1. INTRODUCTION

Rapid industrialization, technological flaws and human negligence have led to untreated discharges mainly from the thermal power and desalination plants as well as from sewage to enter various water bodies. These discharges have been found to cause deleterious effects to the marine environment (Bu Olayan and Thomas, 2004). The increasing expansion of agricultural and industrial activities without proper environmental safeguard can have a severe impact on the environment. The increase in industrial and agricultural activities has hastened the mobilization of numerous chemicals into the environment (UNEP, 1980). Chemicals are among the chief pollutants of the environment and unless their effects are carefully monitored and their use carefully controlled, they can result in serious environmental and adverse health effects (WCED, 1987). Biocides, when discharged in sea water are suspected to alter the hydrological variables and cause synergic toxicity on marine organisms (Marking, 1985; Wayne and Ming, 1998).

Marine environment worldwide is subjected to human impacts on biological and mineral resources, removal and alteration of coastal and sea bed structures. Enormous amounts of industrial and domestic wastes are being continuously dumped into the sea, posing constant threat to marine life. The unceasing flow of sewage and industrial waste along the coastline is destroying the biological communities. A large number of unwanted substances reach to the fresh water systems along with agricultural runoff. The agricultural runoff carries pollutants, including pesticides and chemical nutrients (Gartenstein *et al.*, 2006).

Pesticides play an important role in damaging fresh water ecosystems. Pesticides also reach the water bodies either by direct application or along spray drifts, rain water, sewage and industrial effluents. Water is one of the primary ways pesticides are transmitted from an applied area to other locations in the environment. In addition to the direct impact of pesticides on aquatic life, bioaccumulation of contaminants through the food chain in an organism is another important factor to be considered. Chemicals enter aquatic systems via various pathways and in many cases; the aquatic organisms are exposed to repeated pulses or fluctuating concentrations (Handy, 1940; Reinert *et al.*, 2002).

1.1 Pesticides

Pesticides are substances that are manufactured, sold or used as a means of directly or indirectly controlling, preventing, destroying, mitigating, attracting or repelling any pest or altering the growth, development or characteristics of any plant that is not a pest (Salau, 1993). Pesticides are vitally important for increasing or protecting the quality and quantity of food, commodities, building materials, clothing and ornamentals in improving animal health and in combating diseases transmitted to man. Pesticides constitute an important component in agriculture development and protection of public health. There are about 20 major diseases which have been brought under control by the use of pesticides (Yadawe *et al.*, 2010).

Pesticides are natural or synthetic compounds that are poisonous and can kill pests including insects and rodents. Pesticide use in India dates back to the year 1948 when Dichloro Diphenyl Trichloroethane (DDT) and Benzene Hexa Chloride (BHC) were imported for malaria and locust control. Pesticides are mainly made up of minerals or plants and they are highly toxic to mammals and had long term residual effects, easily causing environmental pollution. The term pesticide covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others (Ecobichon, 1996).

The first known pesticide was elemental sulfur dusting used in Samaria about 4500 years ago. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crops to kill pests. Nicotine sulfate, extracted from tobacco leaves was being used as an insecticide in the 17th century. Two more natural pesticides, pyrethrum which is derived from chrysanthemums and rotenonem, derived from the roots of tropical vegetables, were introduced in the 19th century. However, pesticide contamination of an ecosystem is a relatively recent phenomenon. Synthetic chemical pesticides were hardly used before World War II. During the war period, pesticides began to be used for warfare. Organophosphates were developed as nerve gases, and the herbicides 2-4-D was used to kill the Japanese rice crop (Hayes and Laws, 1991).

1.1.1 Classification of pesticides

There are various ways by which pesticides are classified, according to four distinctive functions, namely, stomach poisoning, contact poisoning, fumigation and systemic action. Pesticides can also be classified by their chemical nature. Organochlorine pesticides are the synthetic organic pesticides that are earliest discovered and used. Their characteristics are broad spectrum, long residual effect and relatively low toxicity (Hu *et al.,* 2010) . However, due to their stable chemical nature, they are hard to break down in the natural environment. Prolonged use in large quantities will easily lead to environmental pollution and accumulation in mammals, resulting in cumulative poisoning or damage. Organochlorine pesticides are therefore replaced by other pesticides. These compounds could pollute the tissues of every life form on earth, the air, the lakes and the oceans (Hurley *et al.*, 1998). Certain environmental hazardous chemicals, including pesticides termed as endocrine disruptors in the body, their long term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Brouwer *et al.*, 1999; Crisp *et al.*, 1998; Hurley *et al.*, 1998).

