Chapter 1

Introduction & Literature Survey
1.1. ORIGIN OF THE TOPIC

Human population has crossed 6 billion mark, and for feeding them, there is tremendous pressure to increase food production because of economic and social reasons. In this regard either we need to increase crop/food production or protection of crops/foods from different pests/weeds etc. As per an assumption, different pests eat more than one third of total food production. Therefore, protection of the crop/food must be increased. Because of this reason, pesticides have considered as an integral part of modern agriculture. But, by this way, we have become odd with the nature, which increased tremendous chemical pressure on the earth or agricultural soil. More than 3 million tons of pesticides applied per year on the almost 5 billion hectares of the world’s agricultural land.

All the pesticides directly or indirectly reach to soil surface after their applications. Pesticides may contain one or more than one coordination sites, and they can interact with metal ions of soil (bounded metal ions or free metal ions), soils oxides, organic matter etc. For example, glyphosate (world top ranked herbicide) has three functional groups (P-O-, NH and COOH), and these groups are responsible for strong coordination chemistry of glyphosate with metal ions at variable pH values. Like glyphosate, all pesticide contain one or more than one active/coordination site. These active/coordination sites make the coordination chemistry of pesticides richer than their pesticidal application.

The coordination of pesticide with organic/inorganic component of soil may either make soluble (in water) or insoluble product(s). Soluble product through water medium may go inside into the plant, while stable insoluble product may remain in the soil for the longer duration. Soluble complex can allow the uptake of toxic pesticides, heavy metal ions and hazardous organic matter. Whereas, non-soluble complex can causes the non-availability of essential metal ions to the crops and humans. Now, both types of complexes are threat for living being including total environment.
Also, it is the well known fact about the interaction of pesticides with organic matter and soil mineral that these interactions take place more rapidly than biodecomposition or microbial decomposition.\textsuperscript{2,6}

More than three billion people are suffering worldwide from deficiency of micronutrients (Manganese (>10%), Iron (>25%) and Zinc (>45%) deficiency)).\textsuperscript{7,8} Use of extensive amount of chemicals might be a reason behind it. As per our assumption, “The surplus and reiterate use of pesticides may result in severe impairments in micronutrients of crops and nontarget plants, possibly due to the formation of poorly soluble to non soluble pesticides-metal complexes in the plant tissues and/or rhizosphere interactions processes”.

Studies regarding the glyphosate (an organophosphate pesticide) applications revealed, significant decrease in root and shoot dry matter production, chlorophyll concentrations of young leaves, photosynthetic parameters and nutrients in tissues.\textsuperscript{2} Micronutrients deficiencies are increasingly being observed in cropping systems or agricultural land with frequent glyphosate applications.\textsuperscript{2} Glyphosate is not a special compound and this fact is therefore not limited to glyphosate only. As we know, in nature, none of the system is isolated, so, when we see the impact of pesticide on soil/plant system, we cannot forget, presence of humic acid, metal ions and pesticides at a time. There are cases of multipesticides application and metal ions at a time on the same field. So, the simultaneous application of different pesticides should also be studied. They may affect the beneficial bacteria also. Consequently, they can harm the plant growth promoting activities like siderophore production, indole acetic acid production, nitrate production etc. Ultimately, these conditions can affect the crop or plant growth. This thesis is target to unfold all such questions. But, because of the limitation of fund and time constraint, we explored our work by taking four organophosphate pesticides (acephate, glyphosate, monocrotophos and phorate).

The selection of these four pesticides (Figure 1.1) were done on the basis of following facts: (1) from commercial point of view, all pesticides are the top selling
pesticides (OPs) worldwide including India\textsuperscript{9,10} (2) from the coordination chemistry point of view; glyphosate can behave strong complexing ligand, acephate and phorate can behave as intermediate complexing ligand, and monocrotophos can behave as weak complexing ligand. (3) from the application point of view glyphosate is an herbicide, acephate and monocrotophos are insecticides, and phorate is a nematide and insecticide. (4) As per HASB Principle; they may behave as both hard base (through coordination with phosphoryl oxygen or carbonyl oxygen), and as soft base (through sulphur and nitrogen as ligating site). Means, these can form complexes with hard as well as with soft metal ions. Phorate can behave as soft ligand through the S atom and it can only interact with soft metal ions. From the best of our knowledge of available literature, no such study is reported in literature till date.

![Structure of Acephate, Glyphosate, Monocrotophos and Phorate](image1.png)

**Figure 1.1:** Structure of Acephate, Glyphosate, Monocrotophos and Phorate.

1.2. INTRODUCTION AND LITERATURE SURVEY

1.2.1. Significances of Pesticides

There was the time after the Second World War when whole world was in the grip of hunger and misery. To overcome with the problem of hunger, mission “Green Revolution” had been introduced. Due to enormous efforts and firm work of the whole world “Green Revolution” had been launched successfully. The major weapons of the
“Green Revolution” were the "synthetic pesticides" *viz.* Organochlorines, Organophosphates and Carbamates. Their use continued and turned towards one of the world’s biggest industrial sector. With the passage of time, pests developed immune against the implemented pesticide and new formulation had taken the place of the old one. The dependency on pesticides increased and expected to be increased in future due to increase in the populations and decrease in the agricultural land. So, the truth behind the scene is, “In the era of 1960s, the use of the pesticides was the obligation but, it is necessity of present and future” because of following well known facts:

1. To nourish the fast mounting population.
2. To ascend the economic growth of agriculture based countries.
3. To increase the quantity as well as quality of agriculture based products.
4. To control the seasonal & epidemic diseases.
5. To overcome the problem of storage of eatable & food grains.

