Chapter 9

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Pollutants' alteration, transformation, and initiation of chemical changes within the soil.

In a highly complicated system of different phases, such as soil subsurface, penetrating substances will go through a myriad of chemical, physical, and biological processes that will determine their fate, besides controlling the degree of their toxicity to the environment. Such subsurface processes are broadly classified into the following groups:

A. Physical processes (processes related to chemical mobility)
B. Chemical processes
C. Biological processes

9.1 Processes related to chemical mobility

These include process in which no net chemical change occurs. They normally affect physical conditions that control phase distribution of the substance i.e. its association with aqueous or solid phases under given environmental conditions. It is due to this that these processes are collectively known as ***distribution processes***. Distribution processes include processes such as advection dispersion and volatilisation. They include besides the processes discussed in the foregoing chapter (chapter 8) all those processes that may affect the ***mobility*** of a substance in the subsurface environment. Of these, the following are most prominent.

- Immiscible phase separation

- Acid-base equilibrium
- Precipitation-dissolution reactions

a) Immiscible phase separation

Depending on their miscibility in water, fluids or gases will separate forming an independent layer in a multi - component system. An example may be given by NAPL's, separating to form a floating layer on the surface of underground water (LNAPL) or sinking to form a deposit on an impermeable bed at the base of the aquifer (DNAPL).

b) Acid-base equilibrium

The Brønstead- Lawry theory defines an acid as any substance that can give or donate a proton. Likewise, the theory defines a base as a substance, ready to accept a proton. Following examples provide illustration of both definitions:

1) Ionisation of hydrochloric acid:

HCl° (neutral) $\leftrightarrows H^{+} + Cl^{-}$

2) Ionisation of sulphuric acid

$H_2SO_4^{\circ}$(neutral) $\leftrightarrows 2H^{+} + SO_4^{2-}$

3) Ionisation of carbonic acid

$H_2CO_3^{\circ}$ (neutral) $\leftrightarrows H^{+} + HCO_3^{-}$

All three reactions are reversible. In reaction 1 (ionisation of hydrochloric acid), the acid completely dissociates to give a proton (H^{+}) and an anion (Cl^{-}), ready in the reverse reaction to recombine with the proton, restoring the original structure of the acid. In this case, Cl^{-} may be called a base according to the definition. In reaction 2, the same happens as in reaction 1 and here also SO_4^{2-} (the sulphate group) plays its role as a base that may restore the acid on recombining with the released protons. Reaction 3 (carbonic acid), though principally the same as the others, shows a basic difference in that the acid does not completely dissociate like the two other cases. It partially dissociates so that on reaching equilibrium, a fragment of the original neutral species (HCO_3^-) remains unionised in the aqueous solution. Such acids, incapable of complete dissociation in one step are generally known as weak acids, while acids completely dissociating in one stage are known as strong acids. Aqueous solutions of weak acids have pH-values ranging from 4-6, while those of strong acids possess pH-values lower than 4.

Bases may also be classified, on the same grounds, into weak and strong bases. Thus, bases like NaOH or Ca OH, capable of complete dissociation to give a cation and an anion (see equation) are also known as strong bases.

$$NaOH^{\circ}\text{ (neutral)} \leftrightarrows Na^+ + OH^-$$

The hydroxyl ion in the last equation might also combine with a proton to form water, thus emphasising its role as a base. Contrary to sodium, potassium, or calcium hydroxides, bases like $Fe(OH)_3$ and $Al(OH)_3$ are described as weak bases because they do not further ionise in water. This may be illustrated by the dissociation of the mineral jarosite, which generally dissolves in water to form ferric oxyhydroxide and K^+, producing a considerable acidity, according to the following equation:

$$KFe_3(SO_4)_2 + 3H_2O \leftrightarrows K^+ + 3Fe(OH)_3\text{ (ppt)} + 2SO_4^{2-} + 3H^+$$

Thus, the mineral is seen here to be a salt of a strong acid (H_2SO_4) and a very weak base $Fe(OH)_3$, which forms a precipitate that almost does not ionise.

Acid-base equilibria in a subsurface environment control the prevailing pH-values and hence the stability and solubility of the substances present. This plays a principle role in determining mobility, fate, and toxicity of the penetrating pollutants.

Buffering capacity:

If a strong acid or base is added to a solution the pH of the solution changes, according to whether H^+ is removed or released in the solution. Some solutions, however, resist changes in their pH upon the addition of small amounts of acid or alkali. Such solutions are called buffer solutions or simply buffers and the degree of resistance to change is called buffering capacity.

To understand this, let us consider the following example of equilibrium between a weak acid (acetic acid) and its Sodium salt (sodium acetate):

$$CH_3COOH \leftrightarrows CH_3COO^- + H^+ \qquad \text{(i)}$$

$$CH_3COONa \leftrightarrows CH_3COO^- + Na^+ \qquad \text{(ii)}$$

If to a solution of acetic acid and sodium acetate a slight amount of HCL is added, the hydrogen ions from hydrochloric acid will combine with a portion of the acetate ions to form unionised acetic acid, which is anyway a weak acid i.e. slightly dissociates. The removal of the added hydrogen ions to form acetic acid means that they will not affect or change the hydrogen ion concentration of the solution and thus will not change its pH.

Similarly if OH^- ions are introduced by the addition of a small amount of an alkali, the OH^- will combine with the hydrogen ions resulting from the dissociation of acetic acid to form water. Removal of H^+ in this way will disturb the equilibrium in equation (i), enhancing the acetic acid to increasing dissociation in order to restore the equilibrium. Thus, the pH of the solution will practically remain unchanged. Other weak acids and their salts (e.g. carbonic acid) will also display the same pattern of behaviour of retaining their pH after addition of small amounts of strong acids or bases. The best examples of these are seawater and other brines containing carbonic acid and its salts, which may resist change of its pH on being exposed to small spills of strong acids or bases. The reaction in this case would be controlled by the following equilibrium:

$$CO_2(aq) + H_2O \rightarrow H_2CO_3 \leftrightarrows HCO_3^- + H^+ \leftrightarrows CO_3^- + 2H^+$$

