INTRODUCTION

Metals and metal compounds are natural constituents of all ecosystems, moving between atmosphere, hydrosphere, lithosphere, and biosphere (Bargagli, 2000). Metals play a role in our lives in many ways. Metals are natural features of today’s society and their use has increased. Ever since the Bronze Age people have made use of metals for their unique properties such as strength and durability. Industrial revolution brought the use of metals in industries, household uses and cosmetics to limelight. The increased use of metals has been a hazard to human health as metals were fashioned into spears earlier and present day exposures involving space age metals and alloys. Around one hundred known elements 77 are metals, 52 of which can be considered of industrial and economic importance. The quantity used varies from millions of tons of iron to ounces of platinum (U.S. Department Of Interior, 1969).

There are 35 metals that concern us because of occupational or residential exposure. 23 of these are ‘heavy metals’. Heavy metals are categorized based on their high density, atomic weight, atomic number. Antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc are the heavy metals. Although there is no clear definition of what heavy metal is, density is in most cases taken to be the defining factor. The heavy metals are generally regarded as having an atomic numbers of 22 to 92 in all groups from period 3 to 7 in the Periodic Table (Waldichuk, 1974). Heavy metals are those having a specific density of more than 5g/cm³. This definition includes over 70 metallic elements, although only a few of them are recognized as potentially damaging (Piotrowski and Coleman, 1980).

Metallic elements form the part of all living organisms and play a variety of roles. They work as structural elements and components of biological structures (e.g. co-factors in enzymes and haemoglobin) or activators or components of biological systems. Some metals are essential nutrients, and their deficiency results in impairment of biological functions. Metals such as iron, zinc, copper and chrome
fulfill essential functions in every living organism. Even though the quantities of these elements are small they are vital for all biological life as well as for all living organisms. Essential metals, when present in excess, may be toxic (e.g. molybdenum) (WHO, 1996). Other metals serve no biologic purpose, while still others have potential to produce the environmental hazards (Heinberg, 2011).

Sources of metal emission and transport:

There is no life without water. However, fresh water from all sources available to biota constitutes only 1%. Fresh water supports the life through smaller but sound ecosystems. Increasing global pressure of population, urbanization, industrial propagation, increasing living standards of human and climate change has increased the demand of fresh water. On the other hand modern life style demands for industries like metal extraction. Most of the rocks having these metals are sparingly soluble hence limit the concentration of heavy metals in natural waters. Still increasing quantity of heavy metals in our resources is currently an area of greater concern, because serious concerns of environment due to rapid urbanization, industrial development, consumerist life style and anthropogenic sources of environmental pollution have been raised during last few decades. The deposition of new technological equipments increase E-waste. If not recycled or disposed appropriately the metals used in it will leach into the surrounding soil, water and air (Rajya Sabha, 2011).

Almost all metals occur naturally in surface and ground water. However, the amount is negligible. Metals are introduced in aquatic systems as a result of weathering of soils and rocks, from volcanic eruptions and from a variety of human activities involving mining, processing and use of metals and / or substances that contain metal pollutants. The point as well as non point source and atmospheric deposition are the several pathways through which metals enter in terrestrial and aquatic environments (Bohlin et al., 2005).

The greater part of metal load emitted into the environment is transported by water, lead being an exception. All forms of metals used in any industry are discharged to aquatic resources like estuaries, rivers, streams, and lakes (Ripley and
Redmann, 1978). These metals dissolve in water, transfer into food chain as are easily absorbed by fish and other aquatic organisms. It then brings about ill effects on the organism. Pesticides, heavy metals, are bioaccumulating substances, can adversely affect the biota and abiotota (Anjaneyulu, 2002). Mining activities and a number of metal involving industrial processes like catalysts in chloroalkali processes are major point sources from which metals are released into the aquatic environment. Deliberate release of metals into aquatic environment takes place through industries producing insecticides. Atmospheric deposition, storm water and ordinary household waste water end up in the aquatic environment.

It is well known that heavy metal pollutants present in water may induce severe ecological consequences generating reorganizations of the biogenesis, changing it and consequently affecting aquatic ecosystems integrity (Vosyliene and Jankaite, 2006). An example of conversion of a metal in the ecosystem to a toxic form and its subsequent concentration in food has been shown by the tragic incident of Minamata. It was subsequently shown that inorganic mercury discharged from industry was converted to methyl mercury by microbial systems in the bottom mud (Nelson, 1971). Recently more attention and concern is given to metal compounds that have toxic effects at low levels of exposure than those that produce overt clinical and pathological signs and symptoms (Kalia and Flora, 2005). Exposure to heavy metals is potentially harmful especially for those metal compounds which do not have any physiological role in the metabolism of the cells. The ingestion of metals via food or water could modify the metabolism of other essential elements like Zn, Cu, Cd, As, Sb, Cr, Ca, Na, Au, Cl, Br (Cook et al., 2005; Florea, 2005). Prolonged exposure to metals and metal compounds could result in dysregulation of cellular pathways causing subsequent toxicity (Fitsanakis and Aschner, 2005). In the aquatic environment, heavy metals are natural trace components, but their levels have increased because of agricultural, industrial and mining activites. So the aquatic animals are exposed to elevated levels of heavy metals continuously (Unlu and Gumgum, 1993; Kalay and Canli, 2000).

The fraction of total metal that can be taken up into an organism is bioavailability (Sanders and Riedel, 1998). Aquatic organisms absorb these toxic
metals from water over the entire body surface, besides from food. Metal compounds are increasingly introduced in the environment and could finally accumulate in a biotic system (Nordberg et al., 1985; Han et al, 2002). Comparative toxicity of the eight most important heavy metals has been listed (Piotrowski and Coleman, 1980; Moore and Ramamoorthy, 1984). But, their toxicity varies from species to species and environment to environment.

**Effects on Animal Health**

Metals and metal compounds interfere with functions of the central nervous system (CNS), hematopoietic system, liver and kidneys. Adverse effects on an organism’s activity, growth, metabolism and reproduction are examples of sublethal effects (Wright and Welbourn, 2002). The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (arsenic is a metalloid, but is usually classified as a heavy metal (Jarup, 2003). They are highly toxic even in minor amounts but other metals are also of concern.

The presence of heavy metals in natural waters has become a significant topic of concern for environmentalists, scientists and engineers associated with water quality and growing awareness of the public. Mercury has received significant attention from the scientific community because of its wide spread environmental abundance and associated toxicity to human and wild life (Scheuhammer et al., 2007). Adverse effects of heavy metals on animal health are known, still exposure to heavy metals continues. For example, in India mercury is still used in pharmaceuticals, paper industry, dental amalgam, arsenic is common in wood preservatives, and tetraethyl lead remains a common additive of petrol in many underdeveloped countries. Use of heavy metals has been decreased at the end of 20\textsuperscript{th} century and hence the emission. In U. K. the emission of heavy metals fell by over 50\% between 1900 to 2000 (Dept. of Env. U. K., 2001). However, it is not the case with developing and underdeveloped countries. Effluents of industries are directly or indirectly coming to the final sinks like river. Heavy metals contamination in river is one of the major quality issues in many fast growing cities, because maintenance of water quality and sanitation infrastructure did not increase along with population and urbanization.
growth especially in the developing countries (Sundaray et al., 2006; Karbassi et al., 2007; Akoto et al., 2008; Ahmad et al., 2010). Heavy metals (i.e. Zinc, Copper, Lead, Cadmium, etc.) rank as major polluting chemicals in both, developed and developing countries (Lloyd, 1992). Heavy metal toxicity is one of the major environmental health problems and is potentially dangerous because of bioaccumulation through food chain. (Aycicek et al., 2008). It causes hazardous effects on livestock and human health (Aschner, 2002). Metals entering the aquatic ecosystem can be deposited in aquatic organisms through the effects of bioconcentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level (Huang, 2003). Heavy metals dissolved in water are readily available to aquatic organisms and cause serious effects on their health. Gross pollution of aquatic bodies by heavy metals makes the water non-potable and brings heavy fish mortality. In recent years bulk fish mortality after release of industrial effluents is a common incidence in many parts of India. In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues (Mansour and Sidky, 2002).

The issue is to monitor heavy metals entries into environment, especially in areas with highly developed industry (Toman et al., 2003). Concern for metal exposure in developing countries was highlighted in South-East Asia (WHO, 2005). Direct toxicity to human and aquatic life and indirect toxicity through accumulation of metals in the aquatic food chain are the focus of the threatening concern. Avoidance of new applications of the metals is of fundamental importance in protecting humans and animals from adverse health effects (Landrigan et al., 2006)

Effects on fish

Heavy metal toxicity is one of the major environment health problems and is potentially dangerous because of bioaccumulation through food chain (Aycicek et al., 2008). It causes hazardous effects on livestock and human health (Aschner, 2002). Heavy metals like mercury, cadmium, arsenic and lead have no known role in biological systems (Sallam K.H. et al., 1999; Schmitt et al., 2005; Has-Schon et al., 2007).
The fish populations are vulnerable because the aquatic environment is the recipient of virtually every form of human waste (Moyle and Leidy, 1992). Many industrial and agricultural chemicals (including heavy metals and alkylphenols) present in the environment have adverse effects on the reproductive function of fish (Popek et al., 2006). Greenberg and Wingfield (1987) reviewed the physiology of the relationships between stress and reproduction in the poikilothermic vertebrates. The toxic effect of pollutants may affect behaviour, cellular metabolism, endocrine regulations and reproduction of aquatic organisms (Rurangawa et al., 1998). Low-levels of pollution may have no apparent impact on the fish itself, however it may decrease the fecundity leading to a long-term decline and eventual extinction of this important natural resource (Krishnani et al., 2003; Burger and Gochfeld, 2005). Salts of heavy metals exert a depressive action on fish. It has been reported that heavy metals affect both quality and quantity of the gametes as well as the endocrine system, disrupting the gametogenesis (Jezierska and Witeska, 2001). Metals exert a diverse toxic effects on biological systems as their chemical reactivity is diverse e.g. Treatment of lead leads to decreased spermatogenesis (Singh and Singh. 1992) and disturbance in secretion of LH a gonadotropin (Singh and Singh, 1992; Buettner, 1993). In the fish body lead can bind important enzymes and inactivate them (Diertert et al., 2007). Effects of heavy metals on fish are multidirectional and manifested by numerous changes in physiological and chemical process of the body system (Dimitrova et al., 1994).