### 1.2.2. Organophosphate pesticides

Organophosphate pesticides (OPs) are the ester forms of phosphoric acid, usually considered as secure for agriculture uses due to their relatively fast degradation rates. OPs inhibit acetylcholinesterase (AChE) activity, not in insects only, but can also affect the nervous system of other organisms. Human beings are not an exception of it. Organophosphate pesticides derived from phosphorus analog PH$_3$ having phosphorus as a core nuclei involved in oxidation states III and V. Basically organophosphate (Figure 1.2) is the general name for esters of phosphoric acid.

\[
\begin{align*}
R^2O^- & \quad P^\equiv Z \\
R^3O & \quad L - R^1 \\
\end{align*}
\]

- $Z = O$ or $S$
- $R = Me/Et$ etc.
- $LR$ = Leaving groups, *viz.*
  - Alkyl, Alkoxy, Alkylthio, Aryl, Hetrocyclic,
  - Aryloxy, Arylthio etc.

**Figure 1.2:** Basic structure of OPs.
Hydrolyzed derivatives of phosphorus formal incorporation of additional oxygen atoms gives phosphinic acid (O=PH$_2$OH) and phosphonic (phosphorus) acid [O=PH(OH)$_2$]. Notably, these species may tautomerize between P(V) and P(III), that is, H$_3$P(O)OH to HP(OH)$_2$. Although, tetrahedral structure [O=PH(OH)$_2$] is more established at room temperature than its isomer phosphonic acid, P(OH)$_3$. The main classes of OPs are phosphates, amidates, thionates, thiolates, phosphonates and phosphinates (Figure 1.3).

![Figure 1.3: Species of Organophosphates.](image)

1.2.3. Mode of action of OPs

Acetylcholinesterase (AChE) inhibition is the most illustrious mode of action of the organophosphates. Inhibition occurs through the irreversible binding of organophosphates to the catalytic site of the enzyme. Three basic types w.r.t. organ system of organophosphate pesticides toxicity are seen i.e. parasympathetic nervous system, Nicotine-receptors system, Central nervous system. In all these three, the basic mechanism of toxicity is inhibition of acetylcholinesterase due to phosphorylated action of OPs, causing AChE inactivation in an irreversible manner. Enzymatic hydrolysis (Figure 1.4) involves nucleophilic addition and acid-based reactions at the catalytic site of the enzyme that involves a catalytic chord i.e. three amino proteins viz. serine, glutamic acid, and histidine.

An enzyme-substrate complexation between acetylcholine (ACh) and the enzyme form by electrostatic attractions between the positive charge on the ACh nitrogen and the negative charge in the acidic site. Then the imidazole moiety of the histidine catalyzes acetylation of the serine hydroxyl group; the acetylated enzyme then allows for nucleophilic attack by water to transpire. The deacetylation reaction results in the
liberated enzyme and release of inactive choline and acetic acid. Catalytic activity of AChE is very high with a turnover number of $>10^{4}$ s$^{-1}$.17

![Diagram of Acetylcholinesterase Hydrolysis of Acetylcholine](image)

**Figure 1.4:** Acetylcholinesterase Hydrolysis of Acetylcholine.

The mechanism of AChE inhibition with organophosphates (Figure 1.5) is analogous to the reaction of enzyme with ACh, excluding for the reaction in which the leaving group of the organophosphates is lost, so the enzyme becomes phosphorylated instead of acetylated.17 Phosphorylation is a two-step addition-elimination reaction in which the addition step is rate-determining, while the elimination process is faster. It should be noted that phosphorylation occurs via a *trigonal bipyramidal* intermediate, whereas in the case of acetylation is anticipated through a *tetrahedral* intermediate.17 The irreversibly inhibited phosphorylated enzyme can no longer hydrolyze acetylcholine. This leads to an accumulation of acetylcholine in cholinergic receptors and consequent continuous
stimulation of the nerve fiber. Phosphorylated AChE having the stable bonding and forces than acetylated one, but it can undergo a possible secondary process. Among these first one is the reactivation-hydrolysis of phosphorylated enzyme. But, the rate of hydrolysis is much slower than in the case of an acetylated enzyme.\textsuperscript{17} Another mechanism is the breaking of the PO-C bond the inhibited enzyme known as “aging”.\textsuperscript{17}

\begin{center}
\textbf{Figure 1.5:} Inhibition and “Aging” of AChE with OPs.
\end{center}