Organophosphate pesticides are characterized by their multiple functions and the capacity of controlling a broad spectrum of pests. They are nerve poisons that can be used not only as stomach poisons but also as contact poison and fumigant. These pesticides are biodegradable, cause minimum environmental pollution and slow pest resistance. Synthetic organic pesticides are used to control weeds, insects and other organisms in a wide variety of agricultural and non-agricultural settings (Co.oper and Dobson 2007). An organophosphate is the general name for esters of phosphoric acid. Phosphates are probably the most pervasive organophosphorous compounds. Many of the most important biochemical are organophosphates, including DNA and RNA as well as many cofactors that are essential for life. Organophosphates are also the basis of many insecticides, herbicides and nerve gases. Organophosphates are widely used as solvents plasticizers and extreme pressure additives. Organophosphates are some of the most widely used pesticide in the world. They are used in homes, agriculture,gardens and veterinary practices. They are not persistent in the environment as they break down quickly. Because of their relatively fast rate of degradation, they have been a suitable replacement for the more persistent organochlorines (Chawla *et al.,* 1977).

Carbamate pesticides also work on the same principle as organophosphate pesticides by affecting the transmission of nerve signals resulting in the death of the pest by poisoning. They can be used as stomach and contact poisons as well as fumigant. Their molecular structures are largely similar to that of natural organic substances, and they can be degraded easily in a natural manner with minimum environmental pollution. Synthetic pyrethroid pesticides are a pesticide synthesized by imitating the structure of natural pyrethrins. They are more stable with long residual effects than natural pyrethrins. Synthetic pyrethroids are highly toxic to insects but only slightly toxic to mammals (Lahr *et al.,* 2000).

Pyrethroids are a group of man-made pesticides similar to the natural pesticide pyrethrum. Pyrethroids are found in many commercial products used to control insects, including household insecticides, pet sprays and shampoos. Some pyrethroids are also used as a lice treatment applied directly to the head and as mosquito repellents that can be applied to clothing. Pyrethroides after spraying, settle onto the ground and flat surfaces. Because pyrethroids are mixed with water or oil before being applied, the amount of residue left on the surface is very small. Pyrethroids are broken down by sunlight and other chemicals in the atmosphere. Often they last one or two days in an environment (Fishel, 2005).

Two classes of pesticides which have been widely used in residential settings are organophosphate and pyrethroid pesticides (Landrigan *et al.*, 1999). With the withdrawal of the organophosphates, pyrethroids are being used increasingly to control pests indoors (Adgate *et al.*, 2000). Synthetic pyrethroids have insecticidal properties similar to the botanical pesticides known as pyrethrins. However, pyrethroids are more persistent in the environment than the naturally occurring pyrithrins and are therefore used indoors as well as in agricultural applications (Todd *et al.*, 2003).

1.1.2 Pesticides in India

The production of pesticide started in India in 1952 with the establishment of a plant for the production of BHC near Calcultta and India is now the second largest manufacturer of pesticides in Asia after China and ranks twelth globally (Wasim Aktar *et al.*, 2009). There has been a steady growth in the production of technical grade pesticides in India, from 5000 metric tons in 1958 to 102240 metric tons in 1998. In 1996 – 97, the demands for pesticides in terms of value was estimated to be around Rs.22 billion, which is about 2% of the total world market (Mathur, 1999). In India, 76% of the pesticides are used as insecticide against 44% globally. The main use of pesticides in India is for cotton crops (45%) followed by paddy and wheat (Wasim Aktar *et al*., 2009). In India, there are 165 pesticides registered for use and there is a sequential rise in the production and consumption of pesticides during the last three decades. During 2003 – 2004, the domestic production of pesticides was approximately 85TMT and about 60 TMT used annually (Anon., 2005), against 182.5 million hectares of land where 70% accounts for DDTs, HCHs and organophosphate pesticides (Bhattacharyya *et al.*, 2009; Nirula and Upadhyay, 2010).

1.1.3 Benefits of pesticides

Pesticide use in developing countries must continue to expand in order to maintain agricultural productivity and to permit farmers to reap the benefits of related agricultural investments. The increased use of agrochemicals was largely responsible for the great increase in food production in India in the last decade. The increase in food production correlates the rate of pesticide use (IAEA, 1982). In tropical countries such as India, when agricultural crops are grown without protection, yields are always reduced. Modern agricultural methods, including the use of high yielding plant varieties, more intensive cultivation, greater use of fertilizer and intensified irrigation practices require increased use of pesticides (Dhanaraj and Ravasaheb, 2012).

