INTRODUCTION

Man-made toxic chemicals are released into the environment during production, transportation as well as utilization, and thus pose a threat to living biota. Pollution is the unfavourable alteration of our environment, largely because of human activities. Environmental pollution, especially water pollution, has been increasing at an alarming rate due to rapid industrialization, civilization and green revolution (Braunbeck, 1994).

Pollutants are substances, which cause pollution. They may be the substances that occur in nature or unnatural substances released into the environment by human handiwork—ego pesticides and herbicides (Kurian, 1997). Aquatic toxicology has been defined as the study of the effects of chemicals and other toxic agents on aquatic organisms with special emphasis on adverse or harmful effects. Chemical contaminants are ubiquitous in nature and a major goal of ecologists has been to understand and predict their impacts on natural communities (Relyea and Hoverman, 2008). The excess amounts of these pesticides and chemicals produce unwanted and unwarranted residues, which pose a great threat to aquatic organisms (Landis and Yu, 2004; Ramasamy et al., 2007). In the present study, toxicity studies of three different, commonly available chemicals, an insecticide, chlorpyrifos, a surfactant alkylbenzene sulphonate
(LAS) and a disinfectant sodium hypochlorite were made against the test animal *Bradinopyga geminata*.

Chlorpyrifos is the largest market-selling and multipurpose organophosphorus insecticide widely used for urban and domestic pest control, including turf maintenance, and as a termiticidal barrier in, around or under buildings. Agricultural uses include cotton, sugarcane, vegetables, cereals, canola, rice, pome fruit, stone fruit, citrus, tropical fruit and grapes. Chlorpyrifos is widely used around the world and has been very well studied.

Organophosphorous insecticides exert their effects by inhibiting the activity of an enzyme known as acetylcholinesterase that is important in the transmission of nerve impulses. Chlorpyrifos belongs to a group of organophosphorous compounds known as the phosphorothioates that do not inhibit acetylcholinesterase directly. Recent findings demonstrate substantial differences among species in terms of water permeability and chlorpyrifos (an organophosphate insecticide) uptake rates. Highly water-permeable insects (dissolved oxygen breathers) have higher chlorpyrifos uptake rates than slightly water-permeable insects (air breathers) (Saouter *et al*., 1991).

Considerable amounts of cleansing materials (surfactants) used in domestic and industrial domains are directly discharged into waterways and on land. Surfactants are amphipathic compounds consisting of both a hydrophobic region (alkyl chains of various lengths, like alkylphenyl ethers and alkylbenzenes) and a hydrophilic region (carboxyl, sulphate, sulphonate, and phosphates) (Cserhati *et al*., 2002). These may pose environmental problems in the ecosystem including
toxicity of the surfactants to fish and invertebrates, foaming and eutrophication (Abel, 2006).

Linear Alkylbenzene Sulphonate (LAS) is the most widely used anionic surfactant in household and cleaning products (Schleheck et al. 2000). Several authors have reported that anionic surfactants (LAS) cause destruction in gill epithelium, impair chemoreceptor organs and damage epidermis and pharyngeal wall (Pozo et al., 2003) and are reported to be acutely toxic to fish and other aquatic organisms at concentrations between 0.4 and 40 mg/l (Abel 2006). Studies also carried out by Lightowlers, 2004; Ghazali and Ahmad, 2004 showed that LAS is poorly degraded in rivers and soils and may be toxic to organisms inhabiting these environments. Other authors who have reported the harmful effects of different types of surfactants on biological indicators include; Schowanek et al. (2007); Madsen et al. (2001); Fuller et al. (2004) and Edward and Bohlen.(1992), Britton (1998) and Ezemonye and Enete(2004), have reported that the use, storage, transportation and disposal of chemicals into the ecosystem threatens the health of the ecosystem and people. Due to its surfactant properties, LAS is adsorbed preferentially onto sediments (Sanderson et al. 2003).

