CHAPTER-IV
DISCUSSION AND CONCLUSION

Aquatic ecosystems are exposed to a number of point and non-point sources of pollutants particularly from industries, sewages treatment plants, drainage from urban and agricultural areas (Walker et al., 2006). Industries are among the most important point source of pollutants and discharge huge amount of waste substances into aquatic ecosystems. They can generate both organic and inorganic wastes, which could be alter all or parts of biological, physical and chemical characteristics of the receiving water bodies (Gomez et al., 2008). Depending on the dose and exposure time, some of these pollutants are toxic to living systems and cause a serious impairment to aquatic life (Walker et al., 2006; Gomez et al., 2008; Ogundrian et al., 2010). This is because, in the receiving water bodies, pollutants could accumulate in water, sediment and living systems. They also accumulate in food chain and thus cause adverse effects in aquatic systems (Walker et al., 2006).

As a result of a great variety of human activities, the aquatic environment is becoming increasingly threatened by an alarming number of foreign chemicals or xenobiotics. Fish populations living in highly polluted areas often have high incidences of gross pathological lesions (Malins et al., 1988), associated with elevated levels of toxic contaminants in the sediments. Toxic compounds or natural anthropogenic are called xenobiotics. With the onset of the epidemiological investigations was to confirm the hypothesis of many xenobiotics to be dangerous to living things, as well as their respective offspring, exerting toxic effects in the short, medium or long term (Reys, 2001). These substances are persistent in the environment eventually absorbed and accumulated by living organisms, toxic effects on various organs and systems. Thus, it was noted that the use of xenobiotics without evaluation of risks to the ecosystem, constituted a potential threat to the health of people, animals and plants (Sanches, 2006).

The biological response of an organism to xenobiotics following absorption and distribution starts with toxicant induced changes at the cellular and biochemical levels, leading to changes in the structure and function of the cells, tissues, physiology and behavior of the organism. These changes can perhaps ultimately affect the integrity of the population and ecosystem. For the biomonitoring and management of the aquatic ecosystems, these biological responses (biomarkers) have been proposed.
to complement and enhance the reliability of the chemical analysis data (Parvéz; Raisuddin, 2005).

Several definitions have been given for the term ‘biomarker’, which is generally used in a broad sense to include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological (WHO, 1993). A biomarker is defined as a change in a biological response ranging from molecular through cellular and physiological responses to behavioral changes that can be related to exposure to or toxic effects of environmental chemicals (Van Der Oost, R et al., 2003). A bioindicator is defined as organism giving information on the environmental conditions of its habitat by its presence or absence or by its behavior and an ecological indicator is an ecosystem parameter, describing the structure and functioning of ecosystems.

The aquatic environment is increasingly being polluted by anthropogenic inputs. The alteration of the ecosphere by human activities may be physical, chemical, biological or radioactive. Chemical alteration of the environment appears to be the major type which threatens the living system extensively. All organisms maintain their "internal milieu" more or less constant by making use of a variety of regulatory mechanisms. When the level of pollutants in the environment exceeds the assimilatory capacity of these regulatory mechanisms, it leads to biochemical changes, finally resulting in death. In recent years, concern has increased over heavy metal pollution, as all heavy metals are potentially harmful to most organisms at some level of exposure. The release of increasing quantities of organometals or heavy metals and their salt/metabolites in aquatic environment and their accumulation in living and non-living systems endanger life. Organotins (OTs) may be considered as a threat to the environment and biota, only if these could be taken up by biota through whatever route (Fent et al., 1996). In the soluble fraction trace metal ions generally exhibit high biological availability. By contrast some chelates or complexes present in solution may be unavailable. Pagenkopf et al., (1974) cited an example of this effect for copper in fresh waters and postulated that the effect of chelation may be responsible for the observed variation in the toxicity of most metals in hard and soft natural waters.

Toxicity can be defined as the inherent capacity of a toxicant to affect adversely any biological activity of an organism. The toxicity of pollutants to an aquatic organism is usually expressed in terms of LC50. The best method for
evaluating toxicity of a toxicant is by the determination of LC50 or LD50. This value represents the amount of a toxicant either in the form of lethal dose (LD) or concentration (LC50), which kills 50% of the population of the test animal within a fixed period of time (Finney, 1971). If the test animals are terrestrial, the toxicant is administered generally either through the oral or intramuscular or inhalation methods and the toxicity is expressed in terms of LD50. Where as if the test animals are aquatic the toxicant will be generally mixed with the ambient medium and the toxicity is expressed in terms of LC50. The period of exposure is considerably important in evaluating the toxicity levels of toxicants in aquatic animals. Generally depending upon the nature of toxicant LC50 values are assessed at 24h, 48h or 96 hours or even more (Spehar, et. al, 1982; David and Philip, 2005). Aquatic toxicity test are performed in order to evaluate the response of aquatic organisms and to detect or measure the presence or effect of one or more substances, wastes or environmental factors, alone or in combination (Yazdandoost and Katdare, 1999).

Evaluation of toxicity of chemical compounds is a complex phenomenon. The bio-assessment of the toxicity of xenobites with reference to aquatic biota is playing a crucial role in establishing the toxicity evaluation with reference to aquatic fauna. The wide spread usage of synthetic substance ultimately pollute the aquatic environment there by affecting the aquatic fauna mainly fishes, which constitute the major economy of the country and valuable source of protein (Muniyan and Veeraragahavan, 1999). The complexities of the interaction of the organometals/heavy metals with the biological systems of the fishes received greater attention of scientists (Tanabe et al., 2007). Acute toxicity is a major subject of research in all research institutes for evaluating the chemical toxicity test for assessing the potential hazards of chemical contamination to aquatic organisms (Muniyan and Veeraragahavan, 1999; Jain and Mishra, 1995). This concern has induced an increased awareness and closer scrutiny about the impact of these chemicals on fishes and fishery resources. In general, the extent of biological effects of chemicals can be seen at molecular, cellular, tissue, organismic, family and population levels through behavioral, physiological and pathological means (Green, 1984; Marigauder, 2009). The main objective of this study was to evaluate acute toxicity of tributyltin oxide to fresh water fish N.botia collected from the river Godavari at Nandhurmadhameshwar Ta, Niphad, District- Nashik.
The indicator organisms selected for monitoring the chemical and biological effects of pollution should possess a number of important attributes. Many authors have mentioned the desirability of using a standard fish species as a bioassay animal for reproducibility of test results (Marking, 1966; Lennon, 1967; Cairns, 1969; Sprague, 1970).

Freshwater fish *N. botia* selected for the present study fulfills most of the criteria listed by the above authors for a standard test fish. They are found in abundance in the rivers, lakes, ponds, ditches etc. of Nashik region in Maharashtra state. They have bio-economic advantage such as quick growth, fewer bones, tasty for eating, easy to reproduce and adaptability to wide range of environmental features, ready acceptance to artificial feed and effectiveness in controlling insect. As it is a sturdy fish it can be easily maintained in the laboratory. They breed throughout the year. The fish is neither too big to maintain in the laboratory nor too small for experimental purposes. It is available throughout the year. Considering all these factors, the fish, *N. botia* has been selected for the toxicological studies.

When selecting the chemicals for this study, their environmental significance was considered important. While the use of tributyltin oxide (TBTO) is restricted today, they or their metabolites are still present in the aquatic environment due to their persistence against biological degradation.

In the present study tributyltin oxide exposed to the fish via the water and this method was similar to those used in previous studies on fish (Holm et al., 1991; Bayley et al., 1999; Toft and Baatrup, 2001). TBTO was dissolved in acetone to increase the solubility and then stock solution was prepared in water and hence the likelihood of uptake. The amounts of acetone were too low to give any effects on the individuals (Martin et al., 1989).

Freshwater fish *N. botia* showed behavioral changes before die when exposed to tributyltin oxide. They tried to avoid the toxicant by loss of equilibrium, irregular erratic swimming, jerky movements, rapid opercular movements, restlessness, frequent surfacing, gulping of air, upside down surface movement, revolving, convulsions and extension of fins. At 96 hrs of exposure an important effect was the discharge of mucus at the gills and on the skin.

The potential of contaminants to upset metabolic processes has implications not only for social behavior but also for schooling behavior. Migration is energetically demanding processes (Katherine and Rod, 2006). The migration of the fish to the
bottom of the tank following the addition of tributyltin oxide clearly indicates the avoidance behavior of the fish. Similar observations were reported earlier; Murthy (1987) observed avoidance behavior in trout on exposure to pollutants. The erratic swimming of the treated fish indicates loss of equilibrium. European eels, Anguilla anguilla, exposed to sublethal concentrations of thiobencarb for 4 days displayed inhibition of AChE activity in the brain, muscle and gill tissues (Fernandez-Vega, et al., 2002; Ezemonye and Temiotan, 2010). Behavioral effects were also observed, including reduced motility, loss of equilibrium, uncoordinated movements and an increase in the levels of respiratory frequency. Santhakumar and Balaji, (2000) observed exciting and erratic movements in Anabas testudineus exposed to organochlorine pesticides. The surfacing phenomenon of fish observed under tributyltin oxide exposure might either be due to hypoxic condition of the fish as reported by Sambasiva Rao and Chandrasekara Rao, (1987) for Channa punctatus. The increased surfacing during the initial periods of exposure to tributyltin oxide concentrations suggests an elevated rate of metabolism. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to the organotin and these activities help the fish to avoid contact with poison and fight against stress (Ray and Munshi, 1987). The increased ventilation rate by rapid, repeated opening and closing of the mouth and opercular coverings accompanied by partially extended fins was observed in the present study. This could be due to clearance of the accumulated mucus debris in the gill region, for proper breathing as suggested by Carlson and Drummond, (1978), cough and yawns seem to be a more extreme effort to do the same (Cairns, et al., 1982). It can be explained by energetic constraints caused by the toxicants (Deva Prakasa Raju, 2000 and Madhab Prasad, et al., 2002). According to Sambasiva Rao and Chandrasekara Rao (1987) behavioral patterns are also influenced by bio-chemical changes at the tissue level. The significant alterations observed in the bio-chemical constituents of gill, liver and muscle in the present investigation corroborate with the above view that bio-chemical change at the tissue level of the treated fish contribute to the abnormal behavior of the fish. The accumulation and increased secretion to mucus in the fishes exposed to organotin may be an adoptive responses perhaps providing additional protection against corrosive nature of the organotin and they avoid the absorptions of the toxicant by the general body surface. This agrees to the earlier scientists
Secretion of mucus was regarded as a defense and excretory response (Benett and Dooley, 1982; Livingstone 2001) which might help in protecting gills and skin from heavy metal toxicity. Suffocation of fish exposed to heavy metals was discernible in the form of air bubbles on the water surface when the fish had been directed towards the water surface. Finally they lost their equilibrium and settled at the bottom before death. Similar abnormal behavioral pattern was also observed when the animals were exposed to Zinc and Cadmium separately (Benoit et al., 1976; Spehar, 1976).

