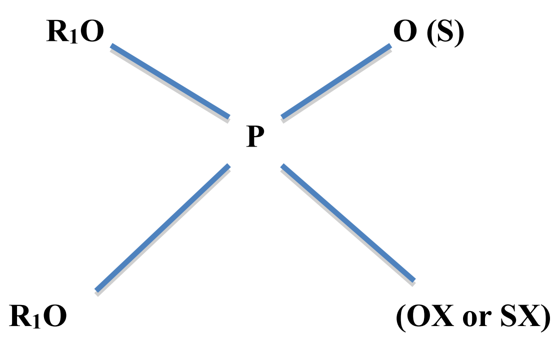
# CHAPTER-2

# LITERATURE REVIEW

## 2.1 OP pesticides

Organophosphates (OPs) are esters of phosphoric or phosphorothioic acid. It manily exist in two forms: -thion (sulfur containing) and -oxon (oxygen containing) (LaDou, 2004). The -oxon OPs have a greater toxicity than -thion OPs. However, -thion OPs readily undergo conversion to -oxons once in the environment. The -thion OPs also undergo conversion into -oxons in vivo (LaDou, 2004). The majority of OP pesticides in use are dimethyl compounds (two [-O-$CH_3$] groups attached to the phosphorus) or diethyl compounds (two [-O-$C_2H_5$] groups attached to the phosphorus) represented by $R_1$ in Figure 2.1.




**Note:** *X is the leaving group*

**Figure 2.1: Generic structure of OP pesticides**

### 2.1.1 The history of OPs

Currently, organophosphorus group forms a major and the most widely used group among the various groups of pesticides that are being used the world over, (Kanekar *et al*., 2004). In 1930, Schrader first developed OP pesticides in Germany, shortly before or during World War II, as a by-product of nerve gas development, in the form of tetraethyl pyrophosphate (Amdur, 1991). The first OP to be used commercially was tetraethlypyrophosphate (TEPP); although effective, it was extremely toxic and not very stable, as it hydrolysed in the presence of moisture (Amdur, 1991). Further development gave rise to parathion (O,O-diethyl-O-p-nitrophenylphosphorothioate, E-605) in 1944 and subsequently the oxygen analog, paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate). Although these chemicals were stable, they "exhibited a marked mammalian toxicity and were unselective with respect to target and non-target species" (Amdur, 1991).

### 2.1.2 OP pesticide half-lives

Unlike organochlorine pesticides, OP pesticides are non-persistent and break down fairly rapidly once in the environment. One way to report the level of persistence of a pesticide is to report its environmental half-life. A half-life is the period of time it takes for one-half of the amount of OP pesticide to degrade. Non-persistent pesticides have a half-life of up to 30 days, moderately persistent pesticides have a half-life of 31 - 99 days, and persistent pesticides have a half-life of 100 days or longer (Deer, 2004).

OP pesticides begin to break down as soon as they are mixed in an application tank. Factors that can influence the rate at which a pesticide breaks down and therefore its half-life include:

- The chemistry of the pesticide;
- Chemical and physical properties of spray additives;
- Chemistry (pH, hardness) of the spray water;
- A multitude of environmental factors (e.g. temperature, humidity, rainfall);
- Factors relating to the plant (surface chemistry, waxiness, etc.); and
- Soil conditions (e.g. microbial populations, moisture, temperature, pH) **(Cornell University, 2005)**.

### 2.1.3 Routes of exposure

OP pesticides can be absorbed via dermal exposure, inhalation (particularly when fine mists, dusts or fumigants are used), or ingestion. In the occupational setting, the dermal route of exposure is often the most significant. The main routes of OP exposure are shown in Figure 2.2. Humans are exposed to OPs via ingested food and drink and by breathing polluted air (WHO, 2001).As OPs and many other pesticides are absorbed across the external surfaces of insects and plants, they are also effectively absorbed across intact human skin. Absorption may be increased during hot weather when the skin is wet with perspiration.

In a human volunteer study conducted by Griffin et al., (1999), the absorption rate through the skin of a 28.59 mg dermal dose of chlorpyrifos was calculated to be 456 $ng/cm^2/h$. The fact that many pesticides, including OPs, have high lipid solubilities and low molecular weights enables them to be absorbed across intact skin (LaDou, 2004). A lower dermal $LD_{50}$ value[1] indicates greater OP absorption across the skin

(LaDou, 1997). For example, the OP,mevinphos has a reported dermal $LD_{50}$ value of 1-10 mg/kg. Several other OPs such as parathion, azinphos-ethyl, and fensulfothion have low dermal $LD_{50}$ values, making them extremely toxic chemicals. Dermal absorption will often go unnoticed until symptoms develop (Broadley, 2000).

