Present research embodied effect of organotin tributyltin chloride on freshwater prawn, Macrobrachium kistnensis. In toxicity evaluation of organotin tributyltin chloride on Macrobrachium kistnensis is highly sensitive to TBTCl toxicity. In the present study the safe concentration was 0.0322 ppm. The respiratory changes in oxygen consumption in the prawn, Macrobrachium kistnensis after exposed to 48hours, 1/3rd and 1/10th lethal and sublethal concentrations for 2, 4, 8, 12 and 24 hours have also been studied. The death of freshwater prawn, Macrobrachium kistnensis might be due to the impact of TBTCl on gill which ruptured the gill lamellae resulting decline in respiratory rate, an inhibition of the electron-transport system (ETS) or an effect on mitochondrial integrity. From these observation it can be inferred that the organotin compound disrupting enzyme-mediated process and / or disrupting cellular structures. From this it can be conclude that the TBTCl is very toxic to the freshwater prawn, Macrobrachium kistnensis. Therefore, the release of organotin compounds in aquatic environment especially in freshwater ecosystem should be controlled.

Pravin Kharat

Dr. Pravin Shamrao Kharat, earned his Ph. D. (Zoology) in 2007. He was receiver of prestigious "Rajiv Gandhi National Fellowship" by UGC, New Delhi, India. To his credit he has two Major Research Projects sanctioned by UGC and SERC. Presently working as Assistant Professor, Department of Zoology, Nutan Mahavidyalaya, Selu, Dist. Parbhani (MS).

978-3-659-19938-7

Pravin Kharat
Kirti Niralwad
Tanvir Pathan

Toxicity Of TBTCL To Freshwater Prawn, Macrobrachium Kistnensis

Physiological Aspects

LAP LAMBERT Academic Publishing

Pravin Kharat
Kirti Niralwad
Tanvir Pathan

Toxicity Of TBTCL To Freshwater Prawn, Macrobrachium Kistnensis

Pravin Kharat
Kirti Niralwad
Tanvir Pathan

Toxicity Of TBTCL To Freshwater Prawn, Macrobrachium Kistnensis

Physiological Aspects

LAP LAMBERT Academic Publishing

Impressum/Imprint (nur für Deutschland/only for Germany)
Bibliografische Information der Deutschen Nationalbibliothek: Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.d-nb.de abrufbar.

Coverbild: www.ingimage.com

Verlag: LAP LAMBERT Academic Publishing GmbH & Co. KG
Heinrich-Böcking-Str. 6-8, 66121 Saarbrücken, Deutschland
Telefon +49 681 3720-310, Telefax +49 681 3720-3109
Email: info@lap-publishing.com

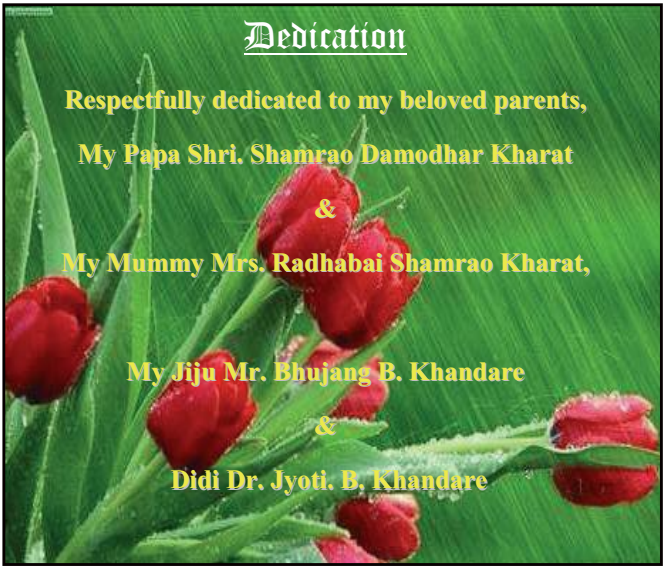
Herstellung in Deutschland (siehe letzte Seite)
ISBN: 978-3-659-19938-7

Imprint (only for USA, GB)
Bibliographic information published by the Deutsche Nationalbibliothek: The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.

Cover image: www.ingimage.com

Publisher: LAP LAMBERT Academic Publishing GmbH & Co. KG
Heinrich-Böcking-Str. 6-8, 66121 Saarbrücken, Germany
Phone +49 681 3720-310, Fax +49 681 3720-3109
Email: info@lap-publishing.com

Printed in the U.S.A.
Printed in the U.K. by (see last page)
ISBN: 978-3-659-19938-7


Dedication
Respectfully dedicated to my beloved parents,
My Papa Shri. Shamrao Damodhar Kharat
&
My Mummy Mrs. Radhabai Shamrao Kharat,
My Jiju Mr. Bhujang B. Khandare
&
Didi Dr. Jyoti. B. Khandare

ACKNOWLEDGEMENTS

I take this opportunity to record my deep sense of gratitude to my research guide Dr. K. B. Shejule Reader, Department of Zoology Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for suggesting such an interesting topic and his constant encouragement, valuable suggestion and guidance throughout the period of my research work.

Sincerely I thanks to Dr. B. C. Ghoble Principal, Milind College of Science, Aurangabad and Karuna B. Ghoble for their valuable & timely suggestion.

I am immensely thankful to Dr. S. S. Kulkarni, Principal, Nutan Mahavidyalaya, selu dist, Pabhani, Y. R. Gaikwad for their timely suggestion.

I take genuine pleasure to express my thanks to my dearest friend Laxmi Ghoble, Dr. R. R. Bainwad, Dr. Vishal Sarode, Dr. Rajendra Kakde and Dr. Rajesh Gaikwad for their support during my work.

It's my pleasure to thanks Dr. K. B. Shejule, Ex- D.S.W for providing me financial backup during my research work through "Earn and Learn Scheme" of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad

My profound thanks to University Grants Commission for providing me "Rajiv Gandhi National Fellowship" during my research work.

No words could do justice to express my deepest sense of gratitude and respects towards my beloved parents and my Jiju (Mr. Bhujang B. Khandare) and Didi (Dr. Jyoti. B. Khandare) who have been an inexhaustible source of inspiration to me and their blessing, love and affection have brought the cherished expectation true.

I find no words to express my feelings towards my brother Mr. Sachin Shamrao Kharat and my sister Sow. Vaishali and her husband Chandrashekhar

Shirsat for their love and affection. I again have no words for my lovable nephew's master Loukik, Prashik, Pranav and Rajveer and my niece Tejal for their love and affection.

I end by expressing endless thanks to all those whom I am able to recall here and also those whom I not able to recall and also those whom I might have left unknowingly.

Dr. Pravin Shamrao Kharat

INDEX

TOXICITY OF TBTCL TO FRESHWATER PRAWN, *MACROBRACHIUM KISTNENSIS*

PHYSIOLOGICAL ASPECTS

[1]Pravin Kharat, [2]Kirti Niralwad and [3]Tanvir Pathan

[1&2]Nutan Mahavidyalaya, Selu, Dist. Parbhani

[3]Kalika Devi Arts, Science and Commerce College, Shirur Kasar, Dist. Beed.

INTRODUCTION

Fresh water ecosystem is under increasing threat due to rapidly expanding population and the subsequent modernization process resulted in inconspicuous exploitation of nature leading to the pollution crisis. Rivers are very vulnerable, since waste in effluents from industries, domestic and farms open directly into them. During the past few decades, rising trends of population explosion, development of modern technology, industrialization and dramatic increase in the production and consumption of large variety of new synthetic chemicals and there by high amount of pollutants were released into aquatic environment.

Pollution enters the food chain through air, water, soil and organism and derived from industrial effluent, agricultural runoff, mining or mineral processing, storm water erosion, erosion of natural rocks. Unplanned development in all fronts leads to the increase in discharge of heavy metal into the aquatic ecosystem. Urbanization and industrialization causes serious deterioration of aquatic environment possess a threat to aquatic life, Chua *et al.,* (1997). Accumulation of industrial effluents in water bodies has become a major concern, FAO (1986).

Ecotoxicology is a natural extension from the toxicology, the science of the effect of pollutants. However, the transitions from the study single organism to that of ecosystems has brought complexities, which has not yet appeared fully, appreciate. Its development had been due to, the extensive use of industrial chemicals, pesticides, antifouling agents and natural resources, more extensive utilization of urban agricultural and recreational space and marine environment and heightened awareness of hazard of chemicals to wild life, domestic animals and people.

In the last two decades ecotoxicology evolved mainly from three different disciplines such as toxicology, applied ecology and environmental chemistry. Ecotoxicology as an interdisciplinary environmental science deals with the interactions between environmental chemicals and biota, thereby focusing on adverse effects at different levels of biological organization. Toxic effects of anthropogenic compounds in biota and ecosystems are investigated in close connection to their environmental chemistry and fate in the environment. The bioavailability of chemicals, which is dependent on biogeochemical processes, is an important factor often neglected in ecotoxicological evaluation and hazard assessment. The bioavailable fraction is the critical parameter for the uptake and ultimately for the concentration at the target sites in organisms, which is the critical parameter for toxicity. Ecotoxicological research on selected pollutants requires an interdisciplinary effort, considering physicochemical, molecular, toxicological, physiological and ecological processes. Whereas practical aspects of ecotoxicology are mainly focused on regulatory issues as registration of chemicals and thus for testing of chemicals in standardized tests, the focus of Ecotoxicological research is aimed at an understanding of toxicological phenomenon in a variety of biota, populations and the ecosystem. Ecotoxicological effects are dependent on the bioavailable fraction of pollutants. Concentrations at the target sites induce molecular effects that propagate to a variety of toxic manifestations in organisms. Thereby, diverse aspects such as mechanisms of

toxic action and ecological processes in contaminated systems are regarded, Fent (2003). Ecotoxicological studies may also focus on ecological and toxicological effects observed in the field of retrospective studies, thereby a causative correlation between effects and chemical residue analysis is, however, often difficult to establish. Ecological investigations such as biomonitoring studies alone do not have sufficient resolving power to identify causative agents. Likewise, chemical analysis of pollutants in ecosystems alone cannot provide evidence for toxicological consequences in biota. Only an integrated approach considering environmental chemical, toxicological and ecological concepts may be suitable for understanding ecotoxicological effects in contaminated ecosystems, Fent (2003). A selection type of biomarker allows a gross discrimination between certain groups of contaminants, e.g. Polyaromatic and dioxin-like pollutants versus heavy metals, as well as toxicological mechanisms or biological functions affected, e.g. genotoxicity versus neurotoxicity or reproductive effects.

The wide spread use of organotin compounds as stabilizer in manufacturing Polyvinylchloride, as catalyst in the production of synthetic organic polymers and as biocidal agent in wood preservation, crop protection and mainly antifouling system has provided several paints of entry for these compounds into aquatic and terrestrial environments. The organotin compounds have been recently found in lacustrine and marine waters, sediments and biota, Tas J. W. (1996). In spite of regulation and prevention act the release of organotin compounds aquatic and terrestrial environments has decrease recently, but inputs, still occur and previously contaminated sites continue to act as source, Ristema R (1994) and Stab J. A. *et al.,* (1995).