Sprague and Drury (1969) reported that organisms have exhibited an avoidance response at 24 hrs concentrations of pollutants. The lethal toxicity of TBTO for fish varies considerably depending on species and age of target individual ( Triebskorn et al., 1994; Fent, 1996). The route of uptake for dissolved TBTO is mainly over the gills, but intake via food may also be of significance (Pärt, 1989; Holm et al., 1991). Experimental studies on several fish species have shown that exposure to commonly used chemicals (i.e. herbicides, cadmium, methylmercury, DDT, TBTO etc.) may severely impair both swimming capacity (e.g. Besch et al., 1977) and activity (e.g. Niki and Farrell, 1993; Triebskorn et al., 1994; Steinberg et al., 1995; Zhou and Weis, 1998; Grillitsch et al., 1999; Dwivedi and Trombetta 2006).

Behavioral effects of TBTO-exposure are poorly documented. It has been suggested that the variation in behavior responses between individuals and pollutant may act as an indicator of pollution (Shulman and Pomory, 2000). The behavioral biomarkers that are dealt with in this study are mainly general biomarkers, i.e. the same behavioral trait can be affected by different chemical agents. The used pollutants may cause biochemical and physiological alterations that might explain the behavioral effects. Some of these alterations, such as altered ATPase, gill structure and osmoregulation have also been reported after exposure to several other substances such as arsenic (Hwang and Tsai, 1993), heavy metals (Sola et al., 1994; Muhvich et al., 1995 Diez 2002 et al.,), acid water (Staurnes et al., 1996), and the preservative agent (Niki and Farrell, 1993). Fish N.botia behavior described may thus be considered as a general biomarker that is triggered by chemical agents acting upon different biochemical variables. Also, altered gill ATPase and gill structure may affect
other behavioral responses such as swimming performances (McGeer et al., 2000). It has been claimed that changes in behavioral characters, such as avoidance behavior occur after exposure to lethal concentrations of chemicals may serve as early indicators of contaminants (Smith and Logan, 1997, Peakall, 1996). However, the importance of using behavior in studying of effects of pollution implies further development of methods that optimize the use of behavioral biomarkers.

Mortality of N.botia is a more sensitive measure of toxicant. The percent survival rate of the fish decreased with increasing concentration and period of exposure. The evaluation of LC50 concentration of pollutants is an important step before carrying out further studies on physiological and biological changes in N.botia. In the present study the N.botia exposed to tributyltin oxide for the study of acute toxicity. It was expressed in terms of LC50 values. The LC50 values for 24h, 48h, 72h and 96 hrs for organotin tributyltin oxide were recorded 0.01852 ppm, 0.0153 ppm, 0.01311 ppm and 0.01099 ppm respectively.

The increase in mortality response of the test fish species with increased exposure and time could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols et al., 2001; James et al., 2003). So it is possible that the cumulative action of copper and organotin at various metabolic sites is responsible for the death of the fish (Basa and Rani, 2003). The main reason of death in fish exposed to pollutants is the hypoxia because the metals act on the gill function and structure causing damage of the gill epithelia, disturbances in osmo-regulation process, decrease of oxygen consumption and then death (Albaster and Lloyd, 1982; Peuranen et al., 1994; Hassan, 2005). The gills are very susceptible to water-born metals and often show various metal induced lesions. This leads not only to osmotic imbalance but may also caused an impairment of the respiratory system function of the fish which differs according to the type of metal and site of action (Jezierska and Sarnowski, 2002; Dobreva et al., 2008).

The lethal toxicity of tributyltin oxide and other xenobites for fish varies considerably. It might be due to change in chemical structure and way of intake of the contaminant used in the present investigation. The route of uptake for dissolved contaminant is mainly through the gills, but intake via food may also be of significance (Pärt, 1989; Holm et al., 1991 Triebskorn et al., 1994).
Various authors (Khangarot and Ray, 1989; Mackie, 1989; Oyewo, 1998; Khunyakari et al., 2001) have similarly observed and recorded differential toxicity of heavy metal compounds against test animals. Acute copper effects on adult fish physiological parameters or copper hazards to invertebrates have been extensively studied and reported by Hogstrand and wood (1996), Svecevicius and Vosyliene (1996), Khangarot (1989) and Eisler (1998). However, the reported LC50 values for this metal by the authors were lower than the values obtained in our study. Generally, there was corresponding increase in mortality response of the test fish species with increased exposure and time.

The evidence indicates that metals cross the cell membrane essentially by a passive transport process although endocytosis may also occur (Viarengo, 1985). Studies by Simkiss (1983) suggest that the metal complex goes through the biological membranes as a lipophilic compound. Moreover in fish, metals like Cu, Hg, and Cd etc. are able to disrupt the ionic balance, altering the permeability characteristics of the cell membrane. Thus they effect passive ion movements as well as the active transport process either by directly inhibiting the activity of Na/K dependent ATPase or as a secondary effect by reducing the availability of ATP (Bouquegneau and Gilles, 1979). When metals cross the cell membrane the metals react with cytosolic components and are usually complexed in different ways (by sulphydrylic binding, chelation and salt formation) to cytosolic compounds such as high affinity specific ligands, substrates, products of enzymatic activity or enzymes themselves. The form of the heavy metal (ionic form, oxidized, reduced, complexed by organic substances, adsorbed to inorganic or organic particulate materials, acting singly or in combination with other cations to which the organisms are exposed is extremely important in its overall toxicity to aquatic organisms and its uptake by them. Metals are taken up by aquatic organisms usually across respiratory surfaces and strongly bound by sulphydryl groups of proteins. Because of this ability, there is a tendency for them to be fixed in tissue and not to be excreted. In other words they have a long biological half life. Metal also changes the structure and enzymatic activities of proteins and causes toxic effects, evident at the whole organism level.

There are many factors which may affect the bioavailability and intake of heavy metals by the organisms, such as variations in the physicochemical parameters in the surrounding water like, temperature, pH, total suspended solids, dissolved organic carbon. Among others (Van Hattum et al., 1996); variations in water flow,
which may cause dilution of the concentrations of pollutants in water (Camusso et al., 1994); and variations in the physiology of organisms (Kraak et al., 1991; Naimo et al., 1992). These factors remain in constant interaction in the environment and these interactions could cause of different intake patterns of heavy metals by organisms.

Floach et al., (1964) calculated LC100 for various species of freshwater invertebrates using tributyltin acetate. The lethal concentration of TBT to Daphnia magna over 24 and 72 hr. were 0.12 mg / lit and 0.06 mg / lit, respectively. The LC100 was 0.15 mg / lit for 72 hr. exposure to Daphnia magna and 0.15 mg / lit. for 96hrs exposure of Cypridopsis. The LC0 was 0.075 mg / l., for both species. However it was found that Daphnia is considerably more sensitive to TBT. Robert et al., (1987) maintained adult oysters, *Crassostrea virginica* in TBT solutions containing 0.05, 0.1, 0.5 and 1 µg / lit for up to eight weeks. He observed that 20% and 30% mortality occurred between second and fourth week of exposure. Meador et al., (1993, 1996) have reported acute toxicity (LD 50 s) for Rhepoxynius abronius, Eohaustorius washingtonianus and Armandia brevis at concentrations ranging from 34 – 89 mg TBT / kg body weight. The 48-h LC50 was 2.3g/ litre; the NOEC has been estimated to be 0.5g/liter based on reversal of normal response to light (IPCS, 1990). Fargasova, (1997) reported long-term toxicity value (21-day NOEC) for Daphnia magna is 0.19 g/liter; the 96hrs LC50 for Tubifex tubifex is 0.1g/liter. Davidson et al., (1986) calculated the 96h LC50 to be 0.42 µg / lit, after exposing the mysid shrimp, Acanthomysis sculpta to a leachate of TBT. Walsh (1986) exposed to mole crab, Emerita talpoida to concentration of 10 µg TBTO / lit of sea water, no effect on crab survival was observed after 7 days of exposure. Effects on common oyster larvae exposed to 0.02-100 µg/L tributyltin acetate were studied by His and Robert (1985), as a result, in the group of larvae exposed to tributyltin acetate at 0.05 µg/L (0.05 µg/L in terms of tributyltin chloride) or over, growth was inhibited and deaths were observed within 10 days. No observed effect concentration on growth was reported to be 0.02g/L (0.02 g/L in terms of tributyltin chloride). Waldock and Thain (1983) exposed C. gigas to TBT oxide (TBTO) for 56 d; they reported that exposed to 0.15 µg/L TBTO did not grow as well as controls and had pronounced thickening of the upper shell, and that spat exposed to 1.6 µg/L TBTO were severely inhibited in terms of growth. Roberts (1987) conducted 48-h acute toxicity tests of the hard clam (Mercenaria mercenaria) and the oyster (Crassostrea gigas), using
tributyltin chloride (TBTCl). The maximum exposure concentration was 1.3µg/L TBTCl. The 48-h LC50 values were 1.13µg/L for clams and 1.30µg/L for oysters. For the larvae of both species, the 48-h LC50 values were 1.65µg/L for clams and 3.96µg/L for oysters. Beaumont and Budd (1984) exposed veliger larvae of the mussel (Mytilus edulis) to TBTO for 15 days. No larvae survived longer than 5 days in 10 µg/L TBTO or longer than 10 days in 1 µg/L TBTO. About half the larvae exposed to 0.1 µg/L TBTO were dead on Day 15 (i.e., 15-d LC50 approximately 0.1 µg/L TBTO), and most surviving larvae were moribund and had grown significantly more slowly than controls.

