E monsoons (http://ces.iisc.ernet.in/energy/wetlands/sarea.html). It has a salubrious climate with an annual mean temperature of 24°C (75.2°F) with extremes ranging from 37°C (98.6°F) to 15°C (59.0°F) (http://www.isec.ac.in/urban%20Governance.PDF). Rainfall gets divided into three valleys viz., Koramangala-Challagatta, Hebbal and Vrishabavathi which are the repository of all the lakes in Bangalore.

The three valleys consist of number of lakes that are interlinked to each other through aseries of lakes giving a cascading effect to the whole system. Many lakes have reportedly springs at the bottom of the lakes, some of which are stated to be choked due to silt, which also feed the lakes (Gowda and Sridhara, 2006). Some of the major lakes that disappeared over the years are as Shoolay lake, Akkithimmanhalli lake, Sampangi lake, Dharmabudhi lake, Challaghatta lake, Domlur lake, Koramangala lake and Jakkarayanakere.

Inland waterbodies can be classified as lotic habitats (running water) including rivers, streams, etc. and lentic (standing water) including lakes, ponds and marshes. Difference between lotic and lentic is the continuous flow of
water in lotic ecosystem. Characteristic of lentic ecosystem (lake) is the presence of vertical stratifications which are of several important features and display marked seasonal variation. A lake is a body of relatively still fresh or salt water of considerable size, localized in a basin that is surrounded by land. They may be artificial, constructed for industrial or agricultural use, for hydroelectric power generation or domestic water supply or for aesthetic or recreational purposes. Lakes have numerous features in addition to lake type, such as drainage basin (also known as catchment area), inflow and outflow, eutrophication and sedimentation. Lakes differ from each other in size, shape, physico-chemical nature, degree of pollution and abundance of aquatic life. Changes in the level of a lake are controlled by the difference between the input and output of water compared to the total volume of the lake. Significant input sources are precipitation onto the lake, runoff carried by streams and channels from the lake's catchment area, groundwater channels, aquifers and artificial sources from outside the catchment area. Output sources are evaporation from the lake, surface and groundwater flows, and any extraction of lake water by humans.

Water is the greatest gift of nature and being the prime necessity of life is the soul and hope of the nature. The role of water in nature is unique not only from the point of human consideration; even the numerous organisms make an aquatic medium their abode. It is required for recharge of ground water, industry, agriculture, fishery, navigation and transport besides the disposal of
waste water and other activities like drinking, cooking, bathing and washing, recreation.

From time to time immemorial, humanity and its civilisation has been flourishing in the vicinity of or along with the water resources only. Humans have exploited this natural resource to a level where controlling water pollution is impossible. In earlier days Bangalore was called as ‘The city of lakes’. The earliest history of creation of lakes in and around the city is traced to the founders of Bangalore – the Kempe Gowda in the Sixteenth century and later by the Wodeyars of Mysore Kingdom and the British. These lakes and tanks were made for purposes of drinking water, irrigation and fishing needs, have also favorably influenced microclimate of the city. The lake waters have also served as “Dhobhi Ghats” or places where washer–men (‘dhobis’ is the locale usage in India), have traditionally used them as a means of livelihood for washing clothes and drying them. They have served to replenish ground water resources in the vicinity, which are tapped through wells for drinking water.

Pollution of water is the contamination of water (e.g., lakes, rivers, oceans, aquifers and groundwater), due to direct or indirect discharge of pollutants into water bodies without adequate treatment for the removal harmful compounds. This affects plants and organisms living in such bodies of water and in almost all cases the effect is damaging not only to individual species and populations, but also to the natural biological communities. Water pollution may be caused due to excessive phosphorus and nitrogen compounds present in industrial, agricultural (pesticides, herbicides) or municipal sources.
(sewage and litter) discharged into lakes (Bomchul et al., 2001; Stephon, 2005). Untreated sewage and domestic wastes including wastes from sanatoriums, hospitals and slaughter houses containing complex organic substances in the form of urine, faeces, paper, soap, detergent, scrap of food and grease etc are also disposed into these water bodies. Lakes often contain high pollution levels relative to the surrounding landscapes and environment. Rivers and streams drain pollutants in the landscape where they concentrate in lakes and other water bodies. Non-point source pollution accounts for most of the contamination in water systems. Most of the lakes in India are facing acute pollution problem e.g., Hussain sagar in Hyderabad, Bellandur lake in Bangalore, Loktak in Manipur, Dal in Kashmir which are choked by algal blooms causing excessive eutrophication.