TBT effects in prosobranch snails are ranked among the best-documented examples of the impact of an EDC on aquatic invertebrates, Matthiessen and Gibbs (1998). These organotin compounds are mainly used as biocides in antifouling paints, but also in various other formulations. They

produce a variety of malformations in aquatic animals with molluscs as one of the most TBT-sensitive groups of invertebrates, Bryan *et al.,* (1989). In the early 1980s the first impact of TBT on nontarget organisms were observed in France, Alzieu *et al.*, (1980). France was the first country to draw up regulations to control TBT emission from antifouling paints and banned the use of TBT-based antifouling on small boats (length <25 m) in 1982. This legislation was adopted later by other countries. Although a consequent drop of aqueous TBT concentrations was expected and reported for some regions, TBT pollution of coastal waters was found to have remained on a high level or even increased further in other areas. Consequently, the International Maritime Organization (IMO) decided in Autumn 2001 to ban the application of TBT-based paints on all boats by January 2003 and the presence on ship hulls by January 2008. Meanwhile, the proposed ban has been inacted to law in many countries worldwide. The first adverse effects of TBT on molluscs were observed in *Crassostrea gigas* at the Bay of Arcachon, one of the centers of oyster aquaculture in Europe with ball-shaped shell deformations in adults, and a dramatically decline of annual spatfall Alzieu *et al.*, (1980). These effects led to a break-down of local oyster production in the bay with marked economic consequences.

In recent years PVC is a much widely used plastic with many applications including flooring, furniture, windows frames, pipes and short life packaging. The use of PVC has increased greatly during recent decades and consumption is predicted to increase still further in the future, Van der, and Thorpe (1998). PVC is always formulated with a range of additives to enhance its properties, Ehrig (1992). Additives for PVC have included hazardous substances such as lead, cadmium, organotins and pthalate plasticisers. The use of such additives from PVC products has generated concern regarding environmental contamination and human health, in part, because of potential leaching of these additives from PVC products. For instance, children's toys made from soft PVC have been showed the content of 10-40% by weight of

pthalate plasticisers (Stringer *et al.,* 2000). Organotins are also used as heat stabilizers in PVC and biocides in industry and in agriculture. Recently it has been reported that the major use of organotins is for the heat stabilization of PVC that represents about 2/3rd of global consumption of these compound, Sadiki and Williams (1999). Both butyltins and ocyltins have been used. The latter group of compounds was specifically developed in an attempt to overcome toxicity problems of generally toxic butyltins, Matthews (1996).

As a conscience of its use in paint for marine shipping, TBT has caused major reproductive problems on some species of shellfish and in some instances has been related to massive population's declines, *Brayanet al.,* (1987); Gibbs (1993). Studies on laboratory rodents have shown that TBT is toxic to the immune system, Kerososien and Rice (1998). A study in Germany raised concern about the presence of comparatively high levels of TBT and other organotins in PVC flooring , Oeko –Test (2000). Research on organotin has suggested that they leach from PVC under laboratory simulated landfill conditions, Mersiowsky *et al.,* (1999) and from PVC water pipes to water, Sadiki and Williams (1999).

Much attention on the release of organotin compounds into the environment has found in the form of tributyltin (TBT) which has been widely used as biocide in paints and coating used for their antifouling application. However, in 1980`s concern about the apparent toxicity of tributyltin to nontarget species led to restricted use among many industrialized countries. Despite such restriction, tributyltin persists in many areas at the level consider to be chronically toxic.

The major objectives of aquatic toxicological studies in laboratory were to identify the mechanism of toxicity and to predict safe contaminant concentration in the environment. Acute toxicity bioassay is the first stage of such problem in the aquatic toxicology, Sprague, (1971). The toxicology of organotin tributyltin compounds to a particular organism is usually expressed

in term of LC50 (Lethal concentration). This value represent the amount of poison per unit weight, which kills 50 % of the particular population of the animal species employed for the tests this in term of represents the median tolerance limit, Finney (1971). The relationship between the dose of a compound and its toxicity is control theme in toxicology. The selected test animal's aquatic lethality is expressed in term of lethal concentration (LC50) and the compound is expressed as parts per million (ppm).

Among the crustaceans the prawns have been used as biomarkers for assessing the aquatic environmental pollution. Nagabhushanam, *et al*., (1990) reported the accumulation of tin oxides in fresh water prawn, *Caradina rajadhari*. Indira (1988) worked on effect of TBTO on freshwater prawn, *Caradina weberi*. Acute toxicity tests using mysids, copepoda, cranonid shrimp, *Listmata ambilnesis*, Daphnia, Sand crab, *Plalamon paulidens*, Pond snail Mussel, European oyster and common oyster at test organism, and reported by Goodman et *al*., (1988); Uren (1983); Thain (1983); Linden *et al*., (1979); Meador (1986); Walsh (1986); and Termink and Everts (1987) respectively. As a result 96 h LC50 was in the range of 1.1317 ng/L in term of organotin tributyltin chloride and the lowest LC50 value was observed for mysids (*Mysidopsis bahia*) .

Among toxicants found in coastal sea water and fresh water organotin tributyltin compound, an ubiquitous contaminants of the coastal environment impacted by navigation activities which is still used in antifouling paints of large ships, although the need for global ban of its used by maritime industry has emerged, IMO (2002); Champ (2000). Although the water concentration of organotin tributyltin compounds should decrease when direct inputs from ship will cease, the organotin tributyltin compounds issue will still be there for decades as contaminated sediments in shallow water should be acting as a long lasting reserviour for organotin tributyltin compounds and its degradation products, Maguire (2000). Calculation based on field data have demonstrated

that near shore and harbor sediments could be significant source of organotins to water column, originating from years of organotin tributyltin compounds accumulation in sediment and its slow degradation in mono and dibutyltins, Amorous *et al*., (2000). The sediment acts, as a reserviour of organotin compounds to the aquatic environments as long as the gradient between the concentrations of organotin compounds in the surface sediment pour water and the water column will be present.

Organotin tributyltin compounds is highly toxic and wide spread contaminant in aquatic environment and has caused world wide imposex (a pseudohermaphrodicic condition), characterized by the development of penis, vasadeferens and seminiferous 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 pseudohermophrodite. At advanced stages imposex results in snails become sterile or dying.

In 1985, the world production of triorganotins with biocidal properties was in range of 8 to 10,000 tons annually, De Mora (1996). Over recent decades, the use and production of organotin tributyltin compounds containing paints has been restricted, resulting in significant reduction in environmental concentrations, Alziue (1998). Despite these restrictive regulations, coastal TBT contamination can still reach upto 200 ng TBT/ L, Michel *et al*., (2001). In order to evaluate effects of pollutants, a number of assays have been developed linking effects at the sub-organismal level with population level effects, Koolsman and Metz (1984); Nisbet *et al*., (1989).

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 california grunion,

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 (1986). Salazar and Salazar (1995) observed accumulation of TBT in blue mussel, *Mytilus species.* Meador *et al.,* (1997) reported that TBTCl strongly affect on amphipod, *Rhepoxynius abronius.* Shah *et al*., (2001) studied TBTO induced alteration in histomorphology of digestive gland in *Otra violaea*. Tim verslyce (2003) revealed that the cellular energy allocation in the esturine mysid shrimp *Neomysis integer* to different TBT exposure, Richard saint Louis (2004) reported that the lost of TBT to atmosphere by volatisation and its effect on biota. Rabbito (2005) have been studied the effect of TBT on Neotropical fish, *Hoplias malabaricus*. Very significant reports are available on the accumulation of organotin in the shellfish, Food standard agency (2005).

Environmental pollution, caused by the development of industries, technology and informal settlements does, however, threaten many freshwater ecosystems. Not only does environmental pollution cause a decrease in water quality, but subsequently affects all living organisms in that system. It is therefore, necessary not only to identify and manage the pollution sources, but also to monitor their effects on the health of aquatic ecosystems. Any pollution, either physical or chemical, cause changes to the quality of the receiving waters, Noble *et al.,* (1971); Wittmann & Förstner (1977); Sanders (1997). These changes may include increased dissolved nutrients which may result in eutrophication, changes in stream temperatures and bottom characteristics which lead to habitat destruction and alteration of species diversity, and the addition of toxic substances may affect physiological and biochemical phenomenon which can have either acute or chronic effects on aquatic organisms, Sanders (1997).

A more prospective approach is based on investigations of potential toxicological effects in laboratory assays that may be used for extrapolation to

the field. Bioassays play an important role in this process, however, more comprehensive studies on contaminated systems and ecotoxicological processes are needed in addition. Often, bioassays do not consider the processes in ecosystems, and neglect environmental factors that influence toxicity. However, they are valuable tools in the characterization of the toxic action of chemicals, and in the understanding the associated toxicity. Despite the usefulness of these tools, it should be noted that the multitude of chemicals in ecosystems, species diversity, biological and ecological functions and structures makes extrapolations necessary for estimating possible effects in ecosystems.

In ecotoxicological research cellular effect studies including knowledge of mechanisms of toxic action are as important as studies in laboratory species, because the primary interaction between chemicals and biota occurs at the surface of or in cells. Whether chemical-induced alterations in cell structure and physiology will develop into an adverse toxic effect depends on many parameters, including adaptive responses. A cellular effect is often, but not necessarily, deterministic for adverse effects at higher levels of biological organization. Factors such as compensatory mechanisms and the presence of indirect effects may influence the relevance of the cellular toxicological response for overall ecotoxicological effects of a given chemical. The relation between cellular toxicological responses to toxicity at higher biological levels is a key question in ecotoxicology. The hypothesis that cellular changes may ultimately influence biological parameters important for populations such as growth, development, health, and reproduction is obvious. Hence, cellular toxicology provides an essential concept in understanding ecotoxicological processes, as it plays a key role in elucidating toxic mode of action, and diagnoses toxicological effects at higher biological levels, but it is not the sufficient one only. Its value will be strongly increased, when it can be integrated more closely with ecological effects.

Organotin compounds are among the most hazardous pollutants known so far in aquatic ecosystems, Fent (1996). Tributyltin (TBT) is of particular importance because of its widespread use as biocide, namely in antifouling paints on ships and in wood protection. Since the late 1970's considerable quantities of TBT were introduced into the aquatic environment and as a result, widespread pollution of marine and freshwater harbours and adjacent areas resulted. Organotin pollution in the aquatic environment is of global concern. Due to the extreme toxicity and the ecotoxicological hazards associated with TBT in antifouling paints, restrictions on its use have been implemented in many countries in the mid to end 1980's. As a consequence, TBT concentrations in harbour waters decreased significantly at many locations in industrialized countries, Chau *et al*. (1997); Fent and Hunn (1995), but this is not or less in the case of other locations, Biselli *et al*. (2000); Takahashi *et al*. (1999), in developing countries, Kannan *et al*. (1995). Still TBT compounds are released into aquatic ecosystem because of heavy use in antifouling paints on large vessels, or by other industries such as plastic. TBT accumulates in aquatic environment for a long period of time and as a consequence, high levels of TBT have been found in freshwater and coastal sediments, with concentrations up to several mg/kg. High contamination of marine harbour waters and sediments with tributyltin occurred long after regulation of TBT antifouling paints. In addition, alternative pesticides such as the s-triazine herbicide Irgarol 1051, which is used as a replacement for the organotins, has also been found in considerable concentrations, Biselli *et al*., (2000).

