1. GENERAL INTRODUCTION

“All things are poison and nothing is without poison. It is the dose that makes a thing poisonous” Paracelsus, 1590 A.D.

The aquatic environment is continuously being contaminated with chemicals from agriculture activities. Hundreds of pesticides of different chemical structures are extensively used to control a wide variety of agricultural pests and can contaminate aquatic habitats due to leaching and runoff water from treated areas. The pesticides may produce an immense disruption of the ecological balance causing damage to non-target organisms including fish of commercial importance (Oruc- and Uner, 1999; Bretaud et al., 2000). Several biochemical and physiological responses occur when a toxicant enters an organism, which may be an acclimation of the organism or may lead to toxicity (Begum, 2004). Fish blood is sensitive to pollution-induced stress and changes in the hematological and metabolic parameters can be used as toxicity indicators of the pesticides and herbicides (Roche and Boge, 2000; Sancho et al., 2000).

Pesticides are often considered a quick, easy, and inexpensive solution for controlling weeds and pests in urban landscapes. Pesticides have contaminated almost every part of our environment. Pesticide residues are found in soil, air, and in surface and ground water. Pesticide contamination poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms to plants, insects, fish, and birds. In fact, weed killers can be especially problematic because they are used in relatively large volumes. The best way to reduce pesticide contamination in our environment is for all of us to do our part to use safer, non-chemical pest control (including weed control) methods (Jess and Becky, 2001). Pesticides presents in aquatic environments can affect aquatic organisms in different
ways. The rate of accumulation of such chemicals depends on the kind of associated food chain, on availability and persistence of the contaminant in the water and most of all, on the physical and chemical characteristics of the agrochemical. Fish and aquatic organism can accumulate pesticides concentrations much higher than those found in the waters where they live, because such chemicals may go through bioaccumulation or connect to the particulate material in suspension, which can be ingested by organisms present in the environment (Bruna de Campos Ventura, et al., 2008).

Herbicides atrazine known to be directly toxic only to plants and also to affect animals in complex communities when an additional stressor was added to the system (Hayes et al., 2002). Atrazine is a systemic triazine herbicide that has been currently used for over 40 years in more than 80 countries. It can be detected in nearly every surface water system in the world, yet a consortium of university scientists concluded that this posed no significant long term ecological risk because of its negligible bio accumulation and biomagnifications (Diana et al., 2000 and Felsot, 2001). The compound atrazine presents physical and chemical properties that favour, in special, the contamination of the underground resources. Its high flow potential that results of its low adsorption potential to organic substances; moderate solubility in water, high half-life in the ground and slow hydrolysis, constitute determining factors for the contamination of aquifers. The interaction of atrazine with the environment is tied with its stability and low solubility. In other words, atrazine can make the aquifers on a region unfit to drink for years. Atrazine affects the vertebrate organisms as a hormone disrupter, particularly as a somatostatin represser, which implies that atrazine poisoning will disturb the mechanisms regulated by somatostatin which include the growth of nervous system. Atrazine concentrations found in surface and groundwater are generally not considered to be ecologically harmful
(Gaido et al., 2000). However, recent investigations have demonstrated that the presence of atrazine may cause sublethal effects such as induction of xenobiotic metabolizing systems (Safe et al., 2002 and Singleton et al., 2004) and may influence the toxicity of other xenobiotics. It is used initially to control the growth of annual broadleaf weeds on such row crops as corn and sugarcane, atrazine is now applied to commercial golf courses, industrial centers, lawns, and along roadsides. According to the Environmental Protection Agency (EPA, 2006) over 76 million pounds of atrazine are applied to crops every year. Despite its widespread use in the U.S., the European Union banned atrazine in 2003, citing it as a major contributor to water contamination. Atrazine is a chlorotriazine and its chemical properties make it prone to leaching and runoff. According to the Agency for Toxic Substance and Disease Registry (ATSDR), once atrazine is applied to the soil, it can remain there for several days or months. It is usually broken down, however, within one growing season. Atrazine that enters the groundwater or surface waterways through run off or leaching can remain for a much longer time because the chemical is slow to breakdown in water. This is one reason for people living near agricultural fields oftentimes find atrazine in their drinking water. If atrazine enters the air, other reactive chemicals which can break it down unless atrazine attaches to dust particles. In this case, breakdown is not expected. When this happens, the wind can carry atrazine-laden dust particles long distances from the original application area. Atrazine has been detected in rainwater more than 180 miles (300 kilometers) from where it was applied (EPA, 2006).