Carbonic acid ***bicarbonate*** ***carbonate***

On attaining equilibrium, the concentration of H^+ remains constant. If the solution is exposed to a strong acid, the concentration of H^+ increases leading to a shift of the equilibrium to the left forming unionised carbonic acid, upon which the excess H^+ ions will be removed keeping the pH of the solution practically unchanged. If OH^- ions are added to the solution by the introduction of a strong base, they combine with H^+ to produce water keeping the pH of the solution at its initial value. The buffer capacity however depends on the initial concentration of carbonates and bicarbonates; since an exhaustion of these will stop the formation of carbonic acid, thus allow an increase of the hydrogen ion concentration, and consequently lead to a lower pH-value.

c) Dissolution-precipitation reactions:

Solubility and precipitation are perhaps the most characteristic phase distribution processes, that take place in the soil environment; for in dissolution there would be a transition from a gaseous or a solid phase into an aquatic one and if at any time precipitation occurs, it will follow just the opposite way. Thus, dissolution obviously induces an increase in mobility, while precipitation inhibits the same.

Solubility of a substance in the soil environment depends on the nature of the substance as well as on physical parameters such as temperature, pressure, pH, and Eh (redox potential). As for the nature of the contaminant substance, we find that organic toxic substances are less soluble than inorganic salts. This is naturally to be expected due to the hydrophobic character of non-polar substances. Another factor determining solubility in water would be the already existing concentration of the same substance in solution and how far it is from the equilibrium concentration at a given temperature. This is manly controlled by the solubility product, which is an expression of the maximum amount of a substance that will dissolve in a solution at a given temperature and pressure. Precipitation occurs when the value of the solubility product is exceeded. In aquatic systems, this may occur due to change of equilibrium conditions such as temperature, pressure, pH, or Eh, making the boundary separating distribution processes from processes encompassing chemical change to fade.

9.2 Chemical transformation processes

In fact, chemical transformation processes and distribution arrangements that may affect the chemical mobility of a substance are complementary in nature. They normally go hand in hand with one or the other implementing the advent of its successor. Non-the less, the two groups of processes are completely different in their mode of action. While, as said before, distribution processes mainly affect the mode of association of a given substance, chemical transformation will in the first place change the chemical structure of the substance, bringing about a net chemical change. Both may happen parallel and it is rarely observed in the soil environment, that one of them occurs without the other following on its heels. This may be illustrated by the case of precipitation, which - as mentioned above - is one of the most typical examples of phase distribution. PERELMAN, 1967 classified processes of metal precipitation from natural waters into the following types which include a great deal of transformation going hand in hand with the processes affecting mobility and phase distribution.

1. Oxidation type:

An example is the precipitation of iron and manganese oxides by the oxidation of reducing waters.

2.Reducing type:

Examples are given by the precipitation of U, V, Cu, Se, and Ag as metals or lower valency oxides by the reduction of oxidising waters. This is usually caused by encounter with organic matter or by mixing with reducing waters or gases.

3. Reducing sulphide type

Sulphate waters carrying ions of Cu, Ag, Zn, Pb, Hg, Ni, Co, As, or Mo may be reduced to precipitate sulphides of these metals. This occurs usually by the action of sulphate-reducing bacteria or on encounter with organic matter.

4. Sulphate and carbonate type:

Alkali metals such as Ba, Sr, and Ca may be precipitated as carbonates following a shift in equilibrium relations. Griffith et al, 1976 have reported about Pb-precipitation from landfill leachates as carbonates.

5. Alkaline type:

Percolation of acidic solutions into carbonates and silicates as well as their encounter with alkaline solutions lead to precipitation of metals like Ca, Mg, Sr, Mn, Fe, Cu, Zn, Pb, and Cd.

6. Adsorption type

This type encompasses all transition metals, which are susceptible to adsorption on clays and other particulate substances.

7. Oxidation – reduction type:

Mobility of trace metals in aquatic solutions is largely influenced by the redox status of their envirnment, even though they generally are not directly involved in oxidation – reduction reactions. Figure 65 shows the stability relations in the system $Zn + S + CO_2 + H_2O$.

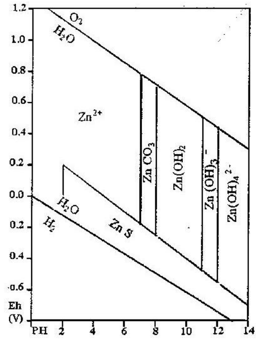


Fig. 65 Stability relations in the system $Zn + S + CO_2 + H_2O$

In this system , three solid phases (Precipitates) are possible: the sulphide, carbonate, and hydroxide of zinc. Under reducing conditions, sulphide and hydroxide are the stable phases at high ph-values. At lower pH-values, however, the hydroxide dissolves and only the sulphide is precipitated.

Oxidising conditions induce precipitation of amorphous iron and manganese oxihydrides, that later as adsorbents tremendously affect the mobility of trace metals in the solution. Reducing conditions induce the reduction of Mn^{+4} to Mn^{+2} and Fe^{+3} to Fe^{+2} thereby solubilising their associated and adsorbed trace metals. Figure 66 summarizes the redox chemistry of iron and manganese.

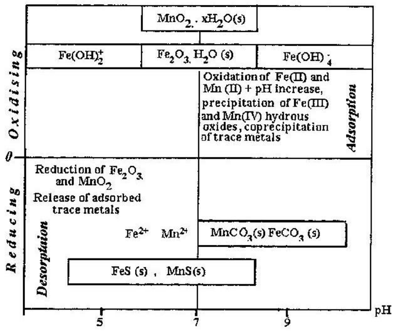


Fig. 66 . Schematic representation of the redox chemistry of iron and manganese

8. Complex formation and chelation:

Most trace elements exist in water as hydrated ions rather than free ones. In the course of hydration, water due to its polar character and the unsatisfied charges on both hydrogen and oxygen, orient its molecules such that oxygen points to the cation while hydrogen points away from it (see figure 67). The water molecules, thus connecting themselves to the cation form a hydration shell, around the cation, producing a species known as cation aquocomplex (copper aquocomplex in figure 67). Each of the water molecules will be called a ligand and as it is seen here, ligands are not attached to the cation through electron sharing, but rather due to electrostatic forces arising from pairs of electrons that exclusively belong to the cation. The so formed species may further be associated to anions that would form an additional sphere at the outer surface of the water shell, forming what is known as ***an outer-sphere complex***. Anions in the outer sphere complex are associated to the cation by long-range electrostatic forces; their association is not strong enough to displace any of the water molecules in immediate contact with the cation.

