CHAPTER - II

REVIEW OF LITERATURE
II. Review of Literature

Pesticides are routinely utilized in agricultural to destroy or repel pests, pathogens, bacteria, fungi and weeds etc. Even with the use of pesticides crop losses amount to 10-30%; without the use of pesticides crop losses may amount to anything from 50% to over 80%. Hence, it has become imperative to reduce crop and stored grain losses by using pesticides. The undue persistence, high mammalian toxicity and developing resistance of the organochlorine, organophosphorus and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries (Wasim Aktar et al., 2009).

Since pesticides as well as some of their active substances, their presence in the environment is of concern. Pesticides from agriculture runoff and other sources into aquatic systems has been increasing their contamination by these products in several areas around the world with potential adverse effects. Unfortunately, after use pesticides do not stay in their place of application but move to the other parts of the environment and ultimately to the aquatic environment through surface runoff. Since pesticides are poisons and are meant to kill, repel or destroy during their sojourn through the different compartments of the globe, they kill a host of other non-target organisms (Altinok et al., 2012).

The pesticide residues are transported to the aquatic environment either through surface runoff or through precipitation into which they get in by evaporation from cropland (David Hernandez-Morenoa et al., 2011). The increasing awareness of the environmental hazards of pesticides necessitated the testing of toxicity of different
pesticides to different aquatic organisms. The acute effects of agriculture chemicals to fish have been worked out by (Reish and Oshida, 1987; Josephine O. Boateng et al., 2006; Mohsen Khalili et al., 2012).

Pesticides may alter the metabolism, activate or inhibiting enzyme, changes in the enzymatic activities and bioaccumulation, in turn cause modification of metabolism, physiological alterations and cellular damage to different organs (Vasylkiv et al., 2011). The uptake of pesticides by aquatic organisms may occur from the water, from the sediments, suspended particulate matter or from the food. The fish plays an important role in the monitoring of water pollution because it responds with great sensitivity to changes in aquatic ecosystems (Sikora and Drastichova, 2004; David Hernandez-Moreno et al., 2011).

II.1.1.Test Toxicants

Two test compounds selected for this study belonged to two categories viz., Organophosphorus (OPs) and Carbamate (CM) pesticides (profenofos and carbosulfan), widely used in agriculture fields.

i. Profenofos: Profenofos is a broad spectrum of organophosphate pesticide and used widely for control of pests in agriculture and household purposes in India. It works by inhibition of acetyl cholinesterase. Insecticide with activity on boll worms, jassids, aphids, Thrips, White flies. Profenofos (50% E.C) was manufactured by Nagarjuna Agrichem Limited., Hyderabad, India.

ii. Carbosulfan: Carbosulfan is a broad-spectrum carbamate pesticide used to control insects, mites and nematodes by soil, foliar and seed treatment applications, mainly on potatoes, sugar beet, and rice, maize, citrus. Carbosulfan (25% EC) was manufactured
by Rallis India Limited, Mumbai, India. Both of the toxicants were purchased from the local pesticide market in Guntur of Andhra Pradesh, India.

According to US Environmental Protection Agency (USEPA), profenofos was first registered in the United States (US) in 1982 and about 775,000 pounds (lbs.) of active ingredient are applied to cotton each year (USEPA, 2012). The toxicity of profenofos is extremely toxic to fish and macro-invertebrates (Akerblom, 2004), its mode of action is by inhibition of acetyl cholinesterase activity (Fukuto, 1990) and also resulting toxicity in humans (Costa et al., 2003).

According to the previous reports from the US Environmental Protection Agency (USEPA) and previous research works, profenofos pesticide is a potential contaminant in a wide range of aquatic and terrestrial ecosystems (Jabbar et al., 1993; Safiatou et al., 2007; USEPA, 2006) and its residues have been found in, different tissues of aquatic animal like fish, foods and vegetables (Parekh et al., 1994; Radwan et al., 2005; Nagaraju and Rathnamma, 2014). It affects not only the target animals but also non-target animals including human beings. Profenofos is of mid range in risk of assessment among the 12 top priority pesticides of cotton (Batley and Peterson, 1992; Tomlin, 2000). The insecticidal activities of profenofos are attributed to inhibition of the acetyl cholinesterase (AChE), which plays an important role in neurotransmission at cholinergic synapses by rapidly hydrolyzing the neurotransmitter acetylcholine to choline and acetate (Ozmen et al., 1999; Fulton and Key 2001). Profenofos is activated to desthiopropyl profenofos through the oxidation of the sulphur in the P-S-N-propyl bond to the more potent AChE inhibitor (Wing et al., 1983). The primary effect of
profenofos in studies of acute, short, and long-term toxicity was inhibition of acetyl cholinesterase activity (Wing et al., 1984).

