CHAPTER-I
INTRODUCTION AND REVIEW OF LITERATURE

Anthropogenic effects, processes, objects or materials are those that are derived from human activities as opposed to those occurring in natural environments without human influences. The term is often used in the context of environmental externalities in the form of chemical or biological wastes that are produced as by-products or otherwise purposeful human activities. Many different chemicals are regarded as pollutants, ranging from simple inorganic ions to complex organic molecules. Every class of pollutants has its own specific ways of entering the environment and its own specific dangers. Organotins or butyltins (BTs) are a group of organometallic compounds that were first synthesized in the 1930s, but did not gain wide commercial use until the 1960s and beyond (Tanabe, 1999).

Water pollution due to xenobiotics is a serious problem due to their toxicity and persistence in the environment. As a result of their usage, they find their way into the freshwater resources with the run-off water from agricultural land or by direct application, spray drift, aerial spraying and by discharge of effluents from factories and sewage. Most environmental problems of concern today are attributed to the production and release of toxic chemicals capable of interacting with the environment and disrupting the ecosystem. Indiscriminate discharge of biocides from agricultural runoff and other sources into aquatic media affects non target organisms such as which is of great economic importance to humans. Triorganotin in the form of agricultural and industrial products are one of the most potentially harmful chemicals liberated into the environment in an unplanned manner. Though they have contributed considerably to the welfare of humans, their adverse effects on non-target organisms are enormous. The impact of chemical environmental contamination on fish health, consequently fish productivity is of economical relevance for fishes as well as aquaculture. Environmental pollutants have been reported to accumulate in fish and have threatened human health either directly or indirectly through the food chain. However, the proper handling and use of biocides in aquatic areas are especially critical, accidental spills or over dose can kill fish or cause other damage to its habitats that may lead to the reduction in the fish population. Majority of the studies concerning the effects of heavy metals on fish have been confined to the acute toxicity test with the death of fish as an end point but it is only due to interference in the
functioning of vital organs like gill, liver and kidney etc (Cullen et al., 2001, Diaz et al., 2002).

Ecotoxicology is the science regarding the adverse effects of toxic agents on living systems such as insects, mollusks, amphibians, fishes and birds. Ecotoxicological science involves the fields of chemistry, ecology and toxicology and is categorized as a new discipline (Richardson, 1993). The concept of ecotoxicology involves the distribution of substances in the environment together with their fate. It is focused on the effects on populations rather than on individuals (Richardson, 1993; Solbe et al., 1998). This science provides important information for legislative and regulatory processes regarding the assessment of the likely impact of new and existing chemicals on the environment (Solbe et al., 1998). The goal of ecotoxicology is to determine processes of toxicity of all chemicals of interest. There are approximately 100,000 compounds released into the environment in quantities that could threaten the environment. Practices reducing the impacts of agricultural pollutants involved the use of buffer zones between the natural ecosystem and agriculture, development of a new generation of pesticides, a wider use of the biological methods for the control of weeds and herbivorous insects and the development of strains which do not require the use of pesticides and fertilizers. Ecological engineering, cleaner technology and global concern have been considered in environmental management methods (Jorgenensen, 1997; Bickham et al., 2000).

The evaluation of acute toxicity is essential for determination of sensitivity of animals to the toxicants and also useful for evaluating the degree of damage to the target organs and the consequent physiological and behavioral disorders. The tests of acute toxicity of various pollutants were undertaken in the 19th century by some workers studying the lethal effects of industrial wastes on the aquatic life. Our industry and agriculture are the two main sources of chemical pollution. Chemical pollution has become particularly a worrying issue in developing countries such as India because thousands of their companies continue to dump untreated industrial waste water filled with different toxic materials and other chemicals in rivers. In India, for instance, more than 40% rivers are considered unsafe for drinking. Industrial pollution is the major source of global air, water and soil pollution and refers to all pollution which can directly or indirectly be linked with industry. Chemicals can cause deleterious effects at one or more levels of biological organization from biochemical, physiological, individual, population and through to
the ecosystem levels. In contrast to the established hypothesis that a pollutant affects the different biological levels in an escalating time dependent pattern, starting at the biochemical level, it is here suggested that biomarkers at the biochemical, physiological and behavioral levels often will respond early and simultaneously in the same individual. Whereas some biochemical responses are specifically related to one class of exposure agents and thus may act as specific indicators of pollution, most behavioral traits may be altered in response to a variety of chemicals. In eco toxicological research the aquatic environment is highly relevant since most pollutants either directly or indirectly ends up into water systems. Since fish as well as other aquatic organisms are constantly exposed to pollutants either directly or via the food chain, they are ideal sentinel species and have been used as model species in eco toxicological investigations. In present study the term “indicator of pollution” is used to describe a biological variable that may indicate exposure to the effects of pollution.

The term is often used synonymous with “eco toxicological biomarker” which is defined by Depledge (1994), “A biochemical, cellular, physiological or behavioral variation that can be measured in tissue or body fluid samples or at the level of whole organism (either individuals or populations) that provides evidence of exposure to and/or effects of one or more chemical pollutants and/or radiations” that can be measured in tissue or body fluid samples or at the level of whole organism (either individuals or populations) that provides evidence of exposure to and/or effects of one or more chemical pollutants and/or radiations.”

The development of eco toxicology has shifted from the measurement of acute lethal effects of chemicals to the assessment of sub lethal and chronic effects (Anonymous, 2001). Acute toxicity is toxicity which arises soon after exposure and unless death occurs, recovery is complete (Aldridge, 1988) or the adverse effects occurring within a short time of (oral) administration of a single dose of a substance or multiple doses given within 24 hours (Chan and Hayes, 1989). While chronic toxicity requires prolong or repeated administration of the substance before the toxicity becomes apparent (Aldridge, 1988). The exposure period in chronic studies may vary, depending on the objective of study, the species selected for the study and the route of administration employed. A generalization which is often made is that chronic studies do not exceed 10% of the animal’s lifespan (Mossberg and Hayes, 1989; Stevens and Gallo, 1989).
Biomarkers are defined as biological responses to environmental chemicals that give a measure of exposure and sometimes also of toxic effect (Walker et al., 1996) in an environmental context, biomarker are biological tools used as sensitive indicators demonstrating that toxicants have entered the organisms, been distributed within the tissues and are eliciting a toxicological effect (McCarthy and Shugart, 1990). Biomarkers are state of the art tools used to estimate the impact of chronic exposure to specific chemicals in the environment (Joregensen, 1997). Biomarkers are physiological alterations or manifestations of stress in organisms. A biomarker is a biological reaction used to monitor exogenous exposure, effects of exposure and early symptoms at the organ or organism level (Schulte et al., 1995). Biomarkers commonly represent biological responses of individual organism to foreign chemicals or xenobiotics. The biological responses may include amongst others, 1) enzyme alterations, 2) immune dysfunction, 3) reproductive disturbances 4) DNA changes, 5) behavioral changes and 6) histopathological lesion (Hokas, 1993). Biomarkers have a great potential for use in environmental monitoring of both marine and freshwater ecosystems and biomarkers have been validated to be included in monitoring programs (Den Besten, 1998). Biomarkers have been classified into two groups namely biomarkers of exposure and biomarkers of effect. Biomarkers of exposure are a demonstration of chemical exposure of organism, but do not give information of any biologically important adverse effects that this exposure may have caused biomarkers of effects or more correctly toxic effects, demonstrate that the adverse effect on the organism has occurred due to exposure to pollutants (Molven and Goksoyr, 1993; Walker et al., 1996).