Tremendous benefits have been derived from the use of pesticides in forestry, public health and agriculture. Increases in productivity have been due to several factors, includingthe use of fertilizer, better varieties and use of machinery. Pesticides have been an integral part of the process by reducing losses from the weed, diseases and insect pests can reduce the amount of harvestable produce (Wasim Aktar *et al.*, 2009). Webster *et al.* (1999) stated that considerable economic losses would be suffered without pesticide use. In the environment, most pesticides undergo photochemical transformation to produce metabolites which are relatively non-toxic to both human being and the environment (Kole *et al.*, 2002). Pesticides have been an integral part of the process by reducing losses from the weeds, diseases and insect pests that can markedly reduce the amount of harvestable produce (Wasim and Paramasivam, 2008). Most of the pesticides, in environment, undergo photochemical transformation to produce metabolites which are relatively non-toxic to human beings as well as the environment (Kole *et al.*, 1999).

Vector-borne diseases are not most effectively tackled by killing the vectors. Insecticides are often the only practical way to control the insects that spread deadly diseases (Ross, 2005; Townson *et al.,* 2005). The transport sector makes extensive use of pesticides, particularly herbicides. Herbicides and insecticides are used to maintain the turf on sports, pitches, cricket grounds and golf courses. Insecticides protect buildings and other wooden structures from damage by termites and wood boring insects (Mathur, 1999).

1.1.4 Hazards of pesticides

If the credits of pesticides include enhanced economic potential in terms of increased production of food and fibre and amelioration of vector borne diseases, then their debts have resulted in serious health implications to man and his environment. Now there is overwhelming evidence that some of these chemicals do pose a potential risk to humans and other life forms and unwanted side effects to the environment (Forget, 1993; Igbedioh, 1991; Jeyaratnam, 1985). No segment of the population completely protects against exposure to pesticides and by high risk groups in each country (WHO, 1990). The worldwide deaths and chronic diseases due to pesticide poisoning is about 1 million per year (Environews Forum, 1999).

In India, the first report of poisoning due to pesticides was from Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with parathion (Karunakaran, 1958). In a multi-centric study to assess the pesticide residues in selected food commodities collected from different stages of the countries (Surveillance and Food Contaminants in India, 1993), DDT residues found in about 82% of the 2205 samples of bovine milk collected from 12 stages. The average daily intake of HCH and DDT by Indians was reported to be 115 and 48 mg per person respectively, which were higher than those observed in most of the developed countries (Kannan *et al.*, 1992).

Pesticides can contaminate soil, water, turf and other vegetation. Moreover, it will kill the insects or weeds and it can be toxic to a host of other organisms, including birds, fish, beneficial insects and non-target plants (Wasim and Paramasivam, 2008). Pesticides can reach surface water through runoff from treated plants and soil. Pesticides were found in all samples from major rivers with mixed agricultural and urban streams (Bortleson and Davis, 1987, 1995). Groundwater pollution due to pesticides is a worldwide problem. Once groundwater is polluted with toxic chemicals, it may take many years for contamination to dissipate or be cleaned up (Waskom, 1994).

Pesticide use in agriculture and the value of negative externalities are well documented in Sri Lanka (Van Der Hock *et al.*, 1998;), Lebanon (Salameh *et al.*, 2004), India (Gupta, 2004), China (Huang *et al.*, 2001), Bangladesh (Rehman, 2003), Philippines (Rola and Pingali, 1993), Mali (Ajayi, 2002), Ecuador (Yanggen *et al.*, 2003), Zimbabwe (Maumbe and Swinton, 2003) and Vietnam (Dung and Dung, 1999). Misuse and overuse of pesticides was often observed in the developing countries (Warburton *et al.*, 1995; Heong *et al.*, 1995; Yedulman, 1998; Pimental *et al.*, 1992, 2009; Rola and Pingalli, 1993).Heavy treatment of soil with pesticides can cause populations of beneficial soil micro-organisms to decline. Indiscriminate use of chemicals might work for a few years, but after a while there are not enough beneficial soil organisms to hold on to the nutrients (Savonen, 1997).

The environment, including water ecosystems is very often contaminated by low concentrations of various chemical compounds of foreign origin (Benova *et al.*, 2007). The foreign substances in low concentrations can interact with some physical factors. The effects can occur atsome time and their sub-acute or chronic effects can be expected (Dvorak and Benova, 2002; Benova *et al.*, 2006). Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man. Unfortunately, this is not so the controversy of use and abuse of pesticides has surfaced (Bhatnagar, 2001). Pesticide usage is excessive in homes (Landrigan *et al.*, 1999) and an often time includes the use of prohibited or restricted use pesticides (Adgate *et al.*, 2000; Surgan *et al.*, 2002). Adverse health effects associated with pesticide exposure from residential use include altered fetal growth from prenatal exposure (Berkowitz *et al.*, 2004 ; Whyatt *et al.*, 2004), childhood cancer (Buckley *et al.*, 2000; Daniels *et al.*, 2001; Flower *et al.*, 2004) and asthma (Salam *et al.*, 2004).