Table II.1 Physical and chemical properties of Profenofos

<table>
<thead>
<tr>
<th>Common name:</th>
<th>Profenofos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer's code number</td>
<td>CGA15324, OMS2004</td>
</tr>
<tr>
<td>Other code numbers:</td>
<td>CAS No: 41198-08-7</td>
</tr>
<tr>
<td>CIPAC No:</td>
<td>461</td>
</tr>
<tr>
<td>Chemical name:</td>
<td>IUPAC: ( O)-(4-bromo-2-chlorophenyl) ( O)-ethyl ( S)-propylphosphorothioate</td>
</tr>
<tr>
<td>CA:</td>
<td>( O)-(4-bromo-2-chlorophenyl) ( O)-ethyl ( S)-propylphosphorothioate</td>
</tr>
<tr>
<td>Formulae:</td>
<td>C11H15BrClO3PS</td>
</tr>
<tr>
<td>Molecular mass:</td>
<td>373.6 g/mol</td>
</tr>
<tr>
<td>Structural formula:</td>
<td><img src="image" alt="Structural formula" /></td>
</tr>
<tr>
<td>Purity Minimum:</td>
<td>91.4% Appearance Clear liquid</td>
</tr>
<tr>
<td>Odour:</td>
<td>like cooked onion</td>
</tr>
<tr>
<td>Colour:</td>
<td>Light brown</td>
</tr>
<tr>
<td>Stability:</td>
<td>No thermal effect found between room temperature and 150 °C</td>
</tr>
</tbody>
</table>

Table II.2 Approved uses of registered pesticide profenofos

<table>
<thead>
<tr>
<th>Crop</th>
<th>Common name of the pest</th>
<th>Dosage/ha</th>
<th>Formulation</th>
<th>Dilution in Water</th>
<th>Waiting Period (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tea</td>
<td>Redspidermite, Pinkmite, Teamosquito bug</td>
<td>400-500</td>
<td>800-1000</td>
<td>400</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Looper caterpillar Thrips and Jassid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton</td>
<td>Bollworm, Jassids, Aphids, Thrips, Whiteflies</td>
<td>750-1000</td>
<td>1500-2000</td>
<td>500-1000</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>1000</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Soya bean</td>
<td>Semi looper &amp; Girdle beetle</td>
<td>500</td>
<td>1000</td>
<td>500</td>
<td>40</td>
</tr>
</tbody>
</table>

Source: Directorate of Plant Protection, Quarantine & Storage Central Insecticides; Board & Registration Committee, Ministry of Agriculture, India, 2011

Profenofos is a moderately severe toxicant, causing relatively delayed respiratory failure and death in mammals (Eddleston et al., 2009). It regulates hepatic and testicular CYP2C11 and CYP3A2 mRNA and protein expression levels as well as decreases the testicular aromatase (CYP19A) mRNA in treated rats (Moustafa et al., 2008). An evaluation of the CYPs inhibition potential of selected pesticides in human hepatic microsomes illustrated that organophosphorus insecticides are potent and extensive inhibitors of CYP1A1/2, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP3A4 (Abass et al., 2009).

II.1.2. Mammalian toxicology

According to reviews of FAO/WHO, series 59(2008) & series 61(2009); oral and acute oral profenofos LD$_{50}$ for rats 358, rabbits 700 mg/kg. Skin and eye acute percutaneous LD$_{50}$ for rat’s c. 3300, rabbits 472 mg/kg. Inhalation LC$_{50}$ of profenofos (4
h) for rats c. 3 mg/l air. No-observable-effect level (NOEL) (using EC formulation 380 g a.i. /l) for rats (2 y) 0.3 mg a.i./kg diet; for lifetime study 1.0 mg a.i./kg diet; for mice 0.08 mg/kg diet. Acceptable Daily Intakes (ADI-JMPR) 0.01 mg/kg body weight, Toxicity class WHO (a.i) II; EPA (formulation) II EC hazard Xn; R20/21/22(USEPA, 1998).

II.1.3. Ecotoxicology

Profenofos is toxic to birds, fish, highly toxic to crustaceans, bees, algae and other aquatic species. The LC50 of profenofos (8 d) for bobwhite quail, 70-200, Japanese quail >1000, mallard ducks, 150-612 ppm (Fink, 1978). LC50 (96 hr) of profenofos to fish rainbow trout 0.08, crucian carp 0.09, bluegill sunfish 0.3 mg/l1.(USEPA, 1998; FAO/WHO, 2008).