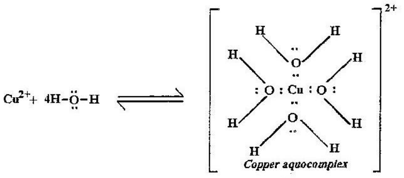


Fig. 67 . Formation of copper aquocomlex

A classical example of the formation of outer-sphere complexes is given by the formation of complex cobalt chlorides. Cobalt forms a simple ionic chloride $CoCl_3$, in which three electrons are transferred from a cobalt atom to chlorine atoms. In the presence of ammonia [NH_3 (aq)], however, this cobalt (III) chloride can form a series of distinct compounds, having up to six ammonia molecules as ligands surrounding the cobalt cation [$(Co\ (NH_3)_6]\ Cl_3$, as shown in figure 68.

A ligand is defined as a species capable of donating an electron pair to a central metal ion at a particular site in a geometrical structure. Therefore, a ligand is essentially considered as a Lewis base and the metal ion as a Lewis acid. If a ligand is capable of donating a single electron pair, as in ammonia, it is called a ***unidentate*** (single toothed) ligand. Some ligands however are capable of donating more than a single electron pair, to different sites in the geometrical structure of a complex ion. These are called ***multidentate ligands***. Most inorganic ligands are unidentate, while multidentate ligands are normally organic ones.

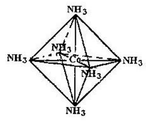


Fig. 68 . octahedral coordination of cobalt with ammonia to form$[Co\ (NH_3)_6\ ]^{3+}$

Humic acids are multidentate ligands, forming cage structures around metal ions when they associate with their complexes (see fig 23). When bonding between a metal and a multidentate ligand results in such ring or cage structures, the process is called ***chelation,*** the species produced, a chelate, and the multidentate ligand is a chelating agent. The expression chelate is derived from the Greek word ***"Chela"*** meaning a crab's claw.

Complex formation in aquatic systems renders metals less bioavailable for organisms. It also influences the adsorption of metals on colloid substances and may increase the solubility of minerals. Solutions with high ionic strengths are favourable media for the formation of complexes, the stability of which will be directly proportional to the cation charge and inversely proportional to its radius.

Hydrolysis:

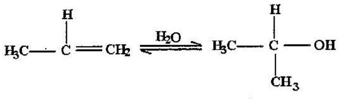
As discussed in 1.2 under chemical weathering, hydrolysis is a pure chemical process during which a proper chemical reaction takes place between water and another substance, to produce or consume a proton (H^+) or an electron (OH^-). A typical example was given by the reaction of the mineral albite with slightly acidic water to produce kaolinite (see equation 1.2).

Organic pollutants percolating through the soil environment may be chemically transformed by hydrolysis, either through water addition to the molecule, or though replacement of some functional groups by water. Examples of replacement reactions may be given by the replacement of halide ions in alkyl halides to form alcohol:

$$RX + H_2O \leftrightarrows R\text{-}OH + HX$$

R = alkyl group, X= halide.

Hydrolysis reactions taking place by addition of water molecules normally proceed more readily than those taking place by replacement. An example of these may be given by the addition of water to alkenes to form alcohol:



$$H_3C-\overset{H}{\overset{|}{C}}=CH_2 \xrightleftharpoons{H_2O} H_3C-\underset{CH_3}{\overset{H}{C}}-OH$$

Propene *Isopropanol*

9.3 Biodegradation and biologically supported transformations

As mentioned before (see section 3.2), organisms (soil biota) form a very important integrative constituent of soil and as such, they play a decisive role in determining the fate of foreign substances added deliberately or accidentally to the soil body. They normally respond to the addition of xenobiotics by initiating two main types of reactions: ***a) Primary metabolic reactions*** (also known as phase I biotransformation), during which the foreign substance is rendered more soluble in water by addition or exposure of functional groups on it ...and

b) Secondary metabolic reactions (phase II biotransformation), through which the products of primary reactions are conjugated with endogenous groups to facilitate their excretion. On passing into solution, the foreign substance will be capable of penetrating the organism with rates, which are specific for organisms and their anatomical and biological peculiarities. In case of high rates of penetration i.e. if a foreign substance enters the organism more quickly than it can be eliminated, it accumulates in some of its organs, and if the substance is toxic, this goes on until a toxic concentration is reached. At normal rates of penetration secondary metabolic transforms will lead, by conjugation with endogenous compounds, to the formation of substances that may be used as energy sources by the organism or to ones that are easily eliminated by excretion. Figure 68 explains this in a schematic way.

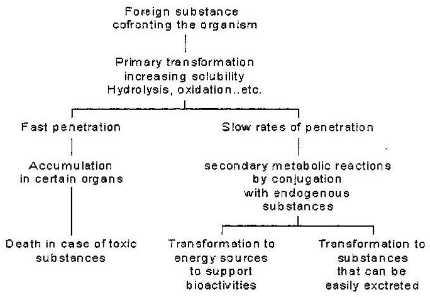


Fig. 69 . Schematic representation of the response of soil biota to foreign substances

From this schematic representation, it becomes clear that the level of metabolism is a principal factor in determining the persistence or degradation of the foreign substance in the soil and thus forms one of the important conditions under which biodegradation succeeds.

As it may be expected, the activation of these reactions requires a high energy demand or at least a source of catalysis to secure their advancement. This catalysis is supplied in organisms by ***enzymes***. These catalytically active high molecular weight proteins enable the activation of biological transformations of substances into energetic sources or easily eliminated chemicals. In order to understand the fundamental action of enzymes in biologically assisted transformations in soil, an outline of the chemical processes involved in enzymatic actions will be shortly explained.

9.4 Enzymatic transformations –A primer on enzymes, their types and mode of action

Enzymes take their specific names from that of the substrate (the substance they help to change) by adding the suffix "ase" to the name of this substance e.g. proteinase, lipase, etc. According to the type of chemical reactions, in which they are usually involved, enzymes are classified into six main groups. Namely, the ***hydrolases,*** those that assist hydrolysis; the ***transferases***, those that help transfer a certain group to another substrate, not usually water; the ***oxidoreductases,*** those that transfer hydrogen or electrons between two substrates, the ***lyases*** those that remove groups from their substrates, the ***ligases (synthases)*** those that catalyse the joining of two molecules (i.e. synthesise a C—C bond) at the cost of chemical energy, and the ***isomerases,*** which are enzymes that catalyse intramolecular rearrangements. Each of the six groups is further classified into sub groups as seen in figure 70.

An enzyme generally consists of two fragments, a protein portion, forming a colloidal carrier and a non protein fragment made of a simple, well defined compound which, unlike the protein portion can be dialysed and is largely stable to heat. The non-protein fragment is known as the ***prosthetic group***, if it is tightly attached to the colloidal carrier and as the ***coenzyme***, if it is loosely attached to the same.

The catalytic activity of enzymes and specifically the special groups upon which this activity depends are subjects of intensive research; for in many cases these groups are not completely well known. In some cases –SH groups play the major role; in some others metals bound to the protein may be the principal factors of catalysis. However, for most enzymes the catalytic activity may be traced to the

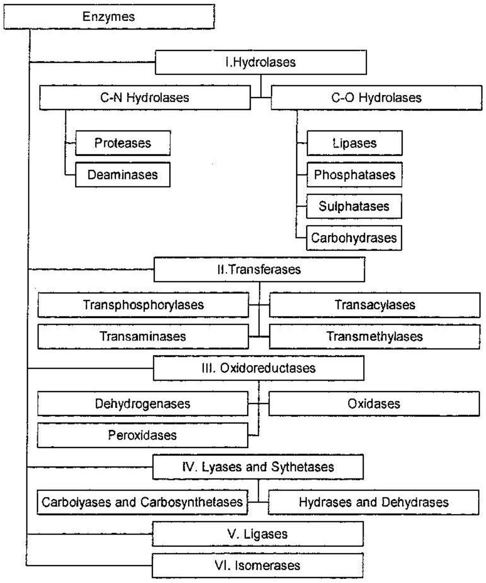


Fig. 70 An outline of enzymes classification

non-protein fragment and may be dependent on certain substances without the presence of which the catalytic activity cannot function. These are called ***cofactors***

I. The Hydrolases

These form about one third of the known enzymes and act mainly on peptides, ester, glycosidic, amide, and similar bonds. They catalyse the hydrolysis of their substrates and may further be classified into C–N and C–O hydrolases according to the type of bond, which may be attacked under their support.

1. C–N - Hydrolases, Specific for the hydrolysis of C–N bond, the C–N hydrolases may be classified according to the C–N bond they hydrolyse into ***proteases*** and ***deaminases.***

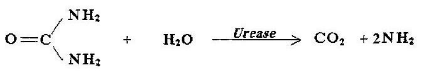
a) Proteases. Are those Hydrolases that help degrade proteins (see figure 17 – breakdown series of the proteins) by hydrolysing the internal peptide bonds along the chain linking the individual amino acids in the protein molecule:

$$-CO-NH- + H_2O \rightarrow -COOH + H_2N$$

Normally, proteases are differentiated into the ***proteinases*** that cleave high molecular-weight proteins into simpler compounds (polypeptides), and the ***peptidases*** that further degrade the polypeptides resulting from protein degradation by prteinases.

$$\textbf{Proteins} \xrightarrow{\textit{Proteinases}} \textbf{Polypeptides} \xrightarrow{\textit{Peptidases}} \textbf{Simpler compounds}$$ *(see figure 16)*

b) Deaminases: These C–N Hydrolases catalyse the hydrolysis of certain types of carbon – nitrogen bond. The most important of them are ***urease*** and ***arginase***. Urease assists the hydrolysis of urea into ammonia and carbon dioxide according to the equation:



$$O{=}C(NH_2)_2 + H_2O \xrightarrow{\textit{Urease}} CO_2 + 2NH_2$$

It occurs in Soya beans, watermelon, moulds, and bacteria. Arginase is principally found in animal liver. It helps splitting arginine (a compound of proteins) into ornithine (α, δ - diaminovaleric acid) and urea. Ornithine in the liver of birds removes the toxic benzoic acid in the form of its dibenzyl derivative.

2.C– O - Hydrolases: C–O hydrolases catalyse the hydrolysis of natural esters. Depending upon the type of their substrates, they may be classified into various groups the most important of which are:

- **a)** ***Lipases:*** These are capable of hydrolysing fats. Lipases of vegetable origin are known as ***phytolipases***. An example of them is castor lipase which is found in the seeds of *recinus communis*
- **b)** ***Phosphatases:*** These C-O hydrolases are capable of catalysing the hydrolysis of phosphoric ester groups. They occur in almost all living cells and their catalytic activity is pH-dependent.
- **c)** ***Sulphatases:*** These are capable of hydrolysing natural sulphuric esters e.g. phenyl sulphuric acid.
- **d)** ***Carbohydrases:*** As it is clear from their name, this group of hydrolases is capable of degrading simple sugars as well as transforming polysaccharides into simple ones. A prominent example of them is ***cellulase,*** which degrades cellulose and is only found in bacteria, fungi, and in the digestive juices of certain snails and worms.

The role of hydrolases in pesticide degradation:

Hydrolases, whether they are specialised on the C–N or the C–O bond, are capable, with their various groups and individual enzymes, of degrading many pesticides containing ester, amide or phosphate linkages as explained in the foregoing section. Examples of such pesticides are various. To mention few of them, one may consider the group of organophosphorus pesticides, the carbamate insecticides, or the urea and carbamate herbicides.

Detoxification of malathion (see table 17 and figure 71) by the action of a C–O hydrolase (carboxylesterase) provides an example for the degradation of an organophosphorus pesticide through cleaving a carboxylethyl linkage.