A large number of studies on the effects of pesticides on different aspects of human health such as on dermatological neurological, behavioural, reproductive and developmental systems, leukemia, solid tumours and other cancers, non-hodgkins lymphoma, genotoxicity, hormone – disrupting effects and effects on children have been provided (Solomon *et al.*, 2000; OCFP, 2004). Exposure to certain pesticides, especially the herbicide 2, 4-D was found to greatly increase the risk of Non-Hodgkin's Lymphoma (NHL) among the occupationally exposed individuals (Gupta, 2008). Synthetic pyrethroids and organophosphates were found to lead to chromosomal aberrations. Incidence of intermediate syndrome in cases of OP poisoning has also been reported (Shailesh *et al.*, 1994; Samuel *et al.*, 1995). There were a number of reports from northern India on the abuse of aluminium phosphide, a grain preservative taken for self-poisoning (Saraswat *et al.*, 1985; Singh *et al.*, 1985; Raman *et al.*, 1991).

Besides the harmful effects on human health, pesticide contamination of ecosystems leading to mortality of wildlife such as reptiles, amphibians, fishes and invertebrates and manifestation of toxic effects in their morphology, physiology and biochemistry, enzyme activity have also been documented the world over (Materna *et al.*, 1995; Pantani *et al.*, 1997; Viran *et al.*, 2003; Koprucu and Aydin, 2004 ; Ural and Saglam, 2005; Khana and Law, 2005; Johansson *et al.*, 2006; Hii *et al.*, 2007; Ezemonye and Ilechi, 2007; Iannacone *et al.*, 2007; Kim *et al.*, 2008) including India (Vutukuru *et al.*, 2007; Gurushankara *et al.*, 2007) and its North-eastern region (Dey and Gupta, 2002).

1.2 Ecotoxicology

Ecotoxicology is the study about the effect of contaminant in environment, in molecule, physiology, individual and ecological level (Kadariae *et al.*, 2012). The purpose of ecotoxicology is to determine and predict the effect of contaminant (Beketov and Liess, 2012). Ecotoxicity parameters which used in ecotoxicology test exposure consisting of lethality, growth ability, respiration rate etc. (Soupilas *et al.*, 2008). Ecotoxicology can be classified such as the agents (elements and compounds), the level of biological organization (organism, population or ecosystem), the duration of exposure (acute, sub-acute or chronic) and the consequences (lethal or sub-lethal) (Moiseenko, 2008). Toxicity test is used to predict the effect of chemical on organism and to compare sensitivity of one or more species (Wei *et al.*, 2006). Toxicity test is needed to do as an implementation of regulation. Toxicity test is also an acceptable base on ease and cost (Hellou, 2011).

Organisms have different manner to response the stressors in an environment. Ecological risk assessment requires an appreciation for complexity and the function of the subunit within structure (Preston, 2002). Zooplanktons are organisms that have high sensitivity to contaminant (Pereira *et al.*, 2007). The organism which was used in this study was *Artemia,* it is an invertebrate organisms of saline aquatic and marine ecosystems (Para *et al.*, 2001), they representatively brine organisms where waste water of fishery industries was thrown.

1.3 Artemia – Morphology and life cycle

Anostracan branchiopod *Artemia* are widely distributed throughout the world (Persoone and Sorgeloos, 1980). In its natural environment at certain moments *Artemia* produces cysts that float at the water surface and are thrown ashore by wind and waves. These cysts are metabolically inactive and do not further develop as long as they are kept dry. Resting stages are specialized for persisting during adverse environmental conditions and for hatching when environmental conditions improve (Dodson and Frey, 2001). When immersed in seawater, the biconcave shaped cysts hydrate, became spherical and within the shell, the embryo resumes its interrupted metabolism.

Resting stages of many branchiopods can become active when exogenous stimuli such as light, rainfall, temperature, oxygen concentration and salinity become favourable (Ivanovski *et al.*, 1981; Van der Linden *et al.*, 1986; Drinkwater and Clegg, 1991; Torrentera, 1993; Triantaphyllidis *et al.*, 1995). After about 20h, the outer membrane of the cyst bursts and the embryo appears surrounded by the hatching membrane. When the embryo hangs underneath the empty shell, the development of the nauplius is completed and within a short period of time, the hatching membrane is ruptured and the free swimming nauplius is born. Induction of diapause in *Artemia* may be under maternal control in response to environmental conditions that determine the metabolic state of the mature embryo (Lochead, 1961; Marcus, 1982, 1984; Gajardo and Beardmore, 1989; Drinkwater and Clegg, 1991). The termination of diapause in *Artemia* and the selection of reproductive mode are mainly under environmental control (Voronov, 1971; Vanhaecke *et al.*, 1984; Van der Linden *et al.*, 1986; Lenz, 1987; Triantaphyllidis *et al.*, 1995).