II.1.4. Environmental fate

Profenofos is not persisting in the environment, particularly in neutral and alkaline soils; profenofos dissipates in neutral and alkaline soils, with a half-life of several days. One of the major degradates 4-bromo-2-chlorophenol, is persistent in the environment, and while the other degradate of O-ethyl-S-propyl phosphorthioate is not well known. Hydrolysis is the primary route of dissipation, profenofos hydrolysis in neutral and alkaline soils with half-life’s 104-108 days at pH 5,24-62 days at 7 pH, and 7-8 hrs at pH 9. The major primary degradates of profenofos under aerobic and anaerobic conditions in soils also its major degradates hydrolysis: 4-bromo-2-chlorophenol and O-ethyl-S-propyl phosphorthioate (USEPA, 1996). A major secondary degrade under both aerobic and an anaerobic condition is 4-bromo-2-chlorophenyl ethyl ether (BCPEE).
A major tertiary degradate under anaerobic conditions is cyclohexadienyl sulphate (FAO/WHO, 2008).

II.1.5. Metabolism of Profenofos

Only a few studies on profenofos metabolism in mammals have been reported (FAO/WHO, 2007). After single and repeated doses of [Phenyl-$^{14}$C] profenofos to rats, unchanged profenofos was detected in the feces, but the amount was very small (approximately 1–2% of the administered dose), and this was probably the proportion of the dose that was not absorbed. Four major metabolites were present in urine and no unchanged profenofos was detected. The major metabolites were the sulfate and glucuronide conjugates of 4-bromo-2-chlorophenol that were formed by hydrolysis of the aryloxy–phosphorus bond followed by conjugation with sulfate or glucuronic acid (WHO, 2009).

The other two metabolites were formed by cleavage of the phosphorus–sulfur bond either by loss of the propyl group or hydrolysis. The 4-bromo-2-chloro-phenol was detected in some urine samples, but probably arose as a result of hydrolysis of the conjugates after excretion (WHO, 2009; WHO, 2013).

In cotton plants the metabolic pathway of profenofos involves cleavage of the phosphorothioate ester bond to yield 4-bromo-2-chlorophenol, followed by conjugation with glucose (Capps et al., 1996). Degradation of organophosphate pesticides is by subsequent biotransformations yielding glycosylated and sulfated derivatives. Profenofos degraded rapidly in aerobic soil conditions. In profenofos cleavage of the phenolphosphorus ester bond is by chemical hydrolysis in sterilized soils, with accumulation of 4-bromo-2-chlorophenol. The metabolic biotransformations of
profenofos in plants and animals are similar and occur via hydrolysis to 4-bromo-2-chlorophenol which is then conjugated by several enzymatic reactions (Fig. II.1), (Saadatullah Malghan et al., 2009; FAO, 2012; Natália Alvarenga da Silva - 2013).

**Figure II.2.1** Proposed metabolic pathway of profenofos in soil, plant and enzymatic reactions  
**Source:** FAO, (2012); Natália Alvarenga da Silva et al.,( 2013)

The predominant metabolic pathway involves stepwise dealkylation and hydrolysis, followed by conjugation. Plants in cotton, Brussels sprouts and lettuce, the compound is rapidly taken up and metabolized. The overall metabolic pattern indicates degradation to polar metabolites. Soil/environment mean half-life in soil is c. 1 week. Profenofos is primarily metabolized in cotton plants to the glucose conjugate of 4-bromo-2-chlorophenol (Capps et al., 1996), in mammal model (Ronald et al., 2008).
II.1.6. Carbosulfan

Carbofuran is the main metabolite of carbosulfan in plants and is itself a pesticide (Guillet et al., 2001) and the other hand, it can pollute environment and become a problem when it reaches water supplies, reported by (Tarig et al., 2010). It is extremely toxic to mammals and including human being, its toxicity is mediated through inhibition of acetyl cholinesterase (Renzi and Krieger, 1986; Giri et al., 2003), carbosulfan has been classified as a moderately hazardous (toxicity class II) pesticide by WHO with a low ADI of 0.0 - 0.01 mg/kg body weight (IPCS, 2004).

The genotoxicity of carbosulfan was investigated in a wide range of tests, it has been reported to induce micronucleus formation, sister chromatid exchange and chromosomal aberrations in human peripheral blood lymphocytes and bone marrow cells of rats (Giri et al., 2003). A survey of literature revealed that very few studies have been carried out on the potential cytogenetic effect of carbosulfan. Studying on the genotoxicity of Marshal (carbosulfan) and its effective ingredient carbosulfan, reported that significantly induced the formation of chromosome aberrations in human peripheral lymphocytes in vitro (Topakata, 1993). Very few published reports could be found on the potential cytogenetic effects of carbosulfan (Topaktas, 1996).
Table II.3. Physical and chemical properties of Carbosulfan