Among the contaminants, organometals constitute one of the main dangerous groups, because they are toxic, persistent and not easily biodegradable. The species and concentrations of metals in water are determined by geochemical processes and large scale releases into the aquatic environment by human activities (anthropogenic activities). Rapid industrial developments as well as the use of metals in production processes have led to the increased discharges of heavy metals into the environment. The harmful effects of heavy metals as pollutants result from incomplete biological degradation. Therefore, these metals tend to accumulate in the aquatic environment. Since heavy metals are non-biodegradable, they can be bio-accumulated by fish either directly from the surrounding water or by ingestion of food. In addition, Heath (1987) indicates that when metals reach sufficiently high concentrations in body cells they
can alter the physiological functioning of the fish. Many of the heavy metals are essential in various metabolism viz. copper is an essential trace nutrient that is required in small amounts (5-20 micrograms per gram (µg/g)) by humans, other mammals, fish and shellfish for carbohydrate metabolism and the functioning of more than 30 enzymes. It is also needed for the formation of haemoglobin and haemocyanin, the oxygen-transporting pigments in the blood of vertebrates and shellfish respectively.

In the 1960s manufactures introduced tributyltin (TBT) as an active substance in antifouling paints (Cato et al., 2007). The substance was very effective but the first deleterious effect emerged at the end of 1970s when the oyster industry at reported severe deterioration in population growth. The toxicity of Tributyltin compounds has become unique focusing point for research because of the extensive uses of TBT includes biocide (fungicide, bactericide, insecticide) in paints and coatings used for marine antifouling applications, preservative for wood, textiles, plastic, paper, leather. The environmental and economic impact of TBT did not become evident until the deformative and reproductive failures of Crassostrea gigas i.e imposex. The environmental impact of organotin as a group of compounds has been the subject of a large amount of research in the past 10 years. Tributyltin (TBT) compounds are organic derivatives of tetravalent tin (Sn⁴⁺) and have the general formula (CH₃-CH-CH₃)Sn⁻ R where R is a covalently linked anion or group. Tributyltin (TBT) was widely introduced into the environment in the 1980s as the bioactive component of antifouling paints which were used to prevent the attachment of barnacles, algae, and other organisms to boat hulls. The commercially important tributyltin derivatives include TBT oxide, TBT benzoate, TBT methacrylate, TBT chloride, TBT hydroxide and TBT fluoride. These compounds were developed to be used as antifouling paints for a wide range of maritime activities. Tributyltin (TBT) compounds are metabolized to dibutyltin (DBT) and at last monobutyltin (MBT). Triorganotin are used as general biocides against microbial and invertebrate pests and in marine antifouling paints (Laughlin and Linden, 1985). The first antifouling paints incorporating an organotin compound as a biocide were developed in 1961. In India, TBT compounds had been used as antifouling agents in paints earlier; however, there is a ban on the usage of these paints which is in force now. There are few studies on the distribution of butyltin residues in water and sediment samples collected from the east coast of India (Rajendran, et al., 2001). Not much is known about the organotin concentrations in
marine and fresh waters of the south Asian region in general and in Indian waters in particular (Bhosle et al., 2004).

Settlement of micro and macro biota on any immersed substrata in water is known as biofouling. This is one of the great problems facing currently by aquatic based industries and is an issue of serious concerns for the entire world (Kill et al., 2002) and known internationally by the term biofouling (Yebra et al., 2004). It is the “process of adsorption, colonization and development of living and non living materials on an immersed substratum (Clare, 1996) which includes the uncommon accumulation of microorganisms included bacterial organisms, plants and or animals on wetted objects (Callow, 1996). Controlling of fouling by applying commercial antifouling techniques of a surface is the antifouling process. It includes mechanical cleaning, biocides, toxic antifouling coatings. Currently the most effective formulations are based on organotin notably tributyltin (TBT), Cu and organo nitrogen group (Willemsen and Ferrari, 1993; Isensee et al., 1994; Yebra et al., 2004). Organotin is known to be the most toxic chemical ever deliberately released into the aquatic ecosystem (Stebbing, 1996; Omae, 2003). Organotin compounds are the important constituents of such paints to the aquatic environment and are known to cause deleterious effects to non-target organisms. TBT compound is highly toxic to many species of aquatic organisms specially fishes. TBT is one of the known endocrine disrupting chemical agents (EDC). Many studies on TBT have shown the effect of TBT on growth (Seinen et al., 1981) and reproduction (Nirmala et al., 1999; Nakayama et al., 2004; Shimasaki et al., 2003). Uptake and elimination of TBT has been observed in the rainbow trout Oncorhynchus mykiss (Martin et al., 1989). Besides, Champ (2000) reported that TBT inhibits growth (Triebskorn et al., 1994), reproduction (Nirmala et al., 1999) and sexual differentiation in aquatic organisms (Shimasaki et al., 2003). Aquatic organisms living in water polluted by organometals/heavy metals especially with organotins pollution may not die immediately. However, these pollutants may damage the vital organs by altering the structure of cells and their functions.

Organotin compounds (OTCs) are man-made chemical compounds, based on a hydrocarbon structure combined with tin. The most well known organotin is tributyltin (TBT), used widely in aquatic antifoulant paints to prevent the growth of organisms such as crustaceans and molluscs on the hull of ships. Other organotins include the mono and dibutyltins (MBT, DBT), octyltins (Monooctyltin, MOT and
Dioctyltin, DOT) and mono, di- and tri- phenyltins (MPT, DPT, and TPT). Organotins have many applications, which include use as stabilisers in PVC, catalysts in chemical reactions, glass coatings, agricultural pesticides, biocides in antifoulant paints and wood treatments and preservatives (Batt, 2006) and in anti odour/anti fungal treatments for textiles and textile polymers (Greenpeace, 2003 and Peters, 2006). The five major commercial applications (biocides, PVC stabilizers, catalysts, agrochemicals and glass coatings) account for approximately 20,000 tons of tin consumption per year (de Brito et al., 2002). TBT is now an omnipresent global contaminant. Extensive use in antifouling paints on watercraft led to the widespread distribution of TBT (and its breakdown products DBT and MBT) in the global marine and freshwater environment, in water, sediment and biota (Kannan et al., 1996, 1999a; Connelly, et al., 2001; Elgethun et al., 2000; Lee et al., 2006, 2005; Strand and Jacobsen, 2005; Sudaryanto et al., 2004; Ueno et al., 2004). The persistence of some organotin compounds means that they remain in sediments, particularly anoxic sediments, for long periods of time e.g. the half-life of TBT in deep sediment has been estimated to be approximately 18 to17 years (Viglino et al., 2004). “Hot spots” of particularly high levels of TBT in water, sediment and biota are normally associated with commercial ports, harbours, shipyards, shipping lanes, marinas and the like (Alzieu, 2000; Shim et al., 2002; Smith et al., 2006), although the situation is slowly improving following legislation banning the use of TBT in antifoulant paints. MOT and DOT are used as PVC stabilisers for food contact materials (Kawamura et al., 2000, RPA, 2005). It is estimated that between 12 to 13K tons of tin is used annually in tin stabilizers worldwide.