The primary mechanism of OPs toxicity is well studied – they function as inhibitors of the enzymeacetylcholinesterase (AcHE).

Human exposure to OPs is most frequently assessed by measurement of decrease in AcHE activity. This method is relevant for professional exposure, where OP concentrations entering to body are relatively high. However, low OP concentrations, which are present continuously, do not cause significantly decreased AcHE activity. Exposure of wider populations must lean on assessment of OP metabolites, such as alkylphosphate in urine (Gupta, 2006).

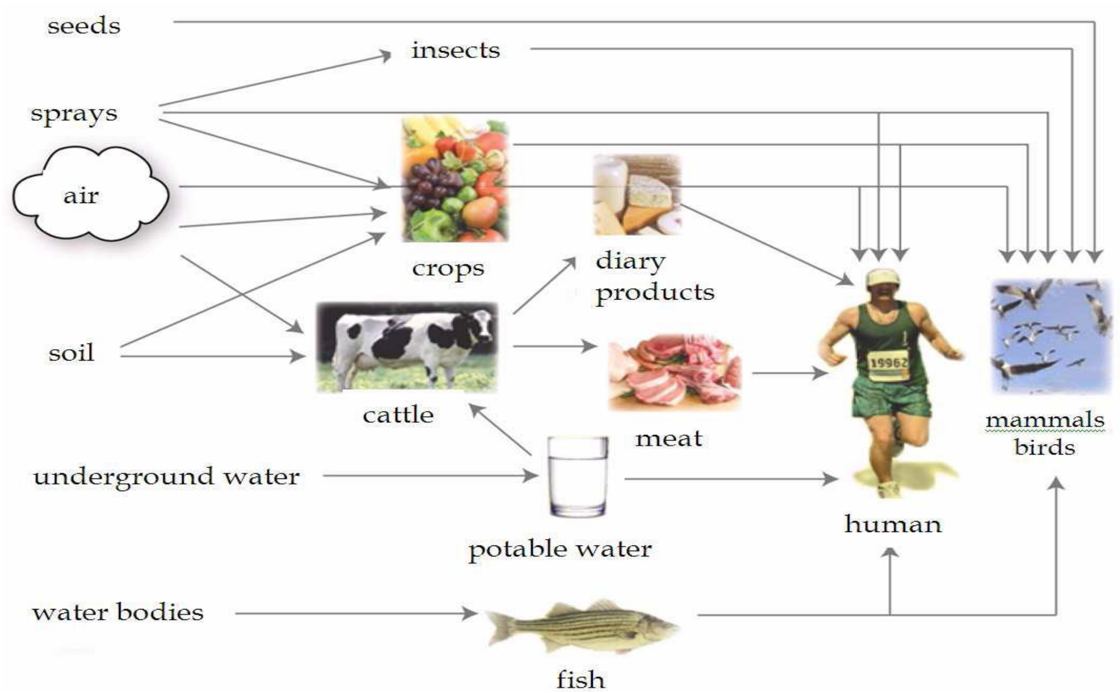



**Figure 2.2: Routes of exposure to OPs (adapted from WHO, 2001)**

### 2.1.4 OP pesticides toxicity

All OPs have the same general structure and mode of toxicity. The mode of toxicity for OP pesticides involves the inhibition, via phosphorylation, of the nervous tissue enzyme acetylcholinesterase (AChE) (LaDou, 2004). OPs are acutely toxic and act by inhibiting acetylcholine esterase, an important enzyme in the nervous system (Kanekar*et al*., 2004). On exposure to OPs, the enzyme is unable to function causing accumulation of acetylcholine, which interferes with the transmission of nerve impulses at the nerve endings.

Exposure to OP pesticides can result in two main types of health effects, acute and chronic. Acute effects occur rapidly after exposure.

The symptoms of acute poisoning are well documented (Klaassen, 2001, LaDou, 2004); they may include blurred vision, lachrymation, salivation, bronchorrhea, pulmonary oedema, nausea, vomiting, diarrhoea, urination, perspiration, incontinence, bradycardia, arrhythmias, heart block, cramps, headache, dizziness, malaise, apprehension, confusion, hallucinations, manic or bizarre behaviour, convulsions, loss of consciousness, and respiratory depression. Acute intoxication may cause death; however, with appropriate emergency treatment, death is less likely.

There are three types of chronic health effects from OP poisoning. These can be due to:

1. repeated exposures over a short time period;

2. an acute poisoning episode; or

3. as a result of low-level, long-term exposure without any acute poisoning episodes.

