

NAME OF THE COURSE WORK
ENVIRONMENT & AGRICULTURAL
MICROBIOLOGY

UNIT-III
WASTE TREATMENTS

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What are Wastes?

Basel Convention Definition of Wastes

“Substances or objects which are disposed of or are intended to be disposed of or are required to be disposed of by the provisions of the law”

Disposal means

“Any operation which may lead to resource recovery, recycling, reclamation, direct re-use or alternative uses”.

Definitions

- ❖ Waste emissions – Includes GHG emissions resulting from waste management activities (solid and liquid waste management, excepting CO₂ from organic matter burned and/or used for energy purposes).
- ❖ Source – Any process or activity that releases a GHG (such as CO₂, N₂O, CH₄) into the atmosphere.
- ❖ Activity Data – Data on the magnitude of human activity, resulting in emissions during a given period of time (e.g. data on waste quantity, management systems and incinerated waste).
- ❖ Emission Factor – A coefficient that relates activity data to the amount of chemical compound that is the source of later emissions. Emission factors are often based on a sample of measurement data, averaged to develop a representative rate of emission for a given activity level under a given set of operating conditions.

Types of Wastes

Non Degradable wastes

- Cant be broken down by other living organisms.
- Not capable of degradation or decomposition
- Ex: plastics, metal and glass.
- Also include dangerous chemicals , toxins as are the plastic grocery bags.

Degradable wastes

- From plant or animal sources
- Broken down by other living organisms
- Ex: municipal waste-green waste, food waste, paper waste and bio-degradable plastics.
- Also include human waste, manure, sewage.

- Infectious waste: Body tissues, body soaked cotton, gauze pieces and body fluids.
- Infectious plastic: disposable needles and syringes, tubings, bottles and gloves.
- Sharps: Broken glasses and metal sharp like scalpals and needles.
- Average of Bio-medical waste/bed/day: 0.075-0.1 kg.
- General waste: packaging materials and food waste.

Sources of Wastes

- Domestic wastes
- Commercial wastes
- Ashes
- Animal wastes
- Biomedical wastes
- Constructions wastes
- Industrial solid wastes
- Sewer
- Hazardous wastes
- E-wastes
- Nuclear wastes

Effects of Waste If Not Managed

- ❖ Affects our health
- ❖ Affects our socio-economic conditions
- ❖ Affects our coastal and marine environment
- ❖ Affects our climate
- ❖ Rise in global temperatures
- ❖ Rise in sea levels

Methods of Waste Management

Disposal methods

- **Land fills**
 - **Convenient inexpensive**
 - **Destruction of food sources**
 - **Desalination**
- **Incineration**
 - **Requires minimum land**
 - **Can be operated in any weather**
 - **Expensive to build and operate**
 - **Continuous maintenance**

Recycling methods

- **Biological reprocessing**
- **Energy recovery**
 - **Key to providing a livable environment for the future**
 - **Expensive**
 - **Some wastes cant be recycled**
 - **Technological push needed.**

Practical Issues with Waste Management in India

- Physical characteristics
 - Unsorted waste of bio-degradable and non-biodegradable
 - Low calorific value
 - High moisture value
 - Presence of hazardous waste
- Lack of awareness
- Unplanned growth and development of cities
- Land availability

Waste Water Treatment Components

- ❖ Water-tight sewer lines
- ❖ Septic tank
- ❖ Disposal system
 - ❖ Subsurface drain field
 - ❖ Absorption trenches
 - ❖ Infiltration chambers
 - ❖ ET/Absorption trenches
 - ❖ Aerobic system with sprinkler
 - ❖ Total retention lagoons
 - ❖ Pre-approved alternative disposal systems

Septic Tank

- ❖ All household wastewater systems will have a septic tank
- ❖ Microbial action digests solid wastes
- ❖ Liquids flow through tank to disposal area
- ❖ Tank size
 - ❖ 1000 gallon liquid capacity (4-BR house or less)
 - ❖ Add 250 gallons per additional bedroom

Septic Tank Additives

Biological

- ❖ Bacteria
- ❖ Yeast
- ❖ Enzymes
- ❖ Combination

Chemical

- ❖ Acids
- ❖ Bases
- ❖ Organic Solvents
- ❖ Flocculants

Chemical Additives

Strong Acids and Bases

- Disrupt normal tank biological activity
- Harm soil structure in the drain field

Organic Solvents

- Clean thin layers of sewer line build-up
- Contaminate groundwater

Bioremediation

What is Bioremediation?