Cadmium reduces the weight of ovaries thereby arresting the development (Szczerbik et al., 2005). Though zinc is an essential element for fish, its higher concentration causes arrest of oocyte maturation and degenerative changes in testis (Patil and Dhande, 2000). Disruption of reproductive function by zinc has been described at different stages of fish development (O’Dell BL 1992, Popek et al., 1997; Popek et al., 2003). Zinc was reported to increase the activity of gonadotropins (Roy, 1961). Chromium exposure of fish causes impairment in hypothalamo-pituitary axis (Mishra and Mohanty, 2009) which results in disturbance in reproduction. Lead has a profound influence on the hormonal profile of fish (Ramesh et al., 2008). Heavy metals accumulate in brain of carp and result in disturbance in spawning (Popek,
Several workers have documented ecotoxicological manifestations of mercury in various organs of fishes, but there are very few records regarding the adverse effects of mercury on the gonadal profile of fish.

**Toxicity Testing**

Aquatic toxicology is a multidisciplinary scoped science branch and interdisciplinary in practice. Toxic effects of natural or artificial substances on living organisms are studied in ecotoxicology (Butler, 1978). Since the inception, aquatic toxicology has provided critical insights into the state of our environment and early warning of the hazards posed by environmental pollutants (Pritchard, 1993). It has made an immense contribution to our understanding of how natural and manmade substances affect the living environment. Toxicity tests can assist in providing a rationale for awareness of aquatic pollution and steps for its control.

**Acute Toxicity**

The purpose of fish acute toxicity is for decision, whether a certain xenobiotic is dangerous for the aquatic environment. Acute toxicity tests are short-term tests designed to measure the effects of toxic agents on aquatic species during a short period of their life span (Ebrahimpour and Rakhshah, 2010). Acute toxicity tests evaluate the relative toxicity of a chemical to selected aquatic organisms after short term exposure to various concentrations of the test chemical. This test determines the test material concentration at which 50% of the test organisms survive for a specified exposure time (usually 24-96 h). The critical concentration of test material is estimated by exposing the test organisms to a graded logarithmic series of concentrations of toxicants and observing their responses (Buikema Jr. et al., 1982).

It is necessary to review the contribution of different workers in the field of toxicology for critical evaluation and comparison of the results of the present investigation. Many investigators have determined the toxicity and LC50 values in response to industrial effluents, heavy metals and their salts and organic substances in fish and other invertebrate and vertebrate organisms (Funes et al., 2006; Nikelje, 2007; Casas et al., 2008; Kale, 2010).
The effect of acute toxicity of mercury and lead compounds on several fish species have been reported by various workers. Ward et al., (1982) observed that fish when treated with any toxic material showed many behavioural changes like erratic swimming, loss of reflex, discoloration and excessive mucous production. Mercury was consistently most toxic metal in the series of Hg, Cd, Cr\(^+6\), Ni and Zn for freshwater annelids, insects and gastropods (Rehwoldt et al., 1972), crustaceans (Wilson and Connor, 1971) and fish (Weir and Hine, 1970). Acute toxicity values of mercury compounds for various marine species are summarized by Eisler (1977) and these values are similar to those reported for fresh water groups (Mackim, 1977). Krishnakumari et al., (1983) conducted acute toxicity tests to fish *Teropan jarbua* and concluded that mercury, vanadium and lead are more toxic to *T. jarbua* than other metals. Hiraoka et al., (1985) reported that Ag, Hg, Cu and Cd are the most toxic metals to medaka fish (*Oryzias latipes*). Dimitrova et al., (1994) commented that effect of heavy metals on fish are multidirectional and manifested by numerous changes in physiological and chemical process of the body systems. Significant alterations in nucleic acids in *Cyprinus carpio* due to heavy metals were reported by Muley et al., (2000).

LC\(_{50}\) values of mercury salts to a fresh water snail *Viviparous bengalensis* were reported by Muley and Mane (1989). LC\(_{50}\) values of copper, mercury, cadmium and zinc to intertidal gastropod *Morula granulata* were determined by Devi (1997). Fishes showed a wide range of LC\(_{50}\) values for mercuric chloride. It is 0.55ppm for *Leuciscus cephalus* (Gul, 2004), 1.43ppm for *Clarius batrachus* (Selvanathan, 2011), 2.113ppm for *Channa punctatus* (Agarwal, 1991). Acute toxicity of zinc to some developmental stages of *C. mrigala* was assessed by Sharma and Sharma (1992). Eggs were found more resistant to zinc than their developing stages. Acute toxicity levels of mercury to fish *Tilapia mossambica* was determined by Menezes and Qasim (1984). Bioassay was carried out for HgCl\(_2\) on catfish *Heteropneustes fossilis* by Singh and Munshi (1992). Dhande and Patil (1998) estimated the LC\(_{50}\) values of mercuric chloride, cadmium chloride and cupric chloride for *C. punctatus*. Variable 96-hr LC50 values of mercury were reported as 0.181, 0.51, 0.13 and 0.51 mgL\(^{-1}\) for *Barbus conchonus*, *Clarius batrachus*, *Etrophus maculates* and *Salmo gairdneri*.
respectively (Veena et al., 1997; Iliopoulou Georgudaki and Kotsanis, 2001). Acute exposure to HgCl\textsubscript{2} and behaviour changes were studied in European fresh water carp \textit{Leuciscus cephalus} by Gul et al., (2004). Acute toxicity of mercury to fingerlings of Indian major carps \textit{Catla catla}, \textit{Laboe rohita} and \textit{C. mrigala} in relation to water hardness and temperature was reported by Kumar (2006). Acute toxicity of Hg as HgCl\textsubscript{2} to a genetically important fish \textit{Capoeta fusca} was reported by Mansouri and Baramaki (2011) and suggested that mercury is more toxic in the soft water than in hard water. The median lethal concentration (LC\textsubscript{50}) of mercury to \textit{C. punctatus} was determined by Jayanthi and Selvakumar (2011).

Davies et al. (1976) studied toxicity of lead to rainbow trout \textit{Salmo gairdneri}. Srivastava and Mishra (1979) reported 96-hr LC\textsubscript{50} of lead as 19.00 mgL\textsuperscript{-1} for a gourami \textit{Colisa fasciatus}. Prasanna and Rao (1994) estimated LC50 values of lead to \textit{Nerita albicilla} a marine gastropod. Bhilave (2000) reported acute toxicity of lead acetate on fingerlings of \textit{Cirrhinus mrigala}. Olaifa et al., (2003) reported that fish exposed to sublethal levels of lead produced dose dependent increase in the concentration of lead in \textit{Oreochromis mossambicus}. Kumar et al., (2004) reported toxic effects of cadmium chloride and lead nitrate on Indian major carp, \textit{Laboe rohita} while determining LC\textsubscript{50} values.

Balawi et al., (2011) studied toxicity bioassay of lead acetate on \textit{Clarius gariepinus}. The acute toxicity of mercury, plumb and zinc was evaluated by Hedayati et al., (2012) on fresh water fish \textit{Rutilus rutilus}. Waterborne metals have generally exhibited their greatest toxicity to aquatic organisms in soft waters of low pH (Penttinen et al., 1995).

**Chronic Toxicity**

Acute toxicity tests have significant limitations due to short-term exposure in revealing the adaptation of fishes to the toxicants. Perkin, (1979) suggested the use of sublethal tests to study the responses of organisms. Aquatic organisms are often exposed to chronic levels of chemicals in their environment (Handy et al., 2003). Though field observations are more useful, are expensive, not standardized and time
consuming hence controlled laboratory studies using single species gained more importance.

The chronic aquatic toxicity test is long-term but definite toxicity which involves repeated administration of a toxicant over a considerable period of time. Chronic toxicity tests are used to study the effects of chemicals on the structure and function of organs, tissues and cells after a prolonged period of exposure. Safe levels of toxicants and secondary effects of chemicals can also be detected by chronic toxicity.

Toxicity effects after exposure to sublethal concentration of inorganic mercury was documented by Panigrahi (1980). Chronic toxic effect of chromium was studied by Sastry and Sunitha (1984) in Channa punctatus. Narayan and Madyastha (1985) reviewed heavy metal pollution and toxic effects on marine ecosystem. Histological microanalysis of Cd and Hg exposure in Brachydonia rerio was recorded by Delamarre and Trunchet (1985). Victor et al. (1986) studied toxicity of mercury and cadmium on differentiation and vitellogenesis of teleost. Radhakrishnaiah (1988) studied exposure of Labeo rohita to copper. Adverse effect of mercury on different fish tissues was reported by Kirubangaran (1988). Chronic toxic exposure to Cd on Puntius ticto was studied by Pundir and Saxena (1990). Mercury taken into the body is distributed and deposited in the endocrine glands as well as in the liver, kidney, brain, and other tissues of humans and laboratory animals (Khayat and Dencker, 1983, Danscher, 1990). Bakshi (1991) reported the effects of copper on Heteropneustus fossilis. Srinivas and Rao (1998) studied chromium induced alterations in fresh water teleost Labeo rohita. Toxic effect of cupric and zinc chloride was reported by Patil and Dhande (2000) in Channa punctatus. Effect of nickel and chromium on Cyprinus carpio was reported by Virk and Sharma (1999). Zikie (2001) observed effect of chronic exposure of fish to metals like Zn, Cu and Cd on enzymes of liver. Ribeiro et al., (2002) described the acute and chronic histopathological effects of inorganic and methyl mercury in Salvelinius alpines. A link between exposure to heavy metals and lesions in liver was cited by Aly et al., (2003) in Clarias gariepinus. The toxicity of various compounds of heavy metals has been mainly
studied in invertebrates, fishes, amphibians and mammals. Gupta and Srivastava (2006) reported necrosis, apoptosis and cell death in fish after exposure to cadmium. Similar observations were reported by Prasanth (2011) in *Cirrhinus mrigala* exposed to cypermethrin and Ghosh (2012) in *Labeo rohita*. Mekkawy, (2011) found an increase in enzymes activity in response to chronic exposure to heavy metals in different fish species.