To date, eco-toxicological effects of anionic surfactants on aquatic species have been studied mainly in juvenile and adult life stages (Okuwosa and Omorogie, 1995; Ribelles et al., 1995; Rosety et al., 2000). Available toxicity data on surfactants largely comprise of works related to mortality, larval development and reproductive capacities (Rosety et al., 2001; Zelimira et al., 2005; Sandbacka et al., 2000; Lewis et al., 1995).
Sodium hypochlorite is a chlorinated inorganic disinfectant used in laundries, swimming pools, ponds, drinking water, and other water and wastewater systems; on food and non-food contact surfaces; and as a post-harvest, seed or soil treatment on various fruit and vegetable crops. The hypochlorite control bacteria, fungi, and slime-forming algae that can causediseases in people and animals. Sodium hypochlorite, better known as bleach, is widely used compound and its chemical and toxicological properties are extensively documented in published literature. These chemicals were first registered as pesticides in 1957.

When in water it is commonly known as bleach, in general, a solution containing 3-8% sodium hypochlorite and 0.01-0.05% sodium hydroxide; the sodium hydroxide is used to slow the decomposition of sodium hypochlorite into sodium chloride and sodium chlorate. In fresh water, the hypochlorites break down rapidly into non-toxic compounds when exposed to sunlight. In seawater, chlorine levels decline rapidly; however, hypobromite, toxic to aquatic organisms is formed.

The disinfection process is an effective barrier to many pathogens in drinking water. However, many studies have detected the presence of mutagens in drinking water, due not only to different pollution sources but also to disinfection treatment (Kraybill, 1981; Miller et al., 1986; Meier, 1988; Vartiainen et al., 1988; Monarca and Pasquini, 1989; Peters et al., 1990; Suzuki and Nakanishi, 1990; Kusamram et al., 1994; Filipic et al., 1995; and Zia'ee, 1995; Rehena et al., 1996; Cantor, 1997; Hofer and Shuker, 2000). It is known that chlorine reacts with organic matter (humic and fulvic acids derived from plant decomposition) present in untreated surface water to give disinfection by-products (Kruithof, 1985;
Sodium hypochlorite is low in toxicity to avian wildlife, but highly toxic to freshwater fish and invertebrates (U.S. EPA, 1991). Thirty-three freshwater species in 28 genera have been exposed to sodium hypochlorite, and the acute LC$_{50}$ values for total residual chlorine (sum of the free and combined chlorine) range from 28 pg/L for *Daphnia magna* to 710 pg/L for three spine stickleback (U.S. EPA, 1986a).

Toxicity tests are used to evaluate biochemical changes and the duration of exposure required to produce the criterion effects (Gary and Rand, 1995). Ecological evaluation of chemicals is important for safeguarding the environment (Ezemonye et al., 2007).

Aquatic insects play fundamental roles in freshwater ecosystems. They play significant roles in nutrient cycling and organic materials processing (Wallace and Webster, 1996). Because of their ecological importance and diversity, aquatic insects are used extensively to evaluate ecosystem health and water quality through field biomonitoring (Hilsenhoff, 1988; Plafkin et al., 1989) and to a lesser extent through laboratory bioassays. Toxicologists have observed marked sensitivity differences among aquatic insect species Stuijfzand et al., 2000). The use of biota as an indicator of pollution is advantageous over chemical analysis as they are ecologically realistic (Pocklington and Wells, 1992).
The present study was undertaken in three different stations of Kanyakumari district where agriculture is a yearlong process. So, much fertilizers, insecticides and pesticides are used here and they find their way into the freshwater bodies as agricultural runoff and anthropogenic activities like laundering, bathing, and even fishing. Washing and cleaning domestic animals are very common where enormous amount of detergents and disinfectants are used. The objective of this study is to evaluate the toxicity of an insecticide chlorpyrifos, a surfactant alkylbenzene sulphonate and a disinfectant sodium hypochlorite on the commonly available Dragonfly nymph B. geminata to predict their ability to tolerate adverse conditions and to prove their biocontrol efficiency even in undesirable ecological condition.
MATERIALS AND METHODS