Triphenyltin (TPT) has been used as co-toxicant with TBT in some antifouling paints, Fent and Hunn (1995), although it's major application lies in agriculture, where it is used as a fungicide for various crops and enters aquatic ecosystems via leaching and runoff from the agricultural fields, St¨ab *et al*., (1996). In wastewater and sewage sludge, Fent and M¨uller (1991), as well as in water and sediment, degradation products of tributyltin (TBT),

dibutyltin (DBT) and monobutyltin (MBT), as well as from Triphenyltin (TPT), diphenyltin (DPT) and monophenyltin (MPT), respectively, occur in addition to the parent compound, Fent (1996).

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 (2000); Fioramonti *et al*., (1997); Hamasaki *et al*., (1993); Horiguchi *et al*., (1997) and Mathiessen and Gibbs (1998). However, the long-term ecotoxicological effects of organotins on the structure and function of aquatic ecosystems are still not well understood, particularly with respect to its mode of action in different physiological and biochemical systems, Guruge *et al*., (1996); Jak *et al*., (1998).

The uptake of dissolved chemicals may take place all over the body surfaces of small and/or soft-bodied organisms as well as at sites of high permeability, such as the gills, Phillips & Rainbow (1994). Uptake can vary tremendously depending on the environmental conditions or physico-chemistry of the medium and the specific bioavailable metal species present, Rainbow & Dallinger (1993); Van Ginneken *et al.,* (1999). Nagabhushnam *et al.,* (1990) reported that the uptake of tributyltin oxide in the freshwater prawn *Caridina rajadhari*. For free (bioavailable) metal ions to bioaccumulate or to elicit a biological response, a metal must first interact with and/or traverse a cell membrane of the organism, Campbell & Tessier (1996). The general importance of the free metal ion activity in determining metal uptake was formulated as the free ion activity model (FIAM). The free or chelated metal ion will bind to a lipophilic cell surface receptor to form a surface complex, followed by internalization Nair & Robinson (2000). Uptake of such pollutant should therefore, display normal Michaelis-Menten kinetics, Van Ginneken *et al*., (1999). The uptake, nutrition or toxicity of the bioavailable metal involves the reaction of the free metal ion concentration (or activity), with the body

surface, rather than the total dissolved or complexed metal concentration Tessier & Campbell (1990).

Uptake is based on the membrane transport (i.e. active, passive and facilitated transport) or the endocytotic processes of phagocytosis or pinocytosis. Direct uptake involves the transport of ionic or complexed pollutants to receptor sites, followed by the transfer through the organism's cell membranes. The chemical pollutants will then interact with the binding site and the extent of binding would depend on the ion activity. It is generally believed that the ionic form (aquo-ion) of most metals is more available to aquatic organisms, Wang & Evans (1993). Access to binding sites may be controlled by competition with the other ions, or by physical changes to the membrane of the organism, Wright (1995). There is no evidence that any organism can prevent the entry of pollutants by changing membrane permeability rapidly, although organisms, such as bivalve molluscs, can temporarily prevent uptake by closing the shell, Bryan (1976).

Responses to sub-lethal toxicity commonly observed in freshwater molluscs include: changes in the respiration rates, growth rates, reproductive capacity, valve closure, enzymatic activity and the production of metal-binding proteins, Elder & Collins (1991). If an organism cannot tolerate, detoxify or excrete a pollutant, it can result in the organism's death, Depledge & Fossi (1994). Tolerance may be due to physiological processes (acclimation) or have a genetic (adaptation). Mechanisms of adaptation to the presence of pollutants, may establish rapidly in many species due to the significant phenotypic and genotypic variability among individuals, Caquet & Lagadic (2000). Detoxification of toxicants is an important survival mechanism for an organism living in a chronically polluted environment, Moore *et al.,* (1986).

Toxicity may be governed by an organism's metabolism, which can be either beneficial (detoxification) or harmful (bioactivation). Biotransformation

and bioactivation are detoxification and metabolic conversion reactions processes present in all the organisms. Bio activation is the conversion of pollutants by cellular enzymes to more reactive intermediate or electrophilic metabolite (e.g. free radicals), which may induce adverse effects on various cellular constituentsWhen assessing biological responses, correlations between dose-effects of pollutants may be difficult to establish, because of the biotransformation of chemicals. Biotransformation involves the mechanism for the excretion of foreign pollutants/chemicals (xenobiotics), such as various pollutants that have become bioavailable. It may govern the toxicity of compounds and will determine the type of activity, the duration of the activity and the half-life of the chemical compound in organisms, van der Oost *et al.,* (2003). This detoxification mechanism entails enzymatic reactions that are involved in oxidation, reduction and hydrolysis (phase I) and conjugation (phase II) processes, to render the initial xenobiotic (generally lipophilic) more polar or water-soluble to be readily excreted from organisms. During phase II reactions, the xenobiotic or its product produced by the phase I reactions, is covalently bound to polar cellular constituents, such as glutathione, sulphate and glucuronic acid, Buhler & Williams (1988); Landrum *et al.,* (1996). However, many of the metabolic processes that are involved in the detoxification of various pollutants are still not well known for the most invertebrate species, Verrengia Guerrero *et al.,* (2002).

In aquatic toxicology, prawns have been widely used as a test species for evaluating the stressors especially the effect on respiratory metabolism. It has been reported that the response in prawns showed many similarities to that of the invertebrates, which involves stimulations of oxygen uptake and transfer. Stress increases the permeability of the surface of the epithelia including gills to water ions, and thus induce systematic hydro mineral disturbances. Toxic stressors are part of the stress literature in crustaceans more than in mammals. This is mainly related to the fact that crustaceans are exposed to aquatic pollutants via the extensive and delicate respiratory surface

of the gills. The high bioavailability of many chemicals in water is an additional factor. Together with the variety of highly sensitive, respective mechanism in the integument, this may explain why so many pollutants evoke an integrated stress response in crustaceans in addition to their toxic effect at the cell and tissue level.

The survival of the living organism is dependent on its breathing capacity of the cell to change the numerous simple compound into complex molecules is necessary for proper cellular function. The energy required for chemical processes and maintenance of the cellular activity must be obtained from oxidation of high-energy phosphate esters. This entire phenomenon is dependent on the availability of molecular oxygen to the cell by the process of respiration. The toxicants find their way into the body of the aquatic animals by means of gills, general body surface, gastrointestinal mucosa and through circulation.

The metabolic response of the organism to environmental changes is overall indicator of adaptive capacity of organism. All environmental factors have marked effect on the physiology and aquatic animals, Waldichuk (1974). Any chemical pollutants constitute one of the important factors affecting the rate of oxygen consumption. In accordance with general practice the bio-energetic pathway or the metabolic rate in poikilothermic animals in ordinarily measure in term and 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 ion concentration, dissolve oxygen content of the median pollutant, etc. All these factors individually and collectively influence the metabolism of animal, Harper (1986).

Respiratory process is a valuable indicator of energy metabolism, Thunberg *et al.,* (1973) on which depends many vital functions for survival of species, O' Hara (1971). Respiratory rates of prawns intoxicated with

antifouling agents vary in their degree and direction of effect. Brown and Newton (1972) suggested that in exceptions circumstances the environment of the fish habitat makes the aquatic respiration impossible, the fish have departed from aquatic respiration through gills and have evolved organ adapted for breathing atmospheric oxygen. The increased demands in the oxygen uptake from air and decreased aquatic contribution could be suggested due to the fish trying to avoid the toxic aquatic medium restoring to safe aerial respiration. It is beyond doubt that respiratory changes are good indicators of general conditions of an animal and has been correlated to stress from factors such as temperature, salinity, starvation and pollutants. Respiratory distress is one of the important manifestations of acute heavy metals toxicity and is known to produce physiological imbalance. Oxidative stress is a harmful process characterized by cellular damage that occurs when the equilibrium between the rate of ROS production and ROS elimination by cellular antioxidant mechanisms is disrupted, Sies (1991). Various stress responses have been observed in crustaceans. These include black gill syndrome, molt retardation, and disoriented behaviour as a consequence of aquatic pollution, Sindermann (1996). Oxidative stress may also result when oxygen availability is low, Storey (1996) and in response to various chemical compounds, Bainy *et al.,* (1996).

In crustaceans gills are one of the vital organs, which comes direct in contact with water, and indicative of any environment stress in prawn, Dhanapakiam *et al.,* (1998). It has been suggested that water born agencies damage prawns gills presumably, by causing breakdown of the gas exchange mechanism with consequent tissue hypoxic conditions, Sarojini *et al.,* (1989). Prawns easily get its tissue damaged due to water pollutants. As pollutants gain entry into the body, the first physiological activity that gets affected is oxygen uptake. Changes in the rate of oxygen consumption serve as a good indicator of stress, and used in the evaluation of changes in metabolism. Sub-lethal concentrations of toxicants are found either to depress or elevate rate

functions. Variations on oxygen consumption and on exposure to toxicants were supposed due to the filtration rate of ciliatory activity. Therefore filtration rate affected by the effluents changes the level of gill irritation which in turns affects the oxygen uptake. High concentration of the toxic substances has been suggested to reduce filtration efficiencies in prawns, Indira (1989).

Studies on the oxygen consumption form a useful tool in assessment of toxicant stress on the aquatic organism and give an index of energy expanditive mechanism for environmental variation, Sultana and Devi (1995). Oxygen consumption is a very sensitive physiological process and changes in the respiratory activity has been used as an indicator of stress animal exposed to toxicant, R. shivakumar and M. David (2007). According to Fry (1957), the rate of oxygen consumption is considered as the most acceptable and meaningful index of the metabolic rate in aquatic animals and it has been consider as an indication of intensity of metabolism. The intimate contact of gill with the water born pollutants may use co-alteration in the respiratory surface area, Singh and Singh (1979), in turn warning the diffusion capacity of gills. There changes are related to the metabolic rate, which is estimated indirectly by oxygen consumption. The measurement of oxygen consumption has been used to determine the effect of toxicant on the animal. Such measurements were made by Cairns and Scheir (1964) and Waiwood and Johnson (1974). The decrease in oxygen consumption appears to be protective measure to insure that coat intake of the toxic substances, Sultana and Devi (1995). Gills are an important source for uptake, biotransformation and excretion of toxicants, Thurnberg *et al.,* (1980). In spite their great physiological importance. Any change or damage in the gills are the toxic effects of pollutants could reduce oxygen consumption in prawns, B. Indira (1989). It is well known that gill lamellae play an important role in the transport of the respiratory gases and perform osmo ion regulatory and excretory functions, James *et al.,* (1992).