The determination of the LC50 value is of immense importance since it provides fundamental data for the design of more complex disposal model. The values obtained are highly useful in the evaluation of safe level or tolerance level of a pollutant. Mary (1984) has reported that the LC50 values depend on the concentrations of pesticides and also with the time of exposure. The 96 hours LC50 value was the low, however the mortality scored was high. Srinivasulu reddy et al., (1985) reported that the LC50 values and the exposure period showed inverse relation. The result shows that the LC50 values decreased with increase in exposure period and vice-versa and also the 95 % confidence limits. Sultana, (1995) reported the toxicity evaluation of heavy metals CuSO4, HgCl2 and ZnSO4 was conducted on the bivalve, L. marginalis and LC10 and LC50 values were calculated, they show the HgCl2 was more toxic than CuSO4 and ZnSO4 respectively. Eldon et al., (1981) studied the effects of low concentrations of heavy metals on the bivalve. Similar kind of results were obtained by Patil, (1993) in L. marginalis when exposed to heavy metals like CuSO4, HgCl2 and CdCl2. Toxicity evaluation of ZnSO4 to the freshwater snail, Viviparus bengalensis. Alam, (1984) heavy metal ions in sufficient higher concentration might kill organisms or cause adverse effects that change aquatic community structure. Catherine and Jayapaul (1993) studied the acute toxicity of Zn to the green mussel, Perna viridis and recorded the highest values of Zn accumulated in the viscera followed by gills and mantle. Ong et al., (2001) studied the toxicity of cadmium, copper and zinc in the clam Donax faba, and observed that the Zn is toxic when excess amount occurs in the body.

The experiments conducted by Holwerda and Herring, (1986) were found that the freshwater clam Anodonta anatine could not survive exposure to tributyltin oxide in a concentration equivalent to 5µg Sn/L for longer than 6 weeks.
Dode, (1993) reported that LC50 values of all the five size groups of fresh water prawn, Macrobrachium kistnensis exposed to different concentrations of cuprous oxide for 24, 48, 72 and 96 hours, they show that relative toxicity increases with increasing exposure time since LC50 values decreased as the exposure period increased. Kungolos et al., (2001) studied the toxicity of four organotin compounds towards freshwater crustacean, Daphnia magna. Tributyltin chloride proved to be the most toxic among all four organotin compounds. Shejule et al., (2006) reported LC50 values of the organotin tributyltin chloride exposed to freshwater prawn, Macrobrachium kistnensis; to 24h, 48h, 72h and 96 hours, LC50 values were found to be 0.33 ppm, 0.26 ppm, 0.17 ppm and 0.09 ppm respectively. They showed the LC50 values decreased with increase in exposure period. Kharat, (2007) shows the same results of LC50 values of the organotin tributyltin chloride exposed to freshwater prawn, Macrobrachium kistnensis.

Addition of toxicant stress increased the demand and thereby the animal becomes sensitive to the toxicants stress. Our observation are supported by Alderidge, (1976) reported that tributyltin compounds influences aquatic invertebrates and suggested that tributyltin compound must be acting on the organized enzyme sites of the cells, in the aquatic concentration initially increases and further decreases the rate of oxygen consumption causes mortality. Wulf and Byington (1975) reported similar observation and stated organotin compound are known to cause variety of effects on mitochondrial which correlates with increase in oxygen consumption at initially and further decreases, oxygen consumption decreases as pollutant concentration increases gradually. Furthermore tributyltin compounds are known to inhibit ions translocating ATPase (Selvin et al., 1970).

Organotins are considered as major environmental pollutants causing ecotoxic, cytotoxic and mutagenic effects in animals (More et al., 2003). Tributyltin compounds are known to cause a variety of effects on mitochondrial, which correlates with increase in oxygen consumption. (Wulf and Byington, 1975; Aldrich, 1976). Furthermore, tributyltin compounds are known to inhibit ions translocating ATPase (Selvin et al., 1970; Selvin 1976). Indira, B. (1989) displays oxygen consumption rate by Caradina weberi shows alteration like increase and decrease oxygen uptake when exposed to different concentration of copper sulphate and TBTO. Organotins are extremely toxic to aquatic biota as demonstrated for a variety of different organisms in vivo and in vitro, Fent, (1996). Many ecotoxicological studies on organisms of
different evolutionary level have been reported. (Alzieu and Heral, 1984; Bryan et al., 1989; Fent and Muller 1991; Fioramonti et al., 1997; Alzieu, 2000 Nesci et al., 2010, Mitra et al., 2014).

Respiration is an essential physiological activity of all living organisms by which they obtain energy for carrying out all other metabolic activities of the body. The degree of metabolism is measured by the rate of oxygen consumption in fish. The vital metabolic activities in organisms require energy, which is obtained by oxidative reactions. The aerobic method depends on the presence of oxygen to go through different stages of metabolic activities to release energy (Lehninger, 1983). The oxygen consumption of an animal is an important parameter. This parameter can be adopted as a useful measure of lethal and sub lethal effects because energy processes are indication of overall physiological status of organism. The total oxygen consumption of an animal reflects the basic metabolic status which in turn evidences the general effects of any environmental stress. Depletion in the oxygen contents occurs in the medium, when hazardous matter entered in to the water body (Jones, 1973). Heavy metals in the sub lethal concentration present in the aquatic environment are too low to cause rapid death directly, but may affect the normal functioning of organism and their behavior. They may also reduce the fitness of organism in nature. In the aquatic environment, one of the most important manifestations of the toxic action of the chemical is the over stimulation or depression of respiratory mechanism (Muirhead Thompson, 1971). Changes in the respiratory activity of fish have been used by several investigators as indicators of repose to environmental stress (Carpenter,1930; Knight;1964; Patil and David,2008; Marigaudar et al.,2009; Gopal and David,2010; Magar and Afsar Shaikh,2012; Jothinarendiran,2012; Maharajanetal,2013; Ram Nayan Singh,2014). Respiration or transferring oxygen from the water in to the cells of a animal is a difficult phenomenon, compared to its concentration in air (200 ppm) oxygen in water is relatively very low (0-14 ppm). Due to this water must be moved very fast and efficiently over the respiratory surface area in fish i.e. gills. A high value and low-pressure pump are required for proper and effective exchange of gases. Low pressure causes resistance to the water flow over the gills.

Unlike the terrestrial environment, in the aquatic environment, the body of the animal get bathed in the water containing the xebobites and hence the effect of toxicants on respiration is more pronounced. Toxicants enters in the body of fish
mainly through the respiratory organ gills and the onset of symptoms of poisoning, the rate of oxygen consumption increases (Holden, 1963). Holden (1973) observed that one of the earliest symptoms of acute poisoning is respiratory distress. The severity of distress may lead to failure of respiratory functioning by affecting the respiratory organ cell structure and ultimately the respiratory centers in the brain which controls the respiration and activity of the gills. Exposure of the lethal and sub lethal concentration is reported to increase the oxygen consumption activity, resulting in increased ventilation and hence increased uptake of the toxicants. Organochlorines have been reported to stimulate the oxygen consumption exposed to sub lethal concentrations and inhibit the oxygen uptake at the lethal concentrations. Organophosphates pesticides generally act as disruption of nerve impulse, transmission in the central and peripheral nerves system by inhibiting acetylcholine esterase that hydrolyse the neurotransmitter acetylcholine (Aldridge, 1971).