Water quality analysis is one of the important aspects in studies on aquatic ecosystem. The physical and chemical properties of fresh water bodies are characterized by the climatic, geochemical, geo-morphological conditions and level of pollution. The quality of aquatic life is dependent on the water quality. In order to utilize fresh water bodies successfully for fish production, it is very important to study the physico-chemical factors which influence the biological productivity of the water body (Sahani and Yadav, 2012). The physico-chemical study reveals the suitability and quality of water for drinking and for agricultural and industrial purposes. A change in the physico-chemical aspect of a water body also brings about a corresponding change in the relative composition and abundance of the organisms in that water. To determine the
quality of the environment, biomonitoring (biological surveillance) is another aspect in which there is a systematic use of living organisms or their responses to its habitat (Rosenberg and Resh, 1993).

Soluble salts commonly found in water resources include chlorides, sulphates, nitrate and bicarbonates of sodium, potassium, calcium, magnesium, iron and manganese etc. Certain soluble salts of calcium and magnesium render the water hard resulting in its unsuitability for certain industrial purposes especially boilers. In small concentrations, they are harmless to fresh water fish but beyond a limit, they may be dangerous to water body. Excess of dissolved solids is also injurious for human consumption besides corrosion of metals and concrete. Sulphates are too bad in the later aspect because they get reduced to hydrogen sulphide which oxidises to highly corrosive sulphuric acid, known to act directly on concrete (Jivendra, 1995). Soluble salts of relatively non toxic metals like iron and manganese cause pollution by reacting with the alkalinity present in water to give rise to a precipitate of unsightly reddish brown insoluble hydroxides. Several insoluble inorganic substances like clay, calcium carbonate and calcium sulphate etc., are undesirable in water bodies because of the resultant increased turbidity and suspended matter. When biodegradable pollutants are discharged into water body, microorganisms (aerobic bacteria) and their respiration rates increase which lowers down the DO. This in turn is harmful to the aquatic life, including fish, since DO is required for their survival. When the degree of pollution is not severe, oxygen demand of the
organic matter is met by the dissolved oxygen supplemented by atmospheric reaeration in the natural body of water (Jivendra, 1995).

1.1. How does pollution affect lakes?

The problem of water pollution is global. Facilities and resources, however, may vary from nation to nation. While advanced countries like USA can afford sophisticated equipments for pollution abatement, a developing country like India has to resort to indigenous know-how suited to its economy and climatic conditions. Regardless of the source, pollution can disrupt aquatic life in many ways. In general, pollution reduces water quality. It can also reduce the diversity of wildlife, especially sensitive species. The toxic substances are not only lethal to human beings, animals and other aquatic life but can also kill bacteria and other biological forms such as fish present in the stream and thus interfering with the self purification capacity of a water body. Such pollutants render the water unfit for normal uses and industrial purposes.