MATERIALS AND METHODS

The fresh water prawns, *Macrobrachium kistnensis* were collected from Kham river near Aurangabad, Maharashtra. The prawns were maintained in aerated plastic troughs containing tap water. The physiochemical characteristic of tap water is temperature 26±1°, pH 7 ± 0.2, dissolve oxygen is 5.6 ± 0.4 ml/lit. They were acclimatized for a week in laboratory conditions. The water in the troughs was changed every 24 h. After every three days the prawns were fed with green algae. The tributyltin chloride was purchased from Spectrochem Pvt.Ltd.Mumbai. 1-ppm stock solution was prepared in acetone Laughlin *et al*., (1983). Matured healthy female prawns were selected for the experiment. Series of statistic bioassay were conducted under laboratory condition as described by Finney (1971). For each experiment twenty healthy female prawns of approximately similar size (2.5 ± 1cm in length) were exposed to different concentration of tributyltin chloride. The resulting mortality were noted of each concentration for the duration of 24 h, 48 h, 72 h and 96 h. Control group was also maintained in acetone with tap water.

For oxygen consumption matured healthy female prawns were selected for the experiment. For each experiment 20 animals of approximately similar size (2.5±1cm in length) were exposed to 0.26 ppm, 0.026 ppm and 0. 086 ppm (48 h, $1/10^{th}$ and $1/3^{rd}$ LC50 0f 48 h) of tributyltin chloride. The oxygen consumption for lethal exposure were measured after 1 h, 2 h, 4 h, 8 h, 12 h and 24 h. Estimation of oxygen consumption was carried by Wrinkler's method as modified by Strickland and Parsons. Before the start of experiment animals were kept on blotted paper and weighed precisely as quickly as possible and then transferred into glass respiratory chambers.

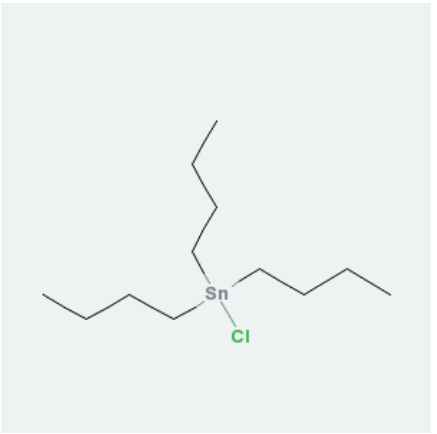
For all observations, final samples were siphoned out after one hour. The rate of oxygen consumption is expressed as ml O_2 / h /g /l body weight. Simultaneously control group was also maintained in tap water with

experimental animal group. The data obtained were statistically analyzed by applying‘t’ test Mungikar (1997). Each experiment was repeated thrice and mean was taken for calculations.

The technical grade, organotin tributyltin chloride was used in present study. The major properties of organotin tributyltin chloride are as follows.

Chemical name	**Tributyltin chloride (TBTCl)**
Cas no.	0120213- Spectrochem India
Molecular formula	C 12 H 27 ClSn
Molecular weight	325.49 g / moles
Appearance	Colourless, transparent liquid, peculier irritating odour
Specific gravity	1.2 (20)
Melting point	- 16
Vapour pressure	0.00927 mm Hg (25)
Min. Assay (G. C)	99 %
Water solubility	0.74786 mg /L (25)

The structural formula of organotin tributyltin chloride is as follows.



Toxicity preparation: -

The stock solution of organotin tributyltin chloride was prepared by dissolving a known quantity of solution of these antifouling agents in acetone and various concentrations were made from this stock solution.

Bioassay: -

Bioassay are classified according to,

a) Duration short terms intermediate, and / or long term

b) Method of adding test solution – static, recirculation, renewal, or flow through,

c) Purpose effluent quality, monitoring, relative toxicity, relative sensitivity, test or odour, or growth rate etc. APHA (1985). In the present bioassay, short term, renewal static and relative toxicity was criterion adopted.

Short term bioassay: -

Range finding tests:-

The known quantity of chemical was added to one litre of test solution to conduct short term (usually 24 h), small scale range finding to determine approximate concentration range to be covered in all full scale short term tests. Twenty healthy female prawns were exposed to a wide range of concentrations of pesticides. The pilot experiments were conducted to choose six test concentrations which, resulted in mortality in the range 10 – 95 %.

Method of adding test solutions:-

Renewal bioassay test was carried out in the present work. Renewal tests are often carried out with fish and macro invertebrates. The test solution was periodically exposed to the test organism after 24-hour interval APHA (1985).

Relative toxicity:-

The relative toxicity of the tested organisms is calculated. The most widely used methods for calculating on LC50 and confidence limits are probit Finney (1971). In the present investigation, James Busvine's regression line relating probits and log dose (1971) was followed;

4) *Statistical analysis*: -

The lethal concentration values (LC50) were calculated using computational procedure for critical analysis of the regression line relating probits and log dose Jame's Busvine (1971). The data were tested for

homogeneity using chi square test. The data were analyzed by two different methods,

Finney`s probit / Log of concentration

Jame`s Busvine Regression line relating probit and log dose.

correction for control mortality:-

The control mortality were corrected according to the Abbot`s formula, since otherwise an appreciable difference would affect the precision of the result and a correction is necessary

$$P1 = \frac{Po - Pc}{100 - Pc} \times 100$$

Where, P1 = Corrected mortality

Po = Observed mortality

Pc = Control mortality

Relative toxicity:-

Relative toxicity was found out and calculated considering 24-hour LC50 values that gives the toxicity value 1.

Lethal doses were calculated due to its importance from industrial point view.

Lethal dose is expressed as :

Lethal dose = LC50 × time of exposure

6) Safe concentration

Hart *et al.,* (1945) have proposed a formula for calculating safe concentrations of toxicants for animal.

$$C = \frac{48\text{ h TLM X } 0.2}{S^2}$$

Where C = Safe concentration

$$S = \frac{24\text{h TLM}}{48\text{ h TLM}}$$

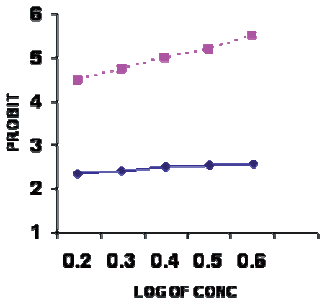
TLM = Median tolerance limit or known as LC50

RESULTS

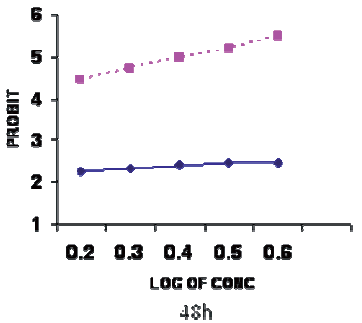
Freshwater prawn, *Macrobrachium kistnensis* exposed to lethal concentrations of TBTCl for 24 h, 48, 72h and 96 h exposures were studied in terms of their general behavior, rate of survival and mortality. The prawns exposed to zero toxicants were observed to have normal activities such as steady balance, normal surfacing phenomenon non aggressive movement or irregular vertical revolving movements. The activity of prawns, *Macrobrachium kistnensis* exposed to lethal concentrations showed minor changes in behavior which were intermediate. Continuous and increased respiratory movement, agitated activity of walking legs and finally autotomy followed by paralysis. Degree of autotomy varied with the time of exposure. Higher concentration induced increased autotomy in prawns with in 12 h , where as in chronic concentrations of TBTCl, atomized its legs within 16 to 20 h. the results of the bioassay testes are presented in table 1.

Percentage survival of freshwater prawn, *Macrobrachium kistnensis* in different concentrations of TBTCl were shown in table no 1. The LC50 values decreased with increased in exposure period. The percentage mortality increased progressively for all the concentration of tributyltin chloride i.e indicated the LC50 values and exposure period showed a direct relationship. The comparative LC50 values of TBTCl to freshwater prawn, *Macrobrachium kistnensis* at 24 h, 48 h, 72 h and 96 h were shown in table 1, 2, 3 and 4 respectively. The 24 h, 48 h, 72 h and 96 h, LC50 values were found to be 0.33 ppm, 0.26 ppm, 0.17 ppm and 0.09 ppm respectively. The LC50 values, regression results, Chi Square, variance and 95% fiducial limits for all concentrations were shown in table 1. From the observed data it appears that freshwater prawn, *Macrobrachium kistnensis* is highly sensitive to TBTCl toxicity.

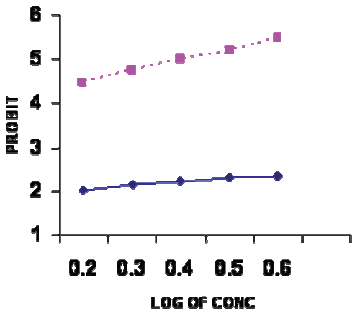
PROBIT REGRESSION LINES SHOWING RELATION BETWEEN LOG OF CONCENTRATION OF TBTCl PROBIT KILL FOR GRAPHICAL DERIVATION OF LC $_{50}$ FOR MACROBRACHIUM KISTNENSIS AT VARIOUS EXPOSURE TIME.



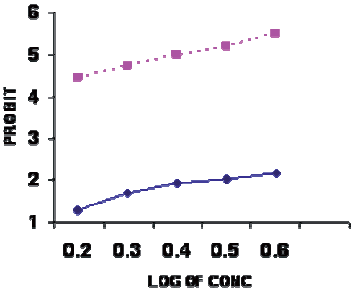
24h



48h


6
5
4
3
2
1
PROBIT
0.2
0.3
0.4
0.5
0.6
LOG OF CONC

72h


6
5
4
3
2
1
PROBIT
0.2
0.3
0.4
0.5
0.6
LOG OF CONC

96h

Table 1: LC50 values calculated for fresh water matured female prawn *Macrobrachium kistnensis* after exposure to TBTCl for a period of 24, 48, 72 and 96 h.