***1.3.1 Artemia* distribution**

The brine shrimp *Artemia* (Branchiopoda, Anostraca) is highly adapted to live in the hypersaline waters of inland salt lakes, coastal lagoons and the evaporation ponds, channels and reservoirs of salterns. Since the initial record of 80 *Artemia* sites by Abonyi in 1915, the number has steadily increased and the branchiopod crustacean *Artemia* has been reported in more than 600 coastal locations and inland waters around the world (Van Stappen, 2002).

The genus *Artemia* comprises a group of seven bisexual species and a variety of parthenogenetic strains of diverse ploidy. In the Mediterranean basin, a bisexual species A. salina (formerly *A. tunisiana*) occurs together with a large number of parthenogenetic populations, one diploid and another tetraploid (Amat *et al.*, 2005). The other old world bisexual species are distributed in Asia, with *A. urmiana* (Gunther, 1980) from Iran, *A sinica* (Cai, 1987) from P. R. China, *A. tibetiana*

(Abatzopoulous *et al.*, 1998; Abatzopoulos *et al.*, 2002) from Tibet and *Artemia* sp. from Kazakhstan (Pilla and Beardmore, 1994). The bisexual *A. persimillis* (Piccinelli and Prosdocimi, 1968), Argentina and Chille and *A. franciscana* (Kellogg, 1906), North Central and South America are endemic to the new world.

Amat *et al.* (2005) reported that the American brine shrimp *A. franciscana* is an invasive species, because of its high biological productivity has been introduced and cultured in several African, European,Asian and Australian locations so a number of habitats have been invaded by *A.franciscana* (Zheng, *et al.,* 2004; Abatzopoulos *et al.,* 2006; Mura *et al.,* 2006; Ruebhart,*et al.,* 2008; Vikas *et al.,* 2012; Ben Naceur *et al.,* 2012b; Salman *et al.,* 2012; Pinto *et al.,* 2013; Zheng and Sun,2013). A.franciscana in the hypersaline ecosystems in the Mediterranean region and replaced the native *Artemia parthenogenetica* and *Artemia salina*. This event was initially seen in Portugan (Amat *et al.*, 2005) and later in France (Thiery and Robert, 1992) in Egypt (Triantaphyllidis *et al.*, 1998) in Italy (Mura *et al.*, 2004) and in Spain and Morocco (Green *et al.*, 2005; Amat *et al.*, 2007).

In India, *Artemia* has been recorded from coastal saltpans Gujarat (Royan, 1979), in the Didwana lake (Alam, 1980 ; Bhargava, 1984) and Sambhar lake (Baid, 1958) in Rajasthan, Gulf of Kutch (Royan, 1979), Great Kann of Kutch (Bowen *et al.*, 1978), Tuticorin area (Royan *et al.*, 1970 ; Achari, 1971; Ramamoorthi and Thangaraj, 1980; Vasudevan, 2012), Veppalodai (Royan *et al.*, 1978), Threspuram, Karapad, Spic Nagar and Pullavalai (Ramanathan and Natarajan, 1987), Thamaraikulam and Puthalam ((John *et al.,* 2004; Vasudevan, 2012) in Kanyakumari.

1.3.2 Habitat of *Artemia*

Artemia, an Anostraca are inhabitants of ephemeral coastal and inland waters. *Artemia* has a wide geographical distribution. The species of this genus possess an uncommon adaptability to extreme conditions, thus being found in environments where other life forms are not sustainable (Triantaphyllidis *et al.*, 1998). The genus *Artemia* is sub divided into six generally recognized bisexual species and a large number of parthenogenetic populations. The different species of the genus *Artemia* present one common characteristic, that is their strong adaptability to hypersaline environments such as permanent salt lakes, coastal lagoons and man-made saltpans where evaporation of seawater results in high sodium chloride concentrations. The brine shrimp are the main zooplanktonic organism inhabiting hypersaline environments worldwide (Triantaphyllidis *et al.*, 1998; Munoz and Pacios, 2010; Green *et al.,* 2013).

Artemia species are extremely osmotolerant and are found in brackish water as well as in supersaturated brine. *Artemia* population are also adapted to widely changeable temperature, to different ionic brine compositions and pH values which they can tolerate vary from neutral to highly saline (Van stapper, 2002). The physicochemical differences influenced their reproductive strategies through ovoviviparous versus cyst production (Camargo *et al.*, 2005) and morphological characters of adult specimens (Gilchrist, 1960; Hontoria and Amat, 1992; Litvinenko *et al.*, 2007; Asem and Rastegar Pouyani, 2008; Ben Naceur *et al.*, 2011a).Species of the brine shrimp, *Artemia* are found in a variety of harsh environments in many parts of the world (Triantaphyllids *et al.*, 1998; Van Stappen, 2002) where they encounter severe hypersalinity, high doses of ultraviolet radiation very low oxygen tensions and extremes of temperature (Persoone *et al.*, 1980; Decleir *et al.*, 1987; Browne *et al.*, 1990; Abatzopoulous *et al.*, 2002).