Tributyltin compounds is highly toxic and wide spread contaminant in aquatic environment and has caused world wide imposex characterized by the development of penis, vasadeferens and somniferous tubules in female in marine gastropods, Defur et al., (1999). Organotin compounds responsible for snail suffering from imposex, due to a serious disturbance of hormone balance. The name imposex is used about the beginning of change of sex where female snail develops a seed duct and a penis, they will become pseudohermaphrodite. At advanced stages imposex results in snails become sterile or dying. Tributyltin (TBT) and its derivatives are endocrine disruptors that induce deregulation of vertebrate and invertebrate endocrine systems (Golub and Doherty, 2004). Aquatic invertebrates, particularly marine gastropods, are extremely sensitive to organotin compounds such as TBT. Consequently they undergo changes
in imposition of male secondary sex characteristics in response to exposure (Maguire, 2000; Oetken et al., 2004; Sternberg et al., 2010), which is caused by a decrease in aromatase activity (the enzyme that converts androgens to estrogens) (Matthiessen and Gibbs, 1998; McAllister and Kime, 2003).

The most toxic OTC, namely TBT and TPhT, are well known to have the main biological impact on the hormonal asset (Iguchi and Katsu, 2008; Delgado Filho et al., 2011), where they act as endocrine-disrupting chemicals (EDC) (Porte et al., 1996; Tabb and Blumberg, 2006). Endocrine perturbations often associated with widespread metabolic shifts (Swedemborg et al., 2009 Alzieu, 2000; Nakayama et al., 2004; Delgado Filho et al., 2011). The most striking in vivo effect of trisubstituted OTC at very low concentrations (1ppb) is imposex, namely the irreversible masculinization of female gastropods, a recognized biomarker of organotin pollution (Iguchi and Katsu, 2008; Delgado et al., 2010). Beside gastropods, OTC at very low tissue concentrations act as EDC in a variety of taxa (Meador, 2011) including bivalve mollusks (Morcillo and Porte, 2000), tunicates (Mansueto et al., 2011), crustaceans (Tang et al., 2009), echinoderms (Sugni et al., 2010), fish (McGinnis and Crivello, 2011), mammals (Nakanishi et al., 2006; Delgado Filho et al., 2011) and fish (Zhang et al., 2009).

The uptake of heavy metals from solution into the bodies of invertebrates at permeable surface is generally considered to be a passive process not requiring any expenditure of energy (Mason et al., 1988). When assimilatory capacity of the system was over loaded either by an excess of essential metal or by presence of unusually high level of rare non-essential metals deleterious effect occurred (Coombs George, 1978). The active substance TBT is highly toxic and also damaging to a multitude of non target species. Numerous bottom dwelling organisms such as snails and mussels take up TBT via their food, water and sediments. The compound accumulates in their tissues and causes damage such as shell deformation, reproduction organ abnormalities and inhibition of larval release (Alzieu et al., 1989) Due to the slow degradation of TBT in sediments (Stewart and De Mora, 1990; De Mora et al., 1995; Evans et al., 1995; Viglino et al., 2004), the large reservoirs of TBT-polluted sediments may also pose a threat, especially to bottom-dwelling fish species such as flounder. Experiments with organotin compounds have shown various toxic effects in experimental animals including effects on different parameters can become valuable biomarkers of effects of xenobiotic substances in fish. Immune system has been
shown to be sensitive to the effects of pollution (Anderson and Zeeman, 1995; Vos et al., 1996), and organotin compounds affect the immune system in particular. Thymus atrophy was induced by TBTO in flounder (Grinwis et al., 1998) and in the guppy (Wester and Canton, 1987; Wester et al., 1990). Also a reduction of the non-specific cytotoxic cell (NCC) activity in European flounder (Grinwis et al., 1998) and channel catfish Ictalurus punctatus (Rice et al., 1995) as well as a concentration-dependent decrease in phagocytic activity of phagocytes in Atlantic croaker Micropogonias undulatus, hogchocker Trinectes maculatus and in oyster toadfish Opsanus tau (Wishkovsky et al., 1989) were noted after exposure to TBT. Exposure to TBT also caused histopathological lesions in liver, kidney, eye and gill epithelium in medaka Oryzias latipes and guppy Poecilia reticulata (Wester and Canton, 1987; Wester et al., 1990), and masculinization in Japanese flounder Paralichthys olivaceus (Shimasaki et al., 2003). Dose-dependent teratogenic effects, delayed hatching and decreased survival have been reported after short-term exposure in minnow Phoxinus phoxinus (Fent, 1996). Both morphological and functional effects on lymphoid organs have also been recorded in the rat, including a reduction in weight and cellularity of the thymus, depletion of T-cell areas in the spleen and lymph nodes (Seinen et al., 1977a, b; Krajnc et al., 1984; Snoeij et al., 1985) and a decreased thymus-dependent antibody synthesis against sheep red blood cells and Trichinella spiralis (Vos et al., 1984, 1990).

Heavy metals/organometals deposited in the environment pose a serious threat to global ecosystems. Organometallic compounds are an increased risk due to their potential reactivity, bio concentration and bioaccumulation. TBTO has low water solubility and binds strongly to suspended material, eventually precipitating to bottom sediment. The high lipid solubility of TBT allows for rapid membrane permeability and affects the intracellular environment, inducing cytotoxicity (Gadd, 2000). TBT exposure was shown to be involved in endocrine disruption, immune suppression and infertility in a variety of species (Blaber, 1970; Evans et al., 2000; Mensink et al., 2002; McAllister & Kime, 2003; Dwivedi & Trombetta, 2006). The deleterious effects seen in invertebrates, including mussels, clams, oysters and lobsters, include shell thickening, growth abnormalities, infertility and death with exposure to dissolved TBT (Huggett et al., 1992). Heavy metals catalyze the cleavage of hydrogen peroxide via the Fenton reaction, producing the hydroxyl radical and labile metal complexes. High valence metal oxidants are the most reactive in biological
systems (Koppenol, 1994). The production of hydroxyl radicals (HO•) by metal ions leads to the disruption of lipid membranes (Roméo & Gnassia-Barelli, 1997). A series of hydrogen abstraction reactions results in the generation of lipid peroxy and lipid alkoxyl radicals, producing membrane degradation. These reactions are thought to be the driving force in lipid peroxidation. The toxicity of TBTO in fish is variable, LC50 values were shown to range from 1.5 to 36 µg/L. A study in Platichthys flesus revealed that a concentration of 17.3 µg/L produced mortality after 7 to 12 d (Grinwis et al., 1997). Recent studies comparing the accumulation of metals in tissues of marine teleosts and elasmobranchs indicate susceptibility of the elasmobranch gill to metals. It was shown that the elasmobranch gill accumulates water-borne copper 40 to 50-fold, as compared to a 3-fold increase in the sculpin (Grosell et al., 2003). Similar results were demonstrated in the exposure of the elasmobranch Squalus acanthias to silver when compared to marine teleosts (De Boeck et al., 2001). Elasmobranchs possess significantly lower antioxidant enzyme activities and a reduced capacity to neutralize hydroxyl radicals (Gorbi et al., 2004).