<table>
<thead>
<tr>
<th><strong>Chemical name:</strong></th>
<th>IUPAC: 2, 3-dihydro-2,2-dimethylbenzofuran-7-yl(dibutylaminothio) methylcarbamate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synonyms:</strong></td>
<td>FMC 35001, Marshal®, Advantage(C)</td>
</tr>
<tr>
<td><strong>Chemical Abstracts Registry Number:</strong></td>
<td>55285-14-8</td>
</tr>
<tr>
<td><strong>Company Code Number:</strong></td>
<td>FMC 35001</td>
</tr>
<tr>
<td><strong>Structural formula:</strong></td>
<td>![Structural formula image]</td>
</tr>
<tr>
<td><strong>Empirical formula:</strong></td>
<td>C_{20}H_{32}N_{2}O_{3}S</td>
</tr>
<tr>
<td><strong>Molecular weight:</strong></td>
<td>380.5</td>
</tr>
<tr>
<td><strong>Physical state:</strong></td>
<td>Viscous brown liquid</td>
</tr>
<tr>
<td><strong>Specific gravity:</strong></td>
<td>1.056g/ml at 20°C</td>
</tr>
<tr>
<td><strong>Solubility:</strong></td>
<td>Water (0.3 ppm), completely miscible in Xylene, Hexane, Chloroform, Methylene Chloride, Methanol and Acetone.</td>
</tr>
<tr>
<td><strong>Volatility:</strong></td>
<td>Relatively non-volatile</td>
</tr>
<tr>
<td><strong>Vapor Pressure:</strong></td>
<td>0.31 × 10^{-6} Torr at 25°C</td>
</tr>
<tr>
<td><strong>Flash Point:</strong></td>
<td>96°C - closed-cup method</td>
</tr>
</tbody>
</table>

*Source: Alvarez, 1995*

Table II.4. Approved uses of registered pesticide Carbosulfan 25% EC

<table>
<thead>
<tr>
<th><strong>Crop</strong></th>
<th><strong>Common name of the pest</strong></th>
<th><strong>Dosage/ha</strong></th>
<th><strong>Formulation</strong></th>
<th><strong>Dilution in Water</strong></th>
<th><strong>Waiting Period (Days)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rice</strong></td>
<td>Greenleaf hopper</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>White plant hopper</td>
<td>200250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Brown plant hopper</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Gallmidge</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Stem borer</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td><strong>Chilli</strong></td>
<td>Leaf folder</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>White aphid</td>
<td>200-250</td>
<td>800-1000</td>
<td>500-1000</td>
<td>8</td>
</tr>
</tbody>
</table>

*Source: Directorate of Plant Protection, Quarantine & Storage Central Insecticides; Board & Registration Committee, Ministry of Agriculture, India, 2011*
II.1.7. Environmental fate

Carbosulfan is rapidly degraded in neutral soil (half-life < 5 days) with the degradation rate increasing as pH decreases. A major breakdown product is carbofuran, which degrades more slowly (half-life approx. 50 days). It is hydrolytically unstable in acid, with stability increasing with increasing pH. The bioconcentration potential of carbosulfan is low with a Log Pow (is a suitable indicator for a screening assessment of bioaccumulation) of 3.3, and a measured BCF (bioconcentration factor) in fish of 990. Carbosulfan and its major breakdown product are unlikely to leach into groundwater (EFSA, 2006).

II.1.8. Metabolism of Carbosulfan

There are few reports about the metabolism of carbosulfan in the environment and in plants. In mammals three primary metabolites, 3-hydroxycarbofuran, 3-keto-7-phenolcarbofuran, and dibutylamine, were detected by TLC in rat in vivo (Marsden et al., 1982; Chaudhry et al., 2002; Soler et al., 2006).

Carbosulfan is metabolized by hydrolysis to the 7-phenol or carbofuran and dibutylamine, and is subsequently further metabolized via hydrolysis, oxidation and conjugation to a variety of metabolites. Metabolites of the dibutylamino moiety may enter the carbon pool and be incorporated into natural constituents of the body. No marked sex-specific differences were observed in rats with regard to the excretion pattern, tissue distribution and metabolite profile of carbosulfan (Pesticide residues, 2003). Moreover, carbofuran and polysulfide derivatives of carbosulfan were detected in rat stomach by TLC (Umetsu and Fukuto, 1982). In male and female rats in vivo, ten metabolites were identified by TLC and HPLC and major metabolites were confirmed by gas chromatography-mass spectrometry (GC-MS) (FAO/WHO, 2003). The in vitro
metabolic pathways of carbosulfan in microsomal hepatic preparations are from seven mammalian species including humans.

![Proposed metabolic pathway for carbosulfan in Rats](Image)

**Figure II.2.2.** Proposed metabolic pathway for carbosulfan in Rats
**Source:** Reproduced from Fang and El Naggar (1995)

The primary metabolic pathways in these *in vitro* studies were the initial oxidation of sulfur to carbosulfan sulfinamide and the cleavage of the nitrogen sulfur bond (N-S) to give carbofuran and dibutylamine. Carbofuran was oxidized to 3-hydroxycarbofuran and/or 7-phenolcarbofuran, which were further oxidized to 3-ketocarbofuran or 3-hydroxy7-phenolcarbofuran, respectively and finally to 3-keto-7-phenolcarbofuran (Abass *et al.*, 2009).