The increasing industrialization, urbanization and developmental activities, to cope up with the population explosion have brought inevitable water crisis. Such activities have altered the water ecosystem which is exposed to all local disturbances regardless of where they occur (Venkatesan, 2007). Discharge of untreated waste water, coupled with massive use of water for irrigation, industrial and domestic purposes, lack of awareness and seasonal variations are the main cause of water quality degradation (Joshi et al., 2006; Trivedi, 2010). The health of lakes and their biological diversity are directly related to health of almost every component of the ecosystem (Ramesh et al.,
Fertilizers and pesticides from agricultural and urban runoff and sewage seepage from the groundwater enter lakes and cause elevated levels of nitrates and phosphates. These can lead to harmful algal blooms and eutrophication, which can be harmful to both aquatic life and human health. Industrial runoff containing heavy metals, such as lead and mercuryfind their way into the food chain. This can cause illness or death to fish, other animals, or humans that consume them. Sediment washed away from construction activities and urban or agricultural activity enter lakes, reducing water clarity and water quality, and can be lethal to aquatic organisms by becoming trapped in gills and ultimately accumulated in various tissues and organs. Aquatic ecosystems are affected by several health stressors that significantly deplete biodiversity. In the future, the loss of biodiversity and its effects are predicted to be greater for aquatic ecosystems than for terrestrial ecosystems (Sala et al., 2000). The quality of water is of vital concern for the mankind since it is directly linked with human welfare. Therefore, monitoring the quality of water is one of the essential issues of drinking water management (Shama et al., 2011).

1.2. Status of lakes in Bangalore

Most of the lakes have vanished due to encroachment and construction activity for urban infrastructure expansion. The city once had 262 lakes of which 81 were live, 46 were disused, more than 7 cannot be traced, many have reduced to small cess-pools of water,18 have been unauthorisedly encroached by slums and private parties, 14 have dried up and are leased out by the Government. 28 lakes have been used by the Bangalore Development
Authority to distribute sites and build extensions for residential areas. The remaining lakes are in fairly advanced state of deterioration. Distribution of lakes/tanks in urban and rural Bangalore was as follows: Bangalore North- 61 lakes, Bangalore south- 98 lakes, Anekal- 44 lakes, Hoskote- 23 lakes, Magadi-11 lakes, Nelamangala-13 lakes and devanahalli-12 lakes. It was reported that Bangalore city lakes were bigger in size as compared to rural ones in the state (Thippaiah, 2009).

Historically lakes in the Bangalore region were managed by a plethora of government agencies such as the Forest Department, Minor Irrigation Department, Horticulture Department, Public Works Department (PWD), Bangalore Mahanagra Palike (BMP), Bangalore Development Authority (BDA), Tourism Department, City Municipal Councils and Panchayats; each organization claiming its own jurisdiction of ownership and maintenance rights resulting in a deficient, inconsistent and uncoordinated approach..

The Government initiated action to establish a separate authority to address the problem of lakes and implement the recommendations of the Expert Committee. The Lake Development Authority (LDA) was thus established in July 2002 (https://www.karnataka.gov.in/ldakarnataka/Pages/home.aspx). In 2004, the LDA began a process of "public-private participation (PPP)" where private companies bid for the lakes to “develop and maintain” them for the next 15 years with the specified Terms of Reference. The tender specified the following Terms of Reference:
• Desilting, dredging, sewage diversions, constructed wetland and bio-
remedial measures

• Construction of water treatment plants and chain link fencing

This was to be followed by beautification of lakes through:

• Landscaping and gardening

• Foreshore and island development

• Creation of tree parks and Rock gardens

• Walkways, Jogging path and cycling track

• Fountains and Children play area

• Electrification for illumination

• Boat jetty

• Eco friendly restaurants

• Urban (joy) fishing, bird watching, Butterfly Park, Aviaries and boating.

The need to maintain the ecology of a water source cannot be undermined. In this context, keeping in view the public health and the ecology of the water body, a critical study of the water sources appear to be a necessity to assess the damage caused by pollution from a particular source in terms of quality and quantity. This would help the concerned to assess and realise the impact of pollution and take the necessary remedial measures to combat the same.