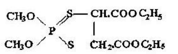


Fig. 71 Malathion

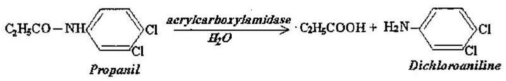
Hydrolysis of the carboxylethyl linkage follows the following reaction path:

$$R—COO—C_2H_5 \xrightarrow[H_2O]{\textit{Hydrolase (Carboxylesterase)}} R—COOH + C_2H_5OH$$

The low toxicity of malathion for some higher animals may be due to the efficiency of this process (Hassall, 1982).

Organochlorine herbicides such as esters of 2,4-D (figure 39 b) are also degraded through the catalytic action of carboxylesterase. After penetrating into the weed, the esters are hydrolysed to release the biologically active dichlorophenoxyacetic acid.

Propanil — an organochlorine herbicide, derived from aniline (figure 40 a), was found to be hydrolysed by the action of a C–N hydrolase (aryl carboxylamidase), found in rice plants (Yih et al, 1968) to produce dichloroaniline.



These were just few examples of the enzymatic degradation of some insecticides and herbicides by the action of hydrolases. The interested reader is referred to (HASSALL, 1982) for a detailed treatment of this theme.

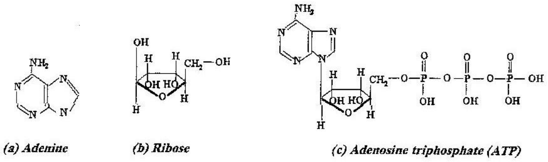
II. The Transferases:

Transferases derive their individual names from that of the transferred group, either with the prefix "trans" and suffix "ase," or followed by "transferase." So one finds names like:

Trans-phosphoryl-ase, trans-acyl-ase, or dihydroxyacetone transferase. Following groups are the most important among the transferases.

1. Transphosphorylases

As mentioned before, some enzymes need to fulfil their action of transforming their substrates into other chemical constitution the assistance of a third substance, normally called the cofactor. Cofactors are usually phosphate esters of sugars or of compounds between sugars and nitrogen-containing bases. A good examples of such cofactors is adenosine triphosphate ATP), formed from the base adenine (figure 72 a), the sugar ribose (figure 72 b) and three phosphate groups.



(a) Adenine *(b) Ribose* *(c) Adenosine triphosphate (ATP)*

Fig. 72 Constitution of adenosine triphosphate (ATP)

Transphosphorylases, being one of those enzymes that need a cofactor to carry out their task, which is adding phosphate groups to their substrate, use ATP as a source for the required phosphate groups

2. Transacylases

Acyl groups (from *acidum* = acid) are groups of general formula, RCO where R is an alkyl group. They may be viewed as fatty acids (R–COOH), lacking a hydroxyl group (OH^-). Examples are ***acetyl*** (CH_3CO), ***formyl*** (H–CO),etc. Enzymes, capable of transferring an acyl group from a donor to an acceptor molecule are known as transacylases. These have a loosely attached non-protein fragment functioning as a coenzyme that cleaves the acyl bond in an intermediate step followed by its transfer to the acceptor group. An example can be given by the transfer of an acetyl group (CH_3CO) attached to a molecule X (donor) to another molecule Y (acceptor). Such a process proceeds along the following reaction path:

$$\text{Acetyl}-X + \overline{CoA}, \; -SH \longrightarrow \text{Acyl}-S-\overline{CoA}, \; +XH$$

$$\text{Acetyl}-S-\overline{CoA}, \; + YH \longrightarrow \text{Acyl}-Y + \overline{CoA}, \; -SH$$

($\overline{CoA}$, = Coenzyme A without –SH group)

Coenzyme A — a coenzyme of transacylases, made of adenine, ribose, phosphate, pantothenic acid, and cysteine plays a decisive role in all biochemical acylations; especially the oxidative degradation of carbohydrates and fats. It also forms a key substance in the conversion of carbohydrates into fatty acids.

3. Transaminases

These enzymes effect a reciprocal process of amination — deamination between keto-acids (mainly pyruvic acid – figure 73 a) and glutamic acid (figure 73) without incurring the presence of free ammonia. In this reciprocal reaction, known as transamination, L-glutamic acid functions as a nitrogen-carrier in the synthesis of amino acids in animal organisms.

In plants, the same role is played by L-aspartic acid. Pyruvic acid, which plays a key role in transamination, is sometimes produced by the degradation of aliphatic herbicides such as dalapon (figure 45).

d) Transmethylases

Like the other transferases, transmethylases transfer a group (in this case methyl) from a donor molecule to an acceptor.

The role of transferases in the degradation of pesticides and other contaminants.

Among the transferases, the system known as glutathione-S-transferase (GST) plays an important role in the detoxification of contaminants by catalysing the conjugation of the endogenous substance (GSH) to an electrophilic site on the intruding xenobiotic material. Glutathione (GSH) is a tripeptide, formed by combination of the three amino acids glycine, cysteine, and glutamic acid as shown in figure 73.

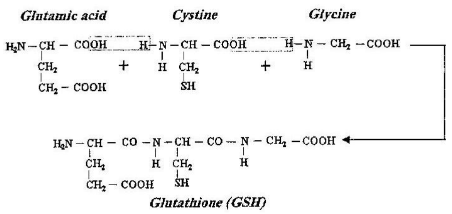


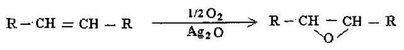
Figure 73 Formation of glutathione

Catalysis of glutathione conjugation on xenobiotics by GST takes place generally on substrates sharing three common features: They must be hydrophobic to some degree, they must contain an electrophilic carbon atom, and they must react nonenzymatically with glutathione at some measurable rate. (T. J. Rees, 1993)

Of the several known glutathione-S- transferases, the following three groups were considered by (HASSALL, 1982) as being among the most important:

a) Glutathione-S-epoxide transferases:

Alkylene oxides or epoxides are groups formed by the oxidation of olefins as shown in the following equation:



$$R-CH=CH-R \xrightarrow[Ag_2O]{1/2\,O_2} R-\underset{\diagdown O \diagup}{CH-CH}-R$$

Epoxides may be formed during phase I biotransformation as intermediate stages in the degradation of numerous unsaturated compounds. A subsequent opening of the ring by conjugation of an endogenous substance on the epoxide leads in most cases to the formation of substances less harmful to life. This may be illustrated by the GST catalysed glutathione conjugation on the intermediate epoxide of allyl phenyl ether (a substance used in polymer synthesis - see figure 74). In this reaction, glutathione conjugation on the substrate leads to opening of the epoxide ring and formation of a glutathione complex with the detoxified original substance as shown in figure 74.

With benzene rings this reaction precedes more readily if the ring contains chlorine atoms and that is why they may be of great help in degrading organochlorine epoxides, used as pesticides, such as dieldrin (figure 35b) and heptachlor epoxide (figure 35 c).

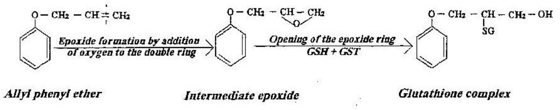


Fig. 74 . Degradation of allyl phenyl ether by Glutathione (GSH)

b) Glutathione –S-aryl transferases

This group effects mainly the elimination of hydrogen halides from their substrates. They are most effective in detoxification of organochlorine pesticides (e.g. triazine derivatives). The chemical mechanism according to which such reaction occurs may be illustrated by the following example (figure 75) of eliminating a hydrogen halide group from a molecule of the herbicide atrazine (figure 43 b).

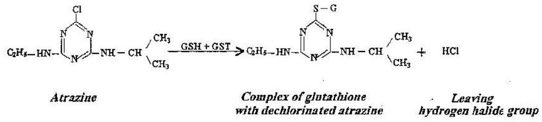


Fig . 75 . Elimination of a hydrogen halide from atrazine by GSH

Various organophosphorus pesticides are also degraded by chemical reactions comprising glutathione conjugation. An example of this is the degradation of the pesticide diazinon, which is strongly adsorbed onto soils. Diazinon is used to control pests that have become resistant to organochlorine compounds. Its cleavage by GST follows the following pathway (Shishido et al, 1972):

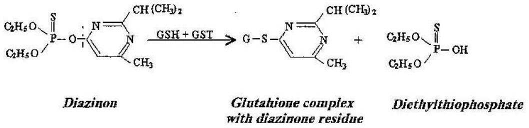


Fig. 76. Cleavage of Diazinon by GSH

c) Glutathione – S – alkyl transferases:

Being capable of reacting with alkyl halides, these enzymes were found effective in removing methyl groups from organophosphorus insecticides, that contain the $CH_3 - O - P$ group. They are less effective in removing ethyl and larger alkyl groups. This may be illustrated by the demethylation of parathion (table 17) as shown in figure 77.

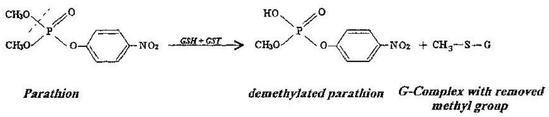


Fig. 77 Demethylation of parathion by GSH

Non-enzymatic glutathione conjugation

Substrates susceptible to glutathione conjugation – as stated before – must be in a position to react nonenzymatically with glutathione (GSH) at some measurable rate. It seems that even in absence of GTS this type of reaction plays an important role in the detoxification of some aromatic pesticides. An example may be given by the degradation of the herbicide propachlor, during which the glutathione is conjugated to an electrophilic site with the elimination of a hydrogen halide to form a complex that subsequently decomposes to an N-acetyl cysteine derivative known as mercapturic acid. The polarity induced by the insertion of COOH group makes such a compound more soluble and hence easy to be excreted in urine or faeces. Figure 78 shows the pathway of such a process. Glutathione levels in soil biota are accordingly used some times as biomarkers in soils i.e. substances that may indicate the level of pollution by relating this to the degree of stress incurred in the organisms. In addition, metabolites such as mercapturic acid may be used for the same purpose.

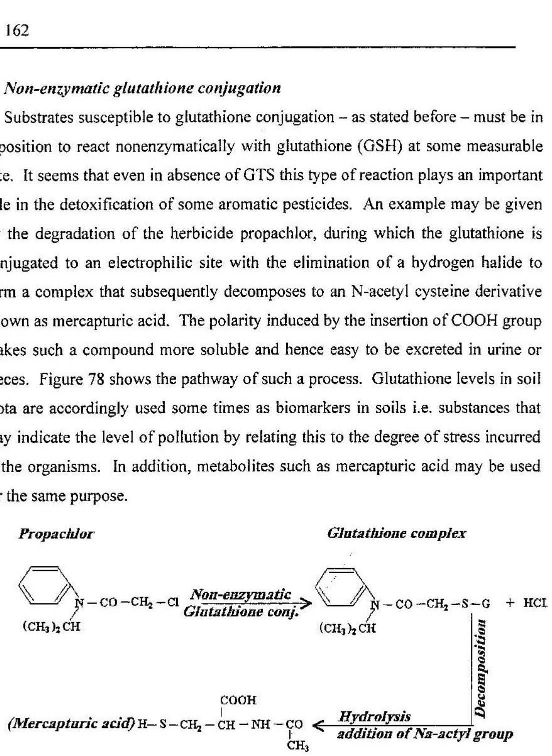


Fig.78 Nonenzymatic propachlor degradation by glutathione

III. The Oxidoreductases

These are enzymes capable of transferring hydrogen or electrons between substrates. They are of three types, the dehydrogenases, the oxidases, and the peroxidases. While dehydrogenases mainly transport hydrogen or electrons to acceptor enzymes or to oxygen to produce H_2O_2, the oxidases react directly with oxygen to give water. The peroxidases, however, catalyse the decomposition of hydrogen peroxide, which has a toxic effect, into oxygen and water.

a) Dehydrogenases:

The catalytic activity of dehydrogenases is characterised by the transfer of two hydrogen atoms from one organic donor to an organic acceptor. This may be schematically represented –as shown in the following two equations in which the enzyme is represented by the box with the letter D.

$$XH_2 + \boxed{D} \longrightarrow X + \boxed{D}\text{-}H_2$$

$$\boxed{D}\text{-}H_2 + Y \longrightarrow \boxed{D} + YH_2$$

As shown by the equations, two atoms of hydrogen were transferred from the donor X to the acceptor Y, while the enzyme as a catalyst was recovered unchanged at the end of the process.