- The use of bacteria and fungi and plants to break down or degrade toxic chemical compounds that have accumulated in the environment.
- The technology used to speed up the natural processes of waste degradation and recycling
- Use of naturally occurring microorganism such as bacteria, fungi, and yeast to degrade pollutants or hazardous substances in soil, water and air into non-toxic or less toxic substances

Principles of Bioremediation

- ❖ Microbes, like all life, need an ecological niche
- ❖ Some get it by brawn (growing very fast)
- ❖ Some by brain (living off material than others can't)
- ❖ Any *abundant, energy-rich* organic material that is hard to degrade thus provides selective pressure to evolve the machinery to degrade it
- ❖ That selective pressure works. Even TNT, PCBs...
- ❖ Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration by regulatory authorities. Conditions: aerobic or anaerobic.

Bioremediation Strategies

- ***In situ* bioremediation**
 - *Bioventing*
 - *Biosparging*
 - *Bioaugmentation*
- ***Ex situ* bioremediation**
 - *Land farming*
 - *Composting*
 - *Biopiles*
 - *Bioreactors*

Requirements for Bioremediation

- ❖ The requirements for biodegradation in descending order of importance. Of prime importance are microorganisms capable of producing enzymes that will degrade hazardous chemicals (target compound). Enzymes degrade compounds through exploitation of the organism's energy needs.
- ❖ Microorganisms gain their energy through oxidation-reduction (redox) reactions, therefore an energy source and electron acceptor are important.
- ❖ Adequate moisture, pH, nutrients and temperature for cellular growth

Advantages of Bioremediation

- ❖ Bioremediation is a natural process and is therefore perceived by the public
- ❖ Bioremediation is useful for the destruction of a wide variety of contaminants.
- ❖ Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the destruction of target pollutants is possible.
- ❖ Bioremediation can often be carried out on-site, often without causing a major disruption of normal activities.
- ❖ Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

Disadvantages of Bioremediation

- ❖ Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- ❖ There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
- ❖ Biological processes are often highly specific. microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- ❖ It is difficult to extrapolate (deduce) from bench and pilot-scale studies to full-scale field operations.
- ❖ Bioremediation often takes longer than other treatment options.

Bioaccumulation

- ❖ When a chemical pollutant enters an ecosystem through accidental or deliberate discharge to the soil, water or air, this chemical may enter the food chain.
- ❖ If it is not readily broken down by organisms, it will accumulate and become progressively concentrated as it enters organisms at higher trophic levels.
- ❖ This concentration is termed bioaccumulation or biological magnification.
- ❖ The accumulation of a contaminant or toxin in or on an organism from all sources (e.g., food, water, air).

Application of Bioaccumulation

- ❖ Assessing bioaccumulation potential
- ❖ setting cleanup goals
- ❖ Evaluating remedial options
- ❖ Estimating risks when limited data are available
- ❖ TSCA (50 ppm for PCBs) (Toxic substance control Act)
- ❖ Ontario Sediment Quality guidelines
- ❖ NOAA Sediment Thresholds(National Oceanic and Atmospheric Administration)
- ❖ Background

Biomagnification

- ❖ The increase in toxin concentration as it passes through successive levels of the food web.
- ❖ DDE accumulates at higher levels in organisms that are higher in the food chain.
- ❖ Most often seen as a problem in raptors and higher aquatic predators.
- ❖ A problem with persistent elements like Cl, Br, metals, and persistent compounds like organohalides.
- ❖ Can be a problem in aquatic systems, far removed from agricultural sites.
- ❖ Results from bioaccumulation but note difference from bioaccumulation.