Atrazine is a herbicide that does not occur naturally. Pure atrazine is an odorless, white powder that is not very volatile, reactive, or flammable and that will dissolve in water. The EPA now restricts how atrazine can be used and applied; only
trained people are allowed to spray it. Atrazine has also been shown to cause changes in blood hormone levels in animals that affected ovulation and the ability to reproduce. These effects are not expected to occur in humans because of specific biological differences between humans and these types of animals. (ATSDR, 2003).

1.1. Impacts of Pesticides Use

The diagram of Figure 1 shows how the misuse of pesticides spreads losses, even on purely economic terms. Pesticides are petroleum derivatives, and to depend on them is to depend on a non-renewable resource. Intensive pesticide use results in water contamination, which damages the overall population health, resulting in productivity loss and heavy social security expenses (Loucks and van Beek, 2005). Intensive pesticide use contaminates the agri-product, which devalues it as an export commodity. Also, as the agri-product is also (frequently) consumed locally, its contamination will also result in more damage to the local population health with associated loss of productivity and increase of social security expenses. As pesticides unavoidably get carried beyond the point of application, they damage the local environments and wildlife (Caza and Bailey, 2000). The losses resulting from this range from proliferation of plagues (from destruction of their local predators) with associated damages to the population health, to business loss from devalued real estate. Last, but not least, one must consider the damage caused to agri-worker's health by pesticides (Maroni et al, 1999). Even from a purely economic point of view, productivity losses and social security expenses are strong negative entry against the pesticide-intensive agri-business (Shiva, 2002). Perhaps the worst impact of the misuse of pesticides on the agribusiness is the addictive effect of using (or misusing) pesticides as if they were some irrefutable solution to pest control problem. In fact, the very "pest control" label exposes the dangerous underlying concept, which does
nothing about the disharmony between the cultivation and the global environment. This disharmony may not be abolishable, at least now, but neither should it be ignored, blamed on "misbehaved" species nor reduced to the problem of eliminating such species. Instead, the pest problem should be recognized for what it is, a symptom of the much wider problem of harmonizing the human agrarian activity with the overall surrounding environment.

Another important impact affects the reputation of the agrarian products. The growing bad name of those is reflected in the growing popularity of the so-called organic products, to be grown free of pesticides. There is also the growing awareness of agrarian products contamination that is reflected in the issuing of more and more restrictive regulations by the countries which import agrarian products (UNEP, 2004).

Worldwide basis, about 20 million tons of organic chemicals enter environment annually of which about 2,000,000 pesticides are being screened which forms the main source for water pollution (Agnihothrudu, 1982).

**Fig 1.** Schematic diagram of impacts of pesticides use
Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment. Almost all pesticides are volatile in nature when applied to crops. These pesticides can be circulated into different ecosystems by different agents after entering into the environment have a deturious effect on fish and subsequently to man (Metelev et al., 1993). The toxicological literature reveals that exposure of chemical can produce unexpected effects (Feron et al., 1995). Many researchers were baffled by these unexpected effects following exposure of individual to low levels of pesticides and other chemical toxins (Chun-Yun et al., 2003). According to Waliszewski et al., (2003), Aronson et al. (2000) and Abdul (2003) most pesticides may enter into the food channels and cause physiological damage. Among all pesticides, the organophosphates (OP) are widely used to control pests because of their rapid effectiveness and easy biodegradation (Mahboob and Siddiqui, 2002).

Pesticides use is known to cause serious environmental problems, especially in the dry season, because during the period, the dilution capacity of the water systems is low thus increasing the risk of high concentration of toxic chemicals. In addition, the dry season is often the critical period of many animals especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season-contamination of water by pesticides either directly or indirectly can lead to silt kills, reduced fish productivity of devoted concentration of undesirable chemical in edible dish tissue which can be affect the health of humans eating these fishes (Adedeji et al., 2009). Pesticides are well recognized as an economic approach to control pests, at the same time such chemicals are highly toxic to other species in the environment. Now there is growing concern worldwide over the indiscriminate use of such chemicals, which result in environmental pollution and toxicity risk to non target
organisms. Most of pesticides find their way into rivers, lakes and ponds have been found to be highly toxic not only to fishes but also to the organisms which contribute to the food chain of fishes (Chen et al., 2004).