Some of the main agricultural products are parathion, methyl parathion, chlorpyriphos, malathion, monocrotophos and dimethoate. Although organophosphates are biodegradable in nature, their residues are found in environment. Considering their toxicity, research on biodegradation of organophosphates is being carried out all over the world.

### 2.1.5 Review of previous studies on OPPs residues in soil, water and food items

Occurrence of pesticide residue in various environments is mainly due to intensive use of pesticide in agriculture, pesticide industries (point source), atmospheric fall out, agricultural runoff, industrial discharges etc. (Babu et al 2005). Recent studies on pesticide residues indicate the occurrence of organophosphorus pesticides in major rivers, cultivable land, and drinking water in India and many other countries.

Jabber et al., in 1993 reported the monocrotophos, cyhalothrin, dimethoate, fenvalerate, cypermethrin, profenofos in the top foot soil of Samudari, a cotton growing area and shallow ground water of Faisalabad district in Punjab, Pakistan.

Mayanglambam et al., (2005) studied the persistence and leaching of quinalphos, an OP widely used in an Indian agriculture for control of pests in various crops under field conditions using soil column. The activity of dehydrogenase and alkaline phosphomonoesterase of soil inhibited by the residues of quinalphos. Activities varied with insecticide applications. It was noted that quinalphos is moderately persistent with half-life of 30 days and is capable of leaching to surface soil, and large concentration of it can cause ground water contamination.

S.A Baig et al., in 2009 determine residues of three pesticides in vegetables, belonging to Organophosphate group, viz. Triazophos, profenofos and chlorpyrifos, which were extensively used in the Southern part of Punjab province in Pakistan. The results showed that 8% of the samples tested contained residues higher than the MRLs.

R. Arjmandi et al., in 2010 determine organophosphorus insecticide residues in the rice paddies in Mazandaran Province. Results indicate that the insecticide, diazinon was used frequently to control stem boring caterpillar of rice. The residuals of this toxic chemical were observed in the majority of stations from the day after the spraying until one to two months later.

Organophosphorus pesticides in soil were determined by rapid and sensitive sample pretreatment technique using dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection by Yang et al., in 2012.

The Concentrations of organophosphorus pesticide residues (dichlorvos, diazinon, chlorpyrifos, and fenitrothion) were determine in some vegetables (spinach, lettuce, cabbage, tomato and onion) and soil samples from different depths within Alau Dam and Gongulong agricultural areas in Borno State, Nigeria by J. C. Akan, L. Jafiya, Z. Mohammed, F. I. Abdulrahman (2013). The concentrations of all the pesticides in the soil samples were observed to be higher at a depth of 21-30cm, while the lowest concentrations were observed at a depth of 0-10cm. The concentrations of all the organophosphorus pesticides in the vegetables and soil samples from the two agricultural areas were observed to be at alarming levels, much higher than the maximum residue limits (MRLs) and acceptable daily intake values (ADIs) set for vegetables and soil by the Cordex 2009 (WHO and FAO).