1.2.4. \textbf{Applications and environmental fate of OPs}
Organophosphate compounds have been in use since 1937 as warfare agents. They were introduced as agrochemicals in 1960.\textsuperscript{16} OPs are now one of the most common pesticides used in agriculture, horticulture, public health protection and domestic activities.\textsuperscript{16} Only USA is using 50,000 tons per year of OPs.\textsuperscript{17} OPs are major part of world economy including other pesticides, because OPs are account for an estimated 34\% worldwide sale. Annually, 65\% of OPs are used as insecticides on worldwide level with an estimated cost of around 150 million pound.\textsuperscript{13,16,17} OPs have following main and common benefits as like other pesticides, they increase (1) crop productivity and yield, (2) economic
development of nation, (3) improvement in food quality and storage, and (4) control of vector disease.\textsuperscript{13}

More than 100 OPs have been reported by World Health Organization (WHO) for different pesticide action, among them only 30 having broad spectrum activity.\textsuperscript{16-18} Commonly used organophosphates are; acephate, glyphosate, parathion, malathion, methyl-parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion, tetrachlorvinphos, azamethiphos, and azinphos methyl. These OPs are tabulated (Table 1.1) in alphabetical order. Toxicity (LD\textsubscript{50}) values of all these OPs are given in table against their name and structures. LD\textsubscript{50} values of the OPs are showing that these pesticides are mild to highly toxic. Various sources (WHO Fact Sheet, MSDS, Chemspider, Sigma Aldrich etc.) have shown that maximum OPs are water soluble in nature and they can easily reach to various compartments of the environment including water and soil. Hence, they can cause toxicity through various medium including water, air and soil. The main mode of toxicity of OPs is the inhibition of cholinesterase.\textsuperscript{18} OPs are very toxic to target and non-target organisms.

The excess use and misuse of OPs can cause impairment of attention, memory loss, alter the functions of a number of enzymes, chronic illness like Gulf War illness, Ginger Jake Paralysis and Alzheimer’s disease.\textsuperscript{19} Approximately one million poisonings are caused by OPs every year.\textsuperscript{19} OPs were usually considered as secure and beneficial for agriculture and domestic uses due to their relatively fast degradation rates.\textsuperscript{16-18} They were considered as replacement of toxic organochlorine pesticides. But, recently few studies have revealed that OPs can also persist in the environment, which were largely unknown.\textsuperscript{17,18} OPs have been detected in soils, river water, river sediments after the long time of their applications.\textsuperscript{16-18} Although, the environmental persistence of OPs belong to the transport processes (adsorption, sorption, volatilization, leaching, runoff etc.), chemical processes (hydrolysis, oxidation/reduction, photolysis etc.) and biological processes (bacterial, enzymatic etc.).\textsuperscript{16-19}
Table 1.1: List of most popular Organophosphate pesticides.