**Selection of Mercury**

Mercury (Hg) is a highly toxic, nonessential, persistent, immutable and non-biodegradable metal and undergoes many changes during transfer through different trophic levels of food chain. Mercury (Hg): atomic weight, 200.6; atomic number, 80; density, 13.6; melting point, -38.9°C; boiling point 356.6°C; crystalline form silver white metallic liquid; oxidation states +1, +2. Mercury occurs in the earth’s crust mainly in the form of sulfides. The red sulfide cinnabar is the most important commercial ore of mercury. Mercury or quicksilver was known in ancient times as hydragyros, hence it’s symbol Hg.

Mercury occurs in three forms, elemental form which is liquid at room temperature, but volatilizes readily. It is rapidly distributed in the body through vapour, but poorly absorbed through GI tract. Inorganic mercury, the dermal exposure which results in toxicity but poorly absorbed through the GI tract and organic mercury which is lipid soluble. It is well absorbed via GI tract, lungs and skin. It is dispersed in all tissues and some amount of it can easily cross the blood brain barrier and the placenta (Clarkson and Magos, 2006). Anaerobic organisms bio-transform the inorganic form to methyl mercury.

Mercury is an interesting metal because its organic form, methyl mercury is the most toxic. Mercury accumulates in the form of methyl mercury. Mercury is known to be the most toxic of all the heavy metals (Taylor, 1979). Mercury (Hg) is one of the oldest toxicants known (Zhu et al., 2000), with considerable risk factors of environment and food chain (Tazisong and Senwo, 2009). Mercury, cadmium and lead are among the heavy metals that are toxic to organisms at very low
concentrations and are never beneficial to living beings (Hilmy, 1985). Mercury was consistently most toxic metal in the series of Hg, Cd, Cr\textsuperscript{+6}, Ni and Zn for fresh water annelids, insects, and gastropods (Rehwoldt et al., 1972), crustaceans (Wilson and Connor, 1971), and fish (Weir and Hine, 1970). Once mercury enters in the organism it draws various immunotoxic effects.

The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other manmade activities (Velez and Montoro, 1998). Heavy metals and chemicals are toxic to animals and many cause death or sublethal pathology of liver, kidneys, reproductive systems, respiratory systems or nervous systems in both invertebrate and vertebrate aquatic animals (Wilbur, 1969). Heavy metals have devastating effects on ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi et al., 2007).

Mercury is generated naturally in the environment from the degassing of the earth’s crust from volcanic emissions. Mercury enters natural waters through industrial discharge (such as chlor alkali industry) where, it is converted into very stable and water soluble methyl mercury ion. This compound is formed in aquatic ecosystems when naturally occurring bacteria methylate inorganic mercury. The reaction takes place at the water sediment interface (Wright and Welbourn, 2002) and is facilitated by low pH and high dissolved organic carbon. Methylmercury dissolves well in water, crosses biological membranes, and persists in fatty tissues of organisms. Hg (CH\textsubscript{3} )\textsuperscript{+} is taken up by fish and through food chain and enters into higher animals and man. Most of the fish today have a mercury conc. of 0.02 to 0.2 ppm. Mercury has been recently proposed as a potential endocrine disrupter (Colburn and Clement, 1992).

Mercury is extremely harmful, even at a concentration of 0.03 ppm in drinking water is not permissible. Mercury levels at the top of the food chain are thousands or millions of times higher than in water or sediments (Wright and Welbourn, 2002). In Japan in the 1950s and 1960s wastes from a chemical and plastics plant containing mercury were drained into Minamata Bay and caused the disaster. Mercury becomes a unique environmental toxicant because of its volatility and
biotransformation. Most industrial countries have banned this use, and the production of alkyl mercury compounds has decreased. Several large episodes of mercury poisoning have resulted from consuming seed grain treated with mercury fungicides or from eating fish contaminated with methyl mercury.

For healthy fish production it is very important to evaluate the harmful effects of heavy metals. (Cunha et al., 2007). The heavy metals may accumulate in the body of fish either through “water path” or “food path” (Feldlight et al., 2008). Like water, mercury can evaporate and become airborne. Because it is an element, mercury does not break down into less toxic substances. Once mercury escapes to the environment it circulates in and out of atmosphere until it ends up in lakes and ocean bottom. Strict regulations have reduced the industrial use of mercury; still high concentrations are present in sediments with industrial applications of mercury (Klaassen et al., 1986). Fish are considered sensitive indicators of aquatic pollution and tend to accumulate both organic and inorganic forms of mercury (Gochefeld, 2003). There are still little data on mercury exposure and its effects in different organs in tropical fish (Mela et al., 2007). A little information is available concerning the underlying mechanisms in the pathogenesis of Hg induced male reproductive dysfunction (Boujbiha et al., 2009). Impact of methylmercury on reproduction of fish have been identified by studies however to understand the underlying mechanisms of these effects, it is critical to look at alterations in neuroendocrine control, because the central nervous system is the primary target for mercury action (Clarkson and Magos, 2006). Problems like infertility, disturbances in the menstrual cycle and inhibition of ovulation arise from ovarian targeting of all chemical forms of mercury (Schuurs, 1999). It has been shown that mercury accumulates in pituitary (Lamperti and Printz, 1974; Erfurth et al., 1990). Convincing evidence in the literature suggesting mercury effects on ovary and hypothalamic pituitary is lacking (Davis et al., 2001). It points up the need for more studies on mercury effects on reproductive system.

Selection of Lead

The Lead is an element and a metal in the carbon group with symbol Pb (Pb is an abbreviation of the latin word plumbum, meaning soft metal) and atomic number
82. It is soft, has a low melting point (327.5°C) and a high density (11.34g/cm³). It is a malleable, corrosion resistant metal naturally found in a variety of minerals including galena, cerussite and anglesite.

Lead is not required by any living organism hence it is one of a limited class of elements that can be described as purely toxic. It has a long environmental persistence and never loses its toxic potential if ingested and persist in bones and teeth for decades. It is oldest and versatile common metal. Archeological research indicates that lead has been used by humans for a variety of purposes for more than 5000 years. It was used by Royal families in Egypt before 3000 B.C.

Lead is rarely found in its native form but it combines with other elements to form a variety of beautiful minerals. Lead is cheap and easy to work with and hence the serious risks of it are often overshadowed by its economic value since a long time.

Early applications of lead include building materials, pigments for glazing ceramics, and pipes for transporting water. In ancient time Romans used it in ornaments, coins, cooking utensils and cosmetics and to sweeten wine as it was found to enhance both the color and bouquet of wine.

Following World War I, the demand for lead increased because of growth in the production in the motorized vehicles, many of which use lead-acid batteries to start their engines. Its density contributes its usefulness in making bullets, weights of various sorts and X-ray shielding. The use of lead as radiation shielding in medical analysis, video display equipment and as an additive in gasoline contributed to an increase in the demand of lead.

By the mid-1980s, a significant shift in the uses of lead had taken place in the United States as a result of compliance of environmental regulations. A change in use of lead in non-battery products, such as gasoline, paints, solders, and water systems had taken place in United States, while by the early 2000s, lead consumption was in lead-acid batteries. Today other significant uses of lead are in ammunition, oxides in glass and ceramics, casting metals, and sheet lead. Lead, combined with bismuth, acts as a coolant for certain types of nuclear reactors. Due to its water-resistive property,
lead sheets are utilized in the construction industry for weathering, roofing and cladding to prevent water penetration.

Lead based paints, leaded fuel for on road vehicles, lead containing toys and other lead products are still widely available in developing countries. Lead is still a major component in some industrial paints, leaded fuel is still used in aircrafts, racing automobiles and marine engines. Fishing gear, batteries, curtain solder, ceramics, hair dyes do contain lead. As a result, considerable lead is disposed off in the aquatic system every year.

The toxic effects of lead have been reported for over 2000 years in both humans and animals (Nriagu, 1983). The first report on lead poisoning was published in USA by Grinnell (1894). However, publication of Rachel Carson’s momentous ‘Silent Spring’ in 1962 refocused attention on the links between chemicals in the environment and the health of people and animals. We know a great deal about the more obvious cases of death and debility caused by lead, but extremely little about the more subtle chronic and sub lethal effects. Acute and sub acute effects are typically caused by relatively larger doses of lead over a short period of days to months. These effects can be dramatic, while chronic effects are quite subtle and non specific but include all body systems. There are written accounts of lead toxicity in Egyptian papyrus scrolls, which indicate that lead compounds were often used for homicidal purposes (Hernberg, 2000). There is no safe threshold for lead.

The report “Lead Hazards to Fish, Wildlife, and Invertebrates, a Synoptic Review” put out by the USFWS in 1988 (Eisler,1988), estimated that U.S. hunters deposited some 6000 metric tons of lead shot annually into lakes, ponds, and estuaries. In Canada, it is estimated that some 500 tons of lost or discarded fishing weights and jigs are deposited into the environment each year. This represents up to 14% of all non-recoverable lead releases in Canada (Scheuhammer, et al 2003). In India, lead consumption has grown at the rate of 10.5% during 1997-2000 while it was 2.5% for all other countries during the same period. The steep growth in lead consumption in India is primarily due to the sharp rise in automobile production as a result of economic and market liberalisation. Substantial increase in use of lead acid
batteries in domestic inverters and UPSs for computers is also a major contributing factor.