Several studies have been performed in order to take advantage of high adaptation patterns of *Artemia* concerning nutrients resources. *Artemia* is able to use several nutrient resources such as wheat bran, soy bean meal, rice bran and whey powder (Dobbeleir *et al.*, 1980; Sorgeloos *et al.*, 1980). Other nutrient sources suitable for *Artemia* production are fish pond effluents (Zmora *et al.*, 2002) or inert commercial diets, such as Nestum (Naegel, 1999). Tolerance to extremely variable oxygen concentrations is another common trait to several species of *Artemia* as stated by Amat (1985), which allows species to successfully face environmental adversities under extreme conditions.

The wide distribution of *Artemia* species and strains can also pose several concerns related to the site where cysts and specimens can be collected (Nunes *et al.*, 2006). Artemia cysts are amoung the most resistant animal life forms, surviving extreme environmental stresses including UV radiation, desiccation, thermal extremes and anoxia (clegg, 2005, Kara *et al*., 2004 The cysts and nauplii biometry and nutritional characteristics of *Artemia* may greatly vary from one strain to another and from different geographical regions (Johns *et al.*, 1980; Leger and Sorgeloos, 1984; Van Stappen, 1996).

Hatching characteristics can vary according to the geographic origin of cysts (Vanhaecke and Sorgeloos, 1982, 1983). Different populations of *Artemia* have different levels of tolerance to water temperature and salinity. The variation in the tolerance levels may be further influenced by the genetic make-up of different strains

(Bowen *et al.*, 1978; Abreu-Grobois and Beardmore, 1980, 1982; Abatzopoulos *et al.*, 2003; Baxevanis and Abatzopoulos, 2004). Several culture studies have revealed that the temperature and salinity conditions vary for different strains (Claus *et al.*, 1977; Vanhaecke *et al.*, 1984; Baxevanis *et al.*, 2005; El-Bermawi *et al.*, 2009). It has also been suggested that there is significant interaction between temperature and salinity for survival of *Artemia* (Browne and Wanigasekara, 2000; Baxevanis and Abatzopoulos, 2004; Kappas *et al.*, 2004).Geographic origin of cysts may also influence several measurable parameters in neonates as described by Vanhaecke and Sorgeloos (1980a), volume and diameter of hydrated, untreated and decapsulated cysts, chorion thickness, length, dry weight, ash free weight and volume index of freshly hatched nauplii. The complete knowledge and control of the origin of cysts is highly important for research.

The brine shrimp, *Artemia* is the dominant macrozooplankton present in many hypersaline environments (Wurtzbaugh and Gliwicz, 2001). Their ability to survive and even thrive in forbidding environments has long been of interest to biologists (Clegg and Trotman, 2002; Eads, 2004). This crustacean, properly called an animal extremophile, has been able to survive in such environments through well-developed osmoregulation involving enhanced Na-K-ATPase activity (Holliday *et al.*, 1990). Although physiologically able to survive and reproduce in salinities near and below seawater, *Artemia* is definitely found at salinities below 100 g/L, but the density may be decreased (Persoone and Sorgeloos, 1980; Wurtsbaugh, 1992).

1.3.3 Use of *Artemia*

To optimize the use of the *Artemia* stocks in the market and to diversify the use of natural resources, a variety of different research initiatives were launched, leading to improved techniques for cyst harvesting, processing and storing and for nauplii applications, and exploitation of new natural resources (Lavens and Sorgeloos, 1996 ; Van Stappen, 2002). After harvesting and processing, cysts are available in cans as storable on demand live feed. After a few hours of incubation in seawater, the free swimming nauplii can directly be fed as a nutritious live food source to the larvae of a variety of marine as well as freshwater fish (Sorgeloos *et al.*, 1998, 2001).

Artemia is considered as an irreplaceable live feed for the larval rearing of most marine fish and crustacean larvae (Sorgeloos *et al.*, 2001; Ben Naceur *et al.,* 2012a). The quality of the *Artemia* product differs from strains to strain and from location to location. Also the nutritional value of *Artemia*, especially for marine organisms, is not constant but varies among strains and within batches of each strain, causing unreliable outputs in marine larviculture (Leger *et al.*, 1987).

1.3.4 Characterization of *Artemia*

Full characterization of biological sample is a fundamental aspect in ecotoxicology testing. The possibility of simultaneously using several different strains of *Artemia*, due to contamination, deficient geographical localization of origin or incomplete characterization of cysts samples can lead researchers to erroneous results (Nunes *et al.*, 2006). Responses of distinct nature and magnitude can be found among strains, when studying acute toxicity and reproductive traits of brine shrimp *Artemia*.