Considerable work has been carried on effect of TBT on aquatic organism, Alzieu et al., (1980) found 100% mortality in pacific oyster, Crassostrea gigas exposed to TBT. Newton et al., (1985) observed significantly enhanced growth and hatching success in Leuresthes tenuis after effect of TBTO in the duration of 10 days. Reproductive abnormalities have been observed by toxic effect of TBT in the European flat oyster, Ostrea edulis, Thain, (1983). Salazar and Salazar, (1996) observed accumulation of TBT in blue mussel, Mytilus species. Meador, (1997) reported that tributyltin chloride strongly affect on amphipod, Rhepoxynius abronius. Tim Verslyce, (2003) revealed that the cellular energy allocation in the estuarine mysid shrimp Neomysis integer to different TBT exposure. Rabbito, (2005) have been studied the effect of TBT on fish, Hoplias malabaricus. The effects of organotin compounds have been extensively studied on experimental animals (Wada et al., 1982; Merkord and Henninghausen 1989; Takagi et al., 1992 Yebra et al., 2004., Okamura et al., 2003, Rodríguez et al., 2007 Nakanishi, 2008, Dollé, 2009; Laursen et al., 2012).

Acute toxicity tests, using Mysids, Copepoda, Crangonid shrimps, Lismata, Daphnia, sand crab, Palaemon paucidens and common oyster as test organisms, are reported by Goodman et al., (1988); U'ren, (1983); Thain, (1983); Linden et al., (1979); Meador (1986); Walsh (1986), and Termmink and Everts, (1987). Effects on
blue mussels exposed to tributyltin chloride were studied by Valkirs et al., (1987) as a result, 66-day LC50 was calculated to be 0.97 g/L. Larval bivalve mollusks appear to be much more sensitive than adults during acute exposures. The 96-hr LC50 of TBT for larval Pacific oysters, Crassostrea gigas, was 1.557 µg/L, where as the value for adults was 282.2 µg/L (Thain 1983). The 96-hr LC50 for larval and adult blue mussels, Mytilus edulis, were 2.238 and 36.98 µg/L, respectively (Thain, (1983). The 96-hr LC50 of 0.01466 µg/L reported by Becerra and Huencho, (1984) for post larvae of the hard clam, M. mercenaria. Juveniles of the crustacean Acanthomysis sculpta were slightly more sensitive to TBT than adults (Davidson et al., 1986a, 1986b; Valkirs et al., 1985).


The concept of increased mucus production is consistent with observations published by various researchers. Several authors noted that exposure to heavy metals is associated with either increased mucus production and/or mucus complexation with the heavy metal to which it was being exposed (Carpenter 1927; Westfall 1945; Skidmore and Tovell1972; Wong, Luk and Choi 1977; Kumar and Pant 1981). Heavy metals enters the body either via the gills or the intestine depending on its form (Roesijadi and Robinson 1994). Published studies (Lauren and McDonald 1985) has indicated that the gills, rather than internal organs are the initial site for metal toxicity and as such may even reduce its impact on the other organs. Within the gills, heavy metals can induce metallothionein (MT) synthesis. Metallothioneins are a specific group of metal-binding proteins found in vertebrates and invertebrates within various organ systems (Roesijadi and Robinson 1994). The heavy metals, once it has passed the gills and irrespective of toxicity there, moves throughout the body via the bloodstream. The exact components involved in this movement are not understood, but it is thought that copper may be bound either to serum MT or other plasma proteins or any combination (Roesijadi and Robinson 1994). MT’s detoxify copper (and other -metals) by first initially binding with that copper which is free within the cells and secondly by actually dislodging copper bound to other sites and binding to the dislodged heavy metals. It is the latter role that is of greatest importance, for some of the other cellular sites are those associated with toxicity.

Allen (1995) investigated soft tissue accumulation of lead in the *Tilapia*. The chronic accumulation profile of lead was determined by analyzing liver, brain, gill filaments, intestine and other tissues. Shafi (1995) investigated sub lethal effects of mercury and lead on monoamine oxidase in different region of the brain in the freshwater teleosts. He reported highest rise in the energy activity in talencephalon with mercury exposure followed by lead. Chaurasia *et al.*, (1996) reported lead induced thyroid dysfunction and lipid peroxidation in the fish *C. batrachus*. Ay O *et al.*, (1999) reported copper and lead accumulation in tissue of the freshwater fish, *T.*

Assessment of toxicity on particular organism exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. Toxicity tests will reveal the organism’s sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem. The toxicity of any pollution is either acute or chronic although the toxicant impairs the metabolic and physiological activities of the organisms; physiological studies alone do not satisfy the complete under toxic stress. Acute toxicity test is used to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organism during a short-term exposure under controlled conditions. All toxicants are capable of severally interfering with the biological systems that producing damage to the structure and function of particular organism and ultimately to its survival majority of the studies concerning the effects of heavy metals on fish have been confined to the acute toxicity test with the death of fish as an end point. Hence in the present study, an attempt has been made to assess the acute toxicity of tributyltin oxide on freshwater fish *N. botia* as bioindicator of local importance from Maharashtra state in Godavari River at Nandhurmadhameshwar village of Nashik district.

Fish contributes to food security in many regions of the world, providing a valuable supplement for diversified and nutritious diets. Fish is highly nutritious. It provides not only high-value protein, but also represents an important source of a wide range of essential micronutrients, minerals and fatty acids. Fish is a vital source of food for people. It is man's most important single source of high-quality protein, providing 16% of the animal protein consumed by the world's population, according to the (FAO) of the United Nations (1997). It is a particularly important protein
source in regions where livestock is relatively scarce fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). The FAO estimates that about one billion people worldwide rely on fish as their primary source of animal protein (FAO, 2000).

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). In some western countries, acute toxicity tests with a fish, Daphnia and an algal species have been made mandatory for the acceptance of new substances (Smeets, 1980). Though the species of fish that should be employed in such tests is not specified, the use of a standard fish has been suggested in order to prove the results and to compare the results of one laboratory with another (Cairns, 1980). Furthermore, the results of tests conducted at different moments in the same laboratory can also be compared by employing a standard fish (Fogels and Sprague, 1977).

The following criteria have been listed by Adelman and Smith (1976) for the choice of a standard test fish:

1. It must have a constant response and have neither high nor low sensitivity to a broad range of toxicants tested under similar conditions.
2. It must be available throughout the year.
3. A constant size group of that species should be available all through the year.
4. It should be easy to collect, transport and handle.
5. The adults should be small enough to facilitate the conducting of acute or chronic tests without imposing undue difficulties in maintaining the recommended loading densities.
6. It should be possible to breed the species in the laboratory.
7. It should complete its life cycle within one year or less.