Recent reports indicated that lead can cause neurological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes in the animals (Park et al., 2006; Berrahal et al., 2007; Reglero et al., 2009; Abdallah et al., 2010; Mobarak and Saraf, 2011). Lead displaces biologically important metals such as calcium, zinc and magnesium interfering with a variety of body’s chemical reactions.

**Cirrhinus mrigala as an experimental model**

The concept of animals use as sentinels of human health is not new. The systemic study of toxic effects in laboratory animals began in 1920s in response to concerns about the unwanted side effects of food additives, drugs and pesticides (Rodricks, 1992). Rachel Carson’s classic book ‘Silent Spring’ almost single handedly created modern society’s fears about synthetic chemicals in the environment and fostered interest in the science of toxicology (Carson, 1962). Initially it was the era of the ‘pickle jar bioassay’ (Doudoroff, 1976) in which toxicity tests with aquatic organisms were conducted in 1-5 gallon jars. Classical studies in toxicity with aquatic organisms include those by Doudoroff and Katz (1950,1953), Hart et al., (1945), Doudofoff et al., (1951), Cairns (1957), Henderson (1957) and Tarzwell (1958). Hart et al., (1945) introduced the single species fish bioassay. Doudoroff et al., (1951) and Cairns and Pratt (1989) demonstrated the usefulness of exposing fish and other aquatic organisms to chemical and wastes in toxicity tests (Sprague, 1985). This work established the acute toxicity test as the cornerstone of methodologies to study and monitor pollution effects (Buikema et al., 1985) a position it still retains. The fish toxicity test was the basis of first protocol of the American Society for Testing and Materials (ASTM 1954). The various studies carried out on different fish species have shown that heavy metals may change the biochemical composition and physiological activities (Basa and Rani, 2003; Canli, 1995; Tort and Torres, 1988).

In order to extrapolate meaningful, relevant and ecologically significant results from aquatic toxicity tests appropriate organisms should be used. Intensive research of
aquatic toxicology has been done in fathead minnow (*Pimephales promelas*), bluegill (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*), sheephead minnow (*Cyprinodon variegates*), gold fish, common carp, zebra fish, snails, rotifers and daphnids (Greene et al., 1988).

Fishes form an important group among vertebrates. Fish is the most susceptible to heavy metal toxicants (Nwaedozie, 1998) so, are more vulnerable to metal contamination than any other aquatic fauna. After reaching sufficiently high concentrations in body cells the metals can alter the physiological functioning of the fish (Heath, 1987). Fishes are selected on the basis of availability, commercial, recreational and ecological importance, past successful use, ease of handling in the laboratory and regulatory use (ASTM 1992 a, b). Their use is encouraged to increase comparability of results and availability of much information about a few species rather than a little information about many species (ASTM 1992, a). Fish are ideal sentinels for toxicological studies due to their ----

1) constant, direct contact with the aquatic environment where chemical exposure occurs over the entire body surface.

2) ecological relevance in many natural systems (Little et al., 1993 a).

3) ease of culture.

4) ability to come into reproductive readiness (Henry and Atchison, 1986) and

5) a long history of use in behavioral toxicology.

Fish occupy a prominent position in the field of toxicology; they have been employed amply in studies concerning both human and ecological health, probably bridging this divide more than any other class of organisms. Fish are the most diverse group of vertebrate, in which 28,000 species have been identified to date and are greater than the combined number for the other classes (Cossins and Crawford, 2005).

The diversity of fish in habitat and species has made the fish available for scientific inquiries. As aquatic food chains are longer than terrestrial food chains many pollutants achieve a greater concentration in aquatic predators (Clements and
Newman, 2002). Fish and amphibians are the only vertebrates with amniotic eggs (lacking a shell or amniotic membrane) and that undergo metamorphosis in surface waters, hence, the embryo, larval stages of these animals are highly sensitive to chemical pollutants (Kendall et al., 2001).

In present study fresh water fish and a major Indian carp *Cirrhinus mrigala* has been used as model/test animal for the studies on effect of heavy metals (mercury and lead) on pituitary gland and gonads. *C. mrigala* commonly known as mrigal distributed in India is a major cultivable fish, highly esteemed as food and used in pisciculture. Dried form used in poultry food. A major percent contribution is given by *C.mrigala* in freshwater aquaculture along the Indian subcontinent.

**Classification of *Cirrhinus mrigala* (Nelson, 1976)**

Phylum – Chordata

Sub-phylum- Vertebrata

  Superclass- Gnathostomata

  Grade- Pisces

  Class- Osteichthyes

  Subclass- Actinopterygii

  Infraclass- Teleosti

  Order- Cypriniformes

  Family- Cyprinidae

  Genus – *Cirrhinus* (Oken, 1817)

  Species – *mrigala* (Hamilton)

*C. mrigala* a major Indian carp found in rivers, ponds and reservoirs with or without aquatic vegetation. It is silver dark grey with golden eyes and caudal fin deeply forked. It is an active and jumpy carp. *C. mrigala* is an herbivore, bottom feeder and a monsoon breeder. Sexes are separate and can be distinguished with advancing maturity and breeding season. Females are larger than males. The females
have smooth to touch pectoral fin and near maturity have a swollen vent, distinctly soft, bulging and rounded abdomen due to evident growth of ovaries. The genital opening is round. The males on the other hand develop fine denticulation on dorsal side of pectoral fin rays. Thus providing roughness to touch. The ripe males ooze milt when their belly is gently pressed. The male genital opening is slit like.

**Study of gonadosomatic index**

Gonadosomatic index (GSI) is the ratio of fish gonad weight to body weight. It measures reproductive strain of fishes by measuring general body weight ratios. It is one of the important parameter of fish biology, which gives the detail idea regarding the fish reproduction and reproductive status of the species particularly in identifying seasons of spawning. Gonadosomatic index and volume of gonads are indicators of gonadal state (Saksena, 1987). The GSI increases with fish maturity and reaches to its maximum at the peak of maturity. Gonadosomatic index helps in ascertaining breeding period of fish, (Ha and Kinzie, 1996; Mohan and Jhajhria, 2001; Shankar and Kulkarni, 2005). Tropical fish that spawned uniformly throughout the year generally showed smaller variations in the GSI value than those that spawned in the short season (Wootton, 1979).

Most of the Indian freshwater teleosts attain maturity and breed during monsoon, hence increase in GSI values is observed during monsoon (Encina and Lorencio, 1997). High values of GSI in rainy season indicate accumulation of large quantity of yolk in ripe ova (Kapil et al., 2011). The lowest values of GSI in winter indicate depletion of gonadal products due to intense spawning, shedding of eggs and resorption of remains of ova in the spent ovaries (Adamassu, 1996). The estimated values of GSI reveal spawning time in a year. It is studied in different fresh water fishes by a number of workers.

Increase in GSI with advanced developmental stages of ovaries was reported in *Barbus longiceps* (Stoumbandi et al., 1993), in *Puntius filamentosus* (Manna et al., 2010). Wilson and Nieland (1994) reported GSI peak in Mexican fish *Sciaenops ocellatus*. *Cirrhinus reba* has a short duration breeding season in July, Qazi (2001) and its GSI is directly proportional to spawning and inversely proportional to post
spawning season (Shendage and Mane 2006). *Cirrhinus reba* from Pakistan was studied by Lashari et al., (2007) and reported same conclusion. Sindhe and Kulkarni (2004) concluded that GSI values are correlated with increased amount of protein and lipids during pre and post breeding seasons in the freshwater fish *Notopterus notopterus*. Narejo et al., (2002) studied the GSI, ova diameter and fecundity of *Mastacembalus armatus* while the same features of reproduction in *Macrognathus aculeatus* were studied by Abujam and Biswas (2011). GSI in gold fish *Carassius auratus* was studied by Ortega-Salas and Bustamente (2006). Sarker et al., (2002) studied it in *Mystus gulio*, Hassanin et al., (2002) and Ghanbahadur (2012) in *Cyprinus carpio* and Brewer et al., (2008) in a small bodied riverine fish. The cyclic changes in GSI of three major carps was studied by Shaikh and Lohar (2011). It was concluded after studies on cyprinid fish *Neolissochilus soroideus* from peninsular Malaysia that the females at the mature stages yielded much higher GSI values compared with the males (Khaironizam and Ismail, 2012). Chandra et al., (2004) suggested that stressors like electroplating effluent affect the reproductive potential by reducing the GSI in fish. A comparative account of GSI and fecundity on two species of ribbon fishes, *Trichiurus lepturus* and *Lepturacanthus savala* was given by Chakravarty et al., (2013). The fall in the GSI is not rapid in fishes of confined waters as they do not spawn there and the gonads are gradually resorbed (Parmeswaran et al., 1972) Seasonal changes in GSI are closely interwoven with gonadotropic potency of proximal pars distalis (PPD) of pituitary (Bhatt and Negi, 1986). Prat et al.,(1996) observed that an increase in GSI and gonadotropin I stimulates the uptake of vitellogenin into oocytes, however, dramatic increase in GSI further decreases plasma gonadotropin I level in rainbow trout. Gen et al., (2000) found a progressive increase in vitellogenin levels with an increase in GSI in red sea bream *Pagrus major*. Kokokiris et al., (2000) found parallel fluctuations in GSI and vitellogenin levels of female red porgy, *Pagrus pagrus*. Singh and Singh (1984) reported parallel fluctuations in pituitary gonadotropin and GSI in preparatory phase in *Cirrhinus mrigala*. A correlation between the GSI and plasma gonadotropin II levels was established by Maitra et al., (2007) in *Cirrhinus mrigala*. The GSI in *C. mrigala* is
considered for study as an indicative of the state of gonadal development and maturity.