Herbicides are widely used for the control of water plants, which may impede the flow of water during the summer, when sudden heavy rain can cause flooding. While the direct effect of herbicides addition is the loss of macrophytes, non-target organisms such as fish may also be affected through loss of habit and food supply (Elia, 2002). Worldwide herbicide usage has increased dramatically during the past two decades, coinciding with changes in farming pesticides and increasingly intensive agriculture (Fung and Mak 2001). As a consequence, residuals amount of herbicides and their metabolites have been found in drinking water and food (Jhohnen 1999; Vander Oost et al., 2003). Since the second half of the last century, the environment has been contaminated by numerous xenobiotics among these pesticides of special concern. This xenobiotic is produced by industries of chemicals, dyes, iron, steel, wood plastic, synthetic fibres, detergents, pesticides, explosives, textiles, petroleum, refinery and pulp mills.

Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pest and disease and also towards clacking the insect-borne disease (malaria, dengue, encephalitis, filariasis, etc.) in human health sector (Rekha et al., 2006). The weed to increase world food production for the rapidly growing population is well recognised (FAO, 2005 and Agoramoorthy, 2008). One of the strategies to increase crops productivity is effective pest management because more than 45% of annual food production is lost to pest infestation. In tropical countries, crop loss is even more served because the prevailing high temperature and
humidity are high conductive to rapid multiplication of pest (Lakshmi et al., 1993). Thus, the application of wide variety of pesticides on crop plants is necessary in the tropics to combat pests and vector borne diseases. However, the sporadic use has been leading to significant consequences not only public health but also to food quality resulting in an impact load on the environment and hence the development of pests resistance (Agnihothri et al., 1999). Atrazine was introduced in the 1950s and since that time has become commonplace in agricultural and forestry application. For instance, atrazine is used to control annual grasses and broad-leaved weeds in selected vegetables and cereal crops, vines, fruits, orchards, citrus groves, sugarcane, grassland and forestry. It is the most widely used herbicide in the world, with between 70,000 and 90,000 tons applied per year (Steinberg et al., 1995). Atrazine is pre-and post emergent broad leaf herbicide that acts an inhibiting the growth of target weeds by interfering with the normal function of photosynthesis (Chapman and Stranger; 1992). To prevent the growth functions of a wide variety of plants, including some species of algae. Atrazine is most effective in wet soils applied after significant winter rain when soils are at field’s capacity. It is therefore also prone to leaching to ground water and surface runoff particularly if a storm event occurs just after the application of the herbicide. Atrazine is up to 20 times more frequently detected in groundwater of the US than any other herbicides (Belluck et al., 1991). Atrazine persist under cool and dry conditions in a stable pH environment. It has been banned in many cool climate countries with fine textured soils.

Atrazine has been suggested to induce activity of aromatase (cytochrome P450 (CYP) 19) (Hayes et al., 2002; Spano et al., 2004). Aromatase is the rate-limiting enzyme in the synthesis of C19 estrogens, such as 17 Beta-estradiol (EZ), from C19 androgen, such as testosterone (T). However, studies have also reported no effects of
atrazine on aromatase activity (Coady et al., 2005; Hinfray et al., 2006) or even reported aromatase inhibition by atrazine (Benachour et al., 2007). Atrazine may also affect hepatic metabolism, phase I and phase II enzyme regulate homeostasis of sex steroids. These enzymes metabolize a large number of exogenous compounds. It also remains to be established whether atrazine metabolism affects the endocrine control of maturation of fish or indeed developmental stages that involve important hormonal changes such as early development and puberty are most prone to endocrine disruption. Atrazine contamination was found in the farmer’s blood and urine exposed to atrazine (Perry et al., 2001). Atrazine could cause damaged the gill epithelium and kidney and increase the renal excretion of sodium chloride and proteins in the rainbow trout (Fisher-Scherl et al., 1991) and carp (Neskovic et al., 1993). In addition, atrazine reduced plasma testosterone, olfactory sensitivity and salinity tolerance in mature male Atlantic salmon (Warning and Moore 2004). Thus, many European and African countries have restricted its use (Coady et al., 2005). The acute toxicity test indicates the relative species sensitivity and lethal concentration which can be used as a basis for long term tests to establish the requirements necessary for the well being of aquatic life. In fishes, biochemical changes are induces by the pollutants before they acquire cellular and systemic malfunction. It is inferred from several investigation that biochemical parameters could be effectively used to detect the effect of pollutants (Ram Narayana Ran and Sathyanesan, 1987).