1.3. Fish as test animal

Biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct
acting toxicants (El-Shehawi et al., 2007). Fish is an indirect non-target organism which is affected due to such changes in the water parameter. It is a suitable model for monitoring aquatic genotoxicity and wastewater quality because of its ability to metabolize xenobiotics and accumulate pollutants, which may damage some physiological and biochemical processes when they enter the organs of fishes (Tulasi et al., 1992). Thus, fish is best model to assess the quality of water. They are excellent subjects for the study of the mutagenic and carcinogenic potential of contaminants present in water since they can metabolize, concentrate, and store waterborne pollutants (Ali and El-Shehawi, 2007). Pollutants transformed in the liver, may be stored there or in fat, which is an extra hepatic tissue or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination (Al-kahtani, 2009). Thus, both these organs have the capacity to store the contaminants and to excrete them thereby reducing the effect of these harmful substances. Fish being at a higher level of food chain, accumulate a significant amount of pollutants and this accumulation depends on the intake and elimination from the body.

1.4. Biomarkers

Biomarkers or biological markers are biological measures of a biological state. By definition, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention." Biomarkers may be used alone or in combination to assess the
health or disease state of an individual. An ideal biomarker has certain characteristics that make it appropriate for checking a particular disease condition. Ideally, an ideal marker should have the following features: safe and easy to measure, cost efficient to follow up, modifiable with treatment and consistent across gender and ethnic groups (http://www.news-medical.net/health/Biomarker-What-is-a-Biomarker.aspx).

Despite the fact that toxic influences in any living tissue are first exerted at biochemical level, biochemical markers are the earliest indicators of toxic potential of any xenobiotics. As a consequence of their ability to identify causal mechanisms potentially responsible for effects at higher levels of organisation, biochemical biomarkers used to be considered the most promising tools for ecotoxicological applications (Adams, 2002). Since these act as good biomarkers of fish health therefore their assessment is necessary in order to check the influence of pollutants under field conditions. Enzyme activities and other sub-cellular components are most commonly included in this group of biomarkers. Much work has been done during the last two decades to establish and promote the application of biomarkers (Adams, 2002).

Proteins are the most abundant biological macromolecules occurring in all cells and all parts of cells. Proteins also occur in great variety; thousands of different kinds may be found in a single cell. Proteins are the molecular instruments through which genetic information is expressed. Most carbohydrates formed in nature occur as polysaccharides, polymers of medium to high molecular weight. Glycogen is homopolysaccharide. The most
important storage polysaccharides are starch in plant cells and glycogen in animal cells. Cholesterol is also a key regulator of membrane fluidity in animals. They are important in the composition of cell membranes and also steroid hormones. The synthesis and utilization of cholesterol must be tightly regulated in order to prevent over-accumulation and abnormal deposition within the body. Several studies have reported alterations in blood glucose and tissue glycogen levels, protein and lipid profile of fishes (Yacoub and Gad, 2012; Cogun and Şahin, 2013). Priya et al. (2014) reported decrease in the protein content in muscle tissue of fresh water fish *Catla catla* while studying the detergent induced toxicity in this fish. A number of significant changes in the carbohydrate metabolism and in glycogen content of liver, brain, muscle and gills tissues of an air-breathing cat fish *Mystus Cavasius* (Ham) after short term exposure to electro plating industrial effluent chromium (Palanisamy et al., 2011). Yousafzai and Shakoori (2011) noted increase in hepatic cholesterol of *Tor putitora* caught from polluted portion of river Kabul. Qualitative and quantitative assessment of heavy metals in the Thermal Power Plant effluent was performed by Javed and Usmani (2013c) to study the impact of their toxic effects on various biomarkers (carbohydrate, protein and lipid profiles) in tissues of fish *Channa punctatus*.