Systematic Position of the test animal

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Odonata
Sub-order : Epiprocta
Infra-order : Anisoptera
Super family : Libelluloidea
Family : Libellulidae
Genus : Bradinopyga
Species : geminata

Collection and acclimatization of the test animal

Nymphs of B. geminata were collected with the standard D-frame net from the cement tanks that are kept in the dragonfly garden in the campus of Scott Christian College, Nagercoil. The nymphs were immediately transported to the laboratory and were acclimatised by holding them in aerated water (non-chlorinated) for at least 48 hours. They were fed ad libitum with laboratory reared dipteran larvae remote from insecticide use, until they were used for toxicity experiments. The animals used for the experiment were putative 12th instar larvae. Only morphologically intact nymphs were chosen and these were placed individually in 1 litre glass beakers. The water used in the experiment had been aerated at least 48 hours prior to use. The animals were not fed during the
experiment. Physicochemical factors of the solutions and the control were not analysed during the experiments.

Range finding tests were carried out before the definitive tests started. In each test four different concentrations of insecticides were made up by diluting the stock solutions. Stock solutions were prepared in dilution water (aerated tap water) from a formulation product. During the LC$_{50}$ test, the nymphs were kept individually to prevent cannibalism.

After the nymphs of the required instar had been held at room temperature at least for 48 h in the controlled environment room, intact animals were randomly allocated to 10 groups (10 concentrations) as evenly as possible. For each test, the beakers containing the test solutions were randomly arranged on a tray, and a single nymph was transferred to each one using a plastic spoon. They were checked for survival 48 hr later. The criterion for death was an absence of response to mechanical stimulus.

In the case of chlorpyriphos, animals sometimes showed a short but unrepeated tremble after being stimulated. These were classified as dead. If it became apparent that the LC$_{50}$ value was not evenly bracketed by the test concentrations, a new concentration (higher or lower than the original concentrations) was included and the most extreme dose on the opposite end of the dose range was excluded in the next test. LC$_{50}$ values were not calculated until a cumulative total of at least 100 nymphs had been tested (i.e. the number of nymphs included in the calculation of each LC$_{50}$ value). The LC$_{50}$ values were then calculated.
Toxicity Bioassay

The toxicity bioassay studies were carried out for chemical chlorine, a pesticide, chlorpyriphos and a detergent Alkyl benzene sulphonate.

Toxicity bioassay for chlorine

About 3 g chlorine was thoroughly mixed in 150 ml of tap water. This solution was maintained as a stock solution. From this stock solution 1ml solution was taken and mixed up with aerated tap water and made into 100 ml. In the same way, 10ml, 40 ml and 60 ml stock solutions were made into 100 ml by mixing with aerated tap water and these solutions with broad range of concentrations were used to fix the range of concentration for the experiment. Finally different concentration of stock solution ranging from 10-20ml (10mg/dl-20 mg/dl) were mixed with ordinary aerated tap water and made into 100 ml test solution in separate glass beakers. For each concentration of test solution (chlorinated water) two replicates were maintained. The mortality of the nymphs was observed after 12, 24, 36, 48, 60, 72, 84 and 96 hours of exposure. The data was presented in the form of mortality table. (Table 2.1 to 2.30).

Toxicity bioassay for chlorpyriphos

About 1ml chlorpyriphos (EC: 20%) solution was thoroughly mixed in 999.9 ml of tap water. This solution was maintained as a stock solution. From this stock solution 1ml solution was taken and mixed up with aerated tap water and made into 100 ml. In the same way, 10ml and 50 ml stock solutions were made into 1000 ml by mixing with aerated tap water and these solutions with broad
range of concentrations were used to fix the range of concentration for the experiment. Finally different concentration of stock solution ranging from 1-21 ml (1 mg/dl-12 mg/dl) were mixed with ordinary aerated tap water and made into 100 ml test solution in separate glass beakers. For each concentration of test solution, two replicates were maintained. The mortality of the nymphs was observed after 12, 24, 36, 48, 60, 72, 84 and 96 hours of exposure. The data was presented in the form of mortality table.