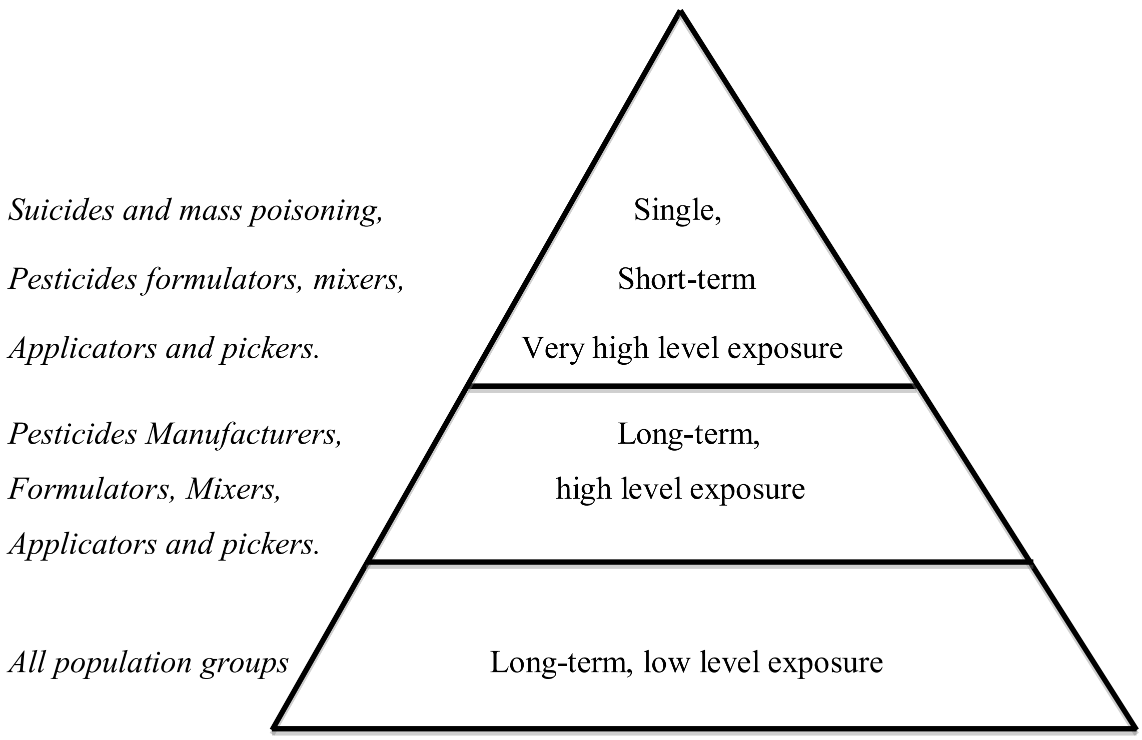
## 2.2 Pesticides poisoning

Despite the large increase in food production brought about by chemicals, pesticides, the agricultural, environmental and health costs arising from pesticides use are high (Wilson, 2000). Mankind enjoyed the benefits of these synthetic agro-chemicals for three decades without knowing their ill effects. The determents to the health of people and the environment were first heard after the twenty years of their use when Carson, 1962, in her curtain-raising publication, “Silent Spring”, has for the first time in the history, has rightly mentioned, “every human being is now subjected to contact with dangerous chemicals, from the moment of conception till death”. Miller and miller (1979) reported on the many environmental chemicals, which are carcinogenic, and 90% of these chemicals were pesticides related. Moreover, the pesticides effects were linked to “the level of chronological (cancer), pulmonary and haematological morbidity, as well as inborn deformities and immune system deficiencies” by United Nation Environment Program (UNEP, 1993).

Since the use of pesticides in agriculture inevitably leads to the exposure of non-target organisms (including humans), an undesirable side effects can appear on whole species, communities as well as ecosystems. Holly et al., in 1992 and Mulder et al., in 1994 have found that the pesticides were responsible with the incidence of Ewing’s bone tumors, lymphomas and leukemia in children.

Farm workers have special risks associated with inhalation and skin contact during preparation and application of pesticides to crops. Occupational exposure (exposure during crop production and during pesticide manufacturing/ formulation) comprises about 60 to 70% of poisonings.

Risk groups associated with the occupational exposure are shown in the following figure 2.3




*Source: Davies, 1984*

**Figure 2.3: Population risk groups**

The World Health Organization and the UN Environment Programme estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (World Health Organization, 1994).

Pesticide-related deaths are a major public health problem worldwide. According to World Health Organization estimates published in 1990, there are around 3 million annual pesticides poisoning cases with 220.000 deaths (American Medical Association, 1997).

A more recent study reported that the number of annual deaths from pesticide ingestion might actually be around 300,000 in China and South East Asia alone. Pesticide poisoning occupied the largest proportion of the deaths by all poisoning in 2001 in South Korea (W J Lee et al., 2009). Pesticides can be dangerous to consumers, workers and close bystanders during manufacture, transport, or during and after use.

### 2.3 Environmental impact of pesticides

Pesticides present the only group of chemicals that are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products. However, the majority of pesticides are not specifically targeting the pest only and during their application they also affect non-target plants and animals. Repeated application leads to loss of biodiversity. Many pesticides are not easily degradable, they persist in soil, leach to groundwater and surface water and contaminate wide environment. Depending on their chemical properties they can enter the organism, bioaccumulate in food chains and consequently influence also human health. Overall, intensive pesticide application results in several negative effects in the environment that cannot be ignored.

The problem of pesticide usage is not over now in many countries the old persistent, bio- accumulative pesticides have been banned. A lot of new substances have been developed and used in large quantities. For many of these substances today we still do not have sufficient amount of knowledge about their possible risks and adverse effects on the environment and human. Several of them appear to have a bad environmental profile.

*Environmental effects of pesticides, in different compartments are:-*

### 2.3.1. Soil contamination

Pesticides enter the soil via spray drift during foliage treatment, wash-off from treated foliage, release from granulates or from treated seeds in soil. Some pesticides such as soil fumigants and nematocides are applied directly into soil to control pests and plant diseases presented in soil.

The transport, persistence or degradation of pesticides in soil depends on their chemical properties as well as physical, chemical and biological properties of the soil. All these factors affect sorption/desorption, volatilisation, degradation, uptake by plants, run-off, and leaching of pesticides.