It has been shown that, carbosulfan is metabolized in the environment, plants, and mammals to carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran. While all are
actually toxic compounds, carbofuran is found to be more persistent and toxic than carbosulfan itself. Carbosulfan (LD 250 and 185 mg/kg, 48-150mg/kg for male and female rats, respectively) is transformed into a more toxic one, carbofuran (LD 8 mg/kg 50-1 for male and female rats (Tomlin, 2000; Trevisan et al., 2004; Iesce et al., 2006).

II.1.9. Ecotoxicological information

In birds acute oral LD_50 of carbosulfan for mallard ducks 8.1 mg/kg; quail, 82 mg/kg; pheasants, 20 mg/kg. For aquatic organisms such as fish LC_50(96 h) for blue gill sunfish 0.015 mg l^{-1}; trout 0.045 mg l^{-1}; toxic to bees; daphnia EC_50(48 h) 1.5 μg l^{-1}, respectively. With LC_50 values between 44 to 46 μg l^{-1} to aquatic arthropods and fish in the laboratory, based on the LC_50 values of carbosulfan is considered highly toxic. Anonymous,(2002). Carbosulfan was highly toxic to water fowl (oral LD_50 = 15 mg/kg) and moderately toxic to upland game birds (oral LD_50 = 104 mg/kg). Carbosulfan is an easily metabolized and reversible cholinesterase inhibitor; recovery from symptoms of a sublethal exposure occurs quickly (JMPR, 2004).

II.1.10. Toxicity evaluation

Aquatic organisms are continually being exposed to various pollutants in the environment. Toxicity of pollutants to plants, animals, fish or wild life can be evaluated simply by exposing a group of organisms under controlled conditions such as evaluation can be performed and is the indices of action. Pesticides can produce adverse effects in a biological system, seriously damaging its structure and function of living system finally leads to death of organism. Those adverse responses may be defined in terms of a measurement as acute toxicity. Pesticides are entering into aquatic ecosystem by
agriculture runoff from land, impairing the quality of the water and making it unfavorable for aquatic life (Tilak et al., 2009).

Toxicity is a relative property of a chemical which refers to its potential to have harmful effects on living organisms. It is a function of the concentration of the toxicant and duration of exposure. The acute toxicity tests were conducted by earlier authors for profenofos and carbosulfan for different species of fish. The toxicity tests provide a measure of the toxicity of compounds to a given species under specific environmental conditions (water quality, pH and temperature etc). The determination of LC$_{50}$ for any period offers more reliable information about the toxicity of a chemical in aquatic hazard evaluation (Alexander et al., 1983).

Nwani et al., (2013) reported that the median lethal concentrations of chlorpyrifos-based pesticide Termifos to African catfish *Clarias gariepinus* were found to be 0.861 mg l$^{-1}$. Toxicity of pesticides to aquatic organisms also has been reported to be affected by dissolved oxygen (DO), size, age, water quality and temperature. The LC$_{50}$ values of carbaryl for *C. catla*, *A. testudineus*, *M. cavasius* and *M. vittatus* are 6.4, 5.5, 4.6 and 2.4 ppm respectively and of 1-naphthol, 4.3, 0.33 and 1.1 ppm respectively. Thus for all the four species of test fish reported by Tilak et al., (1981). Behavior of the animal can serve as the link between physiological and ecological processes; it may be used for studying environmental pollutant effects (Graham and Sloman, 2004). Sublethal and lethal effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival (EIFAC, 1975; Waldichuk, 1979).
II.11. Biochemical changes

Biochemical and physiological parameters are frequently used for detecting or diagnosing sub lethal effects pesticides in fish (Theodorakis et al., 1992). Biochemical biomarkers can provide information about the detoxification process of pesticides in living organisms. When it can contact with an organism, the toxic agent or substance can be transformed by enzymes, which act to make the xenobiotic substance or toxic compounds a less toxic compound and facilitate its excretion (Santosh and Martinez, 2012).

II.11.1. Glycogen

The earlier observation on the effect of pesticides on carbohydrate metabolism in various species indicates an attenuation of the energy reserve under pesticide stress (Levesque et al., 2002; Thenmozhi et al., 2011; Bibha Kumari et al., 2012). It appears that exposure to pesticides leads to enhancement of energy requirement. Since the glycogen is considered to be the first among the organic nutrients, it initially gets effected and decrease under any physiological stress conditions imposed on the animal (Ansari, 1988). A drop in tissue glycogen content may also be either due to decreased synthesis as a consequence of toxic stress or breakdown (Dezwaan and Zendee, 1972; Murthy and Devi, 1982; Ibraik Ohaida and Akrawee, 2010).

