MICROBIOLOGY STUDY MATERIAL

Semester - IV

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ENVIRONMENTAL MICROBIOLOGY (THEORY)

CC9-Unit-V

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
SEMESTER – IV
CC-9: Unit - 5

❖ What are Pesticides?

Pesticide is any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. This use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection product. It is commonly used to eliminate or control a variety of agricultural pests that can damage crops, livestock and reduce farm productivity. The most commonly applied pesticides are insecticides to kill insects, herbicides to kill weeds, rodenticides to kill rodents, and fungicides to control fungi, mold, and mildew.

Meanwhile, in the nineteenth century, researchers focused more on natural techniques involving compounds made with the roots of tropical vegetables and chrysanthemums. In 1939, Dichloro-Diphenyl-Trichloroethane (DDT) was discovered, which has become extremely effective and rapidly used as an insecticide all over the world. However, twenty years later, due to biological effects and human safety, DDT has been banned in almost 86 countries.

❖ Types of Pesticides

Grouped by Types of Pests They Kill:

1. Insecticides – insects
2. Herbicides – plants
3. Rodenticides – rodents (rats & mice)
4. Bactericides – bacteria
5. Fungicides – fungi
6. Larvicides – larvae

Chemical pesticides can be classified in different ways, but one of the most used is according to their chemical composition, which allows to group pesticides in an uniform and scientific way and to establish a correlation between structure, activity, toxicity and degradation mechanisms, among others. Table 1, shows the most important pesticides according to their chemical composition.

Table 1: Classification of pesticides according to their chemical composition

Group	Main composition
Organochlorine	Carbon atoms, chlorine, hydrogen and occasionally oxygen. They are nonpolar and lipophilic.
Organophosphate	Possess central phosphorus atom in the molecule. In relation with organochlorines, these compounds are more stable and less toxic in the environment. The organophosphate pesticides can be aliphatic, cyclic and heterocyclic.
Carbamates	Chemical structure based on a plant alkaloid *Physostigma venenosum.*
Pyrethroids	Compounds similar to the synthetic pyrethrins (alkaloids obtained from petals of *Chysanthemun cinerariefolium*).
Botanical origin	Products derived directly from plants. Not chemically synthesized.

Biological	Viruses, microorganisms or their metabolic products.
Copper	Inorganic compounds of copper.
Thiocarbamates	Differ from carbamates in their molecular structure, containing a S-group in its composition.
Organotin	Presence of tin as a central atom of the molecule.
Organosulfur	They have a sulfur central atom in the molecule, very toxic to mites or insects.
Dinitrophenols	They are recognized by the presence of two nitro groups (NO_2) bonded to a phenol ring.
Urea derivatives	Compounds which include the urea bound to aromatic compounds.
Diverse composition	Triazines, talimides, carboxyamide, trichloroacetic and trichloropicolinic acid derivatives, guanidines and naphthoquinones.

Although pesticides are beneficial in controlling the proliferation of pests, their unregulated and indiscriminate applications for the application of pesticides can cause adverse effects to human health, to different life forms and to the ecosystems, which depend on the degree of sensitivity of organisms and toxicity of pesticides. The continued application of pesticides has increased its concentration in soils and waters, besides; they enter the food chains. Dispersion mechanisms also have increased the level of environmental risk for the occupationally exposed population and the inhabitants of surrounding villages. Despite ban on application of some of the environmentally persistent and least biodegradable pesticides (like organochlorines), in many countries their use is ever on rise. Pesticides cause serious health hazards to living systems because of their rapid fat solubility and bioaccumulation in non-target organisms. The main forms of pollution are direct applications to agricultural crops, accidental spills during transport and manufacturing, as well as waste from tanks where cattles are treated to ectoparasite control.

The effects of the impacts of pesticides can be analyzed from two different points of view:

1. environmental, and
2. public health.

The first occurs when pesticides are introduced to the food chains, for example:

a) producing a change in the decline of populations of phytoplankton and zooplankton (indicators of water pollution);
b) producing carcinogenic, neurotoxic, and on fertility and viability (in invertebrates, fish, amphibians, insects and mammals) of their descendants;
c) the presence of pesticides in the environment have caused the resistance of organisms considered as pests and disease vectors (for example malaria, dengue and Chagas disease), and instead other beneficial insect populations are diminished (like pollinators);
d) alter biogeochemical cycles by decreasing the macro and microbiota,
e) leaching of pesticides pollute water bodies,
f) can be absorbed by pesticides when soil particles interact with positively or negatively charged, thus increasing their persistence in the environment (4-26 weeks).