Blood is the most important and abundant body fluid. Its composition often reflects the total physiological condition. The main route for any pesticides through the gills. From the gills it is transported to various part of the body via the blood stream. Blood provides an ideal medium for toxicity studies. The haematological parameters have been considered as diagnostic indices of pathological condition in
animal. Fish blood can serve as a valuable in detecting changes taking place in animal. Some authors (Chauhan et al., 1994; Agarwal and Chaturvedi, 1995; Nath, 1996) have reported a decrease in haematocrit, haemoglobin and red blood cells values of some fish after their exposure to insecticides. Saxena and Seth, (2002) showed significant changes in the haematology of the common freshwater fish *Cyprinus carpio* on exposure to toxicant. The information suggests that haematological parameters could be used as potential biomarkers of herbicides.

It is well known that pesticides in aquatic environments have a toxic effect on organisms, especially on fish. Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson et al., 1985). Thus, blood parameters such as RBC (Red Blood Cell), HB (Haemoglobin), HCT (Haematocrit), MCV (Mean Cellular Volume), MCH (Mean Cellular Haemoglobin Concentration), and PLT (Thrombocytes) are the most common criteria used in the toxicity studies on fish. As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of some fish. Besides, this type of index reflects certain ecological changes in the environment (Roche and Boge, 1996). The major biochemical response to the effect of diazinon in fish is the inhibition of enzymes Hamm *et al*. (1998) have also observed changes in carbohydrate metabolism in the eel, *Anguilla anguilla*. The glycogen content in the liver and muscular tissues was significantly decreased while glucose and lactate concentration in the blood were significantly increased. Human are exposed to pesticides (found in environmental media such as soil, water air and food) by different routes of exposure such as inhalation, ingestion and dermal contact (Rekha *et al*., 2006).
Exposure to pesticides results in acute and chronic health problems. Pesticides being used in agricultural tracts are realised into the environment and come into human contact directly or indirectly. Increasing incidence of cancer, chronic kidney disease, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and behavioural disorders, especially among children’s have been attributed to chronic pesticides poisoning. Moderate human health hazards from the misapplication of pesticides include paralysis, blindness and even death. Pesticides pollution to the local environment also affects the lives of birds, wildlife, domestic animals, fish and livestock (ICAR, 1961).

The term ‘stress’ mean the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of physical and chemical forces. In other words, stress is a state produced by an environmental or other factor, which extends the adaptive response of an animal beyond the normal range or which disturbs the normal functioning to such an extent that in either case the changes of survival are significantly reduced. A series of morphological, biochemical and physiological changes occur as a result of stress and constitute the general adaptation syndrome (Wedemeyar, et al., 1999). Hence, studying the changes in biochemical constituents and enzyme activities of tissues are important to determine the nature and extent of toxicants effects on organisms can provide an early warning that pollution is reaching harmful levels. With their ability to detect sudden changes in environment and monitor short or long term changes in water quality, fishes thus make efficient biomarkers (Yousef et al., 2006). Protein is the most abundant macromolecules in animals constituting over half of their dry weight. They regulate and integrate numerous physiological and metabolic processes in the body through hormones, enzymes and nucleo proteins. Protein plays a major role in the synthesis of
microsomal detoxifying enzymes and helps to detoxify the toxicants which enter into the animals. Some investigation has been carried out on the impact of heavy metals on protein metabolism. Pazhanisamy, (2002) have reported about the rapid utilization of dietary protein in *Labeo rohita* on exposure to arsenic trioxide. Meyers *et al.*, (1986) have reported the fluctuation in protein content in *Anabas scandens* exposed to lead. In chromium toxicity, the carbohydrates, protein and lipid contents are depleted in *Catla catla* (Vincent *et al.*., 1995). Sorenson *et al.* (1991) have reported similar changes in nickel toxicity also in *Cyprinus carpio* (Linn). Total protein level in liver decreased on exposure to lead nitrate (Jha, 1991). Sharma, (1999) have observed the depression in the protein synthesis of liver in carbaryl exposed *Clarias batrachus*.