II.11.2. Proteins

Assessment of protein levels can be used as a diagnostic tool to determine the physiological status of the cells (Dogan and Can, 2011; Nwani et al., 2013). Detection of changes in activity levels of enzymes is widely used as a quick method to determine the toxic effects of pesticides and other substances (Altinok et al., 2012). Patil and
David, (2009) reported that decrement in total, soluble and structural protein in fish *Labeo rohita* after treated with malathion for 5, 15 and 25 days. Decreased protein content suggests the existence of high proteolytic activity, and impairment in the protein biosynthesis. The decrease in protein levels may also be due to their degradation. The degradation of these proteins is due to oxidative stress.

Decreased protein content in cat fish *Clarias gariepinus* exposed to two sublethal concentrations of malathion i.e. 172 μg l⁻¹ and 86 μg l⁻¹, depletion in protein levels due to hypoproteinemia, impaired food intake, increased energy cost of homeostasis, tissue repair and a detoxification mechanism during toxic stress can also cause protein depletion (Nwani et al., 2013). The decreased trend of proteins and glycogen were observed in fish *Oreochromis mossambicus* due to dichlorvos stress. The reduction in protein content and glycogen may be due to stress caused by the toxicants (Lakshmanan et al., 2013).

The investigations of Anita Susan et al., (1999), indicated that significant decrease protein content in different tissues of *Catla catla* under exposure to fenvalerate. Yeragi et al., (2000), observed decrement of protein content in different tissues of marine crab *Uca marionis* exposed to malathion, Tilak et al.,(2001) observed depletion of total proteins in fish *Labeo rohita* treated with both technical as well as 20% EC of chloropyrifos. Gopala rao et al., (2006), observed decreased protein content under exposure to kelthane in the fish *Channa punctatus*. Decrease in total proteins in liver, muscle, kidney and gills under dimethoate toxicity in fish *Dussumieri* was observed by Rathod et al., (2009). The decrease in the protein levels of pesticide or toxicant exposed fish suggests the disruption of carbohydrate metabolism, destruction or disturbance of
protein synthesis machinery and inhibition of ATP synthesis (Borah, 1996; Ramalingam et al., 2000).

II.1.11.3. Nucleic acids (DNA and RNA)

Borah, (1996), reported that the decreased DNA content of fish indicates the contaminant crude oil interferes with nucleic acid synthesis. RNA content indicates the intensity of protein synthesis in particular tissue. The correlation between RNA and protein, any type of RNA synthesis deficient should have its reflection in corresponding failure of protein synthesis. Thus, the toxicant or contaminant might effect on the synthesis of protein and DNA directed RNA formation.

II.1.11.4. Acetyl cholinesterase (AChE)

AChE inhibition in agrochemical treated fish at sublethal levels has been related to several measures of behavioral changes and neuromotor effects on swimming activity (Pessoa et al., 2011), impact on specific sensorial system like vision and smell (Tierney et al., 2008), proper functioning of swimming skills and sensorial system is essential for detection, attack and capture of prey, as well as for predator evasion (Fuiman et al., 2006; Pessoa et al., 2011).

Alterations in AChE activity can affect locomotion and equilibrium in fish exposed to different types of pollutants, mainly impact on swimming patterns, impair feeding, escape, and reproductive behavior(Bretaud et al.,2000; Miron et al., 2005). Inhibition of AChE induces alteration in the swimming behavior, shaking palsy, spasms and other undesirable effects (Sharbide et al., 2011).

A decrease of AChE activity in brain and blood of silver catfish(Rhamdia quelen) exposed to different types of salinities, acetylcholine is not degraded when AChE
decreases, it accumulates within synapses, which prevents them from functioning normally (Dutta and Arends, 2003). In nerve impulses inhibition of AChE results as nerves become permeable to sodium, allowing sodium to flow into the nerve (Marigoudar et al., 2009). In turn, these impulses release the neurotransmitter ACh, which stimulates other nerves (Eells, 1992); ultimately resulting in buildup of ACh within the nerve synapses leading to a variety of neurotoxic effects and decreased cholinergic transmission (Mileson et al., 1998).

II.1.11.5. Acid phosphatase (ACP)

Dalela et al., (1982) reported decrease in the acid phosphatase in the tissues of \textit{Notopterus notopterus} exposed to three sublethal concentrations (1/10	extsuperscript{th}, 1/15	extsuperscript{th} and 1/20	extsuperscript{th} of 96 hr LC\textsubscript{50}) of phenol and their combinations for 15 and 30 days.

II.1.11.6. Lactate dehydrogenase (LDH)

Elevation of LDH activity in mosquito fish, \textit{Gambusia holbrooki} exposure to clofibric (Nunes et al., 2004). Li et al., (2009) reported that increased LDH activity in fish rainbow trout treated with carbamazepine. Disturbances in their catalytic process due to xenobiotic compounds can cause cellular homeostasis affecting different enzymatic systems, which can lead to effects at higher levels of biological organization such as tissues, organs, or individuals (Orrego et al., 2011).