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Description</th>
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</table>
| Acephate | CAS No. 30560-19-1  
IUPAC Name: (RS)-O,S-dimethyl acetylphosphoramidiothioate  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 700 mg/kg; LD₅₀ Dermal - rabbit - 2.000 mg/kg; Log Kow: -0.90; Log Koc: 1.34. |
| Azinphos methyl | CAS No. 86-50-0  
IUPAC Name: S-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl O,O-dimethyl phosphorodithioate  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 7 mg/kg; LC₅₀ Inhalation - rat - 1 h - 69 mg/m³; Log Kow: 2.53; Log Koc: 1.84 |
| Bensulide | CAS No. 741-58-2  
IUPAC Name: O,O-diisopropyl S-2-phenylsulfonylaminoethyl phosphoro-dithioate  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 27 mg/kg; LD₅₀ Dermal - rabbit - 2.000 mg/kg; Log Kow: 4.12; Log Koc: 3.99. |
| Bilanafos | CAS No. 741-58-2  
IUPAC Name: (2S)-2-amino-4-[hydroxy(methyl)phosphinoyl]butyryl-L-alanyl-L-alanine  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 275 mg/kg; LD₅₀ Dermal - rabbit - 2.000 mg/kg; Log Kow: 3.96; Log Koc: 3.79. |
| Chlorpyrifos methyl | CAS No. 2921-88-2  
IUPAC Name: O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphoro-thioate  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 1.828 mg/kg; LC₅₀ Inhalation - rat - 4 h - > 670 mg/m³; LD₅₀ Dermal - rabbit - > 2.000 mg/kg; Log Kow: 4.31; Log Koc: 3.30. |
| Cyanofenphos | CAS No. 13067-93-1  
IUPAC Name: (RS)-(O-4-cyanophenyl O-ethyl phenylphosphonothioate)  
Toxicity: Toxicity to fish ; LC₅₀ - Poecilia sp. - 0.66 mg/l - 48.0 h; Log Kow: 4.20; Log Koc: 3.87. |
| Diazinon | CAS No. 333-41-5  
IUPAC Name: O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphoro-thioate  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 1.012 mg/kg; LD₅₀ Oral - rat - 696 mg/kg; LC₅₀ Inhalation - rat - 4 h - > 5.400 mg/m³; LD₅₀ Dermal - rabbit - > 2.020 mg/kg ; Log Kow: 3.86; Log Koc: 3.13. |
| Ethion | CAS No. 63-12-2  
IUPAC Name: O,O,O′,O′-tetraethyl S,S′-methylen bis(phosphorodithioate)  
Toxicity: Acute toxicity; LD₅₀ Oral - rat - 13 mg/kg; LC₅₀ Inhalation - rat - 864 mg/m³; LD₅₀ Dermal - rat - 62 mg/kg; Log Kow: 5.07; Log Koc: 4.12. |
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<tr>
<th>Etrimientos</th>
<th>CAS No. 38260-54-7</th>
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<tr>
<td>IUPAC Name: O-6-ethoxy-2-ethylpyrimidin-4-yl O,O-dimethyl phosphorothioate</td>
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<tr>
<td>Toxicity: Acute toxicity; LD₃₀ Oral - rat - 1,800 mg/kg; LD₅₀ Dermal - rabbit - &gt; 500 mg/kg; Log Kow: 2.94; Log Koc: 2.31.</td>
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<th>Fosamine</th>
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<td>IUPAC Name: ethyl hydrogen carbamoylphosphonate</td>
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<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 11,000 mg/kg; LC₅₀ Inhalation - rat - 1 h - &gt; 57,570 mg/m³; LD₅₀ Dermal - rabbit - &gt; 1,660 mg/kg; Log Kow: -2.65; Log Koc: 1.05.</td>
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<th>Glufosinate</th>
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<tr>
<td>IUPAC Name: (2RS)-2-amino-4-[hydroxy(methyl)phosphinoyl]butyric acid</td>
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<tr>
<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 1,620 mg/kg; LC₅₀ Inhalation - rat - 4 h - 1,260 mg/m³; LD₅₀ Dermal - rat - &gt; 2,000 mg/kg; Log Kow: -3.96; Log Koc: 1.53.</td>
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<th>Glyphosate</th>
<th>CAS No. 1071-83-6</th>
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<tr>
<td>IUPAC Name: N-(phosphonomethyl)glycine</td>
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<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 5,000 mg/kg; LD₅₀ Dermal - rabbit - 5,000 mg/kg; LD₅₀ Intraperitoneal - rat - 235 mg/kg; Log Kow: -4.47; Log Koc: 1.27.</td>
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<th>Isoxathion</th>
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<td>IUPAC Name: O,O-diethyl O-5-phenyl-1,2-oxazol-3-yl phosphorothioate</td>
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<td>Toxicity: ----; Log Kow: 3.73; Log Koc: 4.56.</td>
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<tr>
<th>Methamidophos</th>
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<tr>
<td>IUPAC Name: (RS)-(O,S-dimethyl phosphoramidothioate)</td>
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<tr>
<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 7.5 mg/kg; LC₅₀ Inhalation - rat - 4 h - 162 mg/m³; LD₅₀ Dermal - rabbit - 100 mg/kg; Log Kow: -0.82; Log Koc: 0.65.</td>
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<th>Methidathion</th>
<th>CAS No. 950-37-8</th>
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<td>IUPAC Name: 3-dimethoxyphosphinothiolythiomethyl-5-methoxy-1,3,4-thiadiazol-2(3H)-one</td>
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<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 20 mg/kg; LC₅₀ Inhalation - rat - 4 h - 50 mg/m³; LD₅₀ Dermal - rabbit - 196 mg/kg; Log Kow: 2.21; Log Koc: 1.03.</td>
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<tr>
<th>Monocrotophos</th>
<th>CAS No. 6923-22-4</th>
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<td>IUPAC Name: dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate</td>
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<tr>
<td>Toxicity: Acute toxicity; LD₅₀ Dermal - rabbit - 270 mg/kg; Log Kow: -0.35; Log Koc: 2.34.</td>
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<th>Omethoate</th>
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<td>IUPAC Name: 2-dimethoxyphosphinothio-N-methylacetamide</td>
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<tr>
<td>Toxicity: Acute toxicity; LD₅₀ Oral - rat - 30 mg/kg; LD₅₀ Inhalation - mouse - 4 h - 140 mg/m³; LD₅₀ Inhalation - rat - 1 h - &gt; 1,500 mg/m³; LD₅₀ Dermal - rat - 700 mg/kg; LD₅₀ Intraperitoneal - rat - 14,400 mg/kg; Log Kow: -0.74; Log Koc: 1.87</td>
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<td>Chemical Name</td>
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<td>Phenthoate</td>
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<td>Phorate</td>
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<td>Phoxim</td>
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<td>Sulfotep</td>
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<td>Terbufos</td>
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<td>640-15-3</td>
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<td>Thionazin</td>
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<td>Triazophos</td>
<td>24017-47-8</td>
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1.3. EFFECTS OF OPs ON SOIL NUTRIENTS

Soil composition and geographical locations affect the availability of soil’s nutrients to plants. Soil’s nutrients are very significant to obtain high yields and good quality. This relies on appropriate provision of micro and macro-nutrients into plants. Deficiency of these nutrients can cause plant immune disorder followed by yields and quality depletion.\textsuperscript{20}

1.3.1. Classification of soil nutrients

There are sixteen essential nutrient elements crucial for growth of plants/crops. Three essential nutrients are carbon (C), hydrogen (H) and oxygen (O\textsubscript{2}) taken up from atmospheric carbon dioxide and water. The other 13 nutrients are taken up from the soil, often grouped as primary nutrients, secondary nutrients and micronutrients.\textsuperscript{7,20}

Primary nutrients are nitrogen (N), phosphorus (P) and potassium (K), and are obtained from fertilizers apart from soil. They are utilized in the largest amounts by crops, and therefore, are useful at superior rates than secondary nutrients and micronutrients. Secondary nutrients are calcium (Ca), magnesium (Mg) and sulfur (S) these are required in trivial amounts than the primary nutrients. Micronutrients are iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B) and molybdenum (Mo) these are required in even smaller amounts than secondary nutrients.