Respiration is the mostly used tool for understanding the physiological action of the pollutants. The respiration rate of organism is an indicative for the physiological state and changes in the respiration rates may be an indicative for environmental stress. Biological responses of organisms to toxicants in the aquatic environment are usually understood through determining their rate of survival and changes in the levels of various physiological phenomena. Newell, (1973) stated that toxicants act as physiological stressors upon the organism. It is well known fact that the rate of oxygen consumption is used as an important tool for understanding the physiological state of metabolic activity of an organism. In the present study, oxygen consumption of *N. botia* in normal and after exposure to TBTO has been quantified. On exposure to TBTO the respiratory metabolism of freshwater fish *N. botia*, has found to be directly affected. The results obtained clearly indicated that there was decrease in the rate of oxygen consumption in fish, *N. botia* when exposed acute concentration of tributyltin oxide. The rate of oxygen consumption decreased significantly (*p*<0.05) after exposing for 72 h to 96 hrs as compared to control groups. There was a continuous decrease in the rate of oxygen consumption up to 96 hrs when the fish were exposed to all the concentrations of lethal and concentration of TBTO and as the period of exposure increased this uptake gradually but constantly decreased severe fall after 72 h and then continued up to 96 hrs of exposure.
These results clearly indicate that the tributyltin oxide must be acting on the organized respiratory mechanism i.e. damaging epithelial cell layer of gills ultimately altering the elements involved in the respiration mechanism of the gills. The TBTO must be acting on the enzyme sites of cells slowly in the lower concentration initially. Where it might be acting as a stressor in higher concentrations and after prolonged exposure, interfering the physiological activities. This is speculated because there was an obvious decrease in rate of oxygen uptake after 48 h to 96 hrs exposure to all the concentration of TBTO as compared to the first 24 hrs of exposure of TBTO. From these observation it can be inferred that the tributyltin oxide disrupting enzyme-mediated process and / or disrupting cellular structures. The initial elevation in the rate of oxygen consumption showed a compensatory phase to enhance the physiological activity but the continue decrease may be due to the failure of respiratory metabolism. The mechanism of toxic action of organotin compounds appears to be through disruption of oxidative phosphorylation, by a) secondary responses caused by discharge of a hydroxyl chloride gradient across mitochondrial membrane, b) interaction with the basic energy conservation system involve in the synthesis of ATP and c) an interaction with mitochondrial membrane to cause swelling and disruption, Selwyn, (1976); Aldridge, (1976). Thus the decreased rate of oxygen consumption in test animals may be expected because of toxic action of TBTO as reported, Selwyn (1976); Aldridge (1976).

A decrease in oxygen consumption was reported by several authors (Holden,1962; Fergusionetal,1967; RamaMurthy,1988; Vijayalakshmi, 1994; Ramana Kumari,1999; Anthony Reddy,2003; Vineetkumar, et al., 2008; Anita susan et al., 2010; Gopal and David.2010; Das and Gupta 2012; Maharajan et al., 2013; Ram Nayan Singh ,2014). Most of the authors concluded that decline in the consumption of oxygen is due to the damage of respiratory surface area of the gill, as gills are the major respiratory organ and all metabolic pathways depend upon the efficiency of the gills in terms of exchange of gases for their energy supply. Damage to these organs causes a chain of disturbances events leads to respiratory distress (David et al., 2002). Another reason for decrease in the oxygen consumption is autrophy of the respiratory epithelium, enlargement of the water/blood barrier and inhibition of gill ATPase activity (Aldridge, 1971).

In the aquatic environment one of the most important manifestation of the toxic action of chemical is the over stimulation or depression of respiratory activity.
The changes in the respiratory activity of fish have been used by several investigators as indicators of response to environmental stress. The respiratory potential or oxygen consumption of an animal is the important physiological parameters to assess the toxic stress, because it is a valuable indicator of energy expenditure in particular and metabolism in general Franklin et al., 2010. Organotins are indicated to cause respiratory distress or even failure by affecting the tissue involved in breathing or respiratory centers of the brain. The effect of toxicants on the respiration of fishes and invertebrates have received wide spread attention and were reviewed by Hughes, (1979). As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced. Toxicants enter into the fish mainly through gills and with the onset of symptoms of poisoning, the rate of oxygen consumption increases observed that one of the earliest symptoms of acute poisoning is respiratory distress. This serves not only as a tool in evaluating the susceptibility or resistance potentiality of the animal, but also useful to correlate the behavior of the animal (Franklin et al., 2010.)

Tributyltin compounds are known to cause a variety of effects on the mitochondrial membranes, which correlated with the increase in oxygen consumption, Wulf and Byington, (1975). Laughlin, and Linden, (1985) stated that the tributyltin compounds are the active in very low concentration and they are slow acting poison. Sarojini et al., (1989) observed oxygen consumption rate in Caridina weberi showed alteration like increase and decrease in oxygen uptake when exposed to different concentration of copper sulphate and TBTO. Though the antifouling organometallic compounds at the cellular level decrease the metabolism of accumulated toxicant may demand increased oxygen, which is reflected in the upward shift of the oxygen uptake in the prawn. The inhibition of the oxygen uptake in the N. botia might be due to the penetration of the toxicant molecules and its action alters the metabolic cycle at cellular level. Liu and Thomson, (1986) stated that n-butyltin is biologically active due to their ability to stimulate or inhibit the dehydrogenase activity and oxygen consumption. Stimulation or inhibition of the dehydrogenase activity by toxicant is harmful to a living organism as this produces deleterious effects on the organism by interfering with its energy metabolism.

Exposure of fish, N. botia to tributyltin oxide resulted in morphological changes in the gills, (Plate -1 Fig A, B, C, D and E) are reflected in plasma had a
significant effect on the respiration, excretion and osmoregulatory functions of the gills. These changes can be regarded as primary changes, which will inevitably lead to secondary physiologically changes as well as responses that could affect various organ systems. Similar results were described by Ghate and Mulhekar, (1978); Baticodes et al., (1991). The enhancement of oxygen uptake during the initial phase may be due to the excitement and excessive muscular activity caused by pollutants stress, Webb and Brettm (1972). Cremerm (1957) also claims that TBTO affect the oxidative metabolism. TBT compounds are known to causes variety of effect in mitochondria which correlates with and include increases in oxygen consumption, Aldridge (1976).

The excess of heavy metals in the aquatic environment alter the behavior and physiology of living organisms, especially the respiratory physiology. Measurement of rate of oxygen consumption is important parameters to assess the toxicant stress on aquatic organisms since it is also an index of energy expenditure to fulfill the demands due to environmental and biological alterations. The respiratory activity of fish was used as an indicator of response of an organism to water pollution. Nagaratnamma and Ramamuthri (1982) studied the metabolic depression in the freshwater Teleost, *Cyprinus carpio* exposed to an organophosphate pesticide, showing decline in the rate of oxygen uptake. Bodke (1983) observed decreased oxygen consumption in *Barytelphusa cunicularis* exposed to lethal and sub lethal concentration of carbamate. Pawar and Katdar, (1984) have studied the oxygen consumption of *Macrobrachium kistnensis* exposed to lethal and sub lethal concentration of fenitrothion, BHC and carbofuran. Moorthy et al., (1984) observed respiration in freshwater mussel exposed to methyl parathion showing change in the processs. Chaudhari et al., (1988) studied herbicide effect on oxygen consumption of *Bellamya bengalensis* showing change in oxygen uptake. Oxygen consumption is changed when *Thiara torulosu* was exposed to organophosphorus insecticide (Bharathi and Prasad Rao, 1989). Several authors (Rao et al., 2003; Shivakumar and David, 2004; Vutukuru, 2005; Vineetkumar et al., 2008; Shereena et al., 2009; Logaswamy and Remia, 2009) reported that the disturbance in oxidative metabolism leads alteration incompletely animal oxygen consumption in different species of fish exposed to toxic chemicals. The mechanism of toxic action of organotin compounds appears to be through disruption of oxidative phosphorylation, by a) secondary responses caused by discharge of a hydroxyl chloride gradient across mitochondrial
membrane, b) interaction with the basic energy conservation system involve in the synthesis of ATP and c) an interaction with mitochondrial membrane to cause swelling and disruption, Selwyn, (1976); Aldridge, (1976). Thus the decreased rate of oxygen consumption in test animals may be expected because of toxic action of TBTO as reported, Selwyn (1976); Aldridge (1976). Tributyltin compounds are known to cause a variety of effects on the mitochondrial membranes, which correlated with the increase in oxygen consumption, Wulf and Byington, (1975). Laughlin and Linden, (1985) stated that the tributyltin compounds are the active in very low concentration and they are slow acting poison. According to Piver (1973) dialkyltin and trialkyltin compounds are known to be capable of effecting the respiration.

According to Piver (1973) dialkyltin and trialkyltin compounds are known to be capable of effecting the respiration. Devi (1996) studied changes in oxygen consumption of marine fouling dressinid bivalve, Mytilopsis sallei exposed to mercury. Sonawane and Lomte, (2000) studied the effect of heavy metals copper sulphate and mercuric chloride on oxygen consumption of the fresh water bivalve Lamelliden marginalis. Chinni et al., (2000) reported on changes in oxygen consumption, ammonia excretion and metal accumulation in post larva of Penaeus indicus exposed to lead. Manikumar, (1986) also observed changes in oxygen consumption in marine prawn, Penaeus merguiensis exposed to pesticides. Hiltibran (1966) considered that the decreasing oxygen consumption rate due to herbicide brought the process of breaking the link between oxidative phosphorylative processes. This decrease can be also caused by free oxidation in the organism. Kale (2002) observed increased in oxygen consumption rate to first 6 h and decline gradually and steadily after 12 h and continued till they attain normalcy. She observed that stressful effect of cadmium chloride started decline after 6 h of freshwater crab Barytelphusa cunicularis. Vosloo et al., (2002) documented that, in attempt to move away from pollution, the animal’s oxygen consumption rate increases from pre-exposure values to support this additional activity. Ratsamee and Nongnud (2006) documented the changes in the oxygen consumption in the blue swimming crab, Portunus pelagicus (Linnaeus) when exposed to the sub lethal concentrations of copper. They reported that the rate of oxygen consumption per unit body weight tended to decreases when exposed to copper concentrations. He observed the copper affect and the cardiac activity in Portunus pelagicus. He also observed that the heart rate increased with decreasing salinities and increasing copper concentrations. Shivakumar and David
(2007) observed depletion in oxygen consumption in freshwater fish, *Catla catla* exposed to endosulfan and concluded that the decreased rate of oxygen consumption due to disrupt metabolic activities after endosulfan toxicity. Kharat *et al.*, 2010, reported that under stress condition of TBTCI there was an increase in oxygen consumption at initial stage and decrease the oxygen consumption as the exposure period increases.