**Selection of pituitary as a target organ-**

The endocrine master gland pituitary is a complex neuroepithelial structure. It is derived embryologically from a down push of the floor of the hypothalamus (forming the pars nervosa) and an up growth of the roof of the mouth, forming the pars distalis and pars intermedia of the adult (Leatherland and Ferguson, 2006; Roberts and Ellis, 2001). The pituitary gland is situated on the ventral side of brain in a concavity called sella turcica in carps and is covered by duramatter. Sella turcica is generally absent in fishes (Misra and Satyanesan, 1959; Lal, 1964), however, it is reported in fishes like *Hilsa hilsa* (Khan, 1962) and *Cirrhinus mrigala* (Robertson, 1962). The pituitary gland is connected to the brain by infundibulum. In all vertebrates, the pituitary gland or hypophysis consists of two parts, separable on the bases of embryology, structure and function. These parts are neurohypophysis and adenohypophysis (Ross and Pawlina, 2003). Great advances have been made in understanding the central role and physiology of the pituitary during the last century. The small size of pituitary belies its importance and complexity, including its intricate embryology, structural heterogeneity and functional diversity (Amar and Weiss, 2003). The survivors of once numerous groups actinopterygians pituitary gland is of obvious great interest as it helps to clarify the evolutionary developments leading to the highly specialized gland of teleosts. The teleostean pituitary varies in the organization, arrangement and orientation of its different components from species to species. The anterior, intermediate and posterior lobes of the pituitary gland act as separate entities and distinct cell populations, secretory products and regulatory mechanisms characterize each.

On the basis of morphology and following the terminology of Green (1951), the teleost adenohypophysis can be divided into the anteriorly situated pars distalis (PD) and the posteriorly situated pars intermedia (PI). The PD can be further divided into the rostral pars distalis (RPD) and the proximal pars distalis (PPD). Mammals show a hypothalamo - hypophyseal portal system for the transport of neuroharmonal
regulators while teleosts lack such system. Instead they show a direct axonal transport from hypothalamic neurons to pituitary endocrine cells through the hypophysial stalk and the neurohypophysis of pars nervosa (Ball, 1981).

It is well established that gametogenesis in fish like other vertebrates is regulated by the reproductive endocrine system including the brain (hypothalamus), pituitary gland and gonad. Moitra and Sarkar (1976) made the first observation that average weight of the pituitary gland is directly proportional to the state of maturation of the gonads. Great advances have been done in understanding the physiology of pituitary and its central role in governing the homeostatic functions of the body. The pituitary gland of teleost shows typical endocrine cells in the adenohypophyseal region. The morphological characterization and distribution of each cell type have been studied by histochemical, ultrastructural and immunocytochemical techniques (Ball and Baker, 1969; Follenius et al; 1978). The morphological, histological and histochemical studies on the pituitary gland of Cirrhina mrigala were done by Lal (1963). The studies gave a view to gain insight into seasonal changes in cellular composition of pituitary gland. Studies on fish pituitary glands proved that many physiological functions like growth, reproduction, reproduction related behaviour, instinct for nitrification, regulation of body electrolyte balance and gonad development are controlled by hormones that are released from the pituitary gland, in the same way that it functions in mammals (Val-Sella, 1977; Evans, 1998).

The regulation is achieved through the hormones secreted by various types of cells like thyrotropes, lactotropes, adrenocorticotropes and gonadotropes. The pituitary gland primarily through the action of gonadotropins (GtHs) the follicle stimulating hormone (FSH) and leutinizing hormone (LH) plays a central role in initiating reproductive maturation (puberty), maintaining production of sperm and eggs by the gonads and inducing final maturation and gamete release (spawning). Synthesized by the gonadotropes of the pituitary the gonadotropins (FSH and LH) mediate various stages of gametogenesis, oocyte growth, oocyte maturation, spermatogenesis and spermiation.
The pituitary gland contains highly differentiated and committed cells which synthesize unique hormone products. The differentiation of the pituitary cells follow a particular pattern and sequence (Treier et al., 1998). Different cell types of pituitary progress after initial patterning only after the induction of specific transcription factors (Lamolet et al., 2001; Zhao et al., 2001). The gonadotropes vary depending on the moment of the reproductive cycle, age and sex of the animal. Seasonal changes in the gonadotropes during the annual reproductive cycle have been reported in fish (Hifny, et al., 1982; Schimizu et al., 2003; Vongratchranon et al., 2005; Filippa, 2005). Joy and Sathyanesan (1979) demonstrated that the basophils in the pituitary gradually increase in their number as gonads start maturing and as the gonads become ripe these cells from the major component of the proximal pars distalis (PPD). The conclusions were based on the studies made on *Clarius batrachus*. The morphohistology of pituitary gland of two Indian carps *Labeo rohita* and *Cirrhinus mrigala* was studied by Moitra and Sarkar, (1976). A variety of tinctorial techniques were suggested to localize the various cell types in pituitary of roach *Rutilus rutilus* and six glandular cell types were localized and their distribution was discussed by Jafri and Ensor (1980). It was revealed after the studies on pituitary gland of *Padogobius marteusi* (Cinquetti and Dramis, 2006) and *Oreochromis niloticus* (Kasper et al., 2006) that the neurohypophysis ramifies the adenohypophysis. Histochemical investigation of the pituitary glands in three freshwater teleost fish, the perch *Perca fluviatilis*, the roach *Rutilus rutilus*, and the minnow *Phoxinus laevis* was done by Matty and Matty (1959). An anatomical and histochemical examination of the pituitary gland of *Cyprinus carpio* was done by Ekici and Timur, (2013).

Olivereau and Nagahama (1983) have indicated the possibility of two types of gonadotropes in some teleost pituitaries. However, Holmes and Ball, (1974), Putten et al., (1981) believed that the two types of cells actually represent different stages in the secretory cycle of the same cell. Primitive teleost like European eel *Anguilla anguilla* (Queret et al., 1990), Chinook salmon *Oncorhynchus tschawytsha* (Breton et al., 1978) and African catfish *Clarias gariepinus* (Koide et al., 1992; Schulz et al., 1997) show a single gonadotrope. Studies on the perch, *Perca fluviatilis* (Domovska, 1970
and 1977), Anguilla japonica (Yoshiura et al., 1999), cichlid fish (Parhar et al., 2002), Oreochromis niloticus (Kasper et al., 2006) reported two types of gonadotropes in PPD. Ultrastructure of adenohypophysis in a teleost Poecilia latipinna was studied to observe the cell types by Batten et al., (1975). Narayan et al., (1984) reported morphohistology of the pituitary gland in the esturine teleost Valamugil cunnesius for various cell types. Five cell types were identified in the adenohypophysis of Clarias lazera (El-Zoghby et al., 2008) seven in Oreochromis niloticus (El-Gohary, 2001; El-Sakhawy et al., 2011) and eight distinct hormone producing cells were identified and localised in Atlantic halibut, Hippoglossus hippoglossus (Weltzien et al., 2003). Cell types in the adenohypophysis of pituitary gland in relation to the annual reproductive cycle in exotic carp, Carassius carassius were reported by Bhatt and Negi, (1986). Cyclic changes in the pituitary gonadotropes in relation to the ovarian cycle in Puntius sarana were reported by Bhat and Dutt, (1989). Increased gonadal growth brings about relative increase in diameter of gonadotropes (Massoud et al., 1983). A correlation between the activity of the pituitary gonadotropes and the gonads in a popular mariculture rabbit fish Siganus rivulatus was studied by Zaki et al., (1994). Seasonal changes in gonadotropes activity and GSI was correlated in African catfish Clarias gariepinus by Van Oordt et al., (1987). Ursani et al., (2012) detected histological changes in gonadotropes in relation to seasonal changes in ovaries in an exotic fish Oreochromis mossambicus. Gonadotropes from stream fish Nemacheilus mooreh were studied by Kharat and Khillare (2013) and it was observed that gonadotropes became more active with change in nuclear size and cytoplasmic granulation during vitellogenesis.

Immunoreactive gonadotropin (GTH) like material was examined in the hagfish pituitary in correlation with their gonadal conditions by Nozaki et al., (2003). Immunohistochemicel identification and localization of different pituitary cell types in Salminus hilarii females during the annual reproductive cycle was done by Honji et al., (2013). Investigations on the pituitary of the Acipenser baeri, were done by Pelisscro et al., (1988). Vongvatcharanon et al., (2005) studied alteration of gonadotropes in pituitary gland during the reproductive cycle in sand goby Oxyleotris marmoratus by immunohistochemistry. A variety of environmental
contaminants including heavy metals interfere the endocrine axis in fish (Bonga, 1997; Boas et al., 2006; Carr and Patino, 2011). Exposure to cadmium chloride in catfish *Clarias batrachus* caused a significant increase in the ACTH cells, while thyrotropin and gonadotropin secreting cells showed inactivation and accumulation of secretory products (Jadhav et al., 1994). Chromium induced impact on the pituitary-ovarian axis has been demonstrated (Mishra and Mohanty, 2012a, b). Deleterious effects of cadmium on the pituitary gland were reported by Pundir and Saxena (1992). It is reported by Kumari and Gopal (1991) in *Puntius sarana* that high concentrations of CdCl₂ influence the pituitary gonadotropes by bringing about gradual accumulation of secretory granules. Pituitary secretory activity is affected by metals (Ronis et al., 1998). This endocrine gland is particularly sensitive target to cadmium toxicity (Lafuente et al., 2001).

**Selection of gonads for study**

Sexes are usually separate in fishes. Indian major carps breed in rivers once in a year during monsoon months (Ibrahim 1961, Quasim and Quyyum, 1962, Natarajan and Jhingran; 1963). *C. mrigala* is a seasonal breeder hence all reproductive organs undergo cyclic changes. Sexes are separate in *C. mrigala*. Sex differentiation is most remarkable in size. The ripe males ooze milt when their belly is gently pressed, while females have a swollen vent and distinctly soft, bulging and rounded abdomen due to evident growth of ovaries.