**Toxicity bioassay for Alkyl Benzene Sulphonate**

5 mg of Alkyl Benzene Sulphonate (powder form) was thoroughly mixed with tap water and made into 100 ml. This solution was maintained as a stock solution. From this stock solution 5, 15, 25 and 35 ml solution was taken and mixed up with aerated tap water and made into 100 ml and these solutions with broad range of concentrations were used to fix the range of concentration for the experiment. Finally different concentration of stock solution, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 ml were mixed with ordinary aerated tap water and made into 100 ml test solution with the concentration of 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180 mg/dl in separate glass beakers. For each concentration of test solution two replicates were maintained. The mortality of the nymphs was observed after 12, 24, 36, 48, 60, 72, 84 and 96 hours of exposure. The data was presented in the form of mortality table.

**Statistical analysis**

The mortality response of *B.geminata* was further analysed using probit analysis (Finney, 1971). Log dose and probability of mortality values were
calculated. LC$_{50}$ values were derived through probit analysis and the upper and lower confidence intervals were calculated.
RESULTS

Toxic effect of Chlorpyrifos, an organophosphorous insecticide against

Bradynopyga geminta

Toxic effect of chlorpyrifos, an organophosphorous insecticide against B.geminta was recorded in tables 2.1 to 2.10.

In dragonfly nymph exposed to Chlorpyrifos for 12h, 30 percent mortality was recorded at the concentration of 8ml/dl and 50, 60, 70 percent mortality at 10, 11, and 12 ml/dl respectively (Table 2.1). The 12h LC$_{50}$ value was 10.07, the LCL was 8.59 and UCL was 11.79 ml/dl (Table 2.10). The 12h X bar was 1.003 and Y bar, 5.002 (table 2.2).

After 24h of exposure period, 20 percent mortality was recorded at 7ml/dl; 100 percent mortality was recorded at 12ml/dl (Table 2.1). The LC$_{50}$value of 24h exposure was 8.69 ml/dl (Table 2.10).

When the nymph was exposed to chlorpyrifos for 36h, 30 percent mortality was recorded at 7 ml/dl and 90 percent mortality was recorded at 11 ml/dl (Table 2.1). In this exposure, the X bar was 0.919, Y bar was 5.107 and the b value was 8.588(table). The 36h LC$_{50}$ was 8.07 ml/dl (Table 2.10).After 48h of exposure, the mortality recorded at a concentration of 8ml/dl was 70 percent and for the concentration of 9 and 10 ml/dl the mortality rate was 90 and 100 percent. The LC$_{50}$ value for 48 h exposure was 6.95 ml/dl, the LCL was 6.34 and the UCL was 7.60 ml/dl (Table 2.10). The 48h X bar was 0.849 and Y bar was 5.076 and the b value was 11.326 (Table 2.5).
When the dragonfly nymph was exposed to Chlorpyrifos for 60h, 20 percent mortality was recorded at the concentration of 5ml/dl and 40, 60, 80 and 100 percent mortality occurred at 6, 7, 8 and 9 ml/dl respectively (Table 2.1). The 60h LC₅₀ value was 6.23 ml/dl. The LCl was 5.59 and the UCL was 6.93 ml/dl (Table 2.10). The 60h X bar was 0.79 and Y bar was 5.00 (table 2.6).

After 72h of exposure, 20 percent mortality was recorded for Chlorpyrifos at the concentration of 4ml/dl; 50 percent at 6ml/dl and 100 percent at the concentration of 8ml/dl (Table 2.1). Probit analysis of 72h response dragonfly nymph recorded an X bar of 0.75; Y bar of 5.0 and b value of 9.89 (Table 2.7). The LC₅₀ value for 72h of exposure was 5.55ml/dl, the LCL was 4.92 and the UCL was 6.25 ml/dl (Table 2.10).

After 84h of exposure to Chlorpyrifos, the rate of mortality for dragonfly nymph was 30 percent at 4ml/dl; 90 percent at 7ml/dl (Table 2.1). The LC₅₀ value of 84h exposure was 4.69 ml/dl (Table 2.8).