It is also found that fish have little ability to regulate their metabolic rate when faced with adverse environment and as the concentration increases the response is intensified. In conclusion, the response of an organism to the toxic environment is quite evident from the variation in respiratory metabolism and that can also affect several parameters such as the exhausts the biochemical reserve. Hypoxia or anoxia can result from the faulty gaseous exchange. Heavy metals are one class of pollutants which have a disruptive influence on the structural organization of the gill tissue. Gills of rainbow trout exposed to acute lethal concentration of zinc are damaged (Mathiesson and Brafield, 1973; Skidmore and Tovell, 1972). Burton *et al.*, (1972) and Skidmore (1970) found that rainbow trout exposed to Zn$^{2+}$ (40 ppm) die mainly through tissue hypoxia, a major factor being disruption of bronchial respiratory epithelium (Skidmore and Tovell, 1972).

Hughes *et al.*, (1979) have shown that exposure to pollutants causes a reduction in the morphological basis for diffusing capacity of the gills. The gill filaments have a key position in the bodies of fish because of their role in the transport of oxygen. In the secondary lamellae, the circulatory system is separated from the surrounding medium only by a layer of epithelial cell, one or two cells thick, a basement membrane and a thin layer of cytoplasm lining the blood lacunae.

When heavy metal ions exceed a threshold concentration in the aquatic ecosystem, they act as pollutants and create stress in fish. Environmental pollution is reported as one of the major factors causing hypoxemia in animals (Black *et al.*, 1962). The respiratory potential of an animal is an important physiological parameter to assess the toxic stress because it is a valuable indicator of energy expenditure in particular and metabolism in general. This also helps for making valid inferences on its environmental requirements. Basha *et al.*, (1984) found that the activity levels of succinate dehydrogenase and malate dehydrogenase decreased in toxicant-exposed fish suggesting the prevalance of hypoxia. Pesticides, heavy metals and other xenobiotics are known to affect the oxygen consumption and metabolic pathways.
The respiratory system of fish seems to be the prime target of many pollutants. When tissues of the animal do not receive sufficient oxygen they must either reduce the overall energy demand or respire anaerobically. Since glycogen is the ready source of energy even in anaerobic condition, the depletion of glycogen from the tissue is expected to be an immediate manifestation of hypoxemia. During severe hypoxia, flounder reduces its oxygen consumption and partially compensates by increasing anaerobic energy metabolism based on fermentation of glycogen or glucose with lactic acid as the major anaerobic end product (Jorgensen and Mustafa, 1980). This strategy is also employed by other fishes such as carp (Johnston, 1975), goldfish (Van den Thillart, 1977) and trout (Burton and Spehar, 1971). A decrease in the glycogen content confirms the prevalence of hypoxic condition at the tissue level since anoxia or hypoxia increases carbohydrate consumption (DeZwaan and Zandee, 1972) thereby creating a sort of stress in the fish resulting in extra expenditure of energy. Lindahl and Hell (1970) demonstrated the effects of Phenylmercuric hydroxide (PMOH) on the gill tissue and found that the superficial layer of the gill filaments appear somewhat detached from deeper parts. This reduces the diffusing capacity and there is a fall in the oxygen supply to the tissues which becomes hypoxic. Lindahl and Hell (1970) found that $\text{O}_2$ consumption of gill is reduced by 30% after an exposure of the animal to PMOH. Davis (1973) found a decline in arterial oxygen tension in Sockeye salmon following pulp mill effluent exposure.

The increase of death with increasing concentration and increasing of the duration of exposure could be because of the accumulation of metals in different tissues of body especially in the gills which are important sites for the entry of metals, therefore causing lesions and gill damage and failure of metabolic activities (Bols et al., 2001; James et al., 2003). So it is possible that the cumulative action of copper and cadmium at various metabolic sites is responsible for the death of the fish (Basa and Rani, 2003). The main reason of death in fish exposed to heavy metals is the hypoxia because the metals act on the gill function and structure causing damage of the gill epithelia, disturbances in osmo-regulation process, decrease of oxygen consumption and then death (Albaster and Lloyd, 1982; Peuranen et al., 1994; Hassan, 2005). The gills are very susceptible to water-born metals and often show various metal induced lesions. This leads not only to osmotic imbalance but may also caused an impairment of the respiratory system function of the fish which differs
according to the type of metal and site of action (Jezierska and Sarnowski, 2002; Dobreva et al., 2008).

Diffusing capacity of the gills is further reduced following the irritating action of pollutants which causes a secretion of mucus over the gills (Shaffi, 1978b). Interference with gas transfer will reduce oxygen levels within the blood circulating to the brain where responses are initiated by the respiratory centre. The respiratory centre may coordinate cardio-vascular changes and stimulate the hormonal system and erythropoietic tissue to take necessary steps to compensate for the decreased oxygen supply to the tissues. A decreased glycogen level of the body may be a step in that line.

Heavy metals /Organometals are one of the most active polluting substances as they cause serious impairment in the metabolic, physiological and structural systems of the body, when high concentrations are present in the milieu. A continuing study of specific physiological, biochemical, metabolic and enzymatic changes of aquatic organism exposed for short periods to environmental stressors is essential to provide a rational basis for anticipating and understanding the ecological effects in the aquatic environment. Such studies may provide a sensitive method for predicting the effects of chemicals exposure as survival, reproduction and growth. This would allow a relatively rapid evaluation of the acute toxicity of a compound.

In almost all these circumstances the major share of stored energy comes from the carbohydrate or glycogen reserves. Thus carbohydrates form the central point in energy production because of its great mobility in the living systems, together with its capacity to get compartmentalized within cells and tissues. The mobility is provided by glucose and compartmentalization by glycogen and glucose-6-phosphate. It is widely accepted that carbohydrate deposits in the form of glycogen in tissues like liver provide the immediate energy requirements. In teleost fishes under a variety of stressors including exercise (Black et al., 1960, 1961, 1962), physical disturbance (Nakano and Tomlinson, 1967), starvation (Black et al., 1966), environmental hypoxia (Heath and Pritchard, 1965, Narasimhan and Sundararaj, 1971), salinity changes (Bashamohideen and Parvatheswara Rao, 1972). Effects of environmental stress due to chemical pollution on tissue glycogen levels of fish have also been reported (McLeay and Brown, 1975, Mazmanidi and Kovaleva, 1975, Gill and Pant, 1981, Dange and Masurekar, 1982). These studies, involving exposure of fishes to
different pollutants have indicated that the pollution stress stimulates glycogenolysis in teleost fishes under a variety of stressors including exercise.

From a biochemical point of view, life is uniquely characterized by its association with protein. Tissue proteins as energy sources for fishes during thermal stress, spawning and muscular exercise have been demonstrated by several investigators (Fontaine and Hatley, 1953, Idler and Clemens, 1959). Though considerable information is available dealing with the determination of acute toxic levels of several pollutants and their influence on oxidative metabolism, studies on the tissue energy sources are relatively few. Glycogen protein and lipid present in gill, liver and kidney provide energy to the body. Animal under stress deplete the energy sources at different rates. Hence a study was conducted to examine the effect of tributyltin oxide on the glycogen, protein and lipid content of gill, liver and kidney.

Organometal toxicants may induce certain biochemical changes in aquatic organisms and before the drastic cellular and systematic dysfunctions manifest themselves, appropriate biochemical parameters related to proteins, lipids and glycogen etc. could be used effectively to know the gravity of the situation and to check it at the initial stage itself (Aldridge, 1983). Studies on energy metabolism are concerned in the way in which the major carbohydrate, lipid and protein fuels are used by an organism for energy production. Invertebrates, changes in the biochemical constituents are pronounced which are cyclic in reproduction, since a great amount of energy, must be channelized to the gonad during reproduction. This is reflected in deposition or depletion of the nutrients with the advent of departure of the reproductive period (Lambert and Dehnel, 1974). Many aquatic animals meet the metabolic expense of spawing by drawing on response materials accumulated during non-reproductive period. Seasonal changes in biochemical constituents in relation to reproductive cycle of marine invertebrates such as molluscs were studied by Giese, (1969) and bivalve molluscs (Nagbhushnam and Dhamne, 1979, and Nagbhushnam and Talikhedkar, 1977).

Glycogen, protein and lipid present in gill, liver and kidney provide metabolites to the body. Animal under stress deplete the energy sources at different rates. Hence a study was conducted to examine the effect of tributyltin oxide on the glycogen, protein and lipid content of gill, liver and kidney.