Exposure period	Regression equation $Y=(\bar{y} - b\bar{x})+bx$	LC50 Value in ppm.	Variance	Chi square	F.L. upto 95% confidence.	
					M1	M2.
24	3.02306+3.2181x	0.33	0.003307	1.1846	2.3790	2.6044
48	5.9414+4.5612x	0.26	0.001744	0.123531	2.3159	2.4797
72	3.8269+0.5333x	0.17	0.06890	2.6288	1.7016	2.7306
96	2.8714+1.1551x	0.09	0.27199	0.2918	1.5109	2.1574

Figure 1: Rate of oxygen consumption of freshwater prawn, *Macrobrachium kistnensis* exposed to 0.26 ppm 48 hr LC 50 of tributyltin chloride

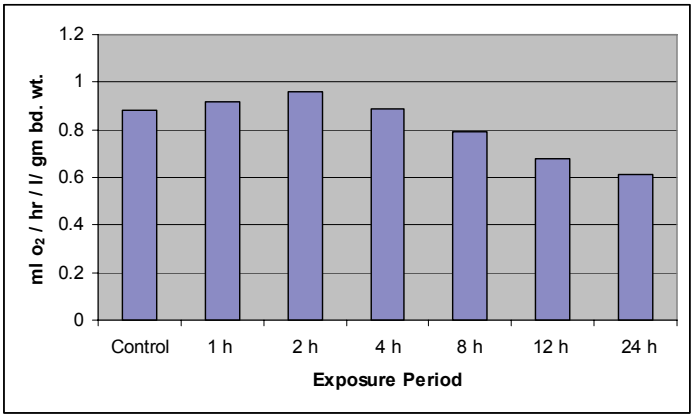


Figure 2: Rate of oxygen consumption of freshwater prawn, *Macrobrachium kistnensis* exposed to 0.086667 ppm of tributyltin chloride

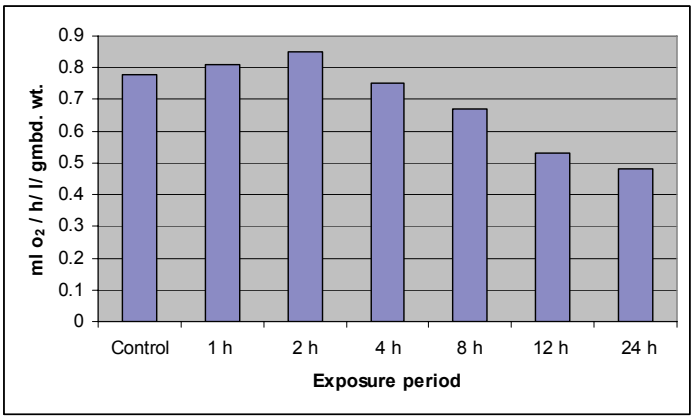
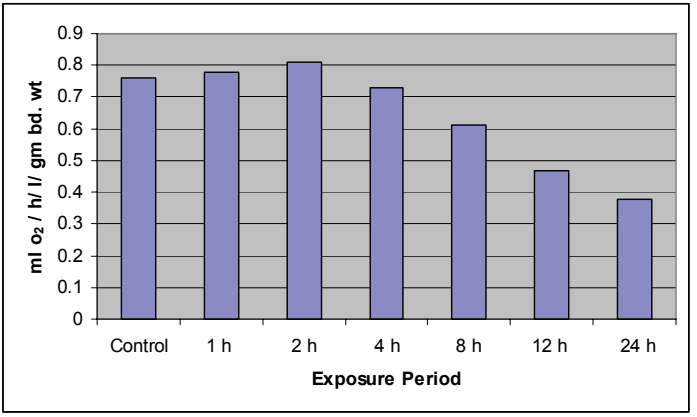


Figure 3: Rate of oxygen consumption of freshwater prawn, *Macrobrachium kistnensis* exposed to 0.026 ppm of tributyltin chloride



DISCUSSION

Tributyltin is a heavy toxic and widespread contaminant in aquatic environments caused several physiological and biochemical imbalances. The main inputs of TBT in aquatic environment are from antifouling paints, PVC industries, paper and pulp industries, as heat stabilizer and biocidal agent in wood preservation, etc. organotin compounds were first developed as moth-proofing agents in the 1920s and later used more widely as bactericides and fungicides, Moore *et al.,* (1991). TBT compounds have introduced since late 1940s, Laughlin and Linden (1985), although use of TBT compound in antifouling paints dates only from the 1960s and then initially as a booster biocide in copper based formulations. As results it's of its effectiveness over copper, Wade *et al.,* (1988). At the same time as its explosive increase in the use, the first observation was made of TBT effects on non target organisms. While toxicity of fouling organism was intentional, its propensity for wider impact on the aquatic environment has been grossly underestimated. An early focus on acute effects especially mortality, Laughlin and Linden (1987), failed to identify sub lethal consequences of prolonged exposure in some aquatic biota. The development of male sexual structure in female (imposex) can be initiated in some gastropod molluscs by TBT in low ng / l.

Industrialization is linked to the economic growth and human progress. It is emphasized that increased industrialization lead to deterioration of environmental quality and subsequently to health hazardous of organisms. The rapid unplanned industrial progresses have added to the problem of pollution. Organotins are noted as one of the significant contributor to water pollution. The organotin pollution in the environment has risen to alarming proportion which has caused great concern among the environmental toxicologist as the organotin residues that are released as non-biodegradable substances. The recent developments in bioinformation on these substances have brought forth the scientist to cause remedial action to this problem. Among the various

toxicants organotins are known to be quite severe in their action. Toxicity is termed as the relative property of chemical pollutants with reference to its potential to cause harmful effects on any organism. The harmful effects depend upon the function of concentration of the chemical and its duration of exposure, Gehring and Rao (1981). These chemicals when enter into water, disturb the normal functioning of cells in flora and fauna which may result in the alteration in the biochemical and physiological mechanism of aquatic animals. Pollutants, because of their potential toxicity are known to produce morphological, behavioural and physiological changes in the vital organs such as respiratory, reproductive, nervous, osmoregulatory etc in different animals, Fingerman (1982). The aquatic animals are particularly susceptible to toxic substances, since their habitats are strictly confined to the water bodies. They also have foot pass a large amount of water over their body surface and hence are exposed more to the toxic compounds which are dissolved in the medium.

The toxic effect of an organism is indicated in the term of mortality, because it is customary to represent lethality to a chemical with respect to the survival rate at various concentration and time. In aquatic organism the pollutant-induced mortality is usually expressed in lethal concentration. The quantitative study of pollutants in aquatic organism offers an interesting challenge to the researchers. Organotin are well known environmental pollutants, they persists, circulate and eventually accumulate through food chain and thus cause a serious threat to non target organisms, Akhter and Mohan (1995). Tolerance is the ability of the organism to show less response to a specific dose of a toxicant than it showed on prior occasion from the same dose. It is observed that the organism had become partially refractory or had developed immunity to the effect of the toxicant by virtue of previous exposure. Enhanced tolerance observed in the organism may results as a failure in translocation of the metal ion, such as absorption or distribution, or enhanced termination, that is enhanced excretion or metabolic alteration of the metal in the organism. Such condition would lead to an effective lowering of

the metal dose as its site of action in the biological system, thereby resulting in a lesser effect from a specific dose. Absorption from aqueous medium by organism involve passive diffusion of the pollutant probably as a soluble complex, down gradient originated by absorption of surface, and chelated by constituents of the surface cells, body fluids and internal organs. Tolerance of pollutants to any organism is determined by the permeability of various ions. Depression in assimilation rate of the toxicants as copper and reduction in toxicity could also happen due to the passive diffusion of the toxic ions along the concentration gradient involving the binding of free copper ion to some carriers were hindered or blocked. The degrees of involvement of different mechanism to explain the observed change in accumulation is the matter of speculation.

The mortality of exposed prawn, *Macrobrachium kistnensis* to antifouling compound tributyltin chloride for the test period is noted and the data was subjected to statistical analysis. Probit analysis is a commonly used parametric technique for handling toxicity data, significant deviation from the log probit model can occur, when the data are not normally distributed. Buikema, *et al*., (1982) has reviewed statistical and experimental approaches used in the acute toxicity testing for aquatic organisms including a discussion on departure of experimental data from log / probit model. Mary *et al*., (1987) analyzed three methods i.e. probit, regression line, trimmed spearman and karber, and found these three methods of toxicity evaluation, do not differ much. In the present toxicity evaluation the critical analyses was carried out by the computational procedure of the regression line relating to probits and log / dose, James Busvine (1971).

In the present study, the prawns treated with tributyltin chloride, the acute toxicity level was expressed in terms of LC_{50} values. The acute value for 24 h for organotin tributyltin chloride was found to be 0.33 ppm,for 48 h found to be 0.26 ppm, for 72 h found to be 0.17 ppm (table 1) and the chronic

value for 96 h for organotin tributyltin chloride was found to be 0.09 ppm (table 1). The result shows that the LC_{50} values decreased with increase in exposure period and vice-versa and also the 95 % confidence limits. Mary (1984) has reported that the LC_{50} values depend on the concentrations of pesticides and also with the time of exposure. The 96 hours LC_{50} value was the low, however the mortality scored was high. The determination of the LC_{50} 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. It is thus significant as a tolerance limit determined here might be useful in conducting chronic studies, since the shrimps are more sensitive to various types of toxicant than fish or molluscs, Couch (1984), and thus may be used as indicator for assaying the water quality.

Floach *et al*., (1964) calculated LC_{100} for various species of freshwater invertebrates using tributyltin acetate. The lethal concentration of TBT to *Daphnia magna* over 24 and 72 h. were 0.12 mg / lit and 0.06 mg / lit, respectively. The LC100 was 0.15 mg / lit for 72 h exposure Daphnia and 0.15 mg / lit for 96 h exposure of *Cypridopsis*. The LC_0 was 0.075 mg / lit., for both species. However it was found that Daphnia is considerably more sensitive to TBT. Robert *et al*., (1987) maintained adult oysters (*Craffostrea virginica*) in TBT solutions containing 0.05, 0.1, 0.5 and 1 µgm / lit for upto eight weeks. He observed that 20% and 30% mortality occurred between second and fourth week of exposure. Laughlin *et al*., (1983) reported LC_{50} value of Zoeae of *Rhithropanopeus harrisii* to TBTO during the twelve days of zoeael development to the 55 nmol / lit. Development of self-polishing antifouling paint containing a chemical TBTO is one of the technological achievement, Karande and Ganti (1999). *Laughlin et al.,* (1985) exposed shore crabs, *Hemigrapsus nudes*, 2 to 3 days after hatching, to TBTO for upto 14 days under static conditions at the highest concentration (500 and 1000 µ g

/ lit.), all the Zoeae were died within two days. Survival time increased as the concentration of TBTO decreased from, 100 and 25 µ g / lit. Most larvae died within 8 days even at 25 µ g / lit. The estimated values of LC_{50} for 100, 75, 50 and 25 µ g / lit, were 3.4, 4.8, 5.8 and 6.2 days respectively. Meador (1986) reported the effect of TBTCl on the photobehavior of water fleas.

In contrast to toxicity levels based on the TBT water concentrations which range over several orders of magnitude for various species. Recent studies on tissue concentrations in Puget Sound organisms indicate that a much narrower range of tissue concentration as associated with adverse effect to these organisms. Different species have widely varying uptake, metabolic and elimination rates for TBT, in part explaining the widely varying sediment and water concentration that yield similar tissue concentration and associated effects.

This finding provides an opportunity to develop tissue TBT concentrations that are directly co-related with observed effects in a wide range of ecologically revalent species. Meador *et al*., (1993, 1996) have reported acute toxicity (LD_{50}) for *Rhepoxynius abronius*, *Eohaustorius washingtonianus* and *Armandia brevis* at concentrations ranging from 34 – 89 mg TBT / kg body weight (dry weight). Tissue concentrations within or above this range would represent a severe adverse effect and sediments associated with these levels would exceed the level at which cleanup would be required, and would also be inappropriate for open water disposal.