1.3.2. Significance of metals ions w.r.t. plants and humans

Trace metal ions are an integral and specific part of many structural and functional components in the plants and human body to complete the healthy life cycle.\textsuperscript{21,22} Some trace metal ions are acting as the major component for humans; such as manganese in arginase, iron in myoglobin, cobalt in vitamin B\textsubscript{12} etc.\textsuperscript{7,11,14,20} As we know that soil is the main source of these micronutrients, and these nutrients ultimately reach to humans’ diet through plants/crops that grew on healthy or nutrient rich agricultural soil.
Now, think for a moment, if there is no manganese (Mn) ion (Z = 25)? There would be no photosynthesis hence “no plants, no oxygen and no life.” Similarly, think, if there is no iron (Fe) ion (Z = 26)? There would be almost zero growth of plants and human beings, and no iron content present in foodstuff, means no myoglobin/no hemoglobin in our body that leading no oxygen transport. Also, in iron deficiency, diseases like anemia become health problems to the whole world. It is not about the Manganese and Iron, but it is about the all essential or trace metal ions because each ion has its utility and functions. Ultimately, deficiency of essential metal ions among the living beings can cause the number of disease, hence, life would become miserable and drastic. Number of living beings looses their health level and their lives directly and indirectly. The major source of metal ions for the humans is the agro-products; so the proper supply of these ions from soils to plant is very important.

1.3.3. Metal Uptake Mechanism of Plants in the Soil

The soil particles generally form electric dipole, whose negative pole is oriented outside which get interacted with positively charged metal ions, which also act as a driving force for the adsorption of metal ions (positively charged) over the surface of the soil. The root hair cells of plant root secrete H⁺ by using the water around nearby soil particles. This smaller H⁺ replaces the larger macro- and micro-nutrient cations from diffusion mechanism. Released cations then get available for uptake into roots. The formation of water-soluble metal complexes occurred at low pH and the uptake of metal ions through soluble complexes is known as active diffusion mechanism or absorption mechanism (Figure 1.6). The non-soluble complexes that can form at moderate acidic to basic pH conditions are uptake by plants through the passive diffusion mechanism or adsorption mechanism. These soluble metal complexes are decomposed, if competitive chelation taken place by the other ligand having greater (affinity towards metal cation), stability and complex formation ability. Among the deficiencies of micronutrients the most
severe deficiencies are in zinc and iron with 50% of world cereal soils deficient in zinc and 30% of cultivated soils globally deficient in iron.\textsuperscript{7,20}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Metal Uptake Mechanism of Plants in the Soil.}
\end{figure}

1.3.4. Effect of OPs on soil nutrients

It is the well known fact; pesticides applied to crop arrive at soil. Their interactions with soil depend upon the sorption values (Log$K_{\text{ow}}$). The interactions of the pesticides with soil organic/inorganic component at the molecular level are central to their bioavailability, bioaccumulation, transport and toxicity in the environment.\textsuperscript{16-18} Profound understanding of the soil’s interactions with pesticides is crucial to understand the soil-pesticide-minerals, soil-pesticide-organic matter, soil-pesticide-plant and soil fertility mechanisms. OPs are known to interact on soil’s mineral surface and organic matter present in soil.\textsuperscript{18} The interaction of pesticides to soil depend upon four main factors; nature of solute (pesticide), solvent (mainly water), soil constituents and pH.\textsuperscript{16-18} In general, mineral surface is positively charged at low pH and negatively charged at high pH values. As a result, positively charged metal ions interact to mineral surface at moderate acidic to basic pH.\textsuperscript{18} The negative charged ligands interact to soil’s minerals surfaces at low pH only.
In literature, the mineral-surface-catalyzed hydrolysis and divalent metal ion-catalyzed hydrolysis of thionate (P=S) and oxonate (P=O) organophosphorus pesticides at different pH values has been examined in light of three possible catalytic mechanisms: (1) metal ion coordination of the thionate sulfur or oxonate oxygen to enhance the electrophilicity of the phosphorus electrophilic site; (2) metal ion coordination and induced deprotonation of water to create a reactive nucleophile; and (3) metal ion coordination with leaving group to facilitate its exit. Results indicate that pH alone cannot be used as a single parameter to predict the stability of OPs, other parameter such as temperature, metal content, etc. also showed significant effect. All these studies have highlights that OPs can be decomposed through the mineral-surface-catalyzed hydrolysis at acidic pH conditions (pH values 1-3) and at highly basic conditions (pH values 9-12) only. There is the significant literature gaps w.r.t. OPs to metal ions interactions over the pH range for health plant growth that is from 5.5 to 8.0.