In toxicological studies of acute exposure, changes the concentrations and enzyme activities which often directly reflect cell or organ damage in specific organs (Casillas et al., 1983). Acid phosphatases act as marker enzymes for the detection of lysosomes in cell fractions and can be altered by
the presence of xenobiotics (Cajaraville et al., 2000), whilst alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of almost all animal cells. Both enzymatic activities have been studied in several organisms and the influence of heavy metals has been reported (Blasco et al., 1993). The effect of lead acetate on acid and alkaline phosphatase activities in the liver tissue of fish *Labeo rohita* was evaluated by Vetrivel et al. (2014) reporting an evidence of inhibition of these enzymes in the liver tissue by the toxicant.

In general, any stress inducing substance will affect the respiratory metabolism of fish. Any alteration in the intermediary metabolism due to stress is bound to affect the activity of oxidative enzymes like SDH, MDH & LDH. SDH is a vital enzyme of the Krebs cycle which catalyzes succinate to fumarate. Malate dehydrogenase is an NAD dependent enzyme which converts malate to oxaloacetate and reversible oxidation of fumarate to malate. It exists in two isozymic forms (a) mitochondrial (b) cytosolic. This enzyme not only converts malate to oxaloacetate but also plays a significant role in CO₂ fixation and in gluconeogenesis. LDH is an important glycolytic enzyme which is present in all animal tissues (Kaplan and Pesce, 2009). The enzyme is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diammantino et al., 2001) and the alterations of normal LDH activity pattern are found to be the O₂ stress after exposure. LDH is a parameter widely used in toxicology and clinical chemistry to diagnose all the tissues and organ damage. Srivastava and Singh (2014) reported a decrease
in SDH and LDH activity in liver and muscle of *Clarias batrachus* when exposed to agricultural fungicide – Mancozeb. Shahid *et al.* (2014) reported decreased level of MDH in rat kidney after sodium arsenate exposure. Kumar and Sunny (2014) stated significant changes in MDH activity in liver and gill of *A. testudineus* when exposed to increasing sub lethal concentrations of Bisphenol A. Palanisamy *et al.* (2011) reported significant accumulation of lactic acid in liver, brain, muscle and gills tissues of air-breathing cat fish *Mystus Cavasius* (Ham).

Several studies (Biswas *et al.*, 2012; Turan *et al.*, 2009) has been undertaken worldwide to assess the accumulation of metals in edible fishes caught from fresh water and coastal waters. There is a growing concern that metals accumulated in fish tissues may represent a health risk, especially for high fish consuming population (Burger and Gochfeld, 2009; Ling *et al.*, 2009). Metals like arsenic, cadmium, mercury and lead are toxic to biota, even in low concentrations, but other metals like Cu, Fe, Mn and Zn are required for physiological activities in biological species (Storelli, 2008). Mallin *et al.* (2011) stated that these metals required in biological systems can also produce toxic effects when taken excessively. Metal effects on central metabolic pathways may have a major detrimental impact on both human and animal life (Pandey *et al.*, 2008). Metal accumulation has also been employed as an impact assessment tool with reference to fish health (e.g. DeViller *et al.*, 2005), fish community structure (Allert *et al.*, 2009) and aquatic ecosystem health (Shinn *et al.*, 2009). Heavy metals, polycyclic aromatic hydrocarbons (PAHs) and
polychlorinated biphenyls (PCBs) were analyzed in muscle tissue of *Engraulis encrasicolus* by Copat *et al.* (2012) to assess the contamination level of the Catania Gulf. Shakir *et al.* (2015) studied bioaccumulation of metals in muscle tissue of a wild carp *Cirrhinus mrigala* from selected sites of a river loaded with municipal and industrial wastes.

Electrophoresis has been used as a tool for examining biochemical variation in a population. It is a technique where charged molecules migrate in a gel medium when an electric field is applied providing a method of size or charge fractionation of the molecules. It is used to isolate individual components of protein or nucleic acids (William and Michael, 2000). The easy determination of some blood parameters is probably responsible for the rise in the use of haematology as a tool for testing of health problems in fish (De Pedro *et al.*, 2005). Alteration in protein bands in liver and gill tissues of *A. testudineus* was noted when exposed to sub lethal concentrations of Bisphenol A (Kumar and Sunny, 2014). Effect of two different habitats on haematological and serum protein profiles of *Mugil cephalus* was reported by Fazio *et al.* (2013) and changes in these parameters were evidently related to exogenous factors such as water quality and seasonal changes.