The male reproductive organs in *C. mrigala* are a pair of testes. Testes are elongated and somewhat fattened structures ventral to kidneys in posterior region of abdominal cavity. A main sperm duct arises from the posterior middorsal surface of each elongated testes and leads to the urinogenital papilla located between rectum and the urinary ducts. The basic and complementary tasks of the gonads of teleosts, like those of higher vertebrates, are to produce fertilizable gametes (i.e. eggs and sperm) necessary for successful reproduction and the pituitary dependent synthesis and secretion of a variety of steroid hormones which regulate the development of germ cells (Nagahama, 1983). The timing of gonadal sex differentiation varies according to species and sex.
Testes

Spermatogenesis is a highly organized and coordinated process, in which diploid spermatogonia proliferate and differentiate to form mature spermatozoa. The process is shorter in fish than in mammals and is influenced by water temperature (Nobrega et al., 2009).

The testes are of the lobular type in *C. mrigala* and composed of numerous tubules which are separated from each other by a thin layer of fibrous connective tissue. The testes consist of thin walled tubules or lobules that contain germ cells- the spermatogonia ectodermal in origin. These are stem cells present throughout the year and divide mitotically giving rise to next generation spermatogonia which then transform to primary spermatocyte. The primary spermatocytes divide by meiosis and give rise to spermatids which differentiate into spermatozoa as the spermatogenesis and spermiogenesis proceeds. Leydig’s cell the interstitial cells are large, polygonal and located within interlobular spaces. The main source of steroids are Leydig cells (Hurk et al., 1978). However, sertoli cells also have enzymes involved in steroidogenesis. Stromal cells around vas deferens epithelium are also steroidogenic (Guraya, 1976b).

In *C. mrigala*, testes are thin, ribbon like in immature fish and develop into somewhat flattened, whitish yellow organs which become relatively solid as the fish advance in maturity. Ovaries in immature fish closely resemble immature testes in appearance. Testes progressively enlarge in length and girth and become somewhat pinkish as the fish progresses in maturity.

A majority of teleosts are seasonal breeders while a few breed continuously. A vast majority of fresh water fishes breed during the monsoon season in Indian subcontinent, when rainfall is heaviest (Jhingran, 1975). *C. mrigala* breeds during monsoon.

The gametogenesis is a process by which gametes are produced from gonia of mature gonads during the reproductive cycle of fish (Lam, 1983, Joy et al., 1999). Several reviews describe the conspicuous cellular, biochemical, molecular and
endocrinological changes during both the oogenesis and spermatogenesis (Wallace and Selman, 1990; Jamieson 1991; Borg, 1994). Available information indicates developmental processes during spermatogenesis in all vertebrates is similar. Undifferentiated spermatogonia give rise to differentiated spermatogonia. Number of morphological changes give rise to rapidly dividing spermatogonia. After several generations the spermatogonia differentiate into primary spermatocytes, from where the developmental stages are: primary spermatocytes (1\textsuperscript{st} meiotic division) - secondary spermatocytes (2\textsuperscript{nd} meiotic division) – spermatids (differentiation without proliferation) – spermatozoa. The number of spermatogonial generations differs between species (Ando et al., 2000). The testicular physiology is regulated by important pituitary hormones, the gonadotropins follicle stimulating hormone (FSH) and leutinizing hormone (LH). The gonadotropins stimulate gonadal sex steroid hormone production directly by activating Leydig cells. LH regulates Leydig cell sex steroid production; FSH regulates Sertoli cell activities, such as the structural, nutritional and regulatory support of germ cell development (Huhtaniemi and Themmen, 2005).

The body growth determines the timing of gonadal developmental events in fishes (Osse and van den Boogaart, 1995). Studies on structure of testicular gland, testis and spermatic duct of Aidablennius sphynx have been carried out by Lahnsteiner and Patzner, (1990a, 1990b). Histological studies on the testes development of species like Mugil seheli (Zaki et al., 1994), Labeo cylindricus (Booth and Weyl, 2000), Labeo victorianus (Rutaisire and Booth, 2004), Epinephelus areolatus (Mahmud, 2009) Labeo parvus (Montchowui et al., 2012) were done. Gonadal development in Zebrafish was studied by Maack and Segner (2003).

Healthy gonads of fish are important determinants of their breeding potential. Thus, adversely affected histoanatomy of gonads by toxicological factors inturn hampers gross production of fishes (Coats, 1979). Number of studies were undertaken to assess the manifestation of insecticides, pollutants and metals on gonadal impairment in fish. Reduction in spermatogenic activity and haemorrhage in the testis have been reported after toxicant exposure by many workers (Srivastava, 2007). Katti and Sathyanesan (1985), observed exposure dependent and concentration-mediated
changes in testis of *Clarias batrachus* treated with lead. Concentration dependent effects of nonylphenol on testicular structure of the fish *Xiphophorus maculates* were reported by Kinnberg et al., (2000). Considerable reduction in size of degenerating spermatids and sperms and necrosis of interstitial cells after exposure to fenthion in *Glossogobius giuris* was reported by Zutshi (2005). Cytotoxic damage in the testis of gobiid fish, *Glossogobius giuris* after fenthion exposure was documented by Zutshi and Murthy (2001). Cytotoxic damage after exposure to anionic surfactant in testis of *Heteropneustis fissilis* were reported by Kumar et al., (2007). Exposure of fish to mercuric chloride caused testicular damage in *Clarias gariepinus* (Kumar et al., 2013). Adverse effects of mercuric chloride on testicular recrudescence was reported by Masud et al., (2003). Joshi et al., (2002) studied the adverse effects of mercuric chloride on haematological changes in *Clarias batrachus*. Testicular growth and spermatogenesis were arrested in murrel after exposure to inorganic mercury (Ram and Sathyanesan, 1986b). Signs of testicular atrophy and arrested spermiation was observed in guppies *Poecilia reticulate*, (Wester, 1991; Wester and Canton, 1992) and medaka, *Oryzias latipes* (Liao et al., 2006) exposed to different concentrations of methyl mercury. Male guppies exposed to methylmercury showed inflammation and fibrosis of the interstitium (Wester, 1991; Wester and Canton, 1992). Seven month exposure to methylmercury decreased spermatogenesis and atrophied seminiferous tubules in male Nile tilapia *Oreochromis niloticus* (Arnold, 2000). After mercury exposure, interstitial cells became inactive and showed signs of degeneration in male walking catfish (Kirubagaran and Joy, 1992). Inorganic mercury interferes the sperm morphology and motility in African catfish, *Clarias gariepinus* (Rurangwa, 1998). Mercury interferes with the function of sperm mitochondria too, resulting in the decrease in energy production (Rao, 1989).

Paucity in basic information on the reproductive physiology of cultivated fishes has caused aquaculture to lag behind agriculture. Such information plays an important role in increasing fish yields. Several reviews have been done on this subject (Fontaine, 1975; Harvey and Hoar, 1979; Peter and Crim, 1979; Devlin and Nagahama, 2002; Yashizaki et al., 2002). Gonadal development in the carp is associated with increasing temperature and spawning occurs when the temperature is
Males of Indian major carps show gonadal recrudescence earlier than females under natural conditions as the threshold temperature for testicular recrudescence is lower than that of ovarian recrudescence.

Morphology of the gonads has traditionally been used to identify annual reproductive cycles, onsets of reproductive maturity, spawning rhythms and various other aspects of the reproductive biology. Excellent reviews discuss the various aspects of developmental biology, endocrinology and environmental factor associated with gonadal differentiation and sex differentiation in teleosts (Nakamura et al., 1998; Baroillier et al., 1999).

Teleosts are becoming increasingly important indicators of environmental health. Considerable information exists suggesting that pollutants may cause serious impacts on fish reproduction: sex differentiation, gonad morphology, rates of gametogenesis and sex phenotype (Devlin and Nagahama, 2002; Arukwe and Goks, 2003). A comparative study of morphology of ovaries and testes as well as oogenesis and spermatogenesis in 17 species of teleost has been done by Fishelson et al., (2013).

**Ovary**

The ovary is a hollow paired organ. It consists of oogonia, oocytes and their surrounding follicle cells, supporting tissue or stroma and vascular and nervous tissue. *C. mrigala* is a cyclical breeder and the ovary varies greatly in appearance at different times in the cycle. *C. mrigala* shows synchronous ovary, which consists of at least two populations of oocytes at different developmental stages. It generally spawns once a year and has a relatively short breeding season.

Changes in various cellular organelles of the oocyte during oogenesis have been described in a number of teleost species (Wallace and Selman, 1981; deVlaming, 1982). Oogenesis is the process of transformation of primordial germ cells (PGCs) into ova, ready to fertilize followed by embryonic development. Knowledge of gonad maturation stages of fishes is required for many purposes that include determination of stocks that are mature and the size or age at first maturity (Benegal, 1978), determination of reproductive potential of fish populations and monitoring of changes
in biological characteristics of exploited fish stocks (Williams, 2007). It is also required in establishing the reproduction period and length of gonadal maturation to allow for accurate implementation of fishery legislation (Goncalves et al., 2006).

Traditionally, there are three stages in oocyte development as primary growth phase (PGP), secondary growth phase (SGP) and finally the maturation including hydration phase (MP). In PGP the ovary shows clusters of oogonia. The SGP shows nests of polygonal oocytes of different developmental stages like chromatin nucleolar stage, perinucleolar stage and yolk vesicle stage. The MP shows previtelogenic and vitellogenic oocytes in different stages of yolk deposition.