However, PSDDA and SMS require consideration of both acute and chronic effects. Chronic effect levels for species of concern in Puget Sound can be found in the literature, Salazar and Salazar (1992, 1995); Widdows and Page (1993); Waldock *et al*., (1992) these values typically fall within a range two to twelve mg TBT / kg body weight (dry weight), with a median value of about 4. Davidson *et al.,* (1986) calculated the 96 h LC_{50} to be 0.42 µg / lit

and NOEL, after exposing the mysid shrimp, *Acanthomysis sculpta* to a leachate of TBT. When 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.

In the present study the safe concentration for freshwater prawn, *Macrobrachium kistnensis* to TBTCl was 0.0322 ppm. The death of freshwater prawn, *Macrobrachium kistnensis* might be due to toxic stress of TBTCl which cause severe physiological and biochemical alteration at cellular as well as organismic level of tested prawn. It might be due to the impact of TBTCl on gill which ruptured the gill lamellae resulting decline in respiratory rate, an inhibition of the electron-transport system (ETS) or an effect on mitochondrial integrity.

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 pesticides 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 this study, oxygen consumption of *Macrobrachium kistnensis* in normal and after exposure to TBTCl media has been quantified. On exposure to tributyltin chloride the respiratory metabolism of prawn, *Macrobrachium kistnensis* has been found to be directly altered. The obtained data clearly showed that there was an increase in the rate of oxygen consumption of prawn, *Macrobrachium kistnensis* after exposing for 1 h and 2 h in both (0.086667 ppm and 0.026 ppm) $1/3^{rd}$ and $1/10^{th}$ sublethal concentrations of 48 h LC_{50} respectively of

tributyltin chloride. The rate of oxygen consumption decreased significantly ($p<0.05$) after exposing for 4 h to 24 h as compared to control groups. It was also observed that at acute exposure, the prawns showed sudden decrease in oxygen consumption followed by an initial increase and then steady decrease as shown in fig. 1, fig. 2 and fig. 3. There was a continuous increase in the rate of oxygen consumption upto 4 hr when the prawns were exposed to all the concentrations of lethal and sublethal TBTCl medium. As the period of exposure increased this uptake gradually but constantly decreased severe fall after 12 h and then continued for 24 h of exposure. These results clearly indicates that the used organotin tributyltin chloride 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 respiratory organ. The TBTCl 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 4 h to 24 h exposure to all the concentration of TBTCl as compared to the first 4 h of exposure to all the concentration of TBTCl. From these observation it can be inferred that the organotin compound 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 continuous 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 of *Macrobrachium kistnensis* may be expected because

of toxic action of TBTCl as reported, Selwyn (1976); Aldridge (1976) in some mammals. The present investigations were supported by many authors.

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 by *Caridina weberi* showed alteration like increase and decrease oxygen uptake when exposed to different concentration of copper sulphate and TBTO. They reported that after 10 days exposure to TBTO the animals could not survive to collect further oxygen consumption data. 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 prawns 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 prawn *Macrobrachium kistnensis* to tributyltin chloride resulted in morphological changes in the gills, are reflected in plasma had a significant effect on the respiration, excretion and osmoregulatory functions of the gills. There 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. Cremer (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, Wulf and Byngton (1975); Aldridge (1976).

According to Piver (1973) dialkyltin and trialkyltin compounds are known to be capable of effecting the respiration. Umadevi (1996) studied changes in oxygen consumption of marine fouling dressinid bivalve, *Mytilopsis sallei* exposed to mercury. Cremor (1957) also claims that TBTO affects the oxidative metabolism. 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. M. 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. 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.

In the present probe the finding related to respiratory mechanism was initial elevation and subsequent decrease in the rate of oxygen uptake. Therefore from the above results it was suggested that the increase in rate of oxygen consumption in different contaminated media might be the reflection of an augmented physiological activity like osmosis at the cellular level in eliminating and / or counteracting the tributyltin chloride stress perhaps when exposed to the different sublethal concentration. It is also found that prawns 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 growth rates in prawns or exhausts the biochemical reserver.

From the above discussion and all the available literature, we can conclude that the TBTCl is very toxic to the freshwater prawn, *Macrobrachium kistnensis.* Therefore the release of organotin compounds in aquatic environment especially in freshwater ecosystem might be controlled.

REFERENCES

Akther, N.M. and P.M. Mohan, (1995). Bioremedation of toxic metal ion from polluted lake water and industrial effluent by fungal biosorbant. *Corr. Sci*. 69, 1028 – 1030.

Alderidge, W.N. (1976). The influnce of organotin compounds on mitochondrial function. In: organotin compounds: New chemistry and application (Zuckrman J.J.eds*.) Amerian chemical society. Wanshigton D.C. 186-196.*

Alzieu, Y. Thibaud, M. Heral and B. Booutier. (1980). Evaluation of the risk of using antifouling paints near oyster zones. *Rev. Trav. Inst. Peches. Marit.* 44: 301- 348.

Alzieu, C. (1998). Tributyltin: case study of a chronic contaminant in the coastal environment. *Ocean coast. Manag.* 40 (1): 23- 36.

Alzieu, C. (2000). Impact of tributyltin on marine invertebrates. Ecotoxicology\ 9, 71–76.

Amouroux, D, Tessier, E, Donard, O.F.X., (2000). Volatilization of organotin compounds from esturine and coastal environment. *Envirol. Sci. technol*. 34, 988-995.

APHA (1985). Standard methods for examination of water and waste water (16th Edition) American public Health Association. Washington. DC.

Bainy ACD, Saito E, Carvalho PSM, Junqueira VBC (1996). Oxidative stress in gill, erythrocytes, liver and kidney of Nile tllapla (*Oreochromis niloticus*) from a polluted site. *Aquat Tox1col.* 34:151-162.

Baticodes, M. Cecilia, L. and Leonor, A. (1991). Effects of gusathion on the survival and shell of guvenile *Penaeus monodon Aquaculture.* 93(1): 9– 20.

Biselli, S., Bester, K., H¨uhnerfuss and Fent, K., (2000). : Concentrations of the antifouling compound Irgarol 1051 and of organotins in water and sediments of German North and Baltic Sea marinas.*Mar. Pollut. Bull. 40, 233–243. Toxicol.* 26: 1–117.

Brown, H.E. and Newell, R.C. (1972): The effect of copper and zinc on the metabolism of the mussel Mytilus edulis. Mar. Biol.,; 16 (2): 108-118.

Bryan, G.W. (1976). Some aspects of heavy metal tolerance in aquatic organisms. In: Effects of pollutants on aquatic organisms, *(Eds.) Lockwood, A.P.M. Cambridge University Press, Cambridge:* 7-35.

Bryan, G.W., Gibbs, P.E., Huggett, R.J., Curtis, L.A., Bailey, D.S., Dauer, D.M., (1989). Effects of tributyltin pollution on the mud snail, *Ilyanassa obsoleta*, from the York River and Sarah Creek, Chesapeake Bay. *Mar. Pollut. Bull.* 20, 458–462.

Bryan G.W., Gibbs P.E., Hummerstone L.G., and Burt G.R. (1987). Copper, Zinc organotin as long term factors governing the distribution of organism in the Fal estury in southwest England Eaturies, 10 (3): 208-219.

Buhler, D.R. and Williams, D.E. (1988). The role of biotransformation in the sediments and its relationship to their accumulation in benthic organisms.

Buikena Jr. A. L. Neiderle Hner, B. R and Carin Hr. A. L. (1982). Biological monitoring 4 toxicity testing. *Water Res*. 16: 229-262.

Cairns, J. and Scheier, A. (1964). The effect upon the pumpkin seed fish, *Lepomis gibbosus* of concentration of dieldrin. *Natnl. Nat., 370.*

Campbell, P.G.C. & Tessier, A. (1996). Ecotoxicology of metals in the aquatic environment: Geochemical aspects. In: *Ecotoxicology: A hierarchical treatment*, (Eds.) Newman, M.C. & Jagoe, C.H. Lewis

Caquet, T. & Lagadic, L. (2000). Consequences of individual-level alterations on population dynamics and community structure and function. In: *Use of biomarkers for environmental quality assessment*, (Eds.) Lagadic, L., Caquet, T., Amiard, J.-C. & Ramade, F. A.A. Balkema, Rotterdam: 291.

Champ M. A. (2000). A review of organotin regulatory strategies pending actions, related coasts and benefits. *Sci. Total Environ*. 258, 21-71.

Chau,Y.K., Maguire, R.J., Brown, M.,Yang, F., Batchelor, S.P., (1997). Occurrence of organotin compounds in the Canadian aquatic environment five years after the regulation of antifouling uses of tributyltin. *Water Qual. Res. J. Can. 32, 453–521.*

Chinni S., Khan R. N. and Yallapragada. (2000). Oxygen consumption, ammonia-N-excretion and metal accumulation in *Penaeus indicus* post larvae exposed to lead. Bull. Environ. *Contam. Toxicol.* 64: 144 -151.

Couch, J.A. (1984). Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica. Mar. Environ. Res*., 14, 525-526.

Cremer J.E. (1957). The metabolism in vitro of tissue slices from rats given triphenyltin compounds*. Biochem. J. 67: 87-96.*

Davidson, B. M., A. O. Vakaris and P. F. Seligman (1986). Acute and chronic effect of TBT on the mysid *Acanthomysis sculpta* (crustacea, Mysidacea) NOSC-TR- 1116 or AD-A 175- 294- 8. National technical information service, spring field, VA.

Defur, P. L., Crane, M., Ingershold, C, Tattersfield, L, (1999). Endocrine disruption in invertebrates: endocrinology, testing and assessment. Society of environmental toxicology and chemistry, pensacola, F. L. USA.

De Mora, S. J. (1996). Tributyltin: case study of an environmental contaminant. *Camb. Envirol. Chem*. Ser. 8, 389.

Depledge, M.H. and Fossi, M.C. (1994). The role of biomarkers in environmental assessment (2) Invertebrates. *Ecotoxicology*, 3: 161-172.

Dhanapakiam P., Ramasamy V. K. and Sompoorani (1998): A study on the histopathological changes in gill of Channa punctatus in cauvery river water. *J. Env. Biol*. 19 (3): 265-269.

Ehrig, R.J. (Ed.) (1992). Plastics recycling. Products and processes. Munich: *Hanser Publishers*, 289 pp.

Elder, J.F. & Collins, J.J. (1991). Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. *Rev. Environ. Contam. Toxicol*., 122: 37-79.

FAO. FAO/UNOP. (1986). Meeting on the effect of pollution on marine ecosystem. *FAO. Fisheries Reports*, 352:20.

Fent, K., and M¨uller,M.D., (1991). Occurrence of organotins in municipal wastewater and sewage sludge and behavior in a treatment plant.Environ. Sci. Technol. 25, 489–493.

Fent, K., and Hunn, J., (1995). Organotins in freshwater harbors and rivers: temporal distribution, annual trend and fate. *Environ. Toxicol. Chem*. 14, 1123–1132.

Fent, K., (1996). Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol. 26, 1–117.*

Fent, K., (2003). ¨ Okotoxikologie. Thieme-Verlag, Stuttgart.