In the present study there were significant depletion of glycogen noted in the gill, liver and kidney of fishes exposed to tributyltin oxide. This indicates that the
metals interfere with the carbohydrate utilization in *N. botia*. The significant decrease in the protein content of gill, liver and kidney of dosed *N. botia* occurred at the end of the exposure period. In the control and exposed fish, *N. botia* the glycogen content was in the order of

Liver > Kidney > Gill

Glycogen levels are found to be highest in liver, as it is the chief organ of carbohydrate metabolism in animals, followed by kidney and gill. Liver glycogen is concerned with storage and export of hexose units for maintenance of blood glucose and that of other organ glycogen is to act as a readily available source of hexose units for glycolysis within the organ itself. A fall in the glycogen level clearly indicates its rapid utilization to meet the enhanced energy demands in fish exposed to toxicant through glycolysis or Hexose Monophosphate pathway. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Decrease in gill, liver and kidney glycogen levels is in corroboration with the reports of earlier workers (Sastry and Subhadra, 1983; Bedii and Kenan, 2005) Significant decrease in the glycogen reserves of both liver and kidney has been reported in *Heteropneustes fossilis* is in response to 25 and 50 ppm of mercury (Qayyum and Shaffi, 1977); in different fishes in response to cadmium, copper, lead and zinc (Shaffi, 1978 a, b; 1979 a, b; 1980 d); in rainbow trout in response to cadmium (Larsson and Haux, 1980); in *Anabas scandens* in response to zinc sulphate (Natarajan, 1982); in *Heteropneustes fossilis* in response to copper, cadmium and mercury (Srinivasthava, 1982); in *Notopterus notopterus* in response to mercury (Verma and Tonk, 1983); in rainbow trout in response to cadmium (Lowe-Jinde and Niimi, 1984); in *S. mossambicus* in response to potassium dichromate (Ghosh and Chatterjee, 1985); in *O. mossambicus* in response to different pollutants (Dange, 1986 a) and in *Barbus conchonius* in response to lead (Tewari et al., 1987). Apart from these, glycogen depletion in the liver is reported in *H. fossilis* in response to lead (Shaffi and Qayyum, 1979); in *Puntius conchonius* in response to mercury (Gill and Pant, 1981); in *Channa punctatus* in response to cadmium (Dubale and Shah, 1981); in *C. punctatus* in response to chromium (Sastry and Tyagi, 1982; Sastry and Sunita, 1983); in *O. mossambicus* in response to 1.5 ppm mercury (Naidu et al., 1984) and in *Clarias batrachus* in response to lithium administration (Goel et al., 1985). However there are also reports that various toxicants caused an increase in the glycogen level of various tissues of different fishes (Grant and Mehrle, 1973; Buckley
et al., 1979; Bakthavathsalam and Reddy, 1982 b; Anderson et al., 1987; Nath and Kumar, 1987). But in some fishes, glycogen levels were not affected by mercury (Sastry and Rao, 1984) or copper (Tort et al., 1987).

Xenobiotics interfere in the various functions of the body. In the present study, it was found that the fish became irritable after exposure to tributyltin oxide and copper sulphate. They get irritated at the slightest provocation and were hyperactive and hyperactivity depletes the stored food materials present in the gill, kidney and liver. Many toxicants are known to oxidize glutathione, Hb etc., damage cell membranes and organelles by lipid peroxidation, inhibit many enzymes and thus disrupt important physiological functions of the body. The body requires large quantities of energy to produce substances like glutathione, metallothionin, glucuronic acid and other substances to remove toxicants by activation, inactivation or conjugation etc. and to repair damaged organelles and replace lost cell constituents. So, in fishes exposed to pollutants the energy demand is very high. This increased energy demand is met by utilizing the stored glycogen in different tissues. The increased metabolic activity of the liver after heavy metalin toxication was reported by Maynard and Loosli (1962). Gill and kidney glycogen is rapidly depleted during intense activity and glucose is mobilized from the liver glycogen stores to supply both raw materials and rebuilding their glycogen. Both glycogenolysis and glycogenesis involves a number of enzymes and is guided by hormones. Hormones increase glycogenolysis during stress to meet the increased energy demand. The alterations in carbohydrate metabolism are produced indirectly by the environmental stresses through primary effect on the endocrine glands exciting them into releasing large amounts of hormones (Hille, 1982; Gluth and Hanke, 1984). To keep the glucose level in the blood relatively high, despite metabolic process following the influence of stressors, glycogenolysis is essential. The elevation in blood glucose may form a part of restorative process in which glucose is mobilized from the liver glycogen stores (Wardle, 1978). It enters the other organ cells supplying both raw materials and energy for rebuilding glycogen. There are reports that the blood sugar levels are elevated in fish during acute exposure to a variety of environmental alterations considered as stressful including exposure to toxicants (Jorgensen and Mustafa, 1980; Larsson and Haux, 1980; Mukhopadhyay and Dehardrai, 1980; Gill and Pant, 1981; 1983; Srivasthava and Singh, 1981, 1982; Sastry and Siddiqui, 1982; Sastry and Tyagi, 1982; Mishra and Srivasthava, 1983, 1984; Srivastava, and Mishra, 1983;
Phosphorylase is a regulatory enzyme of glycogen breakdown. Bhaskar and Govindappa (1986) found that in the red muscle of *Tilapia mossambica* acclimated to alkaline medium, a stepped up glycogen breakdown and an increased phosphorylase activity in the red muscle. They suggested that the phosphorylase activity could be responsible for depleted glycogen content. The depletion of glycogen observed in the present study could be due to this phenomenon also.

Toxic substances are known to inhibit many enzymes. It is known that structural changes of enzymes are induced by heavy metals (Webb, 1966). So it might be possible that copper and organotins inhibited different enzymes of the TCA cycle, like pyruvic dehydrogenase, succinic dehydrogenase, malate dehydrogenase. The inhibition of these enzymes of TCA cycle can prevent the aerobic metabolism. Hence inhibition of the enzymes of TCA cycle indicates the impairment of aerobic metabolism. This also suggests a shift from aerobic to anaerobic metabolism in the fish under pollution stress. An inhibition of the enzymes of TCA cycle in fishes exposed to pollutants was observed by Sastry and Siddiqui (1983); Sastry and Sunita (1983); Balavenkatasubhaiah *et al.*, (1984) and Naidu *et al.*, (1984). In the present study even though no effort was made to find out the activities of TCA cycle enzymes, it would be reasonable to assume that the depletion of glycogen observed in *N. botia* could have been due to such an inhibition also.

The significant decrease in the protein content of gill, liver and kidney of dosed *N. botia* occurred at the end of the exposure period, mainly at 72 h and 96hrs. In the control and exposed fish, *N. botia* the total protein content was in the order of

Liver>Kidney>Gill

This clearly indicates that the body utilizes the glycogen stores first to meet the increased energy demand. When the glycogen stores were decreased, the body utilizes the protein for energy production. This is manifested as a decrease in the protein content in different tissues. There are many reports that the total protein in different tissues of fish decreased after exposure to different toxicants. (Sashikala *et al.*, 1985; Katti and Sathynesan, 1986; Kumar and Ansari, 1986; Yamawaki, *et al.*, 1986; Reddy, 1987; Reddy and Bashamohideen, 1987 and Seshagiri Rao *et al.*, 1987).

The decline in the gill, liver and kidney protein would suggest an intensive proteolysis which in turn could contribute to the increase of free amino acids to be fed
into TCA cycle as Keto acids. Such a possibility is further strengthened by the investigation of Schafer (1967); Mehrle et al. (1971). Shakoori et al., (1976) revealed both qualitative and quantitative variations in the tissue amino acids of fishes exposed to toxicants. In addition, studies by Sakaguchi and Hamaguchi (1975) have also revealed marked variations in the activity of enzymes involved in transamination of fishes in similar situations. Sivaprasada Rao and Rama Rao (1979) found that the decrease in glycogen is due to the immediate utilization in the tissues to meet the excess demands of the energy metabolism. They also suggested that high increase of amino acids they observed in *T. mossambica* treated with methyl parathion is utilized for gluconeogenesis through the transamination and transdeamination reactions to supply the necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during stress There are reports that transamination and transdeamination reactions are prominent under stress (Harper et al., 1977).

Decreased protein content could possibly be due to protein breakdown which increased amino acid pool in the tissue. It is also reported that decreased protein moiety suggests damage to hepatic tissue and an intensive proteolysis (Rao, 1984) resulting in increased amounts of free amino acids to be fed into TCA cycle as keto acids. Loss of weight and elevation of nitrogenous compounds in tissues of fish exposed to benthicarb were reported by Seshagiri Rao et al., (1983) in *S. mossambicus*. Seshagiri Rao et al., (1987) detected an increased protease activity, increased free amino acid content and decreased protein (soluble and insoluble) in liver, muscle, brain and gills of *S. mossambicus*.

In the present study decrease in protein following exposure to tributyltin oxide suggests their possible degradation by increased proteolysis. This increased proteolysis could be attributed to the damage caused to lysosomal membranes thus permitting the leakage of lysosomal enzyme into the cytosol. The lack of alteration of protein level of gill, liver and kidney of *N.botia* exposed to tributyltin oxide and copper sulphate at 24 and 48 h except in the gill of fishes exposed could be that the body utilizes the glycogen of these tissues in the initial period of exposure. The depletion of glycogen in the tissues of *N.botia* after exposure proves this. These findings also support the concept of Fry (1971) that fishes tend to resist a changed situation for a specific period, but will eventually succumb as a result of their inability to adapt. According to Umminger (1970) carbohydrates represent the principal and
immediate energy precursors for fishes exposed to stress conditions while proteins being the energy source to spare during chronic period of stress. Gluth and Hanke (1984) found that changes in plasma protein need time to occur and the reduction of protein can only be found after 70 h of exposure. Radhaiah et al., (1987) observed that amino acids in the kidney increase along with a decrease in the protein values. This proves that intense proteolytic activity in the tissues can increase amino acids in the liver. Such an increase in amino acids after exposure to toxicants in different organisms were found by Girija (1984) and Rao (1984).

A defect in protein synthesis by the action of toxicants can also decrease the protein content in different tissues. An altered relationship between the ribosomes and the membranes of the endoplasmic reticulum may also produce a defect in protein synthesis. Rath and Misra (1980) examined the changes in nucleic acids and protein content in liver, muscle and brain of *Tilapia mossambica* exposed to the insecticide, dichlorovos. Post exposure studies revealed a significant decline in DNA, RNA content of the liver, muscle and brain. They observed that the liver exhibited a greater loss of protein than the gill and kidney. In the present study in *N. botia* exposed to tributyltin oxide, also the liver showed a greater loss of protein than the other tissues. Rath and Misra (1980) also found that the RNA/DNA ratio decreased in exposed fish and it showed a positive correlation with protein. Usually RNA and RNA/DNA ratio of a tissue are considered to indicate the intensity of protein synthesis (Misra and Patnaik, 1974). It is possible that tributyltin oxide and copper may have influenced the protein synthesis in *N. botia* by inhibiting RNA synthesis. Hence a decreased protein synthesis and an increased proteolytic activity might have caused the decline in protein content in the gill, liver and kidney of *N. botia* exposed to the tributyltin oxide.