The amino acids are the building blocks of proteins. Amino acids which cannot be synthesized in the body must be supplemented through diet. Since, the food value of fish is directly dependent on their protein content, the contamination by the toxic substance will reduce their nutritive value (Pugazhendy, 1996). Lipid plays an important role in maintaining the integrity of cell structure and function. The most abundant kind of lipids in animal’s body is fat or triacylglycerols. Lipid forms the storage of chemical energy, exerting an extremely important function in furnishing the energy requisites of the cell. The ability to overcome the stress laid on tissues in animals caused by the environmental contamination mainly depends on the lipid content. Lipid solubility is a major factor for the absorption and accumulation of toxicants in tissues. The affinity of different pesticides for fat is well illustrated by the occurrence of their residues in fish oil, adipose, brain and liver tissues (Pugazhendy, 1996). Carbohydrate supplies a major protein of energy to the living system. Disturbance in carbohydrate metabolism is the most outstanding biochemical lesion arising from the action of toxic compounds. Narendra and Srivastava, (1981) have
suggested that any change in protein and carbohydrate metabolism might cause change in GOT, GPT and LDH activities. Changes in plasma glucose level have been suggested as a useful general indicator of stress in teleost and it is found to be elevated when the animals are subjected to stressful conditions. Glycogen, the food reserve is utilized more to meet the extra demand of energy during stress condition which leads to the decrement (Aruldoss, 2006).

ROS are formed and degraded by all aerobic organisms and can readily react with many bio molecules including protein, lipids and lipoproteins and DNA (Akbarsha et al., 2001). Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to from a complex series of compounds these include reactive carbonyl compounds such as malodialdehyde (MDA). Therefore, the measurement of MDA is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems. Aerobic organisms are protected against free radicals by antioxidant defence systems. Antioxidants include endogenously synthesized compounds such as reduced glutathione (GSH), superoxide dismutase (SOD) (Igbedioh and Akinyele, 1992).

Aerobic organism superoxide anion radical, \( O_2 \) hydrogen peroxide \( (H_2O_2) \) and hydroxyl radical \( (OH) \) as a result of oxidative metabolism. OH can initiate lipid peroxidation in tissues (Halliwell and Gutteridge, 1984). The sensitivity of the cell is attenuated by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxide (GPx), catalase, glutathione reductase (GR). The antioxidant enzymes maintain a relatively low rate of the reactive and harmful OH. Oxidative
stress occurs as a result of the effect of xenobiotics causing the disturbances in the antioxidant enzymes system. Glutathione-S-transferase (GST) is a group of multifunctional enzymes involved in biotransformation and detoxification of xenobitoics (Smith and Litwack, 1980). Highly reactive electrophilic components can be removed they covalently bid to tissue nucleophilic compounds which would lead to toxic effects. Increased glucose concentration in white muscle has been reported in Oreochromis niloticus after cadmium exposure to sublethal concentrations (Almedia et al., 2001). A fall in glycogen levels clearly indicates its rapid utilization to meet the enhanced energy demands in pesticides treated individuals through glycolysis or hexose monophosphate pathway. Decreased glycogen synthesis is attributed to inhibition of enzyme glycogen synthesis.