In 7ml/dl concentration of Chlorpyrifos, 100 percent mortality was recorded at 90h of exposure, 50 percent was recorded in 4ml/dl (Table 2.1). The 96h LC₅₀ value of Chlorpyrifos to dragonfly nymph was 3.940 ml/dl, X bar value was 0.61, Y bar was 5.08 and b value was 5.69 (Table 2.9). The 96h LCL value was 3.29 and UCL value was 4.717 ml/dl (Table 2.10).
**Toxic effect of Alkyl Benzene Sulphonate, a surfactant against B. geminta**

Dragonfly nymphs exposed to lethal concentrations of a disinfectant, sodium hypochlorite containing water for a short-term exposure were studied in terms of rate of survival and mortality.

In 12h exposure, 50 percent mortality was recorded at a concentration of 165 mg/dl, 60 percent mortality recorded at 180mg/dl (Table 2.11). In this exposure, the X bar was 2.18, Y bar was 4.76 and b value was 6.14(table 2.12). The 12h LC$_{50}$ was 164.47 mg/dl (table 2.20).After 24h of exposure period, the mortality recorded at 150,165 and 180mg/dl were 50, 70, and 100 percent respectively (Table 2.11). For 24h exposure the X bar was 2.150, Y bar was 4.901 and b value was b value 13.487. The 24h LC$_{50}$ was 8.69 mg/dl (table 2.13).

In 36 h of exposure, 40 percent mortality was recorded at 120mg/dl and 100 percent mortality at 165 mg/dl. In this exposure, the X bar was 2.103, Y bar 4.874 and b value, 11.780 (table 2.14). The 36h LC$_{50}$ was 8.07 mg/dl (table 2.20).After 48h of exposure, the mortality recorded at a concentration of 120 and 150 mg/dl was 40 percent and 90 percent. The LC$_{50}$ value for 48h was 6.95 mg/dl, the LCL was 6.34 and the UCL was 7.60mg/dl (Table 2.20). The 48h X bar value was 2.06 and Y bar 4.85 and b value was 7.59 (table 2.15).

When the dragonfly nymphs were exposed to Alkyl Benzene Sulphonate for a period of 60h, 40 percent mortality was recorded at the concentration of 105mg/dl and 70, 80,100 percent mortality at 120,135 and 150 mg/dl respectively. The 60 h LC$_{50}$ value was 6.23, the LCL was 5.59 and UCL was 6.93 mg/dl (table 2.20). The 60h X bar was 2.025 and the Y bar was 5.087 (table 2.16).
After 72 h of exposure, 10 percent mortality was recorded at the ABS concentration of 60 mg/dl; 60 percent at 105 mg/dl and 100 percent at a concentration of 135 mg/dl. Probit analysis of 72h response of dragonfly nymph recorded an $X$ bar of 1.97; $Y$ bar of 5.03 and b value of 8.96 (Table 2.20). The LC$_{50}$ value for 72h of exposure was 5.55ml/dl, the LCL was 4.92 and UCL was 6.25 mg/dl (table 2.17).

After 84h of exposure, 30 percent mortality was recorded at 60 mg/dl and 90 percent mortality was recorded at 120 mg/dl (Table 2.11). The LC$_{50}$ value of 84h exposure was 4.69mg/dl (table 2.20).

When the dragonfly nymphs were exposed to ABS for 90h, 30 percent mortality was recorded at 45mg/dl; 100 percent in 120 mg/dl (Table 2.11). Probit analysis of 96h response of dragonfly nymph recorded an $X$ bar of 1.84, $Y$ bar of 5.1, b value of 4.941 and LC$_{50}$ of 3.940 mg/dl (table 2.19 and 2.20).

**Toxic effect of Sodium hypochlorite (NaClO), a disinfectant against B. geminta**

Dragonfly nymphs exposed to lethal concentrations of sodium hypochlorite containing water for a short-term exposure were studied in terms of rate of survival and mortality and the data were recorded in tables 2.21 to 2.30. After 12h of exposure to chlorine 10 percent mortality was recorded at the chlorine concentration of 15 ml/dl. Probit analyses of the toxicity response of chlorine to dragonfly nymph (table 2.21) were used to find out the nhr LC$_{50}$ values and their upper and lower confidence intervals (table 2.30). The LC$_{50}$ value for 12h
exposure was 18.94, LCL was 17.430 and UCL was 20.554 ml/dl and X bar and Y bar were 1.248 and 4.647 respectively (table 2.21 and 2.22).