Moreover, when pesticides are mixed with the soil they absorbed/adsorbed on the soil surface and form the stable complexes, hence the effectiveness of soil and pesticides itself went reduced. These ligands or pesticides may cause the nutrient deficiency to plants through the formation of extra-stable complexes. Only few studies have been reported regarding the stable complexation or chelation of the OPs. Maximum studies out of them have been belonged to herbicide glyphosate and one study was belonged to demeton S. Glyphosate has been reported to form complexes of various stabilities with divalent metal cations due to the presence of amino, carboxylic and phosphonic moieties in its molecule, where each could coordinate separately to metal ions or in combinations as a bidentate or tridentate ligand. Due to such interaction, the presence of several divalent cations including Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Fe$^{2+}$, Ni$^{2+}$ and Zn$^{2+}$ in spray solutions was shown to lower the herbicidal effect of glyphosate. The formation of poorly soluble glyphosate – metal complexes may also be relevant in the rhizosphere, reducing the root uptake and translocation of these nutrients within plant tissues. Most drastically, a study on Demeton S analyzed that it can form the complexes with Hg(II).
As per chelating and HSAB principle, above mentioned pesticides having the binding sites, they can bind through N, O and S atoms. Solubility of particular metal complex or metal salt is governed by the pH factor of soil. As per literature for the healthy growth of the plants taken place at pH range 5.5 to 8.0. At this pH range pesticides also form the stable complexes which are more stable than the complexes of humic acids.

**Literature Gaps:** A huge gap regarding the formation of metal chelates with OPs in wet and solution phase around neutral pH has been found. Since, each moiety of OPs has different structural arrangements/ different chelating sites, their rate and mode of interaction might differ. In addition, none of the system in the world is isolated, environmental factors (pH of medium, temperature, presence of microflora, humic substance etc.) cannot be ignored. This encouraged us to make an attempt to check the feasible interaction of metal ions with OPs from lab to real world in presence of various environmental conditions.

**1.4. HUMIC SUBSTANCES OF SOIL & THEIR ROLES**

Humic substances (HS) symbolize a significant proportion of organic fraction in soils. One of the most significant characteristics of HS is their ability to interact with metal ions to form water-soluble and colloidal complexes. The capacity of HS to interact with metals is recognized to their high contents of oxygen containing functional groups, such as carboxyl (COOH), hydroxyl (OH), and carbonyl (C=O). Almost every feature of the chemistry of heavy metals in soils, waters, and sediments is related in some way to the formation of complexes with HS. Thus, the availability of many metal ions, especially trace elements, to higher plants and to soil microorganisms, as well as their mobility and transport in the environment, are strongly influenced by complexation with HS. Charge characteristics of humic substances depend upon the degree of dissociation of the functional groups. At pH < 3, HS act as uncharged polymers, whereas at pH > 3 they
act as negatively charged polyelectrolytes, due to dissociation of carboxyl groups (3 < pH < 9) and phenolic hydroxyl groups (pH > 9). Chelation enhances the termination of metals from soil minerals.\textsuperscript{28-30}

Though, HS increases crop yield, permeability of plant membranes, uptake of nutrients, enhances growth of soil microorganisms, activates biochemical processes in plants, stimulates root development, enhances phosphate utilization of plants, has a high base exchange capacity and stimulants plant growth.\textsuperscript{28-31} These applications might be disturbed by entering of external chemicals like pesticides into the soil environment. Interaction of OPs with soil metal ions have been studied at different pH by different groups, that indicated complexation, in which coordination sphere was found to be filled by the hydrogen bonding with water. These complexes effected soil and plant activities.\textsuperscript{28-31} A combination of antagonistic and synergistic effects was observed with the selected organophosphate and carbamate insecticides.\textsuperscript{34-42} Humic acid significantly reduced the toxicity of azinophos-methyl, chlorpyrifos, and carbofuran, while enhanced toxicity was observed with methyl parathion and carbaryl.\textsuperscript{37} These results indicate that humic-pesticide interactions can alter the toxicity of agricultural chemicals. Moreover, the influence of humic materials on the toxicity of these chemicals is dependent on temporal relationships and HA concentration.\textsuperscript{34-37}

Herbicides (e.g. glyphosate) can interact with the metal complexes of humic acid through inner sphere complexes.\textsuperscript{38-45} Pesticides can also found to be interacted on clay minerals by forming complexes with interlayer metal ions. The main mode of interaction of herbicides with metal complexes of humic acid and adsorption on clay minerals was through the coordination moieties/ sites of herbicides.\textsuperscript{43-47} Few studies have shown that at low concentration level of herbicides, these can form inner sphere complexes with humic acid through the coordination moieties, by the ligand exchange mechanism, whereas at higher concentration, extra herbicides bound through hydrogen bonding mechanism, to metal-humic acid and already bound herbicide molecules.\textsuperscript{44-47} Strength of binding of
herbicides to metal complexes of humic acid depends upon the chelating ability and number of binding sites of herbicides or pesticides.$^{38-47}$

**Literature Gaps:** After a profound review study, it was observed that pesticides affects the soil physico-chemical properties by the non-covalent interactions, and hinder the crop rhizosphere but exact mechanism behind it is not clear. So there is need to check every kinds of interaction of soils (Humic acid) with metal ions and pesticides before introduction of any pesticide in field. In the same way, we are going to check the interaction of Acephate, Glyphosate, Monocrotophos and Phorate with metal ions in the presence of humic acid. Also farmer use the different kind of pesticides at a time, so there is need to check the Cocktailed Effect of pesticides and fertilizers on the soil.