Finney D. J. (1971). Probit analysis Cambridge University, *Press London. Pp*. 333.

Fioramonti, E., Semlitsch, R.D., Reyer, H.-U., Fent, K., (1997). Effects of triphenyltin and pH on the growth and development of *Rana lessonae* and *Rana esculenta* tadpoles. *Environ. Toxicol. Chem. 16, 1940–1947*.

Fingerman, M. (1982). Keynote address. *All India Symp. On physiological responses of animals to pololutants. Marathwadw university, Aurangabad.*

Floach, H. Deschines, R. and Floch T. (1964). Surles properties molluscicides de, oxyde, et de 1' acetate de tributyletain (prophylaxiedes billharzioses. *Bull. Soc.pathol. Exot*.57:454-465.

Food Standard Agency (2005). The survey of organotin in shell fish.

Fry, F. E. J. (1957). The aquatic respiration of fish. In physiology of fishes (Ed) M. E. Brown, *Vol. 1, pp. 1 – 63 Acad. Press, New york and London.*

Gegring P. J. and Rao K. S. (1981). Toxilogy data exploitation in : Pattys industrial hygiene and toxicology, *3rd Edn. Wiley, New york 567-594.*

Ghate H. V. and Mulherkar L. (1978). Histological changes in the gills of two freshwater prawns exposed to copper sulphate. *All India Symp. Exptl. Zool. Jaipur Abst. 97. pp. 58*

Gibbs, P.E. (1993). A male genital effect in dog-whelk *Nucella lapillus* (Neogastropoda), favouring survival in a TBT-polluted area. *J. Mar. Biol. Associa*. U.K. 73, 667-678.

Goodman, L.R., Cripe, G.M., Moody, P.H. and Halsell, D.G. (1988). Acute toxicity of malathion, tetrabromobisphenol-A, and tributyltin chloride to mysid (*Mysid bahia*) of three ages. *Bull. Enviorn. Contam. Toxicol*., 41, 746-753.

Hamasaki, T., Sato, T., Nagase, H., Kito, H., (1993). The mutagenicityof organotin compounds as environmental pollutants. *Mutat. Res. 300, 265–271*.

Harper, H. A. (1986). Harpers review of biochemistry (Martin. D. W., Mayes P. A. and Rodwell V. W. 20th Edition) *Lange Medical Publications, Maruzen, Asia (Singapur).*

Hart, W. B., Duodoroff, P. and Greenbank, J. (1945). The evaluation of the toxicity of industrial waste, chemicals and othe substances to freshwater fishes. Waste control laboratory. *Atlentic Refining. Co. Philadelphia.*

Hiltibran, R. C. (1966). The effect of 2, 4, D on blue gill: Levels of mitochondrial enzyme system. In: abstract paper presented at the Weed society of America. *St. Lows, 91 – 92.*

Horiguchi, T., Shiraishi, H., Shimizu, M., Morita, M., (1997). Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell, *Thais clavigera*. *Environ. Pollut. 95, 85–91.*

Indira (1989). Effect of antifouling organometallic compounds on the physiologylogy of freshwater prawn, *Cardina weberi.* Ph. D thesis, Marathwada University, Aurangabad.

International maritine organization (IMO), (2002). http//:www.imo.org.

Jak, R.G., Ceulmans, M., Scholten, M.C.T., Van Straalen, N.M., (1998). Effects of tributyltin on a coastal North Sea plankton community in enclosures. *Environ. Toxicol. Chem. 17, 1840–1847.*

James, R., Sampath, K. and Ponmani, K.P. (1992). Effect of metal mixtures on activity of two respiratory enzymes and their recovery in *Oreochromis mossambicus. Ind. J. Exp. Biol., 30: 496-499.*

Kannan, K., Tanabe, S., Iwata, H., Tatsukawa, R., (1995). : Butyltins in muscle and liver of fish collected from certain Asian and Oceanian countries. *Environ. Pollut.* 90, 279–290.

Karande A.A. and Ganti S.S. (1999). Laboratory assays of Tributyltin toxicity to some comman marine organism. In: Recent developments in Biofouling Control. Oxford and IBH Publishing Co. P. Ltd. P. 450.

Kergosien, D.H. and Rice C.D. (1998). Macrophage secretory function is enhansed by large doses of TBTO, but not TBTCl. *Arc. Enviorn. Contam. Toxicol*.34: 223-228.

Kooijman, S.A. L. M., Metz, A. J. (1984). On the dynamics of chemically stressed population : the derivation of population consequences from the effects of individuals. *Ecotoxicol. Envirol. Saf*. 8 (3): 296- 303.

Laughlin R. Frencj, W. and Guard H. F. (1983). Acute and sub lethal activity of TBTO and its putative environmental product tributyltin sulphide (TBTS) to zoeal mud crab. *Water-air, soil Pollut.* 20: 69-79.

Laughlin R. B. and jr. O. Linden (1985). Fate and effects of organotin compounds *Ambio.* 14: 88 – 94.

Laughlin, R.B, Johannesen, R.B. French, W,Guard, H.and Birnkjman, F.E. (1985). Structure-activity relationship for Organotin compound. *Environ. Toxicol. Chem.* 4: 343-351.

Landrum, P., Harkey, G.A. and Kukkonen, J. (1996). Evaluation of organic contaminant exposure in aquatic organisms: The significance of bioconcentration and bioaccumulation. In: *Ecotoxicololgy: A hierarchical treatment*, (Eds.) *Newman, M.C. & Jagoe, C.H. Lewis Publishers, Boca Raton: 85-132.*

Linden, E., Bengttson, B.E., Svanberg, O. and Sundstorm, G. (1979). The acute toxicity of 78 chemicals and pesticides formulation against two brakkish water organism, the black (*Alburnus alburnus*) and the harpacticoid *Nitcora spinipes. Chemosphere,* 8,843-851.

Liu D.and K. Thomson (1986). Biochemical responses of bacteria after short exposure to alkyltins. *Bull. Environ. Contam. Toxicol.,36 : 60 – 66.*

Maguire, R.J., (2000). Review of the persistance, biaccumalation and toxicity of tributyltin in aquatic environments in relation to Canada`s toxic substances management policy. *Water Qual. Res. J. Cal*. 35 (4), 633-679.

Mary Sr. Avelin (1984). Effect of pesticides on some aspects of physiology of freshwater prawn *Macrobrachium lamerrii*. Ph. D. thesis. Marathwada uivicersity. Aurangabad.

Mary, A.S., O.S.M, Tamykodi, P. Sr. Vitalina Mary O.S.M and Sarojni, R. (1987). Toxicity evaluation of five heavy metal and there interactive toxicity in the brine shrimp *Artemia*. Proc .Nat. Symp. Ecotoxic .pp 112-116

Manikumar D. (1986). Effect of pollutant on marine prawn. *Ph. D. Thesis Marathwada University, Aurangabad.*

Matthews, G. (1996). PVC. Production, properties and uses. The Institute of Materials. ISBN 090171659 6.

Mathiessen, P., and Gibbs, P.E., (1998). Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. *Environ. Toxicol. Chem.* 17: 37–43.

Meador J.P. (1986). An analysis of photobehavior of *Daphnia magna* exposed to tributyltin. In procidings of the organotin symposium ocean'86 conference, Washington, Dc., USA, 23-25 September, 1986, New York, The institute of Electrical and Electronic Engineer's Inc. 4: 1213-1218.

Meador, J.P., U. Varanasi, and C.A. Krone. (1993). Differential sensitivity of marine in faunal amphipods to tributyltin. *Marine Biology* 116:231-239.

Meador, J.P., C.A. Krone, D.W. Dyer, and U. Varanasi. (1996) (in press). Toxicity of sediment-associated tributyltin to in faunal invertebrates: Species comparison and the role of organic carbon. Marine Environmental Research.

Meador J.P., Krone C.A., Dyer W., Varanasi U. (1997). Toxicity of sediment-associated tributyltin to infaunal invertebrates: species comparision and the role of oraganic carban. *Mar. Environ*. Res. 43, 219-241.

Mersiowsky, I., Stegmann, I., Ejlertsson, J. and Svensson, B. (1999). Long term behavior of PVC products under soil-buried landfill conditions. Final Report of Research Project. TUHH. 2nd revised edition.

Michel, P., Averty, B., Chiffoleau, J. F. and F. Galgani, (2001). Tributyltin along the coast of corsica (western mediterranean) : A persistance problem. *Mar. pollut. Bull*. 42 (11), 1128-1132.

Monika Kale (2002). Ph. D. thesis, Dr. Babasaheb Ambedkar University, Aurangabad.

Moore, M.N., Lowe, D.M., Livingstone, D.R. and D.R. Dixon, (1986). Molecular and cellular indices of pollutant effects and their use in environmental impact assessment. *Wat. Sci. Tech***.**, 18: 223-232.

Moore, D.W., T.M. Dillon, and B.C. Suedel, (1991). Chronic toxicity of tributyltin to the marine polychaete worm, *Neanthes arenaceodentata*. Aquat. Toxic. 21:181-198.

Nagabhushnam, B. Indira and R. Sarojini, (1990). Toxicity evaluation of the prawn, *Cardina weberi* after exposure to two atifouling organometallic compounds. *Environmental pollution and health hazards*,53-59.

Nair, P.S. and Robinson, W.E. (2000). Cadmium speciation and transport in the blood of the bivalve *Mytilus edulis. Mar. Environ. Res.*, *50: 99-102*.

Newton F., Athum, B david son A. Valkirs and B. Scligman, (1985). Effects on the growth and survival of eggs and embryos of the California grunion (*Leuresthes tenuis)* exposed to stress level of tributyltin NOSC- TI-1040or AD-AL162-445-1. Nat. Tech.. Inform. Sevice. Spring field, VA. 17 pp.

Newell, R.C. (1973). *Am. Zool., 13, 513- 528*.

Nisbet, R., M., Gurney, W. S. C., Murdoch, W. W., Mc. Cauley, E., (1989). Streucturefd population models : a tool for linking effects at the individual and population level. *Biol. J. linn. Soc*. 37 (1-2), 83-100.

Noble, R.G., Pretorius, W.A. & Chutter, F.M. (1971). Biological aspects of water pollution. *S. Afr. J. Sci*. 67:132-136.

O` Hara. J. (1971). Alteration in oxygen consumption by Blue gill exposed to sublethal treatment with copper. *Water Res. 5 : 321 – 327.*

Oeko test (2000) Sondermull i. m. Haus. Oko-test Magazin 5/2000: 74-79.

Phillips, D.J.H. and Rainbow, P.S. (1994). Biomonitoring of trace aquatic contaminants. *Chapman & Hall, London: 86-97.*

Piver, W.T. (1973). Organotin compounds: Industrial application and biological investigation. *Environ. Health prospect. 4 : 61-79.* Publishers, *Boca Raton*: 21.

Rabito I. S., J. R. M. Alvescosta, H. C. Silva de Assis, E. Pelletier, F. M. Akaishi, A. Anjos, M. A. F. Randi, and C. A. Oliveira Ribeiro (2005). Effects of dietary P. B. II and Tributyltin on neotropical fish, *Hoplias malabaricus*; histopathological and biochemical findings. Ecotoxicol. And Environ. Saftey. 60: 147-156.