When the dragonfly naiads were exposed to chlorine for a period of 24 h, 10 percent mortality was recorded at the concentration of 14ml/dl, 20, 30, 60 and 100 percent mortality was recorded at the concentration of 15, 16, 18 and 20 ml/dl respectively. The 24h LC$_{50}$ value was 17.002, the LCL was 16.233 and UCL was 17.79 ml/dl (Table 2.30). The 24h X bar was 1.227 and Y bar was 4.937 (table 2.23).

In 36h exposure, 40 percent mortality was recorded at a concentration of 15ml/dl, 50 percent mortality was recorded at16ml/dl and 80 percent mortality was recorded at the concentration of 18ml/dl. In this exposure, the X bar was 1.198, Y bar was 4.99 and the b value was 18.89 (Table 2.24). The 36h LC$_{50}$ was 15.803 ml/dl (table 2.30).

After 48h of exposure, the mortality recorded at 15, 16, 17 and18 ml/dl were 50, 60, 80 and 100 percent respectively. For 48h period of exposure the X bar was 1.168, Y bar was 5.019 and b the value 16.897 (table 2.25). The 48h LC$_{50}$ was 14.689 ml/dl (table 2.30).

When the nymphs were exposed to the solution of sodium hypochlorite for a period of 60 h, 50 and 100 percent mortality was recorded at 14 and 17ml/dl. In this exposure, the X bar was 1.146, Y bar was 5.131 and the b value was 18.341 (Table 2.26). The 60h LC$_{50}$ was 13.784 ml/dl (table 2.30).
When the dragonfly nymphs were exposed to the chlorine solution for a period of 72h, 100 percent mortality was recorded at 16ml/dl. Probit analysis of 72h response of dragonfly nymphs recorded the X bar value of 1.12, Y bar value of 5.14, b value of 20.238 and the LC₅₀ of 12.816ml/dl (table 2.27 and 2.30).

After 84h of exposure, the mortality recorded at the concentration of 12ml/dl was 50 percent and 15ml/dl was 100 percent. The LC₅₀ value for 84h was 12.017, the LCL was 11.35 and the UCL was 12.712 ml/dl(table 2.30). The 84h X bar value was 1.084and Y bar 5.085 and b value, 18.25 (table 2.28).

At the concentration of 14 ml/dl, 100 percent mortality was recorded after 90h of exposure, 60 percent recorded at the concentration of 12 ml/dl. The 96h LC₅₀ value of chlorine to dragonfly nymph was 11.42 ml/dl, the X barwas 1.064, the Y barwas 5.13 and the b value was 20.11 (table 2.29). The 96h LCL value was 10.76 and the UCL value was 12.103 ml/dl (table 2.30).
DISCUSSION

The wide use of pesticides and fertilizers by farmers may negatively affect water quality because of run-off and leaching. The effect that slightly polluted water has on aquatic organisms is unknown (Charvet, 1988). Biological indices frequently show advantages over chemicals and other pesticides (Wallace et al., 1996; Marneffe et al., 1997). Biological indices are more integrative in time (over the life of the individual), although they may not assess the level of the pollutant exactly.

Comparative toxicity data provide important informations on variations in responses of aquatic species to insecticides. These informations are very useful for determining margins of safety for aquatic biota, either prospectively (before manufacture and use) or retrospectively (after manufacture and use) (Adams 1995; Graney et al. 1994). Laboratory toxicity data also provide insight into expected effects of accidental spills, cropland runoff, pesticide aerial drift, or other events potentially adversely affecting nontarget organisms. Moore et al (1998).