1.5. EFFECTS OF OPs ON THE PLANT GROWTH PROMOTING RHIZOBACTERIA

The majority of the applied pesticides, even if sprayed on foliage of crop plants and weeds, eventually reach the soil, and affect the growth and activity of soil microbial communities or plant growth promoting bacteria (PGPR).$^{48,49}$ PGPR includes the siderophore and siderophore producing microorganisms are an important biological component of the soil ecosystem and play vital roles in soil fertility through their roles in nutrient cycling and organic matter decomposition.$^{48-50}$ Maximum siderophores grows under the supply of metal ions, but that growth is inhibited where the metal ions deprived by other chelating groups (chemicals and microorganism).$^{50,51}$

Siderophores ("metal ions carrier") are low-molecular-weight chelating agents (200–2000 Da), high affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses, known as the strongest Fe$^{3+}$ binding agents. Iron is a very crucial and important nutrient for almost all forms of life.$^{51-58}$ In the aerobic environment, iron occurs principally as Fe$^{3+}$ and is likely to form insoluble hydroxides and
oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms.\textsuperscript{49} these are the siderophores which makes insoluble iron into soluble form by chelating effect. Most of the siderophores are water-soluble and can be divided into extracellular siderophores and intracellular siderophores.\textsuperscript{50} In both Gram-negative and Gram-positive rhizobacteria, Fe\textsuperscript{3+}-siderophore complex on bacterial membrane is reduced to Fe\textsuperscript{2+} which is further released into the cell from the siderophore via a gating mechanism linking the inner and outer membranes. During this reduction process, the siderophore may be destroyed/recycled.\textsuperscript{48,51} Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation.\textsuperscript{49} Not only iron, siderophores also form stable complexes with other metals that are of environmental concern, such as Al\textsuperscript{3+}, Cu\textsuperscript{2+}, Ga\textsuperscript{3+}, and Zn\textsuperscript{2+}.\textsuperscript{57} Binding of the siderophore to a metal increases the soluble metal concentration.\textsuperscript{49,50,59}

Siderophore coordination chemistry is governed by the HSAB (hard and soft acids and bases) principle, originally proposed by Pearson, that is, with preferential coordination between the hard acid (Fe\textsuperscript{3+}) and the hard anionic oxygen Lewis base donor ligands.\textsuperscript{2,50,59} Iron is the most abundance on earth’s crust, but it is not readily bioavailable in aerobic environments due to the low solubility of Fe\textsuperscript{3+} form.\textsuperscript{43} Same time iron is very essential for almost all microorganisms, plants and animals by virtue of its unique chemical properties; the ability to coordinate and activate oxygen and the possession of an ideal redox chemistry (Fe\textsuperscript{II} - Fe\textsuperscript{III} - Fe\textsuperscript{IV}) for involvement in electron transport and metabolic processes, these are the siderophores which makes the insoluble Fe(III) to soluble Fe(II), hence activate various biological and environmental process.\textsuperscript{49,57-60} Siderophores possess a higher affinity (pFe\textsuperscript{III}) for Fe(III) than Fe(II) due to formation of high spin octahedral complexes, with multidentate ligands, hexadentate complex, can account for an increase in the formation constant, K\textsubscript{f}, of up to 6 log units.\textsuperscript{49,59} For Fe(III), favourable entropic contribution and donor–acceptor bond energies largely determine the enthalpy contribution to the stability of the complex.\textsuperscript{49,59}
Hexadentate ligands associate more entropy changes association by the formation of complexes than with tetradebate and bidentate ligands, thus leading to a higher affinity constant for the hexadentate ligand. This effect is concentration dependent.\textsuperscript{49,59} Thus the [Fe\textsuperscript{3+}] values (and therefore the pFe\textsuperscript{III} values) have a dependency on the concentration of free ligand, L. This dependency is 3, 1.5 and 1 for the bidentate, tetradebate and hexadentate hydroxamate siderophores respectively.\textsuperscript{59} The dissociation constant or lability of iron in hexadentate siderophores at neutral pH is low (about 10\textsuperscript{-10} M to 10\textsuperscript{-9} M) or inert, hence it is typical to exchange iron from one to other siderophore at neutral pH. As the pH decreases the lability increases.\textsuperscript{59} The rate of lability is very fast with bidentate, tridentate and tetradebate siderophoric ligand. Basically these siderophores made the iron soluble and hand over it to hexadentate siderophore.\textsuperscript{50,59}

There are numerous studies (\textit{invitro} and \textit{invivo}) has been reported on decomposition of OPs in the presence of microorganisms including some plant growth promoting rhizobacteria (PGPR).\textsuperscript{2,47-49} As, PGPR are classified based on their functional activities as (i) biofertilizers (increasing the availability of nutrients to plant), (ii) phytostimulators (plant growth promotion, generally through phytohormones), (iii) rhizoremediators (degrading organic pollutants) and (iv) biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites).\textsuperscript{48} If we search about the effect of pesticides on PGPR, maximum investigations highlighting the dose dependent effects of pesticides on PGPR, i.e. with the increase of dose adverse effect of pesticides on PGPR increases.\textsuperscript{55-61} The hazardous effect of pesticides on PGPR is directly proportional to the persistence and dose level.\textsuperscript{59-64} Repeated use of pesticides having high persistency level in the soils may further increase the threat. As per above mentioned points siderophore are also part of PGPR. In this context, the chemistry of siderophore and siderophore producing microorganisms is intriguing as a potential metal chelator.