According to Fagr *et al.* (2008), the incidence of micronuclei in fish and other aquatic life serves as an index of these types of damage and counting of micronuclei is much faster and less technically demanding than scoring of chromosomal aberrations. The micronucleus assay has been widely used to screen for chemicals that cause these types of damage (Palhares and Grisolia,
Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage. Because counting of micronuclei is much faster and less technically demanding than scoring of chromosomal aberrations, the micronucleus assay has been widely used to screen for chemicals that cause these types of damage. The MN assay, originally developed with mammalian species (Heddle et al., 1983), is today widely applied in fish and other aquatic organisms, including sea urchin, mussels, oysters, crabs and worms, and in wild and transplanted animals. The large majority of studies or programmes on the genotoxic effect of the polluted environment have been carried out with the use of bivalves and fish. Arslan et al. (2015) assessed the biological damages in some fish caused by various mutagenic agents present in polluted waters of Aliağa Bay. A significant increase in MN frequencies were observed in fish species *Clarias batrachus* induced by fungicide (Srivastava and Singh, 2015).

Although a number of scientists have conducted investigations to assess the water quality of urban water bodies in cities (Offem et al., 2011; Patel and Patel, 2012) other than Bangalore which were affected by anthropogenic activities, a lacuna was observed in the studies on pollution status of Vengiah lake (lake A) and Yellamallappa Chetty lake (lake B) (Plate - II) which were important for recharging of ground water, with respect to presence of toxic
levels of trace metals and other chemical parameters. Therefore, one of the main objectives of the research was to evaluate the extent and type of pollutant load and its effect on the water quality of these lakes during three seasons viz., winter, summer and rainy season. In the present study, the tissues selected for further analyses were muscle and gill tissue, muscle - an important tissue of nutritive value; gill - which is a vital respiratory organ with their extensive surface area directly in contact with water and targeted by xenobiotics (Jianget al., 2012). Various biochemical and metabolic changes relating to the enzyme activity in muscle and gill tissue of freshwater fish *Labeo rohita*, its blood chemistry and genotoxicity during the three seasons were assessed. The statistical correlation and variations between these biochemical constituents and enzymatic activity of the tissues including few haematological parameters of fish with the physico-chemical parameters of the selected water bodies (lake A and B) during winter, summer and rainy seasons were recognised.

1.5. Scope of the study

The topic of research is basically about fish metabolism but is significantly related to various issues of environment and aquatic pollution with respect to winter, summer and rainy season. The burning issue of today is conserving the fauna and flora of the planet for a better future. The research under this study will also give us various hidden reasons of disturbances in the growth and metabolism of aquatic organisms. Further the possible way to revive them back to healthy conditions.
PLATE – I

Karnataka location map
Google image showing location of
Vengaiah lake (Lake A) and Yellamallappa Chetty lake (Lake B)
OBJECTIVES

The present research work was conducted to assess the following objectives:

- Survey of lakes to check their status.
- Seasonal analysis of physico-chemical parameters of Control site, Vengaiah Lake (Lake A) and Yellamallappa Chetty (Lake B).
- Biomolecule contents (protein, glycogen and cholesterol) in the muscle and gill tissue of *Labeo rohita* sampled from fish farm and the selected lakes (A & B).
- Activities of few metabolic enzymes (ACP, ALP, SDH, MDH and LDH) involved in carbohydrate and protein metabolism of Kreb’s cycle in muscle and gill tissue.
- Bioaccumulation of heavy metals in muscle and gill tissue of fish sampled from selected water bodies.
- Comparison of serum proteins in blood of fish from selected water bodies by SDS- PAGE.
- Genotoxic effect in fish by micronuclei test.