The dragonfly nymphs have been used for toxicity studies for the past few decades. In the present study, dragonfly nymph Bradynopyga geminate Rambur was selected to test the toxicity of chlorpyrifos, Alkyl benzene sulphonate and sodium hypochlorite under laboratory conditions.

Chlorpyrifos (CPF) (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), an organophosphate is one of the most widely used insecticides in agriculture worldwide, which is acutely toxic to some species of aquatic invertebrates. Lethal effects were recorded for all the organisms examined at
different concentrations causing mortality. A substantial aquatic toxicity data base for the insecticide chlorpyrifos exists in the literature.

Lowe's chlorpyrifos studies on the invertebrate species, *Callinectessapidus* sp., *Palaemonetes pugio* sp., *Penaeus duorarum* sp., and *Penaeus aztecs* sp., produced 48-h least common multiple values that ranged from 0.2 to 5.2 pg/L. Chlorpyrifos toxicity may be directly related to temperature. Normally, the two penaeid shrimp species are very similar in sensitivity to pesticides. When Lowe exposed the two species to chlorpyrifos at temperatures that differed by 17 °C, *Penaeus aztecs* (Fabricius, 1798) exposed at 29 °C had an ECW (Extracellular water (ECW) compartment) value of 0.2 pg/L, *Penaeus duorarum* (Fabricius, 1798) at 12 °C, 2.4 wg/L. Macek *et al.* (1969) reported the same temperature-toxicity relationship with rainbow trout *Salmo gairdneri*(Walbaum, 1792) exposed to chlorpyrifos at 1.6, 7.2, and 12.7 °C. Studies on chlorpyrifos with freshwater species generally compare favourably with those values that were reported previously.

For example, the 96-h LCM (Least Common Multiple) values for three species of stoneflies, *Pteronarcys californica* (Newport), *Pteronarcella badia* (Hagen, 1874), and *Cliaussenia sabulosa* (Banks 1900) were 10.0, 0.38, and 0.57 pg/L, respectively (Sanders and Cope, 1968). Rainbow trout exposed to the insecticide for 96 h at three different temperatures gave LC$_{50}$ values from 7.1 to 51 pg/L (Macek *et al.*, 1969).

In the present study, LC$_{50}$ values of chlorpyrphos containing water were calculated for 12 to 96 h. In *B.gemita* exposed to chlorpyrphos for 24h, 20 percent
mortality was recorded in the concentration of 7mg/dl and 70 and 100 percent mortality at 10, 12 mg/dl respectively. Similar observations were made by Karim et al. (1985) who reported LC$_{50}$ (96-hour) value for a period of 24h of exposure for the damselfly naiad, *Pseudagrion* sp. was 0.10 μg/L and for dragonfly (naiad), *Crocothemis erythraea* (Brulle, 1832) is 6.0 μg/L for 24h period of exposure. Sanders and Cope (1968) observed LC$_{50}$ of 0.38 μg/L for Stonefly, *Pteronarcella badia* for a period of 96 hours of exposure. For 48-h of exposure, aqueous solutions of chlordane approaching solubility limits (0.1 mg/L) were required to elicit 80–100% mortality in *Chironomus tentans* (Fabricius, 1805) (Moore et al., 1998). During 96-hour toxicity tests, several species of freshwater and marine invertebrates and fishes died at chlorpyrifos concentrations between 0.035 and 0.58 ug/l (Johnson and Finley 1980).

The effectiveness of chlorpyrifos under field conditions, like that of other organophosphorus pesticides, is significantly modified by numerous variables such as formulation, route of administration, pond substrate, dose, and water temperature (Macek et al., 1969; Bailey et al., 1970; Odenkirchen 1987). Current water quality criteria formulated for chlorpyrifos by the U.S. Environmental Protection Agency (EPA 1986) for aquatic life protection seem to afford a reasonable degree of safety, at least during short-term exposure. Specifically, the proposed criteria for freshwater are 0.041 ug/l (4-day average concentration).

Increased oxygen consumption was also noted in atrazine treated midges as compared to control group larvae of the midge *Chironomus tentans*. Sublethal stress can cause increased respiration, resulting in increased uptake and toxicity.
Chlorpyrifos uptake is likely to increase as respiration increases (Belden et al., 2000).