In natural environments, pesticides or their degradation products may be further transformed or degraded by other microorganisms eventually leading to complete degradation by the microbial consortium. However, persistent xenobiotics like pesticides and metabolic dead-end products will accumulate in the environment, become part of the soil humus, or enter the food chain leading to biomagnification (Figure 1).

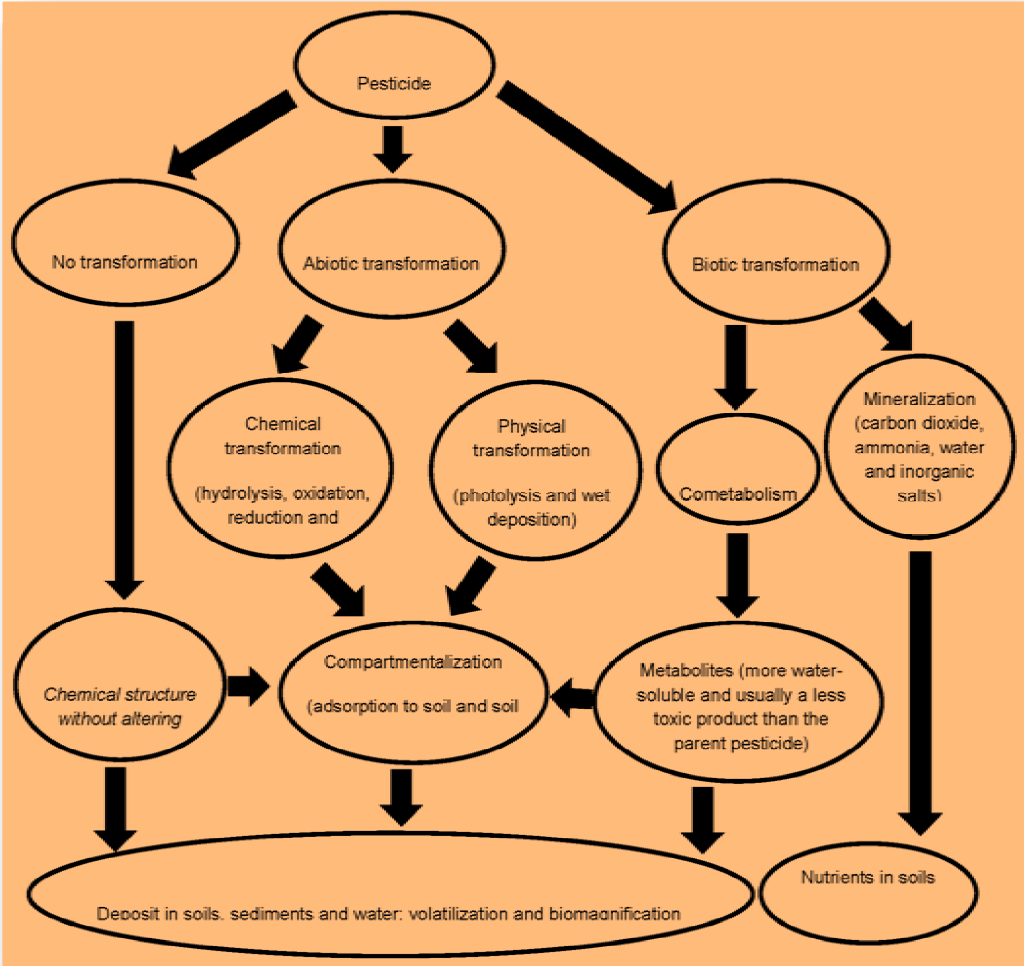

Figure 1: Fate of pesticides in the environment

Pesticides can also be considered as:

- **Biodegradable:** The biodegradable kind are those which can be broken down by microbes and other living beings into harmless compounds.
- **Persistent:** While the persistent ones are those which may take months or years to break down.
- **Chemically-related pesticides:** Another way to classify these is to consider those that are chemical forms or are derived from a common source or production method (details mentioned in Table 1).

Due to the mentioned problems, development of technologies that guarantee their elimination in a safe, efficient and economical way is important. In order to reduce the effects of pesticides on the environment and health, for remediation of contaminated sites and for the treatment of pesticide residues and/or obsolete pesticides, different methods have been developed. The conventional physicochemical approaches are generally expensive and remediation process is often incomplete due to the conversion of the parent compound to metabolites which are more persistent and equally or more toxic to non-target organisms. An alternative pesticide treatment with important global boom is bioremediation, which is conducted through the biodegradation of these chemical compounds. This technique relies on the ability of microorganisms to convert organic contaminants in simple and harmless compounds for the environment. Bioremediation overcomes the limitations of traditional methods for the disposal of hazardous compounds, so it has allowed the destruction of many organic contaminants at a reduced cost.