Liver being the centre for various metabolisms is also rich in proteins. In all the tissues of the exposed fish, the total protein content was found to be reduced. The decrease in the protein content as observed in the present study in most of the fish tissues may be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose or due to directing the free aminoacids for the synthesis of proteins or for the maintenance of osmo and ionic regulation (Schmidt Nielson, 1975). It could also be due to the production of heat shock proteins or destructive free radicals or could be a part of heavy metal induced apoptosis.

Lipids are also the storage form of energy like glycogen. In the present study lipid levels decreased in all the tissues of the fish exposed to the lethal concentration
of tributyltin oxide. Lipids not only provide energy during unfavorable circumstances but also found in various forms in the structural membrane in the cell. As lipids are insoluble, in aqueous, they are mostly found in the form of complex in case of membranes, associated lipids in the form of hydrophobic barrier that permits partition between aqueous contents of the cell and other cell organelles or in association with protein as lipoprotein. Imbalance of lipid metabolism due to toxic stress may cause major lethal problems in the body of animal. Bhilave et al., (2000) stated that considerable decrease in total lipid in tested tissues might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions after glycogen, lipid content may be used for energy production overcome the toxic stress. Similar results were observed in different aquatic animals, Villan et al., (1990); Lomte and Muley, (1993); Sarvana and Geraldine, (1997); Deshmukh and Lomte, (1998); Geraldine et al., (1999); Amanulla et al., (2004); Jagatheeswari J. (2005) and Vijayavel et al., (2006).

The results of the present investigation supported and indicated that N.botia exhibited a differential preference in their utilization of biochemical constituents during time and concentration dependent stress of tributyltin oxide. The decrease in oxygen consumption together with the utilization of lipid and protein during exposure suggests that N.botia might shift to anaerobic metabolism in order to encounter the heavy metal stress in the environment. Sujatha et al., (1996) reported effect of tributyltin oxide induced biochemical changes in estuarine clam. They suggested tributyltin compounds are generally lipophilic and may reduces the lipid level in muscle and digestive gland under stress condition of TBTO in clams; similar results were reported by Lundebye et al., (1997). Shiva Prasad Rao and Ramana Rao (1979) stated that, considerable decrease in the total lipid in muscle might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress conditions and after glycogen lipid content may be used for energy production to overcome the toxic stress. Romeo and Mauricette, (1997) in vitro experiments indicated that cadmium, which does not undergo redox cycling, was found unable to stimulate the lipid peroxidation process, where as copper and mercury may exist under different oxidation states and have detrimental effects on the antioxidant defense system of the Ruditapes decussates. Deshmukh and Lomte, (1998) observed significant depletion in the lipid content in all the tissues tested after acute treatment of copper sulphate.
Teleosts have five pairs of gill arches. In the front four pairs, the slender gill filaments form two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. Numerous secondary gill lamellae are lined up along both sides of the gill filament. The surface of the gill lamellae is covered with epithelial cells and many capillaries separated by pillar cells run parallel along the surface. Numerous secondary gill lamellae are lined up on both sides of the primary gill lamellae. The primary gill lamellae consist of centrally placed rod like structure termed central axis. It consisted of chloride cells and with blood vessels on either side. The secondary lamellae, termed as respiratory lamellae the actual respiratory site of exchange of gases are highly vascularised and covered with a thin layer of epithelial cells (EC). Blood capillaries are innervated into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have a single nucleus which is flattened and the region between the two adjacent secondary gill lamellae is known as inter lamellar region.

Tributyltin oxide exposures have induced marked histological alteration in fish gills structure. The alterations include epithelial lifting, bulging of tips of primary gill filaments, degenerated secondary lamella, curling of secondary gill filaments, atrophy of secondary lamella and fusion of secondary gill filaments. The damage of gills of fish exposed to the 72h and 96h exposers of tributyltin oxide lethal nature was severe. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen. Besides these changes pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also significant.

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants (Poleksic & Mitrovic-Tutundzic, 1994; Mazon et al., 2002; Fernandes & Mazon, 2003). The immediate histopathological responses of the gills of fish exposed to pollutants are often manifested by a significant increase in the secretion of mucous by mucous cells (Dutta, 1997; Hemalatha and Banerjee, 1997a; 1997b). The large quantity of mucous secretion acts as a defense mechanism against several toxic substances documented by (Mazon et al., 1999). This mechanism also helps to remove the bound pathogens,
toxicants and foreign matters (Powell et al., 1992) which adhere to the gills and which can interfere respiration. In the present study also, the slimy coatings of the over the gills showed compositional alterations and sloughed off several times, which might have led to the fusion of the secondary lamellae. Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985; Fernandes & Mazon, 2003). Several other anthropogenic chemical pollutants are also known to induce fusion of the secondary lamellae of gills (Leino et al., 1987; Dutta, 1996; Wendelaar Bonga, 1997). According to Mallatt (1985) induced alterations in gill histology are mostly non-specific in nature, which partially represent the damage and partially the compensatory response of the fish. Examples of the first are necrosis of the epithelial cells of the secondary lamellae, epithelial lifting, dilatation of the blood sinuses of the secondary lamellae and lamellar aneurysm. The main compensatory responses are hypertrophy and hyperplasia of the respiratory epithelial and chloride cells, hyperplasia of the mucous cells (including decrease due to exhaustion, followed by an increase in their density) and infiltration of the dilated intercellular spaces by leukocytes. Dutta (1996) categorised the structural alteration in the gill morphology into two groups: (1) direct deleterious effect of the xenobiotics causing necrosis and rupture of the branchial epithelium. Such type of effect is mostly dose dependent and very often reported under lethal conditions (Mallatt, 1985). They also suggested that death of branchial cells and their rupture usually develops either by autolysis or by rapid lyses caused by the direct action of toxicants on the cells’ constituents (Abel, 1976) and bronchial defense response achieved by mucus hyper secretion, chloride cell proliferation, epithelial lifting, swelling, hyperplasia and lamellar fusion. According to Peuranen et al., (1994) any discontinuity of epithelial lining of the gill due to massive wear and tear may lead to a negative ionic balance and to changes in the haematocrit and mean cellular hemoglobin values of the blood. The formation of an aneurysm is related to the rupture of the pillar cells (Heath, 1987; Martinez et al., 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells.
Hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, disruption of epithelial cells from pillar cells were observed in gill tissues of *Anabas testudineus* exposed to monocrotophos (Santhakumar *et al.*, 2001). Degenerative changes in gills, such as detachment and lifting of the epithelial linings from the surface of the gills, uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, dilation of the blood sinuses of the secondary lamellae, lamellar aneurysm, hemorrhages were noticed after exposure of sublethal concentration of profenofos (Rao *et al.*, 2006). Coutinho and Gokhale (2000) found epithelial lifting in the gills of carp (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*) exposed to the effluents of a wastewater treatment plant.

According to Mallat (1985) such alterations are non-specific and may be induced by different types of contaminant. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. Toxic substances can injure gills, thus reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Saravana Bhavan and Geraldine *et al.*, 2000). However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes & Mazon, 2003).

A number of histopathological changes have been reported in fish exposed to different chemical compounds are on these lines are Vijayalakshmi and Tilak, 1996; Das and Mukherjee, 2000; Rodrigues *et al.*, (2001); Tilak *et al.*, 2001a; Tilak *et al.*, 2001b; Anita susan and Tilak, 2003; Ortiz *et al.*, (2003); Cengiz and Unlu, 2003; Machado and Fanta, 2003; Altinok and Capkin, 2007; Velmurugan *et al.*, (2007), which are in agreement with the observed histopathological changes under tributyltin oxide exposure. In individuals exposed in 10−9 M TBTCl, lamellae showed severe changes: the primary lamellae were often thickened; in the secondary lamellae, often fused, separation of the epithelium from the capillary, epithelial lifting (oedema), dilation and blood vessels congestion were observed (Morcillo *et al.*, 1997). High levels of TBT have been detected in several marinas and harbors (Pickston, 1988; Alzieu *et al.*, 1989; Daly; Fabris, 1993), where damages to aquatic organisms have been described (Gibbs *et al.*, 1988; Byrne *et al.*, 1989; Ellis; Pattisina, 1990; Kingtong *et al.*, 2007), but the toxic mechanisms have not been completely elucidated. Oliveira Ribeiro *et al.*, (2000) described the morphological effects of TBT in tropical fish.
Astyanax sp. and more recently in northern fish Salvelinus alpinus (Oliveira Ribeiro et al., 2007). Gills are a multifunctional tissue involved in respiration, osmoregulation, acid-base regulation and excretion (Heisler 1984; Isaia 1984; Randall & Daxboeck 1984; de Renzis & Bornancin 1984). For this reason, functional impairment of gills caused by protozoan and metazoan pathogens or by chemical irritants, can seriously affect the health of the fish. Zhang et al., (2008) described the occurrence of reactive oxygen species associated with TBT exposure at environmental levels. This, according to the authors, could be the possible explanation to the degenerative effects observed in fish exposed to the organometals. Based on present results, it can be concluded that tributyltin oxide is able to cause various severe damages in gill as reported by Zhang et al., (2008).