Freshwater fish, *N. botia* selected for the present study, fulfils most of the criteria listed for a standard test fish. They occur in a variety of habitats in the rivers, lakes and ponds in Nashik region of Maharashtra State. This species is hardly usually associated with large, slow flowing rivers, usually turbid waters. Sometimes forms large schools. Feeds mainly on aquatic insects and detritus and a surface feeder.

The sources of heavy metals/organometals that reach the ecosystems are given below and listed by Rashed (2001).
1) Natural sources: Metals are found throughout the earth rocks, soil and introduced into water bodies through natural processes such as weathering and corrosion.

2) Industrial sources: Industrial processes such as mixing and processing of metal ores, the finishing and plating of metals, manufacturing of metal object and the paint that contain the metals are main sources of heavy metals.

3) Domestic wastewater: Domestic wastewater contains substantial quantities of metal because some formulations such as cosmetics and cleaning agents contain metals.

4) Agricultural sources: Agricultural discharge contains residues of pesticides and fertilizers, which contain the metals.

5) Mine run off is a major source of heavy metals.

Freshwater fish, *N. botia* selected for the present study, occur in a variety of habitats in the rivers, lakes and ponds in North Maharashtra region of Maharashtra, State. North Maharashtra region particularly Nashik is fast growing in industries included pharmaceutical, pulp and paper, plastic, breweries and automobile. These industries release their effluents in the environment and it reaches in the nearby water bodies, mainly Godhavari river may affect the aquatic animals including fish community.

A behavioral response is a first response of an organism to environmental stress. The behavior of an organism represents the final interrelated result of diversity of biochemical and physiological processes. Thus, a single behavioral parameter is generally more comprehensive than physiological or biochemical parameters. Behavior is considered a promising tool in toxicology (Smita srivastav, *et al.*, 2007) Behavioral changes are the most sensitive indicator of potential toxic effects. Impact of different toxicants on the behavior of fish have been studied by various researchers (Marigaudar *et al.*, 2009; Anita *et al.*, 2010; Nagaraju *et al.*, 2011; Walia *et al.*, 2013; selvam *et al.*, 2014)

Patil and David (2008) in their study on behavior and respiratory dysfunction as an index of organophosphate in *L. rohita*. They reported that the toxicants exposed fish exhibited irregular, erratic and darting movements and loss of equilibrium. This was due to inhibition of acetylcholine esterase activity leading to the accumulation recorded by (Rao *et al.*, 2003; Dube and Hosteti, 2010; Anita susan *et al.*, 2010). The increased cough rate, flaring of gills and increase in the production of mucous on the
gills. Darting movements and striking against the wall of test tanks were noticed in fish *L. rohita* (Dube and Hosteti, 2010). A film of mucous was also observed all over the body and also on the gills. The secretion of mucus could be attributed to the histological changes of gills caused by organophosphate pesticides. The mucus secretion in fish form a barrier between the body and toxic media thereby probably reuses contact with the toxicant so as to minimize the irritating effect or to eliminate it through the epidermal mucus. Similar observations were made by Rao *et al.*, (2003) and Perma de Crpux *et al.*, (2002) in *prochilodus lineatus* under monocrotophos stress. Gulping air at the surface, swimming on the water surface, disrupted shoaling behaviour observed when exposed to lethal and sublethal concentration of biopesticide (Marigoudar *et al.*, 2009; Selvam *et al.*, 2014) Gulping of air may help to avoid contact with the toxicant medium. Finally fish sank to the bottom with the least opercular movement and died with their mouth open (Marigoudar *et al.*, 2009; Abdul naveed *et al.*, 2010; Walia *et al.*, 2013 and Ghazala *et al.*, 2014).

Alteration in the chemical composition of the natural environment usually led to cause effect on behavioral and physiological systems of the inhabitants (Edwards, 1973). Failure of any behavioral response system could be eventually lead to reduced fitness and survival of the organisms resulting in adverse consequences at the population level. Abhilash and Prakasam (2005) observed various behavioral changes, toxic and physico morphological response of *O.mossambicus* exposed to commercial grade endosulfan.

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 an organism 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, 1982). 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, 1927; Knight, 1964; Vineetkumar et al., 2008; Marigoudar et al., 2009; Gopal and David, 2010; Magar and Afsar Shaikh, 2012; Jothinarendiran, 2012; Maharajan et al., 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 gets bathed in the water containing the xenobites and hence the effect of toxicants on respiration is more pronounced. Toxicants enters in the body of fish enters 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. Organophosphophours 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).

A decrease in oxygen consumption was reported by several authors (Holden, 1962; Fergusson et al., 1967; RamaMurthy, 1988; Vijayalakshmi, 1994; Ramana Kumari, 2001; Anthony Reddy, 2015; 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 and events leads to respiratory distress (David et al., 2002). Another reason for decrease in the oxygen consumption is atrophy of the respiratory epithelium, enlargement of the water/blood barrier and inhibition of gill ATPase activity (Aldridge, 1971). The respiratory epithelium get disintegrate when it is exposed to toxicants. Kalawathy et al., (2001) reported that the Dimethoate absorbed efficiently across the gill and diffused in to the blood stream resulting in toxic stress to fish. Reduction in oxygen consumption was reported in Channa striatus exposed to organophosphates (Natarajan, 1981). Author also suggested, it may be due to anoxia in which mucus is lost from the gills, as a result of which absorption of oxygen is affected and termed the phenomenon ‘coagulation film anoxia’. Varma and Dalela (1975) stated that the reduction in oxygen consumption in fish is due to suspended solids present in the effluents which cause mechanical damage to the fish and disturbed the osmotic regulation. Fish are often exposed to highly polluted water and they cause different disabilities, ranging from biochemical changes in single cell to changes in the whole organism. Histological changes are more sensitive and occur early. Histological study provides better assessment of fish health, as well as the effect of pollutants on each biochemical parameter. Histological changes have been integrated with the impact of various stresses such as microbial pathogens, various toxic compounds and environmental conditions (Marchand et al., 2009). The gills which participate in many important function in fish, respiration, osmoregulation and excretion remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water. They are considered primary target of the contaminants (Mazon et al., 2002). According to Leino et al., (1987) the gill of feathered minnows from environmentally polluted water exhibited various cellular, histological and histopathological changes, contributing to problems related to respiration and osmoregulation.

The pollution of aquatic ecosystem by xenobiotic compounds has become a major problem to the flora and fauna and is an immediate concern. These contaminants are released into the water bodies from industrial and agricultural areas
and as most of them are highly persistent. Any change in the behavior and physiology of fish indicates the change in the water quality by the addition of hazardous substances in the aquatic ecosystem, since fish are the biological indicators of water quality. Hence, the present study was undertaken to evaluate the aquatic toxicity of Tributyltin oxide on behavioral and oxygen consumption of the freshwater fish *N. botia*. Decline in oxygen content occurs in the medium when heavy metals, chemicals, sewage and other effluents containing organic matter are released into water bodies. Heavy metals in sub lethal concentrations present in the aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behavior and reduce the fitness of natural population Holden (year). 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. 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.)