Jos et al., (2003) reported that leakage of LDH is a marker of membrane permeability and cell death. They also suggested that an increase in LDH activity may be due to stabilization of cytoplasmic membrane. Tripathi and Singh, (2012), reported that LDH activity in fish \textit{Channa punctatus} significantly increased in skeletal muscle (2.2) fold followed by liver (1.8) fold, gill (1.6) fold and brain (1.4) in response to treatment.
with alphamethrin for 14 days, due to an increase in anaerobic respiratory activity and production of more lactate for completion of metabolic process.

**II.1.11.7. Succinate dehydrogenase (SDH)**

Decrease in Succinate dehydrogenase (SDH) activity was observed in both pesticide treated fish tissues compared to control. Similar decrement in the SDH activity was also observed by the various workers in different species of the fish exposed to different pesticides. Shailendra Kumar Singh *et al.*, (2010), reported decrease in the activities of LDH and SDH in fish *Colisa faciatus* after exposure to cypermethrin. The inhibition in LDH and SDH activities were observed in fish due to toxicity of ethanolic extract of *Nerium indicum* mill latex (Sudhanshu Tiwari and Ajay Singh, 2009).

**II.1.11.8. Malate dehydrogenase (MDH)**

Decreases in the malate dehydrogenase levels were observed in tissues of *Clarias batrachus* on exposure to endosulfan (Rajinikant Mishra and Shukla, 1997). A reduction in MDH activity was observed in *Brycon cephalus* after exposure to Folidol 600 (Lucia *et al.*, 2004).

**II.1.11.9. Aspartate aminotransferase (AAT) and Alanine aminotransferase (ALAT)**

De la Torre *et al.*, (2005) reported that elevation in levels of transaminases ALAT and AAT in the fish *Cnestodon decemmaculatus*. Similar increase in transaminases has been reported in fish tissues exposed to pesticides (Reddy *et al.*, 1991; David *et al.*, 2004; Philip and Rajasree, 1996). The same trend was observed in liver and muscle of *Colisa fasciatus* due to toxicity of ethanolic extract of *Nerium indicum* mill (Lal kaner) latex by (Sudhanshu Tiwari and Ajay Singh, 2009), *Channa punctatus*, under alachlor exposure (Tilak *et al.*, 2009).
II.1.11.10. SDS-PAGE protein profile in different species

Tripathi and shukla (1990a, 1990b) have demonstrated alterations in the cytoplasmic protein pattern of fish *Clarius batrachus* by performing SDS-polyacrylamide gel electrophoresis of the cytoplasmic protein fractions of the liver and the skeletal muscle exposed to endosulfan and methyl parathion for 1 to 28 days. Magdalena Michalczyk, and Krzystof Surowka, (2007) reported that M-protein and troponin bands were reduced, while α-tropomysin and myosin heavy chain when rainbow trout (*Oncorhynchus mykiss*) muscle was subjected to the gravading process. The freezing process of gravid resulted in an increase in the intensity of the six heaviest protein bands, while there was a tendency of most of the bands to decrease compared with fresh gravid.

Jyothirmayee et al., (2005), observed chromium induced changes in the electrophoretic patterns of esterases in kidney, liver, gill and muscle of two freshwater fish *Anabas testudineus* and *Clarias batrachus* and noticed maximum changes in the liver. Jyothirmayee et al., (2006) studied the impact of endosulfan, on the serum protein electrophoretic profile of two important edible fish *Anabas testudineus* and *Clarius batrachus* revealed that these toxicants were transported by serum.

II. 1.11.11. Residue analysis

Essumang et al., (2009) reported that total pesticide residues obtained in fish samples (*Sarotherodon melaanothern*) from the Fosu and Etsii polluted lagoons are 0.0155 mg/kg and 0.0088 mg/kg, respectively. Arnuee Therdreppitak and kittima Yammeng,(2003), studied 16 organochlorine pesticides in the edible portion of 10 different kinds of fish, namely, striped snaked-head (*Channa striatus*), common silver
barb (*Barbodes gonionotus*), tubtim (*Oreochromis niloticus*), nile tilapia (*Oreochromis niloticus*), sand goby (*Oxyeleotris marmoratus*), common carp (*Cyprinus carpio*), grey feather (*Notopterus notopterus*), common climbing perch (*Anabas testudineus*), snake skin gourami (*Trichogaster pectoralis*) and moonlight gourami (*Trichogaster microlepis*). The alpha-BHC, gamma-BHC, delta-BHC, aldrin, endosulfan I, p, p′-DDE and dieldrin were detected in 9 of 10 species. The concentration was delta-BHC for silver barb (*Barbodes gonionotus*) 14.2±0.5 and 35±1 ng g⁻¹ wet weights in *Channa striatus*.