In biological systems, the balance between both endogenous and exogenous pro-oxidant factors versus antioxidant defence can be used to assess oxidative damage induced by different classes of chemical pollutants (Valvanidis et al., 2006) changes of antioxidant enzymes activity may disappear a change in the ROS within the cells. Therefore, these enzymes can be used as biomarkers for oxidative stress (Roche and Boge, 2000). Aquatic organisms are usually more sensitive than terrestrial and may be better experimental subject to evaluate sublethal effect of oxidative stress (Ahmad et al., 2000). Oxidative stress caused by different metals may damage certain tissues and liberate various transamainase into the plasma. Toxicant increases the production of reactive oxygen species (ROS) in tissues and inhibits the activity of some enzymes of the antioxidative defence system (Sies, et al., 1992). ROS include a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H$_2$O$_2$), superoxide (O$_2^-$) and hydroxyl radical (OH) (Datta, 1992).
Alkaline phosphatase (ALP) is a hydrolyse enzyme responsible for removing phosphate groups from many types of molecules, such as, nucleotides, proteins and alkaloids. It is a P-stress marker enzyme most effective in an alkaline environment, that catalyze the hydrolysis is phosphorus compounds and the transfer of phosphoryl groups to an acceptor molecule. The rate of catalytic activity of the enzyme is inversely proportional to the concentration of inorganic phosphate in the ambient environment (Dyhrman and Palanik 1999). This enzyme could serve as a good indicator of intoxication because of its sensitivity to metallic slats (Boge et al., 1992). Acid phosphatase on the other hand is a phosphatase which frees attached phosphate groups from other molecules during digestion. It is a lysosomal, hydrolytic enzyme with an acid pH optimum. It takes part in the dissolution of dead cells and as such serve as a good indicator of stress condition in the biological system (Gupta et al., 1983; Verma et al., 1980).

Histopathological is mainly directed to study the effect of chemical on the structural components of the living system and the ways in which cells and tissues respond to injury. A chemical or a derivative acting directly on the cell or most frequently causes chemical cytotoxicity by altering its environment. The cells in turn respond histopathologically by degeneration, proliferation, inflammation and repair. The chemical organophosphorus affecting the cell by altering the external environment, oxygen and nutrient transport system or the endocrine and immune system (Fanta, 2003). Kumar and Pant, (1984) have stated that histopathological studies are useful to evaluate the pollution potential of pesticides since trace levels of pesticides, which do not cause animal mortality over a given period, are capable of producing considerable original damage. Hence, it is useful to have an insight into histological analysis regarding the extent of damage of the tissue, kidney when
toxicant enters the body (Silva, 2007). Gills are the vital organs for respiration in fish which establish direct contact with the medium through which the pollutant legally enter into the body (Paulose, 2002). The gills are the common site of damage; hence they are the first organs to be affected by the fenvelarate (Anita Susan, 2003). Studies have been shown that histopathological changes occur in the gills of fishes exposed to mercuric chloride in *Channa punctatus* (Sastry and Sachdeva, 1994), mercury and copper in *Salmo gairdneri* (Daoust et al., 1984) and cadmium, copper and zinc in *Anabus testudineus*.

The liver is a very important organ performing vital function such as detoxification, synthesis of several components of blood plasma, glycogen storage and release of glucose to the blood. Morphological, histological and histopathological alteration related to pesticides present in liver of fish have been studies, showing that these substances cause serve damage to the liver cells (Duta *et al.*, 1993; Ortiz *et al.*, 2002). This breakdown is carried out by endoplasmic reticulum of heaptocytes. Due to these reasons, the hepatic cells are damaged severely. The evaluation of biochemical and histological changes in fish liver has become an important tool for monitoring environmental exposure of fish to contaminants in experimental studies (Lagadic *et al.*, 1997). The exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs suitable for histopathological examination in searching for damages to tissue and cells (Rabitto *et al.*, 2005; Oliveria Ribeiro *et al.*, 1999). Many pesticides are not effective and exhibit low toxicity, before they are metabolically transformed into active substances (Loomis and Hayes, 1996). Kidney is a very conspicuous organ for osmoregulation and excretion and is indirectly affected by the toxicant through blood circulation. The histopathological changes in kidney have been reported in different fish *Barbas*
stigma to lead and *Labeo rohita* to mercuric chloride (Jagadeesan, 1994). Further critical studies of the histopathological effects of metals on fishes may help to establish the specificity between the metal and their effects. From a general survey of literature, it is evident that only very few attempts have been made on the impact of pesticides and heavy metals, especially atrazine on the biochemical constitution and histopathological lesion of fishes.