Persistence of pesticides in soil can vary from few hours to many years in case of OC pesticides. Despite OC pesticides were banned or restricted in many countries, they are still detecting in soils (Shegunova et al., 2007; Toan et al., 2007; Li et al., 2008; Hildebrandt et al., 2009; Jiang et al., 2009; Ferencz and Balog 2010). Several studies have been made to detect the contaminated soil in which different kinds of pesticides have been found (Nawab et al., 2003, Zhang et al., 2006). Continuous and heavy application of these agrochemicals and other soil amendments can potentially exacerbate the accumulation of heavy metals in agricultural soils over time (Siamwalla, 1996; Chen et al. 2007).

**Sorption** is the most important interaction between soil and pesticides and limits degradation as well as transport in soil. Pesticides bound to soil organic matter or clay particles are less mobile, bio available but also less accessible to microbial degradation and thus more persistent.

Soil organic matter is the most important factor influencing sorption and leaching of pesticides in soil. Addition of organic matter to soil can enhance sorption and reduce risk to water pollution. However, high yield agriculture depletes soil organic matter and will reduce sorption.

### 2.3.2. Water contamination

Pesticides can get into water via drift during pesticide spraying, by runoff from treated area, leaching through the soil. In some cases pesticides can be applied directly onto water surface e.g. for control of mosquitoes. Water contamination depends mainly on nature of pesticides (water solubility, hydrophobicity), soil properties, weather conditions, landscape and also on the distance from an application site to a water source. Rapid transport to groundwater may be caused by heavy rainfall shortly after application of the pesticide to wet soils.

The geographic and seasonal distribution of pesticide occurrence follows patterns in land use and pesticide use. Streams and rivers were frequently more polluted that groundwater and more near the areas with substantial agricultural and/or urban land use. Pesticides usually occurred in mixture of multiple compounds, even if individual pesticide were detected bellow limits. This potentially can lead to underestimation of toxicity when assessments are based on individual compounds.

More recent studies also reported presence of pesticides in surface water and groundwater close to agriculture lands over the world (Cerejeira et al., 2003; Konstantinou et al., 2006; Gilliom 2007; Woudneh et al., 2009; Anasco et al., 2010).

### 2.3.3. Effects on organisms

***Soil organisms and processes***

**Soil microorganisms** play a key role in soil. They are essential for maintenance of soil structure, transformation and mineralization of organic matter, making nutrients available for plants. Soil microorganisms are also able to metabolise and degrade a lot of pollutants and pesticides and thus are of great concern for using in biotechnology. On the other hand, microbial degradation can lead to formation of more toxic and persistent metabolites. Although soil microbial population are characterized by fast flexibility and adaptability to changed environmental condition, the application of pesticides (especially long-term) can cause significant irreversible changes in their population. Inhibition of species, which provide key process, can have a significant impact on function of whole terrestrial ecosystem.

Organophosphate insecticides (chlorpyrifos, quinalphos, dimethoate, diazinon, and malathion) had a range of effects including changes in bacterial and fungal numbers in soil (Pandey and Singh 2004), varied effects on soil enzymes (Menon*etal*. 2005; Singh and Singh 2005), as well as reductions in collembolan density (Endlweber*et al*. 2005) and earthworm reproduction (Panda and Sahu 1999).

Fungicides were found to be toxic to soil fungi and actinomycetes and caused changes in microbial community structure (Liebich et al., 2003; Pal et al., 2005).

Other bacterial species, such as nitrification bacteria, are very sensitive to pesticides influence. Inhibition of nitrification was proved by sulphonylurea herbicides (Gigliotti and Allievi 2001). Chlorothalonil and dinitrophenyl fungicides such as Mancozeb, Maneb or Zineb have also been shown toxic to nitrification and denitrification bacterial processes (Kinney et al., 2005; Lang and Cai 2009).

A few studies show that some organochlorine and organophosphorus pesticides suppress symbiotic nitrogen fixation resulting in lower crop yields. Authors found out those pesticides Pentachlorphenol, DDT and Methyl parathion at levels found in farm soils interfered signalling from leguminous plant such as alfalfa, peas, and soybeans to symbiotic soil bacteria. This effect, loosely comparable to endocrine disruption effects of pesticides in human and animals, significantly disrupt $N_2$ fixation. As consequence increased dependence on synthetic nitrogenous fertilizer, reduced soil fertility, and unsustainable long-term crop yields occur. (Fox et al., 2007; Potera 2007).