Ibrahim Abu Bakar *et al.*, (2010) investigate the pesticide residues and antibiotic residues in three species of fish were red tilapia (*Oreachromis* sp. red hybrids), Keli (*Clarias* spp.) and patin (*Pangasius sutchii*) from different freshwater aquaculture fish from farms, markets and food premises, pesticide and antibiotic residues were detected in (2.9%) and (5.8%) of fish farm samples.

Joseph R. Fiank *et al.*, (2010) reported that different pesticide residues in fish from Densu river basin in Ghana. The concentration (µg kg⁻¹) of pesticides γ-HCH(0.10-17.65), δ-HCH (0.20-17.66), heptachlor (1.30-21.50), aldrin (0.10-2.90), γ-chlordane (0.25-10.15), α-endosulfan (0.15-16.50), p,p′-DDE (0.10-30.90), dieldrin (0.15-9.90), endrin (0.10-6.95), p,p′-DDT (0.10-12.50), endrin (0.10-6.95) aldehyde(0.10-1.55), endosulfan Sulphate (0.10-10.85), endrin ketone (0.10-6.70) and methoxychlor (0.10-12.60) respectively.

II. 1.11.12. Histopathological changes

Tilak *et al.*, (2006), found anomalies in the gill and liver of the fish *Catla catla* exposed to phenol (5.2 mg l⁻¹) for 12 days. The main alteration found in the gill was
epithelial hyperplasia, lamellar fusion, epithelial hypertrophy, telangiectasia, edema with epithelial separation from basement membranes, general necrosis, increased production and or epithelial desquamation. In liver vaculation, enlargement of nuclei of some cells, enlarged sinusoids with numerous blood vessels and atrophic areas were reported.

Sancho et al., (1997) found that the AChE inhibition of fenitrothion intoxicated fish decreased from 64% to 49% and 37% after 96 hr and 192 hr, respectively of recovery in clean water. The effect of chloropyrifos on the enzyme acetyl cholinesterase (AChE) activity in the freshwater fish Catla catla, Labeo rohita, and Cirrhus mrigala showed maximum inhibition in selected tissues such as gill, liver, muscle, and kidney (Tilak et al., 2005). Majbritt Bolton Warberg et al., (2007), reported that exposed grass shrimp (Palaemonetes pugio) and Oysters (Crassostrea virginica) to sub lethal effects of dichlorvos and obtained results. The results indicated that adult grass shrimps are more sensitive to dichlorvos than juvenile oysters and most notable decreased AChE activity in grass shrimp and oysters exposed to dichlorvos above those considered ecologically relevant.

II. 1.1.13. Oxidative metabolism

Oxidative stress is able to compromise many vital functions, and lipid peroxidation is a major mechanism involved in the cell injury. Oxidative stress occurs if the activities of the antioxidant defense system change by environmental contaminants induce production of reactive oxygen species (Li et al., 2011). The biomarkers of oxidative stress in all living organisms, on the other hand include, superoxide dismutase (SOD) and catalase (CAT), (Elif Oruc, 2010). Superoxide dismutase(SOD) is one of the first enzyme that act in defense against reactive oxygen species(ROS), it was catalyses the
conversion of reactive superoxide anions($O_2^-$) to hydrogen peroxide($H_2O_2$), which is subsequently detoxified by catalase (Santos et al., 2012). Catalase, which protects the tissues from damage by hydrogen peroxide, it was an effective marker of oxidative stress (Vasylkiv et al., 2011).

Ferrari et al., (2007) reported that decreased catalase (CAT) activity in *O. mykiss* after treated with carbaryl and azinophos methyl, both the chemicals affecting the redox balance. Carbamate fungicide Tattoo exposed to goldfish at concentrations of 3, 5 and 10 mg l$^{-1}$, serum protein carbonyls levels in fish were enhanced by 137-184%, the increased protein carbonyls levels in fish due to ROS production during tattoo exposure. The formation of protein carbonyls(PC) is virtually irreversible, causing conformational changes and ultimately resulting in the breakdown of proteins by proteases due to increased susceptibility to protease action or formation of supramolecular aggregates (Kubrak et al., 2012).

The estimation of malondialdehyde (MDA), a by-product of lipid peroxidation, continues to be a reliable method to assess the degree of peroxidative damage to cell membrane (Cini et al., 1994). Increased MDA content, were observed in fish *Cyprinus carpio* exposed to 0.036 ppb concentration of diazinon after 15 days. Hai et al., (1997) reported that increased MDA levels in *C. carpio* and *Ictalurus nebulosus* exposed to dichlorvos. Induction in MDA content in fish *Heteropeustes fossilis* after treated with dichlorvos (Vadha and Hasan, 1986). The levels of lipid peroxidation in tench *Tinca tinca* increased after exposure to carbofuran and deltamethrin. (Hernandez-Moreno et al., (2010).