Surfactants are an important and widely used group of chemicals (Jensen et al., 2009). The toxicities of surfactants to aquatic life have been summarized previously in the scientific literature (Abel, 1974; Koskova and Kozlovakaya, 1978; Margaritis and Creese, 1979; Sivak et al., 1982; Lewis and Suprenant, 1983; Lewis and Wee, 1983; Cooper, 1988). Environmental assessments based on these reviews, however, are out-dated considering the constant development of new surfactants and reformulation of existing surfactant components in detergent products.

Linear Alkylbenzene Sulphonate (LAS) is the most widely used anionic surfactant in household and cleaning products. In the present study, LC$_{50}$ values of alkyl benzene sulphonate containing water were calculated for 12 to 96 h. When B. gemita exposed to alkyl benzene sulphonate for 24 h, 60 percent mortality was recorded in the concentration of 18 mg/dl and 80, 100 percent mortality was recorded at 19, 20 mg/dl respectively.

In fish fingerlings (Cyprinus carpio Linnaeus, 1758) exposed to sublethal concentrations of LAS, Misra et al., (1991) found alterations in the levels of glycogen, lactic acid, sialic acid, and acid and alkaline phosphatases in the gills, liver and kidney at the concentrations of 0.005 mg/L (Hampel et al., 2004). Affected individuals may not be able to perform correct movements affecting both, feeding and/or predation (Hampel et al., 2004).
Sodium hypochlorite is highly toxic to freshwater fish and invertebrates (Taylor, 1993). In the present study, LC$_{50}$ values of sodium hypochlorite containing water were calculated for 12 to 96 h. In *B. geminata* exposed to sodium hypochlorite for 24 h, 30 percent mortality was recorded in the concentration of 135 mg/dl and 70, 100 percent mortality was recorded at the concentration of 165 and 180 mg/dl respectively. The resistance of the dragon fly nymph to different effluents was found to be reduced with increase in concentration and exposure time. The data indicated that decrease in LC$_{50}$ concentration is associated with increase in duration of exposure. Relatively standard flow-through LC$_{50}$ values range from 0.017 mg/L to about 1 mg/L for aquatic invertebrates. While the mechanism of action of aqueous chlorine to aquatic invertebrates is not well-characterized, toxicity is likely due to the oxidizing potential of sodium hypochlorite. Similar investigations were made by Taylor (1993) who observed a concentration of 0.0028 mg/L of sodium hypochlorite which is about 60% of the lowest reported LC$_{50}$ in *Ceriodaphnia dubia* (Richard, 1894).

The toxicity of sodium hypochlorite solution by inhalation is predominantly due to the mixing of bleach with acids and the release of highly irritant gases. Metabolic acidosis may occur in rare cases following significant inhalation of sodium hypochlorite (Racioppi *et al.*, 1994).

Few studies are published on the combined effects of chemical stressors and oxygen depletion in aquatic ecosystems. However, as indicated by Van der Geest *et al.* (2002), periods of the lowest oxygen concentrations often coincide with the presence of the highest contaminant concentrations. The authors postulated that, besides the direct adverse effects of toxicants (Becker, 1987;
Nebeker et al., 1996), low oxygen concentrations influence the response of aquatic insects to environmental contaminants since a variety of physiological processes in the organism may be affected by oxygen deficiency (Penttinen and Holopainen, 1995). The authors explained this fact by a possible increase in gill movements at lower oxygen concentrations, as observed by Philipson and Moorhouse (1974) for aquatic insects. Toxicants could also cause damages to the gill structure and thereby influence the respiratory efficiency.

The combination of nation-wide monitoring data on insecticide concentrations and aquatic macro-invertebrates creates a valuable instrument for the analysis of the impacts of different pesticides and the evaluation of environmental policy. Given the fact that the world-wide use of insecticides is still growing, and given its high leaching potential and its high persistence in water and soil, it is important to sustain and extend chemical monitoring schemes of surface water.