The metabolic response of organisms to environmental changes is overall indicator of adaptive capacity of organism. All environmental factors have marked effect on the physiology of aquatic animals (Waldichuk, 1974). The chemical pollutants constitute on of the important factors affecting the rate of oxygen consumption. In accordance with general practice, the bioenergetic pathway or the metabolic rate in poikilothermic animals is ordinarily measured in terms of oxygen consumption which is highly complex process. Subjected to be influenced by a number of factors such as body size, sex nutritional status, ambient temperature,
salinity, hydrogen concentration, dissolved oxygen content of the medium pollutants etc. All these factors, individually and collectively influence the metabolism of animals (Harper, 1985). Changes in the respiratory activity have been used as the sensitive indicator of stress in animals exposed to pollutants (Anderson, 1971). Various investigators used different types of pollutants such as insecticides (Waiwood and Johnson, 1974), heavy metals (Saraivo, 1973) and studied changes in the respiratory rate of several crustaceans.

Alterations and failure of respiratory metabolism due to acute toxicity is regarded as an early symptom of poisoning (Holden, 1973). The respiratory changes are correlated to stress factors such as temperature, salinity, starvation and pollutants. In the water with waste materials, the dissolved oxygen content supports aquatic life. However, pollutants primarily organic compounds cause a reduction in dissolved oxygen. These pollutants come primarily from untreated sewage, industrial waste and agricultural run-off (Edward, 1973). 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 even at the sub lethal level, resulting in extra expenditure of energy. Mitchell et al., (1978) reported that hyperplasia and separation of the epithelium associated with asphyxiation, partial or complete loss of secretory or excretory function, impairment of oxygen– carbon dioxide exchange and loss of plasma electrolytes or protein. The present study thus attempts to take a step forward in this direction so that aquatic ecosystem can be protected.

It is well known fact that the rate of oxygen consumption is used as an authentic tool for understanding the physiological state of metabolic activity of an
organism (Kulkarni et al., 1983). The rapid industrialization and green revolution increased the use of different chemicals to achieve the growth of economy. Hence the indiscriminate use of different agrochemicals and releases of the chemical in the environment caused hazards to several non target biotas in the aquatic environment. The organic and inorganic chemical and their metallic salts which are released into the environment have threatened the existence of aquatic biota. The excess of heavy metals in the aquatic environmental 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 sublethal concentration of fenitrothion, BHC and carbofuran. Moorthy et al., (1984) observed respiration in freshwater mussel exposed to methyl parathion showing change in the process. 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
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.

Exposing animals to xenobiotics causes alteration at the cellular level and involves modifications of biochemical pathways. The measurable various in biological system are called biochemical markers, commonly referred to as biomarkers (Landis & Yu, 1995). The biochemical changes occurring in the body gives the important indication of stress. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different. Much work carried on toxic effect of heavy metals on specific target and non target aquatic invertebrate and vertebrates with respect to the biochemical changes. There is an increasing need to develop methods for the identification, estimation, comparative assessment and management of risk posed by chemical pollutants discharged into the aquatic environment. Therefore, measuring the biological effect of pollutants in relation to body constituents is essential for assessing the quality of the freshwater environment.

The pollutants act as one kind of stress on an organism and organisms respond to it by developing necessary potential to counter act that stress. The biochemical changes occurring in the body gives important indication of stress. During stress an organism needs sufficient energy which is supplied from reserve materials i.e. protein, glycogen, lipid etc. If stress is mild then the stored glycogen is used as a source of energy but when stress is strong, then the stored lipid and protein may also be mobilized. The mode of action of toxicants and causes for death of poisoned aquatic animal is better understood from biochemical investigations beside mortality studies. Since, the stress conditions caused alteration in metabolic cycles, it is necessary to understand the significance of these variations in the organic components of tissue.

Many researches have worked on the effect of organometals, heavy metals, factory effluents, petroleum hydrocarbons etc. on biochemical changes on aquatic organisms. Nagabushanam et al., (1988) reported altered carbohydrate metabolism in freshwater prawn, Macrobrachium kistnensis exposed to sublethal concentration of naphthalene. Sathyanathan (1988) worked on sublethal effect of heavy metals, copper
and mercury on changes in biochemical constituents of estuarine clam, *Villorita cyprinoids*. Sarojini *et al.*, (1989) found significant decreased in lipids and FFA, where as protein and glycogen significantly increased in crab, *Barytelphusa guerini* exposed to zinc sulphate and copper sulphate. Kewal Jaiswal *et al.*, (1989) observed changes in biochemical constituents such as protein, lipid and glycogen when exposed to naphthalene in freshwater prawn, *Macrobrachium kistnensis*. They suggested that decreased in glycogen content indicated its utilization as countered the naphthalene stress and there was prevalence of anaerobic condition such as anoxia. The observed decreased in protein content was due to enhancement of proteolysis and its possible utilization for other metabolic purpose and increased in lipid content suggested the stress of naphthalene due to which inhibition of lipase activity takes place. Sarojini *et al.*, (1990) studied on effect of cadmium chloride on some biochemical constituents of hepatopancreas of freshwater crab, *Barytelphusa guerini*. Machale *et al.*, (1991) reported depletion in protein, lipid, FAA and glycogen due to cuprous oxide stress in various tissues of freshwater crab, *Barytelphusa guerini*. Khan *et al.*, (1990) observed changes in levels of protein, lipid and glycogen in the muscle of freshwater crab, *Barytelphusa guerini* exposed to copper sulphate. Further they reported that increased metabolism may be the possible reason to the initial rise in various metabolites which might be well accompanied by higher enzyme levels in the muscles. Effect of mercury in carbohydrate metabolism of freshwater fish, *Channa punctatus* was studied by Shuleman (1974). High levels of cadmium caused alteration in tissue glycogen content in fish, Shafi (1978 a, b) and Das and Kaviraj (1993) studied individual and interactive effect of heavy metals, cadmium and cobalt chloride on glycogen level of common carp, *Cyprinus carpio*. Das (2000) studied the chronic effect of cadmium on changes in biochemical constituents of catfish, *Clarias batrachus*. Copuzzo J. M. and Lancaster (1981) investigated hydrocarbon accumulation of organotin compounds in oyster and its effects on their different tissues and biochemical constituents. Shah *et al.*, (1998) investigated changes in glycoprotein contents in estuarine *Anadara rhombea* exposed to TBTO. Shah *et al.*, (2001) reported altered lipid metabolism in earthworm edible blood clam, *Anadara rhombea* exposed to TBTO. Vijayakumar and Kannupandi (1989) observed changes in protein, carbohydrate and lipid exposed to organophosphate insecticide phosphomidan in mangrove crab, *Sesame andersonii*. Kamal Das and Sudip Bannerjee (1980) studied cadmium toxicity and its effect on biochemical constituents in fishes. Veena Shaktivel (2002) studied effect of

The toxic metal pollutant gives rise to alteration in the metabolic and physiological activity on both short and long term exposures. The biochemical changes in the organs of animal exposed to heavy metals have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract toxic stress. To investigate the physiological changes after heavy metal treatment, point is to study the changes in the biochemical constituents like carbohydrate, protein and lipid as they are important metabolites to provide energy for different life processes. Organotins compounds are responsible for water pollution and hence non target organisms in contaminated water bodies come in continuous and direct contact with organotin compound. After penetrating in the body, these organotin compound attacks on the biochemical composition of tissues in the organisms due to pollution stress.