Vitellogenin is a large glycoprophospholipoprotein synthesized by the liver, transported via the blood stream to the ovary, and taken up by the growing oocytes (Wallace, 1985; Mommesan and Walsh, 1988). Vitellogenin binds to a specific receptor on the oocyte surface (Stifani et al., 1990). It then enters the ovarian follicle. Proteolytic cleavage of vitellogenin into yolk proteins occurs. Depending on the species, yolk proteins are sorted in yolk globules or platelets throughout the ooplasm (Wallace and Selman, 1990; Blazer, 2002). Vitellogenin uptake is stimulated by FSH but not by LH (Tyler et al., 1997). Vitellogenin produces two yolk proteins lipovitellin and phosphovitin (Wallace, 1985). Lipovitellin is an important nutritional source of aminoacids and lipids that support embryonic development. Phosphovitin provides the minerals necessary for developing embryos for skeletal development and metabolic functions. The maturation phase oocyte further becomes translucent and hydrated recognized by nuclear movement.

Ovarian development in different species of fish during active reproductive phase is studied by number of researchers. Six stages of maturation in the ovary in *Tilapia mossambica* were reported by Dadzie (1974). A cyclic histomorphological change in the ovary of catfish *Pimelodus grosskopfii* was reported by Cala (1996) and that of catfish *Clarias mossambicus* was reported by Dadzie and Owiti (1998). Oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zilli* was reported by Coward and Bromage (1998). Maddock and Burton (1999) observed ovarian development and related condition changes in American plaice. The process of ovarian
development and maturation in *Acanthopagrus latus* was described by Abu-Hakima (1984) and Abou-Seedo et al., (2003).

Ovarian development in *Labeo dyocheilus* and sequence of oocyte development process in Indian major carp *Labeo rohita* was reported by Singh et al., (2005, 2008). Ovarian maturation cycle in a cyprinid *Barbus grypus* was reported by Dorostghoal et al., (2009). Chakrabarti and Chowdhury (2013) described the seasonal changes in the ovary of *Notopterus notopterus* and stated that the changes occur according to variations in GSI.

Aquatic pollutants and a variety of effluents cause abnormalities in the ovarian structure (Johnson et al., 1988; Mc Comic et al., 1989; Davis and Cook, 1993; Farmer et al., 1995; Kumar et al., 2000). Agents like ionizing radiations, electric current, parasitic infections, mechanical injuries, xenobiotic toxicants cause histoanatomical abnormalities in ovaries (Sarojini and Victor, 1985). Exposure to high temperature causes dysfunction of ovaries and disturbances in ovulation (Davies and Bromage, 2002). Temperature limits the sexual activity and breeding in fish (Chmilevsky, 2000; Davies and Bromage, 2002). Organochlorine insecticides impair the reproduction and development in fish (Olsson et al., 1999). Several degenerative changes in testis and arrest in oocyte maturation in *Channa punctatus* was reported after exposure to cupric and zinc chloride (Patil and Dhande, 2000). Degenerative changes like nucleolar necrosis and atresia in follicles were observed in inorganic mercury treated groups (Ram and Sathyanesan, 1986; Dey and Bhattacharya, 1989). Mercury affects the reproductive function in female fish by altering the ovarian morphology, delaying oocyte development and inhibiting steroid hormone synthesis (Crump and Trudeau, 2009). A great alterations due to mercuric chloride in ovarian biochemical constituents and activity of specific enzymes in *Clarias batrachus* were reported by Saksena and Agarwal (1991). Gonads of *Colisa fasciatus* showed a decreased protein content after exposure to arsenic (Shukla and Pandey, 1984). Effect of mercuric chloride on RNA/DNA ratio in prespawning ovary of *Labeo rohita* was reported by Aditya et al., (2002). Histological changes in gonads and pituitary gland of *Channa punctatus* after exposure to mercuric chloride were described by Ram and Sathyanesan (1983). Hossain et al., (2002) reported effects of insecticides on ovarian
tissue of *Heteropeustis fossila*, *Anabas testudiens* and *Channa punctatus*. *Cyprinus carpio* exposed to mercuric chloride showed histophysiological changes in testis, ovary and liver (Masud et al., 2001,2003).

Serum vitellogenin levels decreased in catfish after exposure to inorganic mercury (Kirubagaran and Joy, 1995). Fecundity of fish populations decrease after exposure to various heavy metals either indirectly via accumulation in the reproductive organs or directly by acting on sperm and ova (Rurangwa et al., 1998). Decrease in fecundity was followed by exposure to a mercurial fungicide in Zebrafish (Kihlstrom et al., 1971). Heavy metal contamination directly affects fish health, disrupts the normal steroidogenesis pattern, impair hormone production in both male and female fish and decrease quality and quantity of sperm and ova production (Ebrahimi and Taherianfard, 2011).

Impacts of methylmercury on reproduction of fish have been identified by studies, however to understand the underlying mechanisms of these effects, it is critical to look at alterations in neuroendocrine control, because the central nervous system is the primary target for mercury action (Clarkson and Magos, 2006).

Low levels of lead pollution could cause some adverse effects on fish health and reproduction (Delistraty and Stone, 2007). In Atlantic croaker plasma estrogen appreciably decreased after exposure to lead (Thomas, and Trant, 1989; Thomas, 1990). Absorption of lead through different organs in fish may lead to high mortality and cause many biochemical and histological alterations in survived fish (Coetzee, 1996). The gonads of teleosts are affected by lead followed by alterations in reproductive behaviour (Weber, 1993). *Puntius conchnofus* exposed to copper, zinc and lead resulted in disappearance of oocytes from the ovaries (Kumar and Pant, 1984). Lead affects the older oocytes first and induces atresia in ovary (Mokhtar and Abd-Elhafeez, 2013; Adeyemo, 2008; Mazrouh and Mahmoud, 2009). Detection of causes of fish mortality after exposure to lead need studies on central nervous system (Van Dyk et al., 2007).
**Gonadotropins**

Gametogenesis in fish is regulated by the reproductive endocrine system including the brain, pituitary and gonad. Pituitary gonadotropins both FSH (Follicle stimulating hormone) and LH (Leutinizing hormone) play a crucial role in regulating gametogenesis and the production of gonadal hormone. Removal of pituitary in fish causes cessation of gametogenesis in both the sexes (Pickford and Atz, 1957).

In fish there are two distinct gonadotropins, GTH I and GTH II that are chemically related to FSH and LH, respectively, in mammals (Suzuki et al., 1988; Swanson, 1991). Studies on physiological functions of GTH I and GTH II have shown that they have similar roles to those of FSH and LH respectively. Gonadotropins play a key role in regulating gonadal maturation. GTH I plays a role in the initiation and maintenance of gonadal growth and GTH II is crucial in the regulation of the final stages of maturation and ovulation/spermiation. Fish gonadotropins have been found to be similar to tetrapod FSH and LH respectively (Swanson, 1991). Work on validation of radioimmunoassay for gonadotropins (Prat et al., 1996) concluded that the seasonal change of plasma GTH I and GTH II in rainbow trout, mimic the ovarian cycle of FSH and LH in mammals. Thereafter GTH I and GTH II are called fish FSH and fish LH, respectively.

Fish FSH and LH are heterodimeric glycoproteins like tetrapod gonadotropins consisting of a common alpha (α) and a hormone specific beta (β) subunits that are non covalently linked (Biome et al., 1999). The GTHs act via binding to specific receptors in membrane. Studies in salmon demonstrated two types of GTH receptors, type I receptor which binds with both GTH-I and GTH-II and a type II receptor which binds with GTH-II but not GTH-I (Yan et al., 1992; Miwa et al., 1994). Determination of serum/plasma levels of these hormones helps in understanding the vertebrate/fish reproductive physiology.

With the availability of radioimmunoassay techniques for measurements of plasma levels of gonadotropins, a number of studies (Billard et al., 1978; Peute et al., 1978; Oordt and Ekengren, 1978) on seasonal variations in gonadotropin levels in the pituitary and plasma have been made in rainbow trout and some fresh water fishes
Lamba et al. 1983, Hussain et al. 1995, Kotteswaran et al. 1995 and Tiwari et al. 2001). Ovarian state and serum gonadotropin level in adult carp during annual reproduction cycle was studied by Bieniarz, (1978). Studies done on trout and salmon showed that, plasma FSH increases early in oogenesis, particularly during the transition from primary to early secondary oocyte growth and during early stages of spermatogenesis. In trout, levels of FSH decline during late secondary oocyte growth and rise again post ovulation during recruitment of oocytes for the next cycle. In contrast to FSH, plasma LH levels are low and unchanging until final oocyte maturation in females and production of mature spermatozoa in males (Swanson, 1991; Prat et al., 1996; Davies et al., 1999; Gomez et al., 1999; Santos et al., 2001).