The surface of liver is covered with serous membrane and some connective tissue extends inward into parenchyma. It is composed of parenchymal cells (hepatic cells) and lattice fibers. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures known as hepatic cell cords. Bile canaliculus, is centrally located in each cord. Fairly large quantities of lipid glycogen granules are also observed in the cytoplasm of fish hepatic cells (Plate 2, Fig A). Hepatic cells have many vital functions. Other than the secretion of bile, they play an important role in protein, lipid and carbohydrate metabolism. They serve as storage sites for some nutrients and detoxification is another function attributed to them. Lethal concentrations of tributyltin oxide exposures have induced discrete pathological changes in the liver tissue of the fish N.botia. These changes include degenerated hepatopancreatic tissue, Blood cells among hepatocytes (BC), formation of vacuoles, along with atrophy, necrosis and disappearance of hepatocytic cell wall and disposition of hepatic cords (Plate 2, Fig B, C, D and F). The degenerative changes are intensified in 96h exposures.

The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply (Van der Oost et al., 2003). It is also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998). The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds and could subsequently result in structural damage (Brusle & Anadon, 1996). Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions.
Some of these environmental factors include biotoxins, parasites, infectious germs, physiochemical parameters and pollutants, for example pesticides, hydrocarbons, PCB’s (polychlorinated biphenyls) and heavy metals (Brusle and Anadon, 1996). Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver. Depletion of the glycogen in the hepatocytes is usually found in stressed animals (Hinton & Lauren, 1990; Wilhelm Filho et al., 2001), because the glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations (Panepucci et al., 2001). Significant changes were observed in the liver tissue in lethal concentrations of tributyltin oxide where marked swelling of the hepatocytes in places with areas of diffuse necrosis (Plate 2, Fig B, C, D and F). Sinusoids in most cases were distended and central veins appeared severely damaged due to marked swelling and degeneration of the endothelial lining cells. A study by Yildirim et al., (2006) in Nile tilapia (*Oreochromis niloticus* L.) fingerlings exposed to 5 µg L-1 deltamethrin revealed severe morphological alterations in liver, where hydropic degenerations in liver was observed. The liver of individuals incubated in 10−9 M TBTCI seemed more enlarged than that of controls and the blood vessels were more prominent and the big ones destroyed. In individuals exposed to 10−9 M TBTCI, a loss of liver normal architecture with cord disarray was evident. Sinusoids were dilated and congested. Melano-macrophage aggregates were present, appearing collapsed in fish *Astyanax* sp. (Oliveira Ribeiro et al., 2007).

TBT is a strong endocrine disruptor presenting various effects on fish sex differentiation. These damages are associated among others with the genotoxic role of TBT to aquatic organisms (Ferraro et al., 2004; Micael et al., 2007; Zhang et al., 2007). The damages in sinusoids as enlargement found after TBT exposures suggest a physiological response of the circulatory system or a consequence of the hormonal unbalance once TBT is a known endocrine disruptor agent (Matthiessen and Gibbs, 1998), or still an inflammatory response supported by the high occurrence of necrosis areas found in liver as target organs. Genetic modifications can also be interrelated as a source of cell damage occurrence, mainly if associated with apoptosis (Filipak Neto et al., 2007). On this way the necrosis described here can be tentatively explained by both, the presence of reactive oxygen species and genotoxic damages. In addition, the physiological changes also found in this work such as the enlargement of vessels could be explained by the wide potential toxicity of TBT and metabolites as DBT.
The lesions found in target organs suggest a cumulative and dose related effect when comparing both tested groups. According to Guruge and Tanabe (2001), higher concentrations of organotins were observed in liver classifying this as a target organ and consequently more affected when exposed to this compound. The higher incidence of necrotic areas in liver in individuals from the longer acute exposure showed that the TBT or metabolites residual time is important to establish the lesion severity. Necrosis and hyperplasia as described by Funahashi et al., (1980) is one of the most evident effects of organotin exposure. The incidence of hepatic vacuolated cells tissue exposed to TBT certainly is an indicative of metabolism disturbances. This lesion was more evident after 96h in individuals exposed to TBT confirming the toxic potential of this organotin form as described by Oliveira Ribeiro et al., (2007). The study of *Brachydanio rerio* hepatic tissue showed morphological alterations in individuals exposed to organophosphates, even when they were exposed to sublethal doses considered safe (Rodrigues and Fanta, 1998). Ansari and Kumar, (1987) reported significant alterations in the hepatic cell count and the nucleocyton plasmic index in the liver of zebra fish *Brachydanio rerio* (cyprinid) exposed to 0.9 mg L-1 concentration of malathion. According to Ansari and Kumar (1987) and Gill et al., (1988), the liver is an organ that frequently undergoes changes when exposed to pesticides at sublethal doses. Rashatwar and Ilysas (1984) reported that in teleost fish *Nemachelius denesoni* (Day) exposure to phosphamidon caused highly vacuolated and cloudy swelling and even the connective tissue was damaged in liver. Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossilis* while subjecting them to acute thiodan toxicity.

Tributyltin oxide induced pathological changes in the liver observed in the present study might affect the physiological activity of the fish such as reduction in enzyme synthesis and reduces the functional ability of liver which indirectly affects all metabolic activities of the organism. Teleostean kidney consists of head and body kidneys. Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. Body kidney is composed of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major haematopoitic tissue in the body. Each nephron consists of two parts, the glomerulus and the urinary tubule. The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. Renal tubules consist of single layer of epithelial cells. Renal tubules are thin and short in the neck segment.
The proximal convoluted segment is divided into two parts i.e. segment I and segment II. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In segment II, renal tubules are composed of cuboidal epithelial cells. Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of proximal convoluted segment. Thus, it is easy to distinguish between proximal and distal convoluted segments under light microscopy (Oguri, 1982).

Kidney of the fish *N. boitia* exposed to tributyltin oxide showed marked pathological changes. Highly degenerative changes were observed in haemopoietic tissue which include Shrinkage of glomerulus (SG), Expansion of space inside Bowman’s capsule, Hypertrophied cells and Lumen tubules diminished, Cytoplasmic vacuoles in epithelial cells of renal tubules. Degenerating haemopoietic tissue with erythrocytes more prominently observed in 72h and 96h exposure period. Besides the above changes severe necrosis, cloudy swelling in renal tubules and granular cytoplasm was also observed. From the body of fish, the waste products are eliminated through kidney. The non-detoxified organometal metabolites like DBT and MBT molecules must be eliminated through the kidney of fish and hence, it is susceptible to chemical compounds when exposed to lethal doses. Tributyltin oxide, while it was eliminated through kidney might have caused degenerative changes in renal tubule and glomerulus. (Oliveira Ribeiro *et al.*, 2005; Valdez Domingos *et al.*, 2007).

The posterior kidney of freshwater fishes is largely dedicated to the production of copious dilute urine and it has little responsibility for ion or acid-base balance. In marine fish, where they are opposite osmotic gradients, urine flow is severely reduced by elimination of all but the proximal tubules. In some marine species, water loss is further reduced by elimination of glomerular filtration altogether and renal function depends solely on tubular secretion. The kidney of the fish receives the vast majority of post branchial blood, and because of that, we can expect renal lesions in the fish when toxicant agents exist in the environment. Therefore, a study of these possible kidney changes may be expected to be a good indicator of environmental pollution (Wester and Canton (1987).

In the present study, kidney of the fish showed cloudy swelling in tubule cells. This alteration can be identified by the hypertrophy of the cells and the presence of
small granules in the cytoplasm. Initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton & Lauren, 1990; Takashima & Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima & Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to lethal doses of tributyltin oxide.

Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman’s space (Takashima & Hibiya, 1995). Following exposure of fish to toxic agents such pesticides, histological alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al., 1997; Thophon et al., 2003). Similar alterations were found in fishes exposed to organic contaminants (Veiga et al., 2002) and mixed environmental contaminants (Schwaiger et al., 1997; Pacheco & Santos, 2002). The toxic sub lethal concentration of fenvalerate technical grade in the kidney of Crrirhinus mrigala showed changes in haemopoietic tissue which included severe necrosis, vacuoles around renal tubules and hemorrhage. (Anita Susan and Tilak, 2003) Similar report by Tilak et al., (2001c) in kidney tissue of the fish Ctenopharyngodon idellus when exposed to technical and sub lethal concentration of 20% EC fenvalerate was observed with tissue damage like necrosis, vacuolar degeneration and atrophy. Gupta and Dalela (1987) reported histological changes in kidney of Notopterus notopterus, exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds. Anitha Kumari and Shreeram Kumar (1997) observed mild activity of carbohydrates in the cytoplasm, nuclei and the luminar border of the proximal and distal tubules in the kidney of the freshwater teleost Channa punctatus under exposure to the polluted water of the Hussain Sagar Lake.

The present observations are in agreement with the reports of Goel and Veenagarg, 1980; Mandal and Kulshreshtha, 1980; Dubale and Awasthi, 1982; Malaya Guptha et al., 1988; Dhanapakiam and Premlatha, 1994; Tanabe et al., 1994 Ramana Kumari, Morcillo et al., 1997;1999; Yacobu, 1999; Tilak et al., 2001a and
2001b; Tilak et al., 2005a and 2005b and Tilak et al., 2007; Kington et al., 2007; Oliveira Ribeiro et al., 2007; Valdez Domingos et al., 2007; Ferraro et al., 2004; Micael et al., 2007 and Zhang et al., 2007) who observed renal damage, rupture in the glomeruli, reduced renal tubules and its lumen in different fish exposed to different toxicants. Thus, when fishes are exposed to organometals, they suffer irreparable architectural changes in various vital organs making the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish such as release of various enzymes and the metabolic processes. Thus, the histological changes observed in the gills, liver and kidney of the freshwater fish *N. botia* exposed to tributyltin oxide indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress.