

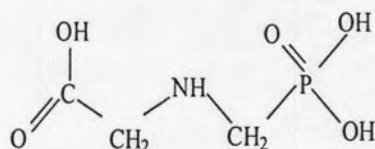
## CHAPTER II

### BACKGROUND AND THEORY

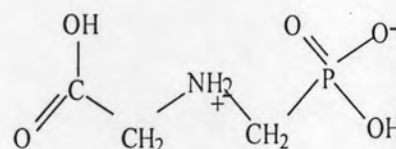
#### 2.1 General Information of Glyphosate Herbicide

##### 2.1.1 Physical and chemical properties of glyphosate

Glyphosate [N-(phosphonomethyl) glycine] molecular formula is  $C_3H_8NO_5P$  with the structure as shown in Figure 2.1.



Glyphosate structure (normal form)



Glyphosate structure (zwitterion)

**Figure 2.1.** Structure of glyphosate.

Glyphosate has 4 pKa: pKa<sub>1</sub> 0.8(1<sup>st</sup> phosphonic), pKa<sub>2</sub> 2.3(carboxylate), pKa<sub>3</sub> 6.0 (2<sup>nd</sup> phosphonic), and pKa<sub>4</sub> 11.4 (amine). It is a dipolar molecule, termed a zwitterion, so glyphosate readily to release protons from its acid group and receive protons in its basic group [22]

**Table 2.1. Physical properties of glyphosate.**

Parameters	Value
Molecular weight	169.1g
Vapor pressure	$7.5 \times 10^{-8}$ mm Hg
Melting point	$189 \pm 0.5$ °C
Boiling point	> 200°C
Octanol-water coefficient log (Kow)	-3.5
Soil adsorption coefficient (Kd) (average over several pH levels and soil)	61 g/m <sup>3</sup>
Henry's constant	$< 2.1 \times 10^{-7}$ Pa m <sup>3</sup> mol <sup>-1</sup>

### 2.1.2 Fate of glyphosate in environment

The solubility of glyphosate in water is 12 g/l and has low solubility in common organic solvents. Even glyphosate has high solubility in water, glyphosate is strongly adsorbed on soil particles too; and this characteristic of glyphosate prevents it from immobility into groundwater.

The half-life of glyphosate in natural water is 35-63 days. Photodegradation can occur under natural conditions [18, 19, and 20]. The half-life of glyphosate in soil ranges from 3 to 130 days depending on the climate conditions and soil structure such as soil texture, type and amount of soil microbial [18, 19, 20, and 22]. Glyphosate can be degraded by microorganism in soil to AMPA which eventually converts to carbon dioxide (CO<sub>2</sub>) [20 and 22]. And glyphosate does not evaporate in air [20].

## 2.1.3 Toxicological data of glyphosate

### 2.1.3.1 Acute toxic

The lethal dose (LD<sub>50</sub>) illustrates the acute effect of chemical toxicity that make population dead 50% of total people who expose to this chemical and generally, the acute toxic express in 3 exposure pathways that is by oral dermal and inhalation. The acute toxic of glyphosate herbicide shown as below:

- LD<sub>50</sub> exposed by oral in rat is 4,320 mg/kg
- LD<sub>50</sub> exposed by dermal (in female rabbit) is 794 mg/kg
- LD<sub>50</sub> exposed by inhalation is not available data because glyphosate does not evaporate.

### 2.1.3.2 Chronic toxic

The chronic toxic illustrates the human health effect by exposure to the toxic in the long term. Chronic toxic of exposure to the glyphosate herbicide are:

- Carcinogenic: EPA has classified glyphosate as group E “non-carcinogenic for human” (*Remark: It's not enough information in long term health effect to human*)
- Reproduction: none effect
- Mutagenicity: shown negative

### 2.1.3.3 Human health effect

The effect of glyphosate herbicide to human health only reported in acute effect due to lacking of the long term data of human exposure to the glyphosate herbicide. An acute toxic of glyphosate to human is irritation to eye and skin during mixing and application.

#### **2.1.4 Application and the mechanism of glyphosate herbicide**

Glyphosate is the post-emergence and broad-spectrum herbicide and is used in both the agriculture and landscaping for control many species of grasses, herbaceous plant including deep rooted perennial weeds, brush, some broadleaf trees and shrubs, and some conifers. Thus, this is the reason making glyphosate become to the most widely used herbicide in the world [7 and 18].

The application of glyphosate can be done by several methods such aerial spraying, spraying from a truck, backpack or hand-held sprayer, wipe application, frill treatment and cut stump treatment. After glyphosate was applied, it is adsorbed by foliage and then rapid translocation through the plant. Then it kills plants by inhibiting the enzyme-5-enolpyruvylshikimic acid-3-phosphate synthase (EPSP), which is necessary for the formation of the aromatic amino acids that are important for the synthesis of protein in the chloroplast in plants [7, 18, 19, and 20].

### **2.2 Regulatory Standard Issue of Glyphosate**

#### **2.2.1 Regulatory standard of glyphosate in drinking water**

The US.EPA has been set up the Maximum Residue Limit (MRL) of glyphosate herbicide in the drinking water is not exceed 0.7 ppm [22] and The European Union (EU) has been set up the Maximum Residue Limit (MRL) of any herbicides in drinking water is not exceed 1 ppm [19].

#### **2.2.2 Regulatory standard of glyphosate in food**

The Commonwealth of Australia has been set up the Maximum Residue Limit (MRLs) for food and animal feed commodity that is published in the Agriculture and Veterinary Chemicals Code Act 1994 [16].

The MRL for food product are: 5 mg/kg for wheat, 20 mg/kg for wheat bran, 10 mg/kg for Barley, 2 mg/kg for broad beans (dry), and 5 mg/kg for field peas (dry).

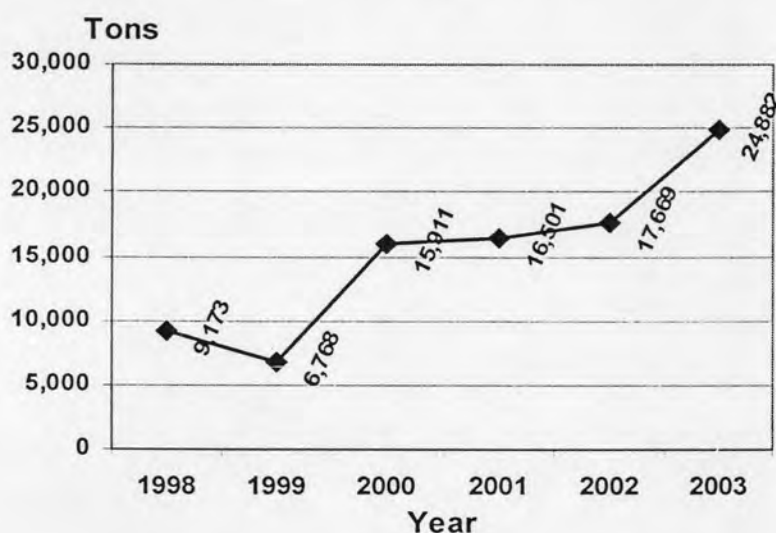
The MRL for Animal feed Commodity in primary feed commodities (other than cotton forage, rape forage, rape seed meal, soya bean hulls, and aspirated grain fractions) is 100 mg/kg.

The FAO/WHO's Codex Alimentarius Commission has been set up the Acceptable Daily Intake (ADI) for an average 60 kg man at 0.3 mg/kg/day.

However, regulatory standard of glyphosate both in drinking water and food have not set up in Thailand.

### 2.3 Status of the Application of Glyphosate in Thailand

The data of herbicide imported in Thailand provided by Department of Agriculture had report that glyphosate is one of the highest imported among herbicide and its imported was increasing every year since 1998 to 2003 as shown in Figure 2.2. The glyphosate herbicide used in Thailand both for agriculture and landscaping. Round up<sup>®</sup> is the popular trade name as glyphosate used in Thailand.



<sup>a</sup> Values provided by the Department of Agriculture

Figure 2.2. Amount of glyphosate imported in Thailand.

## 2.4 Adsorption of Glyphosate Herbicide

Since the structure of glyphosate contains three functional groups (amine, carboxylate and phosphonate), it shows that glyphosate easy to form complex or react with other chemicals. Some researchers have looked into the effects of metals on the adsorption of glyphosate in soil. In 2002, Morillo *et al.*, (2002) studied the effects of dissolved glyphosate upon the sorption of copper in three selected soils. In 2004, Zhou *et al.*, (2004) studied the adsorption and cosorption of Cd and glyphosate on two soils with different characteristics. And recently, Wang *et al.*, (2006) studied the co-sorption of Zn and glyphosate on two different soil characteristics. All of the research groups have studied how soil characteristics, such as cationic exchange capacity (CEC), organic matter, Al and Fe oxides, clay content and pH, affect the sorption of glyphosate on soil with heavy metals. These studies reported similar results, i.e., glyphosate adsorption increased in the presence of heavy metals in soil, and the adsorption was higher in soil that contained more Fe and Al oxides, and a lower pH though its had lower CEC and clay content. Morillo *et al.*, (2002) explained that the adsorption of glyphosate in the presence of Cu was related to the Fe and Al oxides. This was identical to the conclusion of Wang *et al.*, (2006) who studied the adsorption of glyphosate in the presence of Zn. For this phenomenon, Zhou *et al.*, (2004) described that in the presence of Cd, the equilibrium pH of soils decreased and Cd reacted with glyphosate to form complex and was easier to adsorb on soil surface.

For the adsorption of heavy metals in the presence of glyphosate in soil, the study of Morillo *et al.*, (2002) found that glyphosate decreased the adsorption of Cu on soil, and the equilibrium pH decreased because the soil surface had more protons then reducing Cu adsorption. The study of Zhou *et al.*, (2004) and Wang *et al.*, (2006) worked on Cd and Zn respectively; the results of both studies agreed with Morillo *et al.*, (2002), i.e., heavy metal adsorption on soil decreased when glyphosate in soil increased, which decreased the soil pH and the metal tended to form complexes with glyphosate.

## 2.5 Leaching of Glyphosate Herbicide

In 2005, the leaching of glyphosate and its metabolite, AMPA, through undisturbed soil columns were studied by Landy *et al.*, (2005). The aim of their study was to compare the amount of glyphosate leaching in bare soil and vegetated soil. They found that glyphosate appeared earlier in the leachate from vegetated soil in the initial monitoring period. This result was caused by the improved soil structure due to the grass cover. During the 1-year monitoring period, glyphosate was detected in longer and higher concentrations in the leachate from bare soil in the vegetated soil. The concentrations of AMPA should show similar results as those of glyphosate, as it appeared earlier in the leachate of the vegetated soil due to the rapid degradation rate of glyphosate and better flow in the vegetated soil. Similar to the results of glyphosate, AMPA was measured at higher concentrations in the leachates of the bare soil than in the vegetated soil. For the distribution of glyphosate and AMPA in soil profile, it was found that no extractable glyphosate could be detected in the bare soil and vegetated soil columns. These results were in agreement with the rapid degradation rate of glyphosate under the outdoor condition. In contrast, AMPA was distributed throughout the column and reached the 20 cm depth. At the depth of 0-2.5 cm, AMPA in vegetated soil was higher than in the bare soil because the grass cover of the vegetated soil appeared to favor the adsorption of AMPA on the soil surface. For the deeper layers, AMPA in bare soil was slightly higher than in the vegetated soil. This could be related to the concentration of AMPA in the leachate of the bare soil.

Since many research groups have studied the adsorption of glyphosate on soil with regard to different soil characteristics as described above, in 2001, Veiga *et al.*, has studied the behavior of glyphosate in soil with relation to its properties. Their results showed that both glyphosate and AMPA quickly reached 30 cm of soil depth, and the concentrations of glyphosate in both layers (0-20 and 20-35) were quite similar. The soil studied was moderate clay with high organic matter content, which is known to adsorb glyphosate quite strongly. The iron oxide and high value of phosphorus sorption of this soil confirmed the high adsorption capacity for glyphosate. The high concentration of glyphosate in both layers proved that

glyphosate and AMPA had high mobility through soil. The degradation rate of glyphosate to AMPA was found to be quicker in the surface layer due to higher biological activity. The glyphosate concentration along the monitoring period was high in the first sampling date, which indicated a fast translocation into the soil. However, the glyphosate concentration decreased during the study period.

## 2.6 Degradation of Glyphosate Herbicide

Since the half-life of glyphosate in soil had been conducted by many studies that is ranking from 3-130 days [18, 19, 20, and 22]. The half-life of glyphosate in soil is related to soil persistence, varying microbial activity and extent of soil-binding at the different soil study site. Since glyphosate widely used as "Roundup" trade name, there are some studies on degradation and half-life of Roundup in soil. Monsanto (2005) has studied the degradation of glyphosate as "RoundUp" at 8 different sites across the U.S. in 1992-1993. This study revealed that the half-life of glyphosate in some sites was short (1.7, 7.3, and 8.3 days) and some sites were longer, up to 141.9 days. The average half-life of glyphosate at the 8 study sites was about 40 days [14]. In 2000, the effect of glyphosate on soil microbial activity and biomass has been studied by Haney *et al.* (2000) by using silt loam. This study concluded that glyphosate as Roundup was rapidly degraded by soil microbes, even at high application rate, without adversely affecting microbial activity. The degradation of glyphosate was measured by C and N mineralization. Soil samples which have higher organic matter tended to mineralize glyphosate more quickly initially than soil samples which have lower organic C, possibly due to a greater microbial biomass. And other studies on glyphosate degradation were conducted. In 1991, degradation of the herbicide glyphosate by members of the Family *Rhizobiaceae* had been studied by Liu *et al.*, (2001). The seven organisms in family *Rhizobiaceae* which are *Rhizobium meliloti* strains, *Rhizobium leguminosarum*, *Rhizobium galega*, *Rhizobium trifolii*, *Agrobacterium rhizogenes*, and *Agrobacterium tumefaciens* were used in this study. This study found that, during degradation process, sarcosine was found to be the immediate breakdown product, indicating that the initial cleavage of glyphosate was the C-P bond. These results suggest that *Rhizobium* and *Agrobacterium* strains were able to utilize glyphosate and other phosphonates as their sole P source. Thus, glyphosate degradation seems to be a



general property of the Rhizobiaceae family. The degradation pathway of glyphosate in soil studied by Liu *et al.*, (2001) as shown in Figure 2.3.

In 2004, Joanna *et al.* has studied the glyphosate degradation by *Spirulina* sp. This study found that *Spirulina* sp. is able to degrade glyphosate by using glyphosate as sole source of P and N even if glyphosate was applied at high concentrations. Glyphosate was converting to aminomethylphosphonic acid (AMPA) which was further mineralized into inorganic phosphate.

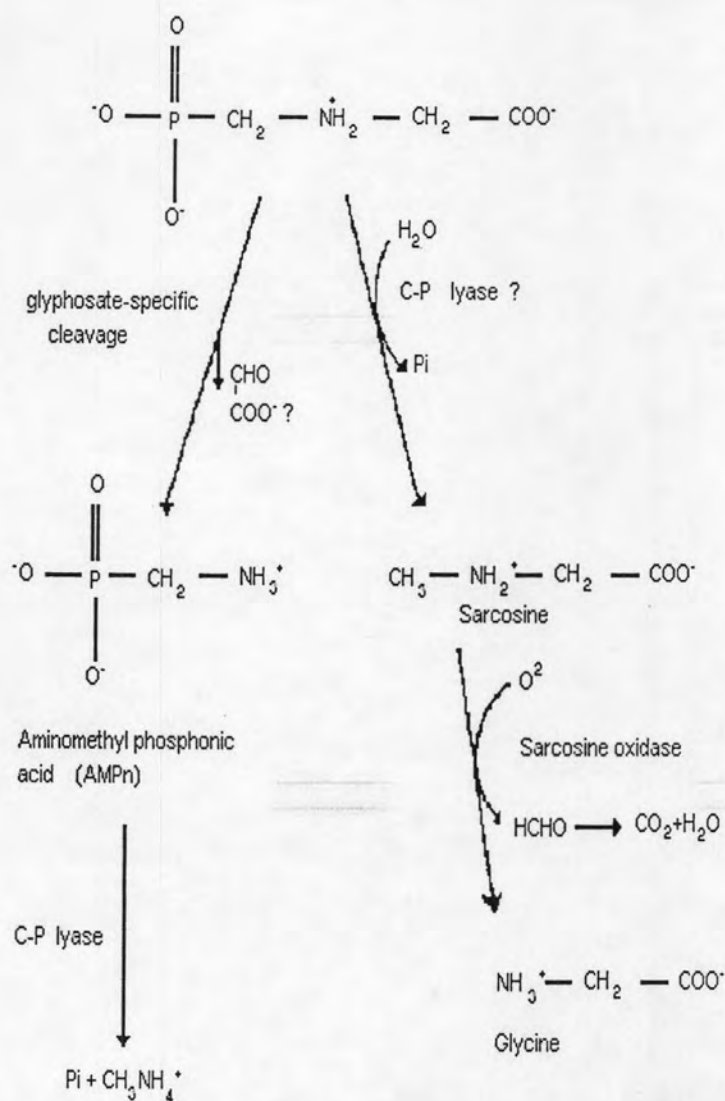


Figure 2.3. Degradation pathway of glyphosate in soil.

## 2.7 Soil Science

For the leaching study, besides the properties of chemical, the characteristics of soil both physical and chemical are important also. So this study provides the basic information of soil science that is advantage to clearly understand the leaching study.

The factors effect the leaching of glyphosate are;

- Soil texture.
- Structure formation and stability.
- Ion exchange interactions.

The soil texture and structure formation and stability are the physical properties of soil that affect on the flow of water through the soil column. The ion exchange in soil is the chemical properties of soil that is including the exchanging all of ions and cations in soil. The detail of the soil properties were provided as the next section.

### 2.7.1 The physical properties of soil

#### 2.7.1.1 Particle size and texture

Due to soil consist of different size of minerals which are clay, silt and sand ordering by the smallest particle respectively. These characteristics of soil minerals give its chemical and physical properties.

##### Particle size;

There are several systems for classification the particle size of soil by using the equivalent diameter. The commonly used schemes are the International Society of Soil Science (ISSS) and the United States Department of Agriculture (USDA). Sand particles range from 2 mm to 0.02 mm (or less than 0.02 mm for USDA) and can be further classified as fine and coarse for ISSS and additional medium for USDA. Silt particle ranges from 0.02 mm to 0.002 mm for ISSS and more than 0.02

mm for USDA. Clay particle less than 0.002 in size same both ISSS and USDA schemes [1, 4, and 13].

Sand mineral mainly consist of the quartz and smaller other minerals such as feldspar and micas. Sand minerals are inactive chemically except when coated with clay minerals. By its characteristic, sand minerals are bad for supply and storage the water. On the contrary, it is good for drainage and aeration.

Silt mineral normally provides the medium water storage if not coated with clay minerals.

Clay mineral is the most surface-active therefore it has high ability to adsorb and hold the nutrient as well as to hold the water. Nevertheless, it is poorly drainage the water and difficult for cultivate.

### *Soil texture;*

The soil texture is the relative proportions of sand, silt and clay. It is an important characteristic because it can use to indicate the properties of soil such as water or nutrient storage and drainage.

Normally soil texture defined by texture triangle as shown in Figure 2.4. The proportions of sand, silt and clay will be use for classified the soil texture by reading the approximately percentage on the axis.

The good soil texture is “loam” which having the uniform of proportions of sand, silt and clay that is suitable for agriculture. In contrary, the soils that have extremely one fraction (sand or clay) are difficult to manage.

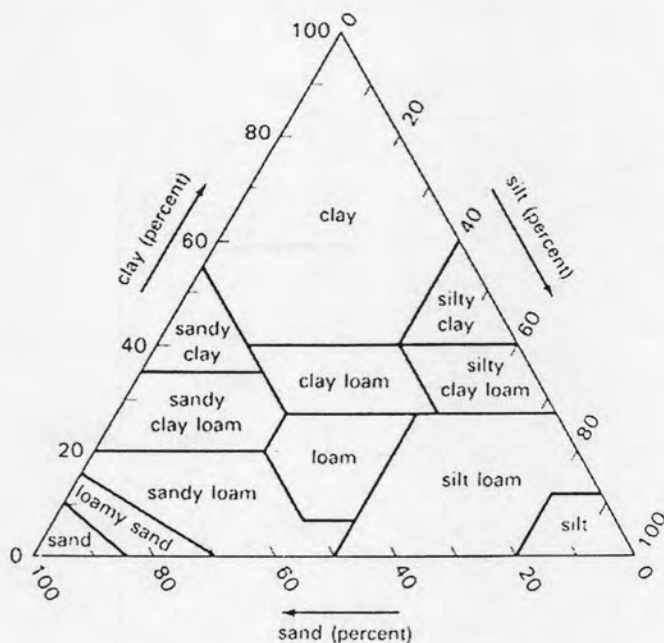


Figure 2.4. Texture triangle of soil.

### 2.7.1.2 Structure formation and stability

In order to study the leaching, it is important to understand the basic of the formation of the soil structure and the stability of the soil. The structure formation of soils is important because it will be influence on the characteristics of soil such the interaction of ions contained in soil and the capability to storage and drain the water. The formation of soil structure divided into 3 scales which are atomic or molecule scale, microscopic level and the macroscopic or the visible scale.

At the molecule scale, it can be use to describe the formation between the mineral and soil particle. Normally, soils (e.g. clay and organic matter) have the negative charge, then it can attract to the cations of mineral such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  or the dipolar molecule of water. On the other hand, the dipolar molecule of water can act as the bridge to link the cations and soils together.

Sand and silt particles have no charge by itself, but they can link together and form the micro-aggregates when they have coated with clay or organic matter on their surface. Then the micro-aggregate bound together into the macro-aggregates that caused by the fungal hyphae and plant root.

## 2.7.2 Chemical properties of soil

### 2.7.2.1 Ion exchange interaction

The soil, both inorganic and organic, contains the charge which will be neutralized by the opposite charges that are attracted to, and accumulated at, the surface of soil particle. Most soil contains the predominance of negative charge which will be balanced by the positive charge are referred to as *exchangeable cations*. But some soil also contains the significant positive charge which will be balanced by the negative charge is referred to as *exchangeable anions*. However, soil can have both exchangeable cations and exchangeable anions.

#### Cation Exchange Capacity (CEC);

The cation exchange capacity of soil is measure ability of soil to hold exchangeable cations. In another word, it indicates the quantity of negative charge present per unit mass of soil. The unit of CEC presents in centimoles of positive charge per kilogram weight of soils ( $\text{cmol kg}^{-1}$ ).

#### Anion Exchange Capacity (AEC);

Although most soils carry the net negative charge but some sites on the soil surface have the positive charge especially in soils containing with inorganic colloid such iron and aluminum oxide. Similar to the cation exchange, this soil will be balance the positive charge on soil surface by attracting to the negative charge. The anion exchangeable can be expressed by the quantity of anions which the soils have ability to hold.

## 2.8 Background of the Behavior of Phosphorus in Soil

The behavior of glyphosate sorption in soil is related to the behavior of phosphorus in soil. Thus, the theory background of the behavior of phosphorus in soil is provided to get the clearly understanding on the mechanism between glyphosate and phosphorus occurring in the soil.

### 2.8.1 Formation of phosphorus in soil

Normally, the total content of phosphorus in soil ranges from 0.02-0.15%. Phosphorus which is depends on the parent material from which soil has developed. The phosphorus presents in soil in both organic and inorganic forms. However, the large proportion of phosphorus content in soil generally present in the organic form around 20-80% of total phosphorus content in soil. The organic phosphorus originated from the death and decomposition of plant cells, animal and microorganisms. By the naturally occurring, the organic forms of phosphorus including esters (compound with C-O-P linkage) of *ortho*-phosphoric acid ( $\text{H}_3\text{PO}_4$ ) can be identified into 3 main groups including inositol phosphate, nucleic acids and phospholipids.

Soil inorganic phosphorus are occur in forms of calcium phosphate [ $(\text{Ca}(\text{H}_2\text{PO}_4)_2)$ ,  $(\text{CaHPO}_4)$ ,  $(\text{Ca}(\text{PO}_4)_2)$ ] and iron and aluminum phosphates (Al or  $\text{Fe}(\text{OH})_2\text{H}_2\text{PO}_4$ )

### 2.8.2 Adsorption of phosphate in soil

Phosphate ions ( $\text{PO}_4^{3-}$ ) can be adsorbed on the soil particle at the positive charge site by anion exchange. However, the soil surface contains with oxide of iron or aluminum can act as the amphoteric surface, depending on the pH. The adsorption of phosphate by the oxide of iron or aluminum considers to takes place predominantly by specific adsorption mechanism. The adsorption mechanism of phosphate and oxide of minerals involves the exchanging the ions of phosphate ( $\text{H}_2\text{PO}_4^-$  or  $\text{HPO}_4^{2-}$ ) with the oxide of mineral ( $\text{Fe-OH}$ ,  $\text{Al-OH}$ ,  $\text{Fe-OH}^{2+}$  and  $\text{Al-OH}^{2+}$ ) through the ligand exchange. The adsorption mechanism of phosphate is shown in Figure 2.5

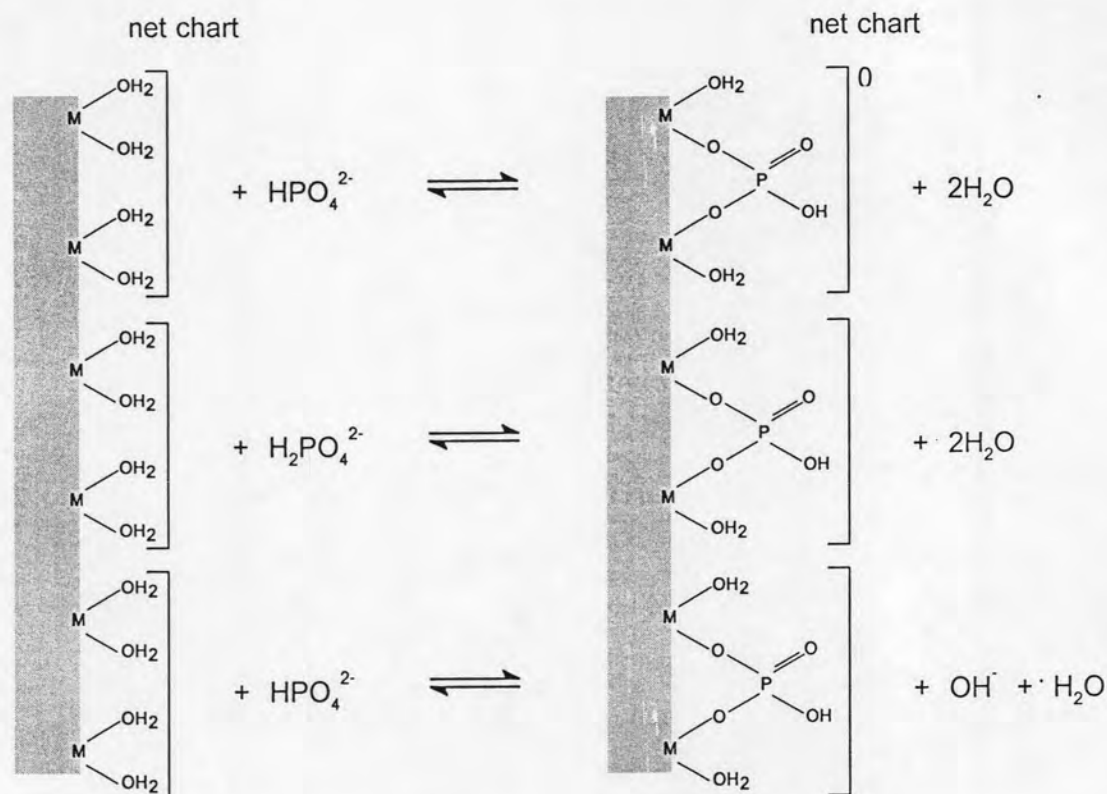


Figure 2.5. Adsorption mechanism of phosphate in soil.

## 2.9 Fertilizer Application

The most commonly formulation of fertilizers applied under agriculture field is the 16% of N, P and K fertilizers (containing with 16% of total nitrogen, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O) because it can use in many purposes to increase the agriculture production.

The application of fertilizers depends on the kind of plant, normally, the fertilizers will be applied in several time during planting. The application method depends on the fertilizers form either in solution or solid forms. If fertilizers contain in the solution form, it normally apply by the spraying method. If the fertilizers contain in the solid form, it will be applied by putting into the soil surface and adding with the water in order to dissolve the fertilizers.