b) Oxidases

This group of enzymes, also known as aerobic electron transferases, help complete reduction of oxygen into water. They thus differ from the majority of dehydrogenases, which carry the reduction of oxygen only to the peroxide stage. They are metalloprotein compounds carrying heavy metals and are accordingly classified into cytochrome oxidases (Fe-proteins), and cuproprotein oxidase.

c) Peroxidases

These are, as mentioned before, enzymes that attack hydrogen peroxide; they are present in animal organs and most plants and are made of iron porphyrin compounds. Peroxidases catalyse reactions between hydrogen peroxide and other substances according to the equation:

$$XH_2 + H_2O_2 \xrightarrow{Peroxidase} X + 2H_2O$$

A similar catalytic function is exerted by ***catalases***, which are also found in almost all animal organs, cells and tissue fluids as well as plant tissues. They effect the decomposition of the cell toxin, hydrogen peroxide, into water and oxygen according to the equation:

$$2\,H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$$

The role of oxidoreductases in soil formation and biodegradation of contaminants

Decay of plant debris and other natural organic matter forms one of the main sources of soil humus; among other processes involved in this process biodegradation of lignin holds a central position. This degradation process is initiated by several oxidoreductases excreted by white rot fungi. Examples of such enzymes are haem-containing peroxidases, lignin peroxidase (L.P), manganese dependant peroxidase (Mn P) as well as copper containing phenol oxidase, laccase (Camarero et al, 1999)

Further research revealed that lignin-degrading enzymes, excreted by white rot fungi, are also capable of oxidising high-molecular-weight polycyclic aromatic hydrocarbons (PAH); this directed attention to them as potential agents for bioremediation of contaminated soils (Michiel et al, 1998).

Various oxidases use nicotinamide adenine dinucleotide (NAD) or its phosphate derivative nicotinamide adenine dinucleotide phosphate (NADP) as coenzyme. NADP-dependent reactions were found, as it will be shown in the following examples, to be important for the biochemical degradation of many insecticides.

a) Hydroxylation of carbaryl:

Carbaryl (figure 79), a carbamate insecticide, which is used to control pests mainly on maize and soybeans may suffer ring hydroxylation by NADP assisted oxidases to give a mixture of 4-hydroxy and 5-hydroxy carbaryl as shown in figure 79.

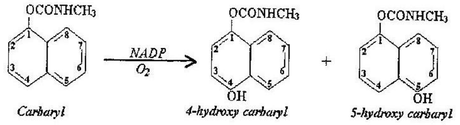


Fig. 79. Ring hydroxylation of carbaryl

b) O-dealkylation of methoxychlor:

Methoxychlor (figure 80) is a minor organochlorine insecticide similar to DDT, yet differing in that it has p, p′ - dimethoxy groups instead of chlorine atoms. It can be easily dealkylated by NADP-dependent enzymatic reactions to produce the polar compound demethyl methoxychlor that can undergo further conjugations, facilitating its removal from animal bodies.

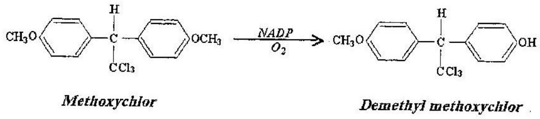


Fig.80. O-dealkylation of methoxychlor

In his book on the chemistry of pesticides, Kenneth Hassall (Hassall, 1982) quotes the eminent toxicologist Barnes with the words that *"if methoxychlor had been marketed instead of the slightly cheaper DDT, the persistence of organochlorine insecticides may never have been regarded as a factor of major ecological importance".*

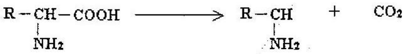
IV. The Lyases:

This group helps mainly the cleavage of a C–C bond. They are subdivided according to the bond they split into the carbolyases and the dehydrases.

a) Carbolyases: an important member of this group is α-carboxylase (pyruvate decarboxylase). It splits pyruvic acid into acetaldehyde and carbon dioxide according to the equation:

Pyruvic acid *Acetaldehyde*

Another important member of the group is amino acid decarboxylase that occurs in microorganisms, in higher animals, and in many plant tissues. It plays a key role in the putrefaction of proteins by catalysing the anaerobic decarboxylation of amino acids into amines and carbon dioxide according to the equation:



$$\underset{\displaystyle NH_2}{R-\underset{|}{CH}-COOH} \longrightarrow \underset{\displaystyle NH_2}{R-\underset{|}{CH}} + CO_2$$

Amino acid *Amine*

b) Dehydrases

This group catalyses the elimination of water. It plays an important role in citric acid cycle. Its function may be illustrated by the action of the enzyme ***aconitase*** (citric-isocitric isomerase) that helps changing citric acid into cis-aconitic acid according to the equation:

$$HOOC-CH_2-\underset{\displaystyle COOH}{\underset{|}{C}}(OH)-CH_2-COOH \rightleftharpoons HOOC-CH=\underset{\displaystyle COOH}{\underset{|}{C}}-CH_2-COOH$$

Citric acid ***cis-aconitic acid***

V. The Ligases

Ligases (Synthases) catalyse the linking of two molecules; they are sometimes classified according to the type of bond formed under their catalytic action. Thus, we may have carbon-sulphur bond forming, carbon oxygen bond forming, or carbon-carbon forming ligases.

VI. The isomerases

This class of enzymes catalyse intramolecular rearrangements. An example may be given by D-arabinose isomerase, an enzyme that catalyses the intramolecular rearrangement of D-arabinose to give D-ribulose.

9.5 Transformations assisted by bacterial action

In the foregoing sections, it was shown that soil organisms assisted by their natural metabolic processes create an environment in soil that allows the whole system to develop and to avoid conditions that would bring it to collapse. It should, however, be reminded that the main target of all metabolic processes is to secure the energy required for an organism to continue its life activities. Energy production in plants is reached through a complex of light-assisted biochemical processes known collectively as photosynthesis. In animals, cleavage (mostly oxidation) of complex organic material fulfils the same purpose; the whole process –in this case- is known under the collective name chemosynthesis.

Bacteria and other low organisms use both processes, yet some bacteria are also capable of energy production through chemical transformation of inorganic material and thus play a role in determining the fate of inorganic pollutants in soil.