Microorganisms involved in the biodegradation of pesticides:

Different biological systems, as microorganisms, have been used to biotransform pesticides. It has been reported that a fraction of the soil biota can quickly develop the ability to degrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors for certain soil microorganisms establishing a way for the treatment of pesticide-contaminated sites. Furthermore, the isolated microorganisms capable of degrading pesticides can be used for bioremediation of other chemical compounds to whom any microbial degradation system is known. However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes, but also a wide range of environmental parameters. Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants.

There are different sources of microorganisms with the ability to degrade pesticides. Since pesticides are mainly applied to agricultural crops, soil is the medium that mostly gets these chemicals, besides pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides, and even some live organisms. In general, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of sites contaminated with some kind of pesticide. New analytical and molecular tools (ranging from sequencing the DNA of biodegrading microorganisms) have deepened our insights into the mechanisms (how), the occurrence (what), and the identity (who) of active players that effect biodegradation of organic environmental pollutants, (Figure 2).

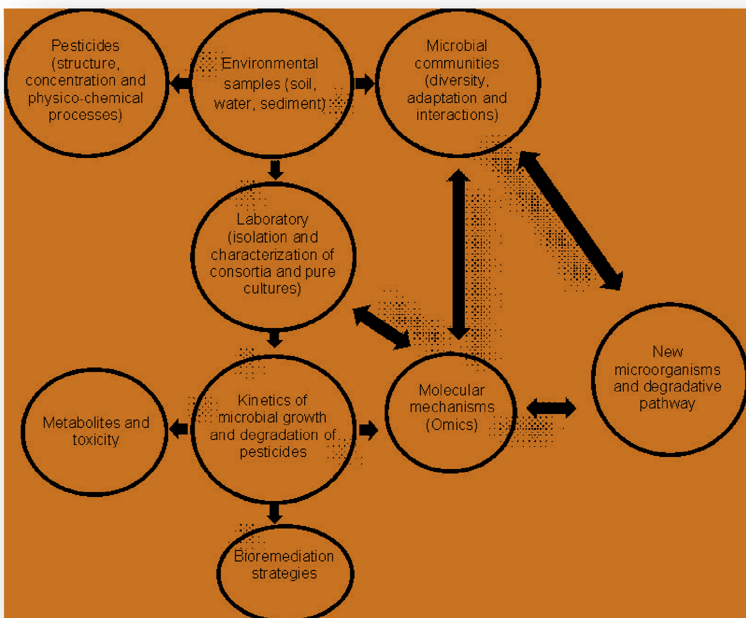

Figure 2: *Representation of the relationships between pesticides, microbial communities, and the discovery of new biodegradation processes*

Some examples of microbial pesticide degradation:

1) *Pseudomonas*, is the most efficient bacterial genus for the degradation of toxic compounds. The ability of this bacteria to degrade these compounds, is related to the contact time with the compound, the environmental conditions in which they develop and their physiological versatility. Three *Pseudomonas* species have been specifically identified for the biodegradation of the herbicide aroclor 1242, showing that these bacteria have a great ability to degrade it, according to their degradation percentage, 99.8, 89.4 and 98.4 respectively.
2) Various isolated fungi species from Algerian pesticide contaminated soils. Observing that the most frequent species isolated were *Aspergillus fumigatus, A. niger, A. terreus, Absidia and Rhizopus microsporus.* 53 of the isolated species were noted for their ability to degrade the herbicide metribuzin in liquid medium. It was demonstrated, at the same time, that the herbicide promoted the *Absidia* and *Fusarium* genera growth; these genera were capable to eliminate 50% of the compound after 5 days. Moreover, the species *Botrytis cinerea* could eliminate the linuron and metroburon herbicides almost completely, and other 31 isolated species also could eliminate metroburon. The fungi *Trichoderma viridae* has the ability to degrade endosulfan and methyl parathion pesticides.
3) Experiments have demonstrated the efficiency of the bacterium *Rhodococcus sp.* to degrade triazines to nitrate. A test conducted to study the atrazine herbicide transformations resulting from microbial decomposition. After microbial action this compound was transformed into nitrite (30%), nitrous oxide (3.2%), ammonia (10%) and formaldehyde (27%) respectively.
4) Several bacterial genera are adapted to grow in pesticide contaminated soils. These microorganisms have enzymes involved in the hydrolysis of

P-O, P-F, P-S and P-C bonds, which are found in a wide variety of organophosphorus pesticides. Some bacteria isolated from the soil are capable of degrading pesticides as ethyl-parathion and methyl-parathion.