Artemia species have been investigated and characterized in the Mediterranean area (Triantaphyllidis *et al.*, 1993, Amat *et al.,* 2007), in France (Scalone and Rabet 2013) while in the south of the Mediterranean basin, where promising *Artemia* sites exist as in Tunisia (Van Ballaer *et al.*, 1987; Aloui, 2003; Ben Naceur *et al.*, 2008,

2013) in Egypt (El-Bermawi, 2003), in Algeria (Kara *et al.*, 2004; Samraoui *et al.*, 2006), in Siberia (Litvinenko and Bayko, 2008), in Portugal (Vieira and Bio 2011, Rodrigues *el al.,* 2013 and in Iran (Abatzopoulos *et al.*, 2006; Asem *et al.*, 2009; Asem *et al.*, 2010). In India, *Artemia* study have been conducted and recorded by Royan (1979), Bhargava (1987), Ramanathan and Natarajan (1987), John *et al.* (2004), Arulvasu and Munusamy (2009) and Vasudevan (2012).

Man has been responsible for several *Artemia* transplantations in South America and Australia, either for aquaculture or for production purpose (Sorgeloos, 1977). A lot of characterization studies were done in *Artemia* species found in new world, Africa, China etc. but *Artemia* populations from India, have not been extensively characterized. Different methods have been used in order to characterize the species of *Artemia*. A study that used morphological characterization of *Artemia* (Gilchrist, 1960) proved that wide differences among populations and between males and females can be found even when the animals are cultured in the same medium, and it is possible to identify each population using body and appendage measurements. Hence a study on the biometrics of cysts and larvae, morphometric and meristic characteristics of adult, survival and growth of the *Artemia* populations was undertaken.

1.3.5 *Artemia* Bioassy

The brine shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity (Carballo *et al.*, 2002; Solis *et al.*, 1993). In most of the literature references deals with Artemia bioassay (Michael *et al.*, 1956; Corner and Sparrow, 1956, 1957; Hood, 1960; Wiseley and Blick, 1967; Tarzwell, 1969; Brown Ahsanullah, 1971; Harwich and Scott, 1971; Zilloux *et al.*, 1973; Prick *et al.*, 1974;

Vanhaecke *et al.*, 1981; Sleet and Brendal, 1983).*Artemia* nauplii and it has been used for the detection of fungal toxins (Harwig and Scott, 1971), plant extract toxicity (Mc Lauglin *et al.*, 1991) Medicinal plants (Mirzaei and Mirzaei 2013, heavy metals (Martinez *et al.*, 1998), cyanobacteria toxins (Jaki *et al.*, 1999), pesticides (Barahona and Sanchez-Fortun, 1991) and cytotoxicity testing of dental materials (Pelka *et al.*, 2000, Milhem, *et al.,* 2008).

The majority of work performed so far involving *Artemia* as test organism in Ecotoxicology is related to the use of cysts (Migliore *et al.*, 1993, 1997) and cyst hatched naupli of *Artemia* (Vanhaecke *et al.*, 1980; 1981; Persoone and Vanhaecke, 1981; Persoone and Wells, 1987). For the past 30 years, the *Artemia* nauplii have been used to detect general toxicity (Persoone and Wells, 1987), in teratology screens (Sleet and Brendel, 1983, 1985; Acey and Tomilson, 1988; Kersetter and Schaffer, 1983) and in ecotoxicology (sorgeloos *et al.*, 1978; Persoone and Wells, 1987). A good relationship has been found with the brine shrimp lethality tests to detect antitumoral compounds in terrestrial plant extracts (Solis *et al.*, 1993; Meyer, 1982; Mackeen *et al.*, 2000). The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts (Sam, 1993; Krishnaraju, 2005). In the past, research has focused on the use of *Artemia* nauplii as test organism for a wide variety of contaminants, such as metals (Sarabia *et al.*, 1998b; Hadjispyrou *et al.*, 2001; Brix *et al.*, 2003), trace elements (Petrucci *et al.*, 1995), S-triazine herbicide (Varo *et al.*, 2002), acrylonitrile (Tong *et al.*, 1996), carbamates (Barahona and Sanchez-Fortun, 1998), toxic cyanobacteria (Vezie *et al.*, 1996; Beattie *et al.*, 2003), endosulfan (Varo *et al.*, 1997), organophosphorous insecticides (Sanchez-Fortun *et al.*, 1996).