Isolation and characterization of two distinct gonadotropins from pituitary glands of a marine fish Katsuwonus pelamis was done by Koide et al., (1993). A radioimmunoassay (RIA) for annual cycle of pituitary gonadotropin in Cyprinus carpio was done by Zhao et al., (1983). Plasma profiles of FSH and LH in male sea bass, Dicentrarchus labrax during the reproductive cycle was studied by Moles (2011) by species specific ELISA. Quantitative and qualitative information on FSH in vital moments of reproductive cycle has been provided by this assay. Steroidogenic activity of the ovary and quantitative profile of metabolites was done by radioimmunoassay in a marine teleost Pagrus pagrus (Kokokiris, 2000). Circulating FSH levels in female of wild spring Chinook salmon (Oncorhynchus tshawytscha) during migration in 2 successive years were measured by Slater et al., (1994). FSH remains at low levels during early vitellogenesis and a surge in LH and FSH during oocyte maturation is reported by Breton et al., (1998). Plasma levels of gonadotropins in female maiden and repeat spawning of Atlantic salmon when exposed to higher temperature were quantified by Anderson et al., (2012) and reported an elevated level of FSH from mid-vitellogenesis. Plasma FSH concentrations in female rainbow trout undoubtedly decreased, while plasma LH changes were of small magnitude in female rainbow trout after exposure to nonylphenol (Harris, 2001). In salmon, both FSH and LH are equipotent in stimulating the androgen production (Planas and Swanson, 1995). In rainbow trout FSH stimulates the incorporation of vitellogenin (Tyler et al., 1997) while LH becomes more potent at final maturation and spawning in both male
and female (Planas and Swanson, 1995). Anderson (2012) assessed gonadotropin levels in Atlantic salmon by RIAs developed to quantify coho salmon FSH and LH (Swanson et al., 1989), he also reported that plasma levels of LH remain unaffected by temperature. Fish reared at 22°C showed raised plasma levels of FSH compared to those reared at 14°C (Pankhurst et al., 2011). Lower plasma levels of FSH resulted in rainbow trout and Sea bass (*Dicentrarchus labrax*) after estradiol treatment (Mateos et al., 2002). In salmonids LH remains at significant levels during preovulatory period (Breton et al., 1998; Oppen-Berntsen et al., 1994). Plasma levels of LH in juvenile stages of male African catfish (Schulz et al., 1997) and black carp (Gur et al., 2001) are detectable and pituitary LH increase with the progression of gametogenesis reaching highest levels in fully mature fish. Studies on annual variations in gonadotropins in relation to gonadal growth are lacking in *Cirrhinus mrigala*.

**Endocrine disrupters**

The endocrine system, nervous system and immune system are the three important integrating and regulatory system in the animal body. Pituitary is one of the major endocrine gland. Hormones are the secretary products of endocrine glands and travel through blood to affect the target organs. Hormones are responsible for maintainance of homeostasis, reproduction, development and behaviour of the organism.

Endocrine disrupters (EDs) have been defined as exogenous agents that interfere with the production, release, transport, metabolism, binding action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock et al., 1996). ECs have serious effects on the ability of that organism to reproduce and its offspring to survive and eventually reproduce. Endocrine disruptors show variety of biological effects on human as well as on animal life. Many of the investigations into EDs in the aquatic environment have involved fish because of similarities in the endocrine system to higher vertebrates (Bond, 1979) (Kim, 1999). Heavy metals such as lead, cadmium, arsenic, nickel, zinc and mercury are also reported to have an endocrine disruptive potential. Therefore, they are also on the list of "known and suspected
hormone disruptors" published by The World Wildlife Fund". Endocrine disruptors interfere with endocrine functions in different ways.

Methyl mercury induces gonadal regression and inhibits ovulation and oviposition in the Japanese medaka (*Oryzias latipes*) possibly by blocking the release of gonadotropin (Chan, 1977). Methylmercury exposure can affect behaviour, biochemistry, growth, reproduction, development and survival in fish (Weiner and Spry, 1996; Sorensen, 1991). Negative impacts on reproduction in various fresh water fish species have been demonstrated in last three decades using sublethal concentrations of mercurials (Kime, 1998). Mercury in its methylated form impairs reproduction and disrupt the expression of estradiol and testosterone among the vertebrates (Fredrick and Jayasena, 2011; Tan et al., 2009).

Mercury affects the endocrine systems through, accumulation in endocrine systems, cytotoxicity of endocrine tissues, changes in hormone concentrations, interactions with sex hormones and up or down regulation of enzymes within steroidogenesis pathway (Tan et al., 2009). Animals and humans exposed to mercury were found to have deposition of mercury in their pituitaries (Nylander, 1986). Mercury taken into the body is distributed and deposited in the endocrine glands as well as in the liver, kidney, brain, and other tissues of humans and laboratory animals (Khayat and Dencker, 1983, Danscher, 1990). In fish, gonadotropins released from the pituitary control the annual cycle of gonadal growth, ovulation in females, sperm release in males and production of sex steroids in both sexes (Weltzien et al., 2004; Kamei et al., 2005). Mercury inhibits the gonadotropic activity, which thereby leads to alterations in gonadal development (Crump and Trudeau, 2009). It is acceptable that mercury acts primarily on the ability of the pituitary to secrete gonadotropin hormones altering release of sex steroids, thereby impairing the reproductive behaviour (Tartu et al., 2013).

Gonadotropins are important factors for the prolonged growth phase of teleost oocytes (Nagahama, 1983). Exposure to mercury results in decreased secretion of gonadotropins thereby impairing the proliferation and growth of oocytes and resorption of yolk in *Channa punctatus* (Saksena and Agarwal, 1986). Hg induces
decreased secretion of gonadotropin which further results in inhibition of vitellogenesis (Joy and Kirubagaran, 1989). It is necessary to identify the gaps in our knowledge of mercury’s health risks to animal and human life.

Joy and Kirubagaran (1989) studied serum levels of gonadotropin in mercury treated fish, the gonadotropins were found to be decreased after three weeks of exposure. Reports with the topic of endocrine disruptive effects of mercury are much fewer than those dealing with cadmium (Zhu et al., 2000). Various animal models exposed to mercury caused impairments in all endocrine glands. Animal studies with low-level long-term exposure and well-designed laboratory animal studies are helpful to confirm the endocrine disruptive effects of mercury (Zhu et al., 2000). A population of white suckers (*Catostomas commersoni*) exposed to bleached craft mill effluent was studied for endocrine disruption (Munkittrick et al., 1991; Mc Master et al., 1991). Alterations like abnormal gonadal development, delayed age of maturity and altered steroid levels were prominent in exposed animals (Mc Master et al., 1991). Van Der Kraak et al., (1992) reported decreased ovarian steroid synthesis and reduced gonadotropin secretion in the same species.

Pb contamination may alter endocrine regulated processes such as longevity, development, sexual receptivity, fertility and locomotion (Bogdon et al., 2011). Gonads of teleosts are affected by lead pollution which thereby affect reproductive behaviour (Weber, 1993). Lead accumulation in brain of some fish species resulted in decreased reproductive potential due to alteration in hypothalamohypophyseal ovarian function (Tulasia, 1989). Secretary activity of pituitary has been shown to be affected by metals (Ronis et al., 1998) and is proved to be a soft target for cadmium. Alterations in the pregnancy in female and morphological alterations in spermatozoa and sperm count in males have been linked with Pb exposure (WHO, 1995). Pb related reproductive impairments are due to its effect on endocrine system (Lavicoli et al., 2009). Ronis et al., (1996) suggested that Pb affected the hypothalamus-pituitary-gonadal axis at multiple action sites.
**Aim of the present research**

Regulation of endocrine glands function is a complex matter and is done by central nervous system. The chemicals target the brain cells not the hormone. Chemical interaction with the brain cells leads to endocrine disruption.

Recent investigations of endocrine disruptions in fishes include intersex fish (Jobling et al., 1998), elevated levels of a female egg protein in male fish (Lye et al., 1998, Janssen et al., 1997, Folmar et al., 1996) and degeneration of gonadal tissue (Lye et al., 1998). Yellowfin sea bream exposed to various concentrations of mercury changed hormone activities in serum (Aliakbar and Abdullah, 2012). Mercury based compounds disrupt steroidogenesis, including sex hormones synthesis, male and female fertility as well as the hypothalamic-pituitary thyroid axis and the hypothalamic –pituitary-adrenal axis (Tan, 2003). Most data available indicate the fact that mercury may act as a major endocrine disrupter (Darbre, 2006; Frynn-Aikins, 2011). Zn, Pb, Hg and As interfere with sex hormones and adrenal cortex hormones steroidogenesis to alter reproduction and sex differentiation (Bogdon et al., 2011). Effects of Pb on 17B-estradiol, testosterone and cortisol are biphasic, with stimulatory effects after low level exposure and inhibitory effects after high level exposure (Chaube, et al., 2010).

Aquatic toxicology for a long period of 25 years has provided an early warning of toxic threat to the organisms, their ecosystem and indirectly the human. Unless the mechanism of toxic action are understood prediction of environmental toxicity will remain empirical (Lederberg 1981). Utilization of a greater variety of species and models would help in determining execution of toxicity of various agents. Since long, use of non mammalian models in toxicology has proven vital. Aquatic animals when used as models help in better understanding of fundamental principles which underlie toxicity in all species. The acute and chronic toxicity studies in aquatic species has documented the susceptibility of individual species to different pollutants (Malins, 1991). The toxicity studies even served to highlight important process like bioaccumulation, biomagnification and importance of physical and chemical properties of each agent in determining this process (Hamelink, 1977). Heavy metals,
such as arsenic, cadmium, copper, lead and mercury are known aquatic toxicants and cause deleterious effects on density, diversity and productivity of aquatic organisms (Moore and Ramamoorthy, 1984).

Reproductive effects of heavy metals in combination have received little attention (Spehar et al, 1978). Various heavy metals are often present in the same polluted environment at the same time, studies of their interactive effects on gonadal activity would be more meaningful. Mercury is widely recognized neurotoxin and its neurotoxic effects have been extensively studied in mammals (Chang, 1977). However, such reports are few in fishes (Ram and Sathyanesan, 1985, Ram and Joy, 1988, Kirubagaran and Joy, 1990).

Considering all these evidences, the present work was undertaken with following objectives.

1. To study the seasonal variations in the pituitary gland of *Cirrhinus mrigala* by Azan’ stain (modified after Koneff, 1938).
2. To study the seasonal changes in the pituitary gland of *C. mrigala* by using immunohistochemical method.
3. To investigate the changes in pituitary gland of *C. mrigala* after exposure to heavy metals such as lead and mercury.
4. To estimate the gonadotropin levels in *C. mrigala* during different seasons by using radioimmunoassay (RIA).
5. To study the seasonal histological variations in gonads of *C. mrigala* by Ehrlich’s haematoxylin.
6. To study the effect of heavy metals lead and mercury on histomorphology of gonads of *C. mrigala*. 