Some pesticides (Benomyl, Dimethoate) can also negatively affect symbiotic mycorrhizal fungi, which facilitate plant nutrient uptake (Menendez et al., 1999; Chiocchio et al., 2000). Moreover, agricultural practises such us tillage, crop rotation, fertilization, pesticide application, irrigation can also reduce root colonisation by myccorhizal fungi (Jansa et al., 2006).

***Soil invertebrates***

Nematodes, springtails, mites and further micro-arthropods, earthworms, spiders, insects and all these small organisms make up the soil food web and enable decomposition of organic compounds such as leaves, manure, plant residues and they also prey on crop pests. Soil organisms enhance soil aggregation and porosity and thus increasing infiltration and reducing runoff.

**Earthworms** represent the greatest part of biomass of terrestrial invertebrates (>80 %) and play an important role in soil ecosystem. They are used as bio indicator of soil contamination providing an early warning of decline in soil quality. They serve as model organisms in toxicity testing. Earthworms are characterized by high ability to accumulate a lot of pollutants from soil in their tissues, thus they are used for studying of bioaccumulation potential of chemicals.

A recent review of pesticides effects on earthworms showed on negative effects on growth and reproduction by many pesticides (Shahla and D'Souza 2010).

Glyphosate, nonselective herbicide, and chlorpyrifos, insecticide, belong to the most worldwide used pesticides, especially on transgenic resistant crops. An integrated study on a Roundup resistant soya field in Argentina showed deleterious effect of these pesticides on earthworm population. (Casabe et al., 2007).

Other soil species are also often affected by pesticides application and non-target soil community structure can be strongly affected. Decrease in number of spiders and diversity, and species richness of Collembolan after application of insecticide chlorpyrifos has been reported on grassland pasture in UK (Fountain et al., 2007).

In the review article from 2010 authors investigated the impact of pesticide restriction in arable crop edges on naturally occurring terrestrial invertebrates. Authors conclude that the restriction of herbicides in crop edges has a positive influence on arthropod populations, especially for chick-food insects, Heteroptera and other herbivores. Predatory insects may be affected indirectly by the exclusion of herbicides alone, or as a result of changes in their prey availability (Frampton and Dorne 2007).

***Other non-target species***

Effect of pesticides on bees are closely watched because their crop pollination. However, little is known about the impacts of pesticides on wild pollinators in the field. In recent study conducted in Italian agricultural area, authors monitored species richness of wild bees, bumblebees and butterflies were sampled after pesticides application. They detected decline of wild bees after repeated application of insecticide fenitrothion. Lower bumblebee and butterfly species richness was found in the more intensively farmed basin with higher pesticide loads (Brittain et al., 2010).

Several articles reported negative effects of pesticides and intensive agriculture on butterflies populations (Longley and Sotherton 1997; White and Kerr 2007; Adamski et al., 2009), and showed positive impact of organic farming (Saarinen 2002; Feber et al., 2007).

***Water organisms – invertebrates, amphibians, fishes***

Pesticides can enter fresh water streams directly via spray drift or indirectly via surface runoff or drain flow. Many pesticides are toxic to freshwater organisms. Acute and chronic effects are derived from standard toxicity tests. Within the ecological context, sublethal effects of toxic contaminants are very important. For example, downstream drift of stream macro invertebrates in common reaction to various types of disturbance, including chemical contamination and may result in significant changes of structure of lotic communities.

Several studies reported toxicity of pesticides on aquatic vertebrates – *amphibians and fishes.* For example, Carbaryl has been found toxic for several amphibian species, additional combination with predatory stress caused higher mortality (Relyea 2003). Also herbicide Roundup, glyphosate, caused high mortality of tadpoles and juvenile frogs in an outdoor mesocosms study (Relyea 2005b).

Impact of Malathion a broad-spectrum insecticide, on aquatic ecosystem has been demonstrated. Malathion is the most commonly applied insecticide around the world and can be legally directly sprayed over aquatic habitats to control the mosquitoes. This study showed that relatively small concentration of malathion caused direct and also indirect effect on aquatic food web. Changes in plankton and periphyton abundance and composition consequently affected growing of frog tadpoles and reduced predation rates on amphibians (Relyea and Hoverman 2008). Moreover, repeated low dose application caused large impact than single exposure (Relyea and Diecks 2008). Similar results were observed also for insecticides carbaryl and herbicides glyphosate and 2,4-D (Relyea2005a; Boone et al., 2007).

**Mixture of pesticides** is commonly detected in the environment and exposition to organisms can be more dangerous than individual effect.