Changes in the soil environment by bacterial action are brought about mainly by organisms, known as the lithotrophs (Rock eaters). The Russian microbiologist Sergei Winogradsky, who first described them in 1885, gave them this name. As the name indicates, they are capable of covering their need of energy by oxidising soil inorganic compounds. This process may be pure chemotrophic as explained before, or it may be a phototrophic one, using visible light as a source of energy. Fig. 81 summarises these relations.

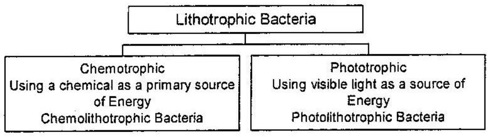


Fig. 81 . Classification of lithotrophic bacteria according to their energy sources

The major groups of lithotrophic bacteria, playing principle roles in modifying the soil environment, are the following:

a) Sulphur bacteria
b) Iron oxidising bacteria
c) Nitrifying bacteria
d) Hydrogen oxidising bacteria
e) Methane bacteria

a) Sulphur bacteria

The natural ecological community of sulphur bacteria is generally known under the collective term *sulfuretum,* coined by Baath-Becking in 1925.

A sulfuretum exists normally in oxygen-deficient environments and may even flourish under extreme conditions such as in the case of the highly alkaline sediments of Wadi Natrun in North-western Egypt (Trüper, 1982). Organisms living in such a community must not essentially follow the same trophic mode– some may be chemotrophic, while others could be phototrophic. As examples of both types, we may mention the following:

1.Photolithotrophic sulphur oxidising bacteria:

A good example of these is provided by the so-called *thiospirills that* belong to the family *Rhodospirillaceae.* They embrace two subgroups of which the first uses H_2S or S as proton donor in photosynthesis, while the other uses H_2 derived from the breakdown of organic substances such as fatty acids, for the same. To differentiate between them, the first group is called the group of *thiorhodaceans* while the second is known as the group of *athiorhodaceans*.

2. Chemolithotrophic sulphur oxidising bacteria:

Two prominent examples represent this group — *Beggiatoa sp.*, and the often-mentioned *thiobacillus denitrificans.* Beggiatoa has a peculiar thread like appearance and is always in a continuous state of worm-like motion (see Fig.82)

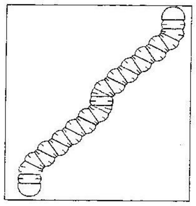


Fig. 82 . Beggiatoa

Sulphur organisms of the beggiatoa species use both Hydrogen Sulphide and elemental sulphur (S^0) for their chemotrophic oxidation processes according to the following equations:

$$H_2S + 2\,O_2 \longrightarrow SO_4^{-2} + 2H^+$$

$$S^0 + 2\,H_2O \longrightarrow SO_4^{-2} + 4H^+$$

Thiobacillus denitrificans is capable of oxidizing hydrogen sulphide, using nitrate $(NO_3)^-$ as an oxidizing agent instead of oxygen. As a result of this process, molecular nitrogen (N_2) and sulphuric acid (H_2SO_4) are released.

Some bacteria species are also capable of reducing sulphate to produce sulphide under anaerobic conditions; of these we may mention *desulfovibrio sp.* And *Desulfotomaculum sp.* Sulphite reduction to sulphide is carried out by the anaerobic bacterium *Clostridium pasteurianum* (Trüper, 1982)

b) Nitrifying bacteria

These widely distributed bacteria, represented by two groups living in a symbiotic community – the nitrite bacteria (e.g. *nitrosomonas europaea*), and the nitrate bacteria (e.g. *nitrobacter agile, nitrobacter winogradskyi)* are very important soil dwellers that cause important chemical changes in the soil environment. The first group changes NH_4^+ (ammonium) into NO_2^- (nitrite), while the second group oxidises nitrite further into nitrate (NO_3^-), according to the following reactions:

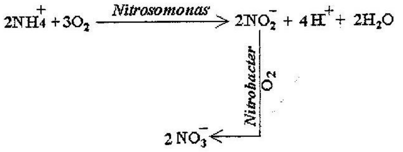


Fig. 83 .The combined work of nitrosomonas and nitrobacter

Nitrosomonas europaea was first discovered and described by Winogradsky in 1890. It flourishes under aerobic conditions in arable soils, where ammonia (NH_3), resulting from the breakdown of organic substances, is continuously oxidised to nitrite $(NO_2)^-$ according to the equation:

$$2NH_3 + 3O_2 \longrightarrow 2HNO_2 + 2H_2O$$

The energy set free in this reaction supplies the major part of energetic requirements for chemosynthesis of carbon dioxide by this group of bacteria.

c) Iron oxidising bacteria

Many species, belonging to the order *Caulobacterials* possess the capacity of oxidising Fe (II) into Fe (III), whereby the oxidation product will be stored in their mucous cells, giving them the characteristic rusty brown colour of Fe (III)-OH. This may in some cases supply an explanation for the appearance of rusty horizons (patches) in soils and marine sediments, inhabited by bacterial colonies such as *gallionella ferroginea* or *siderocapsa treubii.* The first of these, flourishes near iron rich springs, and may also grow in shallow marine environments.

d) Methane oxidising bacteria.

It seems that anaerobic methane oxidation goes hand in hand with sulphate reduction; for it was postulated by many authors that methanogenic bacteria (e.g. *Scarina methanica*) may be producing and oxidising methane at the same time. *Scarina methanica* uses carbon dioxide as a proton acceptor to change alcohols to methane according to the equation:

$$2CH_3CH_2OH + CO_2 \longrightarrow CH_4 + 2CH_3COOH$$

Acetate, produced in this reaction, allows and supports the growth of sulphate reducing bacteria, which may, in turn, assist the anaerobic oxidation of methane into carbon dioxide and water.

e) Hydrogen bacteria

This group, assisted by the catalytic effect of the enzyme hydrogenase, is capable of oxidising molecular hydrogen into water. *Hydrogenomonas* – a species belonging to the family *Nitrobacteriaceae* is capable of oxidising molecular hydrogen to produce water according to the equation:

$$2H_2 + O_2 \longrightarrow 2H_2O$$