The biochemical changes occurring in the body gives the important indication of stress. Different tissues and organs have different activities and metabolic rates and therefore their responses to the same toxicant may be different. Much work carried on toxic effect of heavy metals on specific target and non target aquatic invertebrate and vertebrates with the biochemical changes. Higher concentrations of toxicant in aquatic environment cause adverse effect on aquatic organism at cellular or molecular level and ultimately it leads to disorder in biochemical composition (Waykar and Lomte, 2001). Further they reported that increased metabolism may be the possible reason to the initial rise in various metabolites which might be well accompanied by higher enzyme levels in the muscles. Copuzzo and Lancaster, (1981) investigated accumulation of organotin compounds in oyster and its effects on their different tissues and biochemical constituents. Bayne and Thompson, (1970) determined the biochemical composition of mantle, gonad and somatic tissues of *M. edulis*. Veena Shaktivel, (2002) studied effect of phosphomidan on glycogen, protein and lipids of *G. affinis*. Since the liver of teleosts is important in the maintenance of internal homeostasis and the metabolism of xenobiotics (Chambers and Yarbrough, 1976) and has also been shown to accumulate foreign compounds (Statham *et al.*, 1978) and to
be susceptible to damage by hepato-toxic agents (Racicot et al., 1975; Gingerich et al., 1978) the functional integrity of the liver in fish can be affected by xenobiotics (Gingerich, 1982). The liver is specifically affected by a large number of chemical agents. Backstrom (1967) observed that liver is one of the most important heavy metal accumulating organs in animals. According to Buck (1978) liver is the first line of defence against copper poisoning. Copper becomes toxic only when the high binding capacity of the liver is exceeded and copper is released into blood stream. In fish also, liver is the major storage organ for copper (Buckley et al., 1982; Shearer, 1984). EIDomiaty (1987) found that highest concentration of copper was in the liver and kidney of *Clarias lazera* after exposure to copper and suggested that the liver and kidney are vital organs in the regulation of metal as they are detoxification centers. It is assumed that decrease in glycogen content may be due to the inhibition of hormones which contribute to glycogen synthesis. Decrease in liver and muscle glycogen levels is in corroboration with the reports of earlier workers (Bedii and Kenan, 2005; Dubale and Punita shah, 1981; Sastry and Subhadra, 1984).

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 toxicants inhibit different enzymes of the TCA cycle, like pyruvic dehydrogenase, succinic dehydrogenase and malate dehydrogenase. The inhibition of these enzymes of TCA cycle can prevent the aerobic metabolism. 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), Balavenkata subhaiah 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.

Several workers have reported that the impact of aquatic pollutants on carbohydrate metabolism of different aquatic animals. Nagabhushanam and Kulkarni,(1981) suggested that the decreased in carbohydrate level after prolonged exposure to the heavy metal due to the inactivation of the enzyme involve in the carbohydrate catabolism and anabolism. Reddy and Rao, (1991) observed considerable decreased in total carbohydrate level exposed to phosphomidan toxicity to marine penaeid prawn, *Metapenaeus monoceros*. Bhagyalakshmi et al., (1984) studied the alteration in carbohydrate metabolism in selected tissues of freshwater
crab *Oziotelphusa sensex* when exposed to the crab to fenvalerate a pyrethroid insecticide and noticed an increase in phosphorylase ‘a’ and decrease in aldolase activity level. These results suggested increased glycogenesis and decreased glycolysis in the hepatopancreas and muscle of crab during the pesticide toxicity. Rani *et al.*, (1989) reported changes in carbohydrate metabolism of *Clarias batrachus* when exposed to organophosphorus insecticides. Dhanapakiam and Ramasami, (2001) reported toxic effects of copper and zinc mixture on some hematological and biochemical parameter in common carp, *Cyprinus carpio*. Ramalingam and Indra, (2002) showed decrease in glycogen level when exposed to copper sulphate toxicity on tissue phosphatases and carbohydrate turned over in *Achatina fulcia*. Satyaparameshwar *et al.*, (2006) reported decreased carbohydrate metabolism in freshwater mussel, *Lamelidens marginalis* exposed to copper sulphate and observed decrease in carbohydrate content level in labial palp, gill and mantle.

All metabolic activities are controlled by enzymatic reactions and enzymes are nothing but proteins. So protein play vital role in various metabolic activities essential for growth, development and reproduction in an organism. Proteins are the most abundant chemical compounds in the organisms and important class of biological macromolecules. They are versatile, complex and fragile macromolecules with high molecular weight. The unique role of these biological macromolecules can lead to changes in physiological functions of the metabolically important toxicological studies, for overall homeostasis, since it determines whether there is net uptake or release of amino acids by the tissues. The range of function mediated by proteins resulted from diversity and versatility of amino acids.

Proteins are complex substances with high molecular weight forming not only the structural framework, but also gears and levers of the operating machinery in the living body. Proteins are nothing but polypeptide chains of amino acid molecules. The proteins are useful for the transport and storage. Specific proteins transport many small molecules and ions. For example haemoglobin transports oxygen in erythrocytes, whereas myoglobin a related protein transports oxygen in muscles. The primary function of protein food is to supply the amino acids needed for the growth, repair and general maintenance of the structural and catalytic machineries of living cells.

Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Yeragi *et al.*, 2003; Remia
et al., 2008; Pang-Hung et al., 2008). The percent decrease was found to be greater in all exposures in liver tissue. The reduction of protein may be due to proteolysis and increased metabolism under toxicant stress (Remia et al., 2008). Dalela et al., (1981) observed decrease in protein content in Mystus vittatus under pesticide exposure and reported that the depletion of protein may be due to the excretion of proteins by kidney due to kidney failure or impaired protein synthesis as a result of liver disorders. A number of workers have reported decline in protein level of various organs and tissues under toxic stress of various chemicals. Lipids are the predominant organic reserves of animals and serves as an alternate source of energy, particularly during stress conditions, Gilbert and O’connor, (1970). The function and importance of lipid in the life is well known, they not only play a metabolic role and provides precursor for the endocrine processes to produce steroidal hormones but they are also important in maintaining the structural and physiological integrity of cellular and subcellular membranes, Shigmatsu and Takatika, (1959). Their role is to transfer the substrate via the circulatory system in both vertebrate and invertebrate is a vital one. The variation in the content and composition of depot-lipid is a function of both external and internal central system.

Gupta and Bhide, (2001, 2004) observed pesticidal effect on protein profile due to the intoxication of pesticides in snail, Lymnaea stagnalis. Satyaparameshwari et al., (2006) reported decrease in protein content of adductor muscle, gill and mantle in freshwater mussel, Lamellidens marginalis exposed to chromium. Zambre, (1991) studied the reflections in protein content of freshwater bivalve, C. striatella due to heavy metal exposure. Mule, (1991) observed the alteration in protein content after exposure to monocrotophos, cypermethrin and some heavy metals. Mahajan and Zambre, (2001) studied the depletion of protein levels in the different tissue such as gonad, gills and digestive gland of freshwater bivalve, Corbicula striatella, after exposure to chronic dose of copper sulphates and mercuric chloride. Depletion of the protein contents in different tissues such as gonad, gill and hepatopancreas of bivalve, after exposure to HgCl$_2$ and CuSO$_4$.