A study published in Environmental Health Perspectives gave evidence of synergistic and additional effects of pesticide mixture. Author investigated in vivo effects of combination of common used organophosphate and carbamate pesticides on acetylcholin enzyme inhibition in the brains of juvenile coho salmon. All tested pesticides resulted in sublethal inhibition effects; exposures to binary mixtures of OP and CB pesticides produced significant additive or synergistic effect and lower lethal concentration. Synergistic effect was greater at higher exposure concentrations (Laetz et al., 2008).

***Birds***

Decline of farmland bird species has been reported over several past decades and often attributed to changes in farming practises, such as increase agrochemical inputs, loss of mixture farming or unfarmed structures. Besides lethal and sub lethal effects of pesticides on birds, concern has recently focused on the indirect effects. These effects act mainly via reduction of food supplies (weeds, invertebrates), especially during breeding or winter seasons. As consequence insecticide and herbicide application can lead to reduction of chick survival and bird population. Time of pesticides application plays also important role in availability of food.

Several practises (generally Integrated crop management techniques) can be used to minimize unwanted effects of pesticides on farmland birds, such as use of selective pesticides, avoiding spraying in during breeding season and when crops and weeds are in flower, minimise spray drift or creation of headlands. Evidences of this important indirect effect of pesticides has been reported e.g. by Moreby and Southway 1999; Boatman et al., 2004; Taylor et al., 2006).

### 2.3.4. Effects of pesticides and farming practises on biodiversity

Intensive pesticides and fertilizers usage, loss of natural and semi-natural habitats and decreased habitat heterogeneity and all other aspects of agricultural intensification have undoubted impact on biodiversity decline during last years.

***Intensive agriculture***

A current Europe-wide survey in eight West and East European countries brought alarming evidence of negative effects of agricultural intensification on wild plants, carabid and bird species diversity. Authors demonstrated that, despite decades of European policy to ban harmful pesticides, pesticides are still having disastrous consequences for biodiversity on European farmland. Insecticides also reduced the biological control potential. They conclude that if biodiversity is to be restored in

Europe, there must be a Europe-wide shift towards farming with minimal use of pesticides over large areas (Geiger et al., 2010).

A recent study, conducted in agriculture area in Netherlands, estimated impact of insecticides, herbicides and fungicides drift on terrestrial biodiversity outside treated area. Researches demonstrated that under scenarios based on wider buffer zones and 'best available practise the pesticide impact could be cut to zero. This study suggests that increasing unsprayed buffer zones around crops is critical to the success of any new strategy to prevent the harmful impact of pesticides (de Jong et al., 2008).

**2.4 Evidence for biodegradation of pesticides**

Availability of different pesticides in field provides exposure of several different kinds of microorganisms to pesticides. Most of the organisms die under toxic effect of pesticides but few of them evolve in different ways and use pesticide compounds in metabolism.

Pesticide degradation is the breaking down of toxic pesticides into a nontoxic compounds and, in some cases, down to the original elements from which they were derived. The most common type of degradation is carried out in the soil by microorganisms, especially the fungi and bacteria. Hence the degradation process of pesticides in the different ecosystems universally takes a large space of interest. Recently the use of microbes for effective detoxification, degradation and removal of toxic compounds from contaminated soils has emerged as an efficient and cheap biotechnological approach to clean up polluted environments (Strong and Burgess, 2008). From the great microbial population existing in soil, some bacterial strains show the capability to degrade some types of pesticides thru specific paths, such using it as a source of nutrients and the most common one to use it as carbon and energy sources due to the chemical nature (Aislabie and Lloyd-Jones, 1995).

Several reports are available indicating degradation of different pesticides when they are available in nature in excess (Horvath, 1972; Hussain et. al., 2007 and Lakshmi et. al., 2009). Successful removal of pesticides by the addition of bacteria (bioaugmentation) had been reported earlier for many compounds, including chlorpyrifos, endosulfan, malathion, parathion, quinalphos, ethoprop, and atrazine (Singh et. al., 2004). Most bacterial species, which degrade pesticides, belong to the genera *Flavobacterium, Arthrobacter, Azotobacter, Burkholderia and Pseudomonas*.Several fungi such as *Agrocybesemiorbicularis, Auriculariaauricula, Coriolusversicolor,Dichomitussqualens, Flammulinavelupites, Hypholomafasciculare, Pleurotusostreatus, Stereumhirsutum, and Avatha discolor* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated and organophosphorus compounds (Bending *et al.,* 2002).

**2.5 Microbial degradation of organophosphorus pesticides**

Insecticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation (Agnihotri*et al*., 1981). Microorganisms play an important role in degrading synthetic chemicals in soil (Alexander, 1981). They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source.