Naloxone is an opiate antagonist, which has been used as an emergency treatment for the management of opiate overdose in hospitals for several decades. The expansion of the use of naloxone to include its administration in the community has been suggested in recent years by many drug treatment workers in an effort to reduce or prevent fatal opiate-related overdoses. This review has been commissioned to evaluate the potential usefulness of naloxone in the overall management of opiate dependence syndrome in the Irish setting.

Naloxone is an opioid antagonist, with little or no agonist activity. It is not active orally because of extensive first pass metabolism. Potential pharmacokinetic interactions may occur when co-administered with medications that are extensively metabolised via glucuronidation. Overall, the propensity for clinically relevant pharmacokinetic drug interactions is thought to be relatively low. Naloxone has been used as an emergency room treatment for suspected opiate overdose for many years. However, there are few published controlled trials in this setting. Cases of convulsions, cardiovascular problems and pulmonary oedema have been reported rarely with use. These may be due to occurrence of overshoot phenomena due to the antagonism of opioid effects by naloxone or due to pre-existing disease and or concomitant use of other drugs of abuse by the patient. Furthermore naloxone causes
acute withdrawal symptoms in many recipients which make the experience unpleasant. Similar concerns were raised about the provision of clean needles and syringes to users in the past but these fears have not been borne out by the evidence. Since naloxone would be provided in conjunction with first aid training, it is likely that this information would increase the recipients’ awareness of the dangers of overdose rather than encourage use of naloxone in a risky manner. (Ashworth and Kidd, 2001).

The carp Cyprinus carpio was selected as the focus the present inquiry because, as a widely distributed bottom-dwelling fish whose feeding habit expose them to many different types of environmental contaminants, as well as their ease of capture and importance for the study of these aquatic systems. Measurements were taken in three different seasons, so as to compare the effects of both time and location on pollutant levels. The biochemical markers chosen for the study include a set of parameters connected to oxidative stress as nonspecific biomarkers of exposure to pollutants and also as indicators of toxicity (Livingstone, 2001; Meyar et al., 1986, Gorbi et al., 2005; Monserrat et al., 2007). Their induction can be regarded as an adaptive response to an altered environment; correspondingly, their inhibition could mean cell damage and toxicity.

1.2. Objectives of the study

Hence an attempt has been made to investigate the toxicity of atrazine and protective effect of naloxone on haematological, biochemical, enzymological and histophological changes in freshwater fish, Cyprinus carpio.
The present study deals with following aspects.

1. Evaluation of acute toxic range ($LC_{50}$) of the atrazine in *Cyprinus carpio* to evaluate the sublethal concentration (120 hours $LC_{0}$) and lethal concentration (24 hours $LC_{100}$).

2. To observe the blood parameters like red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb), packed cell volumes (PCV), mean cell volumes (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haematocrit concentration (MCHC) in the freshwater fish of *Cyprinus carpio* exposed to sublethal concentration of atrazine and supplement feed naloxone for the period of 24, 48, 72, 96 and 120 hours.

3. To observe the biochemical studies like Glucose, Glycogen, Amino acid, Protein and Lipid in the freshwater fish of *Cyprinus carpio* exposed to sublethal concentration of atrazine and naloxone supplemented feed for the period of 24 to 120 hours.

4. Estimation of the activity of alkaline phosphatase (ALP), acid phosphatase (ACP), reduced glutathione (GSH) and glutathione peroxide (GPx) enzymes levels in gill, liver, kidney in *Cyprinus carpio* exposed to sublethal concentration of atrazine and supplemented naloxone exposed to the period of 24, 48, 72, 96 and 120 hours.

5. Estimation of antioxidant enzyme activity in lipid peroxidation (LPO), superoxide dismutase (SOD), and catalase (CAT) in gill, liver and kidney of *Cyprinus carpio* exposed to sublethal concentration of atrazine and naloxone for the period of 24, 48, 72, 96 and 120 hours.
6. To estimate the levels of acetylcholine (ACh), and acetylcholinesterase (AChE), activity in brain, gill, liver and kidney of *Cyprinus carpio* exposed to 120 hours sub lethal concentration of atrazine and supplemented feed naloxone for the period of 24, 48, 72, 96 and 120 hours.

7. To observe the effects of herbicide atrazine on histopathology of gill, liver and kidney of the freshwater fish *Cyprinus carpio* exposed atrazine and naloxone supplemented feed for the period of 120 hours.