Including humic substances, siderophores are strong chelators. Alike humic substances, these siderophores promote the termination of metal-oxide bonds through the
formation of surface complexes. During this process H\(^+\) ion of humic substances or siderophores was exchanged to bounded metal of oxide, consequently metal get reduced and form the soluble complexes with siderophores and humic substances. These soluble complexes allow the uptake of essential metal ions to plants through active and passive methods. The process of metal uptake from soluble complexes of siderophores or humic acid is time consuming process. During this process, if external ligands like pesticide get interacted; it may leads to formation of non-soluble or toxic or extra stable complexes and can inhibit essential metal ion uptake. To verify this, stepwise analysis was very important, which can be divided into following homogeneous steps; (1) interactions of metal-pesticide, and metal-pesticide-humic acid systems, (2) interactions siderophore-metal and siderophore-metal-pesticide systems, and (3) affects of pesticides and pesticide-metal on selected crops.

**Literature Gaps:** Direct and excess applications of pesticides including OPs may decline the growth of siderophore and siderophore producing microorganisms. Also, it is anticipated that pesticides containing O, N, S atoms would be a high affinity ligand as like siderophores for a variety of metal ions and that coordination to the metal ions would likely have an effect on the stability behavior of the siderophore-metal chelates. Unexpectedly, no studies are available in the literature on this topic.

1.6. **SCOPE OF STUDY**

With the mount of the growth rate of population, the use of pesticides has become inevitable. We acknowledge the pesticide for the same but on other side our lure for more productivity leads us towards the misuse of the pesticides, which get recognized after the adverse effects on the living being. Still, we have ignored the fact that these pesticides contains the active site which generally have the ability to get interact with the transition metal ions of the soil and leads to the formation of the complexes and may results into
either decrease or totally damage the fertility of soil in terms of essential metal ions, and siderophore and siderophore producing microorganisms.

The importance of current work is exhibited from the multidisciplinary approach of the work. This study has wide axis, from chemistry to environment, and microbiology to agriculture. Current study is even more important because of following reasons; (1) The metal ions under current study are very important for the health of humans and plants. The major source of these ions is soil. All these metal ions (Mn, Fe, Co, Ni, Cu and Zn) have their own significances. Among these ions, millions of peoples are suffering from the deficiency of Fe and Zn, and concept behind this is still unknown. (2) Humic acid is a principal component of humic substances. The presence of carboxylate and phenolate groups gives the humic acid the ability to form complexes with metal ions. The formation of complexes is an important aspect of the biological role of humic acid in regulating bioavailability of metal ions. The presence of pesticides may change the whole chemistry of complexation of humic acid with metal ions through the axial, hydrogen and competitive bonding with humic acid-soluble/ non-soluble metal complexes. (3) Soil microorganisms are also responsible for soil fertility, and these microorganisms are dose dependent to pesticides. Moreover, these microorganisms can produce the siderophores. These siderophores are the active participants to uptake the essential metal ions from soil to plants. Now pesticides can suppress these microorganisms activity directly or indirectly. Directly, pesticides can kill the soil microorganisms, indirectly, chelating pesticides can compete with siderophores produced by soil microorganisms for the complexation with metal ions or simple pesticides can bind axially to the metal complexes of siderophores. H-bonding and weak interactions are also a considerable mechanism of such binding. (4) Various plant growth experiments by different authors have proved that pesticides can inhibits the plant growth. Current study is the extended versions with wheat growth experiments under different designing to confirm the role of metal ions in the plant growth. (5) Current study is also performed under “cocktailed effect” or mixture of pesticides. As per our best information, this study is performed first
time. All the parameters like, metal complexation, effect of soil microorganisms and plant growth are investigated under the “cocktailed effect”.

![Diagram](image)

**Figure 1.7**: Scope of Study.

We are involving ourselves into the purposeful work of investigating the reactivity of the OPs, and to overcome with the problem of interaction of OPs with the essential metals for the better scope of the fertility of the soil. As hundreds of OPs are known, and study on all of them at a time is not possible. So, we focused our job on four OPs, i.e. Acephate, Glyphosate, Monocrotophos & Phorate
1.7. AIM & OBJECTIVES

1. To study the interactions, mechanistic pathway and stability of Acephate, Glyphosate, Monocrotophos & Phorate with essential metal ions in presence and absence of Humic Acid.

2. To study the interactions of metal ions with different OPs (Acephate, Glyphosate, Monocrotophos & Phorate) on their simultaneous presence i.e. study of the “Cocktailed Effect”.

3. To study the interactions of OPs (Acephate, Glyphosate, Monocrotophos & Phorate) with siderophore producing microorganisms.

4. To study the effect of Acephate, Glyphosate, Monocrotophos & Phorate on plant (wheat) growth.