Pollutants produced by incineration plants (Knulstand Sodergren, 1994), antifouling compounds (Oka-mura *et al.*, 2000; Hadjispyrou *et al.*, 2001), pharmaceuticals (Touraki *et al.*, 1999; Para *et al.*, 2001), antifouling agents (Panagoula *et al.*, 2002), mycotoxins (Panigraphi, 1993; Hlywka *et al.*, 1997) and organic solvents (Barahona –Gomariz *et al.*, 1994) are also used in *Artemia* nauplii toxicity testing. *Artemia* nauplii have also been used in marine toxicity testing in coastal areas (Wells, 1999; Nipper, 2000) and for the establishment of alternative toxicity tests (Calleja *et al.*, 1994; Para *et al.*, 2001).

Artemia is used to assess the toxicity of phenolic compounds (Guerra, 2001), cyanobacterial blooms (Tothill and Turner, 1996), effect of composts resulting from industrial waste (Kapanen and Itavaara, 2001). Potassium dichromate and Cadmium chloride (Benova *et al.*, 2006), methanolic extracts of *Euphorbia hirta* (Rajeh *et al.*, 2012) and methanol extract of *Calliandra portoricensis* (Ogugu *et al.*, 2012). Various modification of the biotests on *Artemia* species have been used for the acute or rarely chronic testing of a large number of inorganic substances such as cadmium (Hadjispyrou *et al.*, 2001), Strontium (Hadjispyrou *et al.*, 2001), Zinc (Brix *et al.*, 2003), Copper (Browne, 1980, Brix *et al.*, 2006), Arsenic (Brix *et al.*, 2003), Potassium permanganate, Potassium bichromate and Silver nitrate (Boone, 193), Phenolic compounds (Guerra, 2001) and trace elements (Petrucci *et al.,* 1995).

The organic substances tested on *Artemia* were acrylonitrile (Tong *et al.*, 1996), antifouling agents (Okamura *et al.*, 2000), oil dispersants (Zillioux *et al.*, 1973), phorpbol esters (Kinghorn *et al.*, 1977), phthalates (Vanwezel *et al.*, 2000), atropine (Barahona and Sanchez. Fortun, 1998), anesthetics (Robinson *et al.*, 1965), antihelminthes (Oliveirta-Filho and Paumgartten, 2000), herbicides, insecticides pesticides (Varo *et al.*, 1997, 2002), mycotoxins (Schmidt, 1985; Panigrahi, 1993; Hlywka *et al.*, 1997), pharmaceuticals (Touraki *et al.*, 1999; Parra *et al.*, 2001), pollutants (Knulst and Sodergren, 1994), opiates (Richter and Goldsten, 1970), various plant extracts (Caceres *et al.*, 1998) or toxins (Granade *et al.*, 1976; Vezie *et al.*, 1996; Beattie *et al.*, 2003).

The tests on the genus *Artemia* have also been used to specify biological effects of some physical factors such as ionizing radiation (Grosch and Erdman, 1955; Easter and Hutchinson, 1961) gamma radiation (Dvorak and Benova, 2002), radionuclide (Boroughs *et al.*, 1958) or UV (Dattilo *et al.*, 2005). The effects of cosmic irradiation on cysts of *Artemia franciscana* were studied by Ruther *et al*(1974), Graul *et al* (1975) and Heinrich (1977). Crustaceans like crabs and shrimps are of commercial importance and have a high economic value and hence, they could not be sacrificed in large quantities for laboratory analysis (Bu Olayan and Thomas, 2006). Therefore a representative of crustacean like brine shrimp, *Artemia sp* was chosen for the present study.

Artemia is one of the most striking examples of organisms well adapted to laboratory practice, as long as a strict control over laboratory producers and methodologies is maintained (Nunes *et al.*, 2006). Its use as test organism is representative of the effort to reduce the scale of test organisms, with concomitant reduction in test volumes, amount of produced waste, and space needed to perform testing protocols (Blaise, 1998). *Artemia sp*. was chosen for the present study since it could be easily cultured in the laboratory (Sorgeloos *et al.*, 1977), has a short life cycle period and are found receptive to wide range of salinity and any pollutant in their system (Vanhaecke and Persoone, 1984; Varo *et al.*, 2000).

1.4 OBJECTIVES

The present study was under taken with the following objectives

1. To study the different morphometric and meristic characteristics of *Artemia* species.
2. To find out the median lethal concentration (LC50) values of Organochlorines (Lindane, Endosulfan), Organophosphates (Monocrotophos, Malathion), pyrethroids (Cypermethrin, Fenvalerate) and Carbamate (Carbaryl, Carbofuran) pesticides.
3. To identify the life stages of *Artemia* those are more sensitive to OCs, OPs, pyrethroid and carbamate.
4. To study the effect of sublethal concentration of pesticides on *Artemia* species.
5. To assess the acute and Chronic toxicity effect of OC, OP, pyrethroid and Carbamate on the life history and reproductive characteristics of *Artemia* species.