When organophosphates are released in the environment, their fate is decided by various environmental conditions and microbial degradation. Microbial degradation is the major reason of disappearance of these pesticides. Bacterial enzymatic detoxification of organophosphorus pesticide has become the focus of many studies, because it is economical, environment friendly and effective. However, the use of microorganisms for bioremediation requires an understanding of all physiological, microbiological, ecological, biochemical and molecular aspects involved in pollutant transformation (Iranzo *et al* 2001).

In 1973, the first bacterium to degrade OP compounds was isolated from a soil sample from the Philippines and was identified as *Flavobacterium*sp. ATCC 27551 (Sethunathan & Yoshida). This is the first report of the isolation of OP-degrading bacteria from the environment. A number of other microorganisms with similar degrading capabilities have since been isolated. Since then, several bacteria, a few fungi and cyanobacteria, have been isolated that can use OP compounds as a source of carbon, nitrogen or phosphorus.

The worked on microbial degradation of organophosphates has been reviewed wherein the degradation is reported in plants, soils, water and animals (Beynon et al., 1973), in flooded soil and in anaerobic cultures (Sethunathan, 1973) and by soil microorganisms (Laveglia and Dahm, 1977). Bacteria from flooded soil were found to hydrolyze selected organophosphorus insecticides (Adhya et al., 1981).

Rosenberg and Alexander (1979) studied the microbial cleavage of various organophosphorus insecticides. Two strains of *Pseudomonas* sp. isolated from diazinon and malathion enrichments were most versatile. They demonstrated that the breakdown of these chemicals resulted from an induced enzyme system.

Degradation of malathion by microorganisms isolated from industrial effluents was reported by Singh and Seth (1989). The bacterial strain *Pseudomonas* sp. N-3 was found to degrade malathion up to 150 ppm only in the presence of ethanol (1% v/v) as a co-substrate.

Rangaswamy and Venkateswarlu (1992) studied the degradation of selected insecticides, Quinalphos and monocrotophos by *Azospirillumlipoferum* and *Bacillus* sp. isolated from black soil following enrichment culture technique. By the end of 7 days, about 40 per cent of monocrotophos supplemented to mineral salts medium was degraded by *A.lipoferum*and *Bacillus* sp. Nearly, 56 percent and 76 percent of quinalphos was degraded by *A. lipoferum*and *Bacillus* sp. respectively.

Sharungbam*et al.* (2003) reported the degradation of quinalphos by bacteria isolated from soil. It was observed that 11 isolates degraded up to 92 per cent of the insecticide at higher concentrations (8 ppm and 12 ppm) on the 10th day of incubation.

B. Singh, R. Kaur and K. Singh (2009) reported the isolation of malathion degrading bacteria and showed that isolated culture were rod shaped *Bacillus thuringiensis* and *Bacillus cereus.*

Quinalphos use and its impact on biomass of microbial cells and their metabolic activities in clay loam soil were observed by Sengupta et al., 2009.

A.Ratna Kumari, et al., (2012) isolated malathion degrading bacteria from soil. The analysis showed that the isolated strain belongs to *Bacillus species*.

S. Kavi Karunya and D. Reetha ( Received Mar 2012; Revised 2012; Accepted 17 Jun 2012) carried out the isolation of pesticide degrading bacteria and the bacterial isolates were identified as *Pseudomonas fluorescens*, *Bacillus subtilis* and *Klebsiella sp*. The growths of these three pesticide degrading isolates were assessed in Minimal salt broth containing 2 different pesticides *viz*., Malathion and Parathion which were used in the study and found that among the three bacterial isolates, the bacteria *Klebsiella sp.* utilized the pesticides effectively and showed maximum growth.

Tamer M.A. Thabit and Medhat A.H. E L-Naggar (2013) have conducted studies on malathion degradation by soil isolated bacteria and detected the degradation product by GC-MS. 5 species of bacteria were isolated, *Pseudomonas aeruginosa, Bacillus amyloliquefaciens, Staphylococcus sciuri, Bacillus pseudomycoides* and *Bacillus licheniformis* from newly reclaimed agricultural soil. Two main degradation product resulted from bacterial degradation, namely malathion monocarboxylic and malathion dicarboxylic acid, the first one may convert to the latest one over-time.

K.R.Pawar and G.V.Mali (2014) have conducted studies to determine the degradation potential of *Pseudomonas* species isolated from grape rhizosphere soils towards the organophosphorus insecticide Quinalphos. A total number of 14 *Pseudomonas* strains have been isolated and screened for their tolerance level against various concentrations of Quinalphos. Results indicated that out of 14 isolated *Pseudomonas* strains, only one strain could tolerate and degrade the highest concentration of Quinalphos.