Rainbow, P.S. and R. Dallinger, (1993). Metal uptake, regulation, and excretion in freshwater invertebrates. In: *Ecotoxicology of metals in*

invertebrates, (Eds.) Dallinger, R. & Rainbow, P.S. Lewis Publishers, Boca Raton: 121.

Ristema, R. (1994). Dissolved bitultin in marine water of the Netherlands three years after the ban. *Appl. Organomet. Chem*. 8: 5- 10.

Richard Saint Louis and Emilien Pelletier (2004). Sea–to-air flax of contaminants via bubbles bursting. An experimental approach for tributyltin. *Mar. Chem*. 84: 211-224.

Roberts, M. H. (1987). Acute toxicity of TBTCl. to embryos and larvae of two bivalve mollusc *Crassostrea, virginica and Mercenaria mercenaria*. *Bull. Environ. Centam. Toxicol.* 39: 1012-1019.

Sadiki, A.I. and D.T. Williams, (1999). A study on organotin levels in Canadian drinking water distributed through PVC pipes. *Chemosphere* 38(7): 1541-1548.

Salazar, M.H. and S.M. Salazar. (1992). Mussel field studies: Mortality, growth, and bioaccumulation. In:Tributyltin - Environmental Fate and Effects, Part III. M.A. Champ and P.F. Seligman, Eds. Elsevier.

Salazar, M.H. and S.M. Salazar. (1995). In situ bioassays using transplanted mussels: I. Estimatingchemical exposure and bioeffects with bioaccumulation and growth. In: Environmental Toxicology andRisk Assessment, 3rd. Vol. J.S. Hughes, G.R. Biddinger and E. Mones (Eds.). ASTM STP 1218. American Society for Testing and Materials, Philadelphia, PA. pp. 216-241.

Sanders, M.J. (1997). *A field evaluation of the freshwater river crab, Potamonautes warreni* as a bioaccumulative indicator of metal pollution, *M.Sc. Thesis, Rand Afrikaans University, South Africa, pp.1-288.*

Sarojini, B. Indira and R.Nagabhushnam (1989): Effect of antifouling organometallic compounds on oxygen consumption at lethal and sublethal

levels on the prawns, *Cardina weberi. I. J. INV. Zool. & AQUA. BIOL.* 1: 68 – 72.

Selwyn M.J. (1976). Triorganotin compounds as inopheres and inhibitors of ion-translocating ATPase. In: Organotin compound: New chemistry and applications Zuckrman J.J. (ed.) American Chemical society Washington D.C. 204-226.

Sies H (1991). Oxidative stress: from basic research to clinical application. *Am J Med 91(3C):31S-38s.*

Sindermann, C.J. (1996). Ocean pollution and shellfish diseases. In: Sindermann CJ (ed) Ocean pollution. Effects on living resources and humans. *CRC Press, Boca Raton, FL, p 63-82*

Singh, S.R. and Singh, B.R. (1979). Changes in oxygen consumption of siluroid fish, *Mysius vittatus* put to different concentrations of some heavy metal salts. *Ind. J. Exp. Biol. 17 : 274 – 276.*

Shah, D. S. M., Rajaramani, V, Sathiyapriya, R.C. and Sathick, O. (2001). Effect of TBTO on lipid metabolism in earthworm edible blood clam, Anadara rhombea (Born) *J. Envir. Pollut*. 8 (1): 7-11.

Shivakumar, R. and M. David, (2007). Toxicity of endosulfan and its impact on the rate of oxygen uptake in the freshwater fish, *Catla catla. J. Ecobiol.* 20(1) 79-84.

Sonavane, S.M. and Lomte, V.S. (2000). Effect of heavy metal CuSo4 and HgCl2 on oxygen consumption of fresh water bivalve, *Lamellidens marginalis Symp.Environ. Issu. And Sustain. Develop. Abst.2. p. (5-6)*

Sprague, J.B. (1970). Measurement of Pollution toxicity of fish II utilizing and applying bioassay results. Wat. Res. 4, 3. 32

Sprague, J.B. (1971). Measurement of Pollution toxicity of fish III sublethal effects of and safe concentrations. *Wat. Res*. 5: 245-266.

Stab, J.A., Frenay, M, Frericks, I.L., Brinkman, UAT, Cofino, W. P. (1995). Survey of nine organotin compounds in the Netherlands using the zebra mussel, *Dreissena polymorpha* as Biomonitor. Environ. Toxicol. Chem. 14: 2023 – 2032.

Storey, K.B. (1996). Oxidative stress: animal adaptations in nature. *Bras J Med Biol Res 29:1715-1733.*

Stringer, R., Labunska, I., Santillo, D., Johnston, P., Siddorn, J. and Stephenson, A. (2000). Concentration of pthalate esters and identifications of additives in PVC children toys. *Enviornmental science and pollution research.* 7 (1): 27-36.

Sultana, R., and Uma Devi, V. (1995). Oxygen consumption in cat fish, Mystus gulio (Ham 1, exposed to heavy metals. J. Environ. Biol.,; 16 (3): 207-210.

Tas, J.W., Keizer, A., Opperhuizen, A. (1996). Bioaccumalation and lethal body burden of four triorganotin compounds. *Bull. Environ. Contam. Toxicol*. 57: 146- 154.

Takahashi, S., Tanabe, S., Takeuchi, I., Miyazaki, N., (1999). Distribution and specific bioaccumulation of butyltin compounds in a marine ecosystem. *Arch. Environ. Contam. Toxicol. 37, 50–61.*

Temmink, J. and Everts, J.W. (1987). Comparative toxicity of tributyltin oxide for fish and snail. In prociding of Seventh World Meeting of the ORTEP- Association. Amsterdem, 7-8 May, 1987, *vissingen-Oost, The Netherlands, ORTEP-Association*,6-20.

Tessier, A. and Campbell, P.G.C. (1990). Partitioning of trace metals in responses of marine biota to pollutants. Editor, Vernberg, F. J. Calabrese, A. Thurberg F. P. and Vernberg N. B., *Academic press, New York.*

Thain, J.E. (1983). The acute toxicity of bis(tributyltin) oxide to the adults and larvae of some marine organism. Copenhagen. International counseling for the exploration of the sea (ICES). 5, report no. C,M, 1983/E.13.

Thain, J. E. (1986). Toxicity of TBT to bivalves ; effectgs on reproduction growth and survival in : Oceans 86, vol. 4 Proc. IntNat. Symp.. Mar. Technol. Soc. Washington, D. C. Pp 1306-1313.

Thurnberg, F. P. and Dawson, M. A. and Collier, R. S. (1973). Effect of copper and cadmium on osmoregulation and oxygen consumption on two species of estuarine crab. *Mar. Biol.* 23: 171- 175.

Thurnberg, F. P. A., Calenbrese, E., Gould R. A., Grieg, M. A., Dawson, and Tucker, R. K. (1980). The use of physiological techinique in monitoring pollution. A consideration of its problems and current research. *Rapp. P. V. Reum. Cons. Ind. Explo. Mer.* 179: 82-87.

Tim Verslycke, Jordy Vercauteren, Christophe Devos, Luc Moens, Pat Sandra, Colin R. Janssen (2003). Cellular energy allocation in the estuarine mysid shrimp *Neomysis integer* (Crustacea: Mysidacea) following tributyltin exposure. *Journal of Experimental Marine Biology and Ecology* 288 (2003) 167– 179.

Umadevi, V. (1996). Changes in oxygen consumption and biochemical composition of the marine fouling dressinid bivalve *Mytilopsis sallei* (Recluz) exposed to mercury. *Ecotoxicol. Environ. Saf.* 39 (3): 168-174.

Uren, S. C. (1983). Acute toxicity of bis tributyltin oxide to a marine copepod. *Mar. Pollut. Bull*, 14: 303 – 306.

Van Der Oost, R., Eyer, J. and Vermeulen, N.P.E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Chem.*, ***13: 57-149*.**

Van der Naald, W. and Thorpe B.G. (1998). PVC Plastic: A looming waste crisis. *Greenpeace International*. ISBN. 90-73361-44-3.

Van Ginneken, L., Chowdhury, M.J. & Blust, R. (1999). Bioavailability of cadmium and zinc to the common carp, Cyprinus carpio, in complexing environments: A test for the validity of the free ion activity model. *Environ. Toxicol. Chem.,* 18(10): 2295-2304.

Verrengia, Guerrero, N.R., Taylor, M.G., Davies, N.A., Lawrence, M.A.M., Edwards, P.A., Simkiss, K. and Wider, E.A. (2002). Evidence of differences in the biotransformation of organic contaminants in three species of freshwater invertebrates. *Environ. Pollut.*, *117: 523-530*. Videla LA,

Vosloo A., W. J. Aardt and L. G. Mienie (2002). Sublethal effects ofcopper on the freshwater crab, *Potamonauts warreni. Comp. Biochem. Physiol. Part A,* 133, 695 – 702.

Wade T.L. B. Garcia – Romero and J. Brooks (1988). Tributylytin contamination in bivalves from united states coastal esturies. Environ. Sci. Technol. 22: 1488-1493.

Waidwood, K.G. and P.H. Johnsen, (1974): Oxygen consumption and activity of the white sucker *Catestomus commersoni* in lethal and sublethal of the organochloride insectide, Methoxychlor. *Water res, 8: 401*.

Walsh, G.E. (1986). Organotin toxicity studies conducted with selected marine organisms at EPA's environmental research laboratory, Gulf Breeze, Florida. In proceedings of the organotin symposium ocean'86 conference, Washington, Dc., USA, 23-25 September, 1986, New York, The institute of Electrical and Electronic Engineer's Inc., 4,1210-1212.

Waldock, M.J., M.E. Waite, J.E. Thain, and V. Hart. (1992). Improvements in bioindicator performance in UK estuaries following the control of the use of antifouling paints. International Council for Exploration ofthe Sea, CM1992/E:32.

Wang, Y. and Evans, R.D. (1993). Influence of calcium concentrations on cadmium uptake by the freshwate r mussel Elliptio complanata. *Can. J. Fish. Aquat. Sci.*, *50: 2591-2596.*

Wittmann, G.T.W. and Förstner, U. (1977). Heavy metal enrichment of mine drainage. IV. The Orange Freestate Goldfield*. S. Afr. J. Sci.* 73:374-378.

Widdows, J. and D.S. Page, (1993). Effects of tributyltin and dibutyltin on the physiological energetics of themussel, *Mytilus edulis*. *Mar. Environ. Res*. 35:233-249.

Wright, D.A. (1995). Trace metal and major ion interactions in aquatic animals. *Mar. Pollut. Bull.*, 31(1-3): 8-18.

Wulf, R.G., and Byington, K.H. (1975). On the structure-activity relationship of organotin induced no-energy dependent swelling of liver mitochondrial arch. *Biochem. Biophys.* 167:176-185.