Various authors studied reduction of lipids in various tissues. Ram and Sathyanesan (1984) observed a decreased lipid level in the liver of Channa punctatus exposed to emisan. Srinivas et al., (1991) has showed decreased lipid content in T. mossambica on exposed to atrazine. Gradual depletion in lipid content of liver and muscle when exposed to Malathion was analysed by Mishra et al., (2004).
Arockia and Milton (2006) have showed declining trend of lipid content in the tissues like brain, gill, kidney, liver and muscles upon exposure to carbamate in the fish *Oreochromis mossambicus*. Saradhamani and Selvarani (2009) showed significant decrease in cholesterol content in the tissues of the freshwater fish, *Tilapia mossambica* exposed to metribuzin.

Lipids are the predominant organic reserves of animals and serves as an alternate source of energy, particularly during stress conditions, Gilbert and O’connor, (1970). The function and importance of lipid in the life is well known, they not only play a metabolic role and provides precursor for the endocrine processes to produce steroidal hormones but they are also important in maintaining the structural and physiological integrity of cellular and sub-cellular membranes, Shigmatsu and Takatika, (1959). Lipids are also the storage form of energy like glycogen. The lipid levels also decreased in the tissues of the fish exposed to the sub-lethal and lethal concentration of cadmium chloride. Effect of cadmium on the lipid content was reported by earlier investigators (Levesque *et al.*, 2002; Dubale and Punita shah, 1981; Fabien Pierron *et al.*, 2007).

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. Bhilve *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.

Some toxicologist focused certain attention on the impact of pollutants on the lipid reserves of aquatic animal. Nagabhushanam *et al.*, (1972) reported decrease in lipid level in hepatopancreas of the freshwater prawn, *Macrobrachium kistnensis* in response to pesticidal toxicity. Villalan *et al.*, (1990) observed the reduction in lipid content in muscle due to chromium stress in *Macrobrachium idella*. Lomte and Muley, (1993) reported the decrease in lipid level in the freshwater snail and bivalve, *Thaira tuberculata* and *Parresia corrugata* exposed to copper toxicity. Sarvana and

Though considerable information is available dealing with the determination of acute toxic levels of several pollutants and their influence on metabolism, studies on the tissue energy sources are relatively few. Glycogen, lipid and protein 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 lipid and protein content of gill, liver and kidney in *N. botia*.

Heavy metals undergo metabolic activation that provokes a cellular change in the affected fish. The tissue lesions and apoptosis arise from bioaccumulation, infections, diseases and parasites stimulate necrotic alterations in the fish with an inflammatory defensive reaction (Roganovic – Zafirova *et al.*, 1998). It is imperative that histological biomarkers are the indicators of pollutants in the overall health of the entire population in the ecosystem (Velkova – Jordanoska and Kostoski, 2005). Well documented reports can be found in organs of fish exposed to heavy metals, chemical pollutants and microorganisms (Adham *et al.*, 2002, Olojo *et al.*, 2005, and Farombi *et al.*, 2007). The freshwater fish, *N. botia* is the most common fish widely consumed worldwide. Therefore, it can be a good model to study the responses to various environmental contaminations. The investigation of histological changes in organs of fish is an accurate way to assess the effects of xenobiotics compounds in experimental
studies. Hence, this study was undertaken to examine the effect of tributyltin oxide at lethal and sub lethal concentrations on histology of gills, liver and kidney of freshwater fish _N botia_.

Histological analysis appears to be a very sensitive parameter and is very important method in assessing cellular changes that may occur in target organs such as the gills, liver and kidney (Dutta, 1996). A histological analysis may therefore prove to be a cost effective tool to determine the health of organisms, hence reflecting the health of an entire aquatic ecosystem. The histology of fish liver could therefore serve as a model for studying the interactions between environmental factors which include biotoxins, parasites, infectious germs, physicochemical parameters and pollutants such as hydrocarbons. Histopathology is used to study the impact of toxicants on fish as it provides the real picture on the toxic effects of xenobiotics in vital functions of a living organism. Toxicants produce pathological changes in fish such as necrosis in the liver, tubular damage of kidney and gill lamellar abnormalities (Ramalingam, 1985). Therefore, histopathological studies with light microscope are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants (Mayers and Hendricks, 1985). Besides, biochemical parameters are the best indicators of stress.

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. The histopathological changes in fish depend on the chemical concentration, exposure time, uptake routes, species and other environmental features (Oliveira Ribeiro _et al._, 2005; Valdez Domingos _et al._, 2007). Zhang _et al._, (2008) described the occurrence of reactive oxygen species associated with TBT exposure at environmental levels could be the possible reasons to the neurodegenerative effects observed in fish exposed to the organometals. 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 and DBT exposures suggest a physiological response of the circulatory system or a consequence of the hormonal unbalance.
According to Guruge and Tanabe (2001), higher concentrations of organotins were observed in liver as a target organ and consequently more affected when exposed to organotin and described higher incidence of necrotic areas in liver and posterior kidney with the longer chronic exposure to the TBT. The incidence of vacuolated cells in epithelial tissue from ducts in posterior kidney after exposed to DBT indicated osmoregulation or metabolism disturbances. These authors concluded that histotoxicity of both TBT and DBT increases with time and even DBT appeared more toxic than TBT for *H. platessoides*. The potential toxicity of TBT to aquatic organisms and the risk to human population was recently very well documented by Rudel *et al.*, (2007) in fish species.

The histopathological changes in fish depend on the chemical concentration, exposure time, uptake routes, species and other environmental features (Oliveira Ribeiro *et al.*, 2005; Valdez Domingos *et al.*, 2007). However, the use of butyltins for decades contaminated most marine aquatic ecosystems in different coastal regions around the world (International Marine Coatings, 2008). Tributyltin (TBT) is a compound added to antifouling paints widely used in the past to protect small and large ships from fouling and constituted the main source of organotins detected in estuarine ecosystems (Fent, 1996). 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 neurodegenerative effects observed in fish exposed to the organometals. The programmed cell death is an acceptable hypothesis to explain the lost of tissue function. These authors suggest both TBT and its metabolites like DBT are able to cause severe damages as necrosis associated with cell death as reported by Zhang *et al.*, (2008). The damages in sinusoids as enlargement found after TBT and DBT 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 both target organs.

Environmental factors of both natural and anthropogenic origins have been known to induce alteration of different magnitudes in the physiological and biochemical status of animals (Shilov, 1981; Vosyliene and Kazlauskienes, 1999). Therefore, biomarker parameter assessment is a means of environmental monitoring, with the advantage of providing quantitative response as valuable information on
ecological relevance as well as on the acute/chronic adverse effects caused by water pollution.

Specific aims of the study is to assess toxicity to *N.botia* used as a bioindicator evaluating the acute concentration of tributyltin oxide, to study oxygen consumption, histopathological and biochemical responses detectable in a comparable time frame following to tributyltin oxide.