❖ Biodegradation Mechanisms

Biodegradation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient and cost-effective technology. It is a process that involves the complete rupture of an organic compound in its inorganic constituents. The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (cometabolism). In natural environments, biodegradation involves transferring the substrates and products within a well-coordinated microbial community, a process referred to as metabolic cooperation. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the environment, where it is susceptible to further degradation by bacteria.

Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence. White rot fungi have been proposed as promising bioremediation agents, especially for compounds not readily degraded by bacteria. This ability arises from the production of extracellular enzymes that act on a broad array of organic compounds. Some of these extracellular enzymes are involved in lignin degradation, such as lignin peroxidase, manganese peroxidase, laccase and oxidases. Several bacteria that degrade pesticide have been isolated and the list is expanding rapidly. The three main enzyme families implicated in degradation are esterases, glutathione S-transferases (GSTs) and cytochrome P450.

Enzymes are central to the biology of many pesticides. Applying enzymes to transform or degrade pesticides is an innovative treatment technique for removal of these chemicals from polluted environments. Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods. Enzymes are central to the mode of action of many pesticides: some pesticides are activated *in situ* by enzymatic action, and many pesticides function by targeting particular enzymes with essential physiological roles. Enzymes are also involved in the degradation of pesticide compounds, both in the target organism, through intrinsic detoxification mechanisms and evolved metabolic resistance, and in the wider environment, via biodegradation by soil and water microorganisms. So it can be stated that

(i) the central metabolism of the global biodegradation networks involves transferases, isomerases, hydrolases and ligases,
(ii) linear pathways converging on particular intermediates form a funnel topology,
(iii) the novel reactions exist in the exterior part of the network, and
(iv) the possible pathway between compounds and the central metabolism can be arrived at by considering all the required enzymes in a given organism and intermediate compounds.

For pesticides degradation, three main enzyme systems are involved: hydrolases, esterases (also hydrolases), the mixed function oxidases (MFO), these systems in the first metabolism stage, and the glutathione S-transferases (GST) system, in the second phase. Several enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage. The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability.

Metabolism of pesticides may involve a three-phase process:

1. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent.
2. Phase II involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide.
3. Phase III involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc.

Table 2: Relevant enzymes in the bioremediation of pesticides

Enzyme	Organism	Pesticide
Oxidoreductases (Gox)	*Pseudomonas* sp. LBr *Agrobacterium* strain T10	Glyphosate
	Monooxygenases:	
ESd	*Mycobacterium* sp.	Endosulphan and Endosulphato
Ese	*Arthrobacter* sp.	Endosulphan, Aldrin, Malation, DDDT and Endosulphato
Cyp1A1/1A2	Rats	Atrazine, Norflurazon and Isoproturon
Cyp76B1	*Helianthus tuberosus*	Linuron, Chlortoluron and Isoproturon
P450	*Pseudomonas putida*	Hexachlorobenzene and

		Pentachlorobenzene
Dioxygenases (TOD)	*Pseudomonas putida*	Herbicides Trifluralin
E3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester
Phosphotriesterases : OPH/OpdA	Agrobacterium radiobacter Pseudomonas diminuta Flavobacterium sp.	Insecticides phosphotriester
Haloalkane Dehalogenases: LinB	*Sphingobium* sp. *Shingomonas* sp.	Hexachlorocyclohexane (β and δ isomers)
AtzA	*Pseudomonas* sp. ADP	Herbicides chloro-s-trazina
TrzN	*Nocardioides* sp.	Herbicides chloro-s-trazina
LinA	*Sphingobium* sp. Shingomonas sp.	Hexachlorocyclohexane (γ isomers)
TfdA	*Ralstonia eutropha*	2,4 - dichlorophenoxyacetic acid and pyridyl-oxyacetic
DMO	*Pseudomonas maltophilia*	Dicamba

For the biological degradation of pesticides, it is important to understand the molecular mechanisms involved in enzymatic catalysis, which will be possible to design new alternatives and/or efficient tools for the treatment of pesticide residues or for the bioremediation of contaminated sites. This information could be used in the future to treat pesticide residues in the field (such as waste resulting after washing pesticide containers), or the obsolete pesticides. Moreover, in implementing strategies to increase the efficiency of degradation, such as cell immobilization (bacteria or fungi), we may have tools to abate the existence of obsolete pesticides and waste generated, it will reduce the danger of pesticides on the environment and health.