Biodegradation

- ❖ Breakdown of pesticides by soil microflora.
- ❖ A normal process in soil.
- ❖ Recycles pesticide residues to harmless and useful elements like N, P, S, etc.,

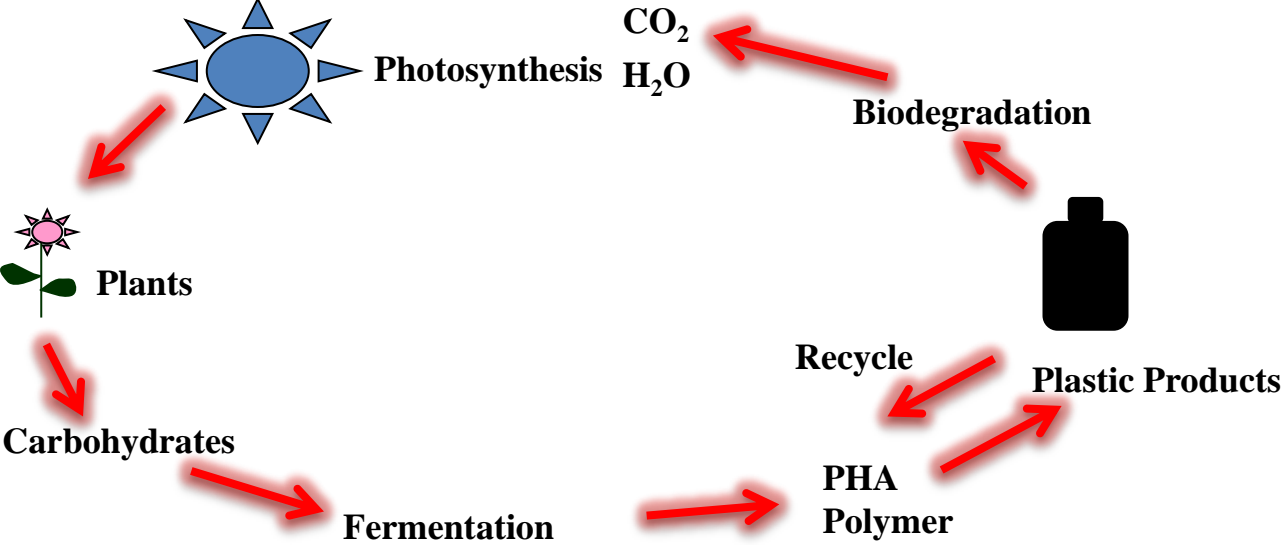
Biodegradation Microbial Metabolism

- ❖ Need nitrogen, phosphorus, sulfur, and a variety of trace nutrients other than carbon
- ❖ Carbon is often the limiting factor for microbial growth in most natural systems
- ❖ Acclimatization period - a period during which no degradation of chemical is evident; also known as adaptation or lag period
- ❖ Length of acclimatization period varies from less than 1 h to many months
- ❖ Acclimatization of a microbial population to one substrate frequently results in the simultaneous acclimatization to some structurally related molecules

Summary of Metabolism Modes in Degradation

Reductant electron donor	Oxidant electron acceptor	End products
Aerobic respiration		
Organic substrates (benzene, toluene, phenol)	O ₂	CO ₂ , H ₂ O
NH ₄	O ₂	NO ₂ ⁻ , NO ₃ ⁻ , H ₂ O
Fe ²⁺	O ₂	Fe ³⁺
S ²⁻	O ₂	SO ₄ ²⁻
Anaerobic respiration		
Organic substrates (benzene, toluene, phenol, trichloroethylene)	NO ₃ ⁻	N ₂ , CO ₂ , H ₂ O, Cl ⁻
Organic substrates (benzene, trichloroethylene)	SO ₄ ²⁻	S ²⁻ , H ₂ O, CO ₂ , Cl ⁻
H ₂	SO ₄ ²⁻	S ²⁻ , H ₂ O
H ₂	CO ₂	CH ₄ , H ₂ O
Fermentation		
Organic substrates	Organic compounds	Organic compounds, CO ₂ , CH ₄

Carbon cycle of Bioplastics



What are Bioplastics? and Advantages

- ❖ Degradable polymers that are naturally degraded by the action of microorganisms such as bacteria, fungi and algae
- ❖ 100 % biodegradable
- ❖ Produced from Natural, renewable resources
- ❖ Able to be recycled, composted, or burned without producing toxic by-products.

Applications of Biodegradable Plastics

❖ Industry

- ❖ Products, films, paper laminates & sheets, bags and containers

- ❖ Automobiles

❖ Medical

- ❖ Sutures, ligament replacements, controlled drug release mechanisms, arterial grafts...

❖ Household

- ❖ Disposable razors, utensils, diapers, feminine hygiene products, containers

Polyhydroxy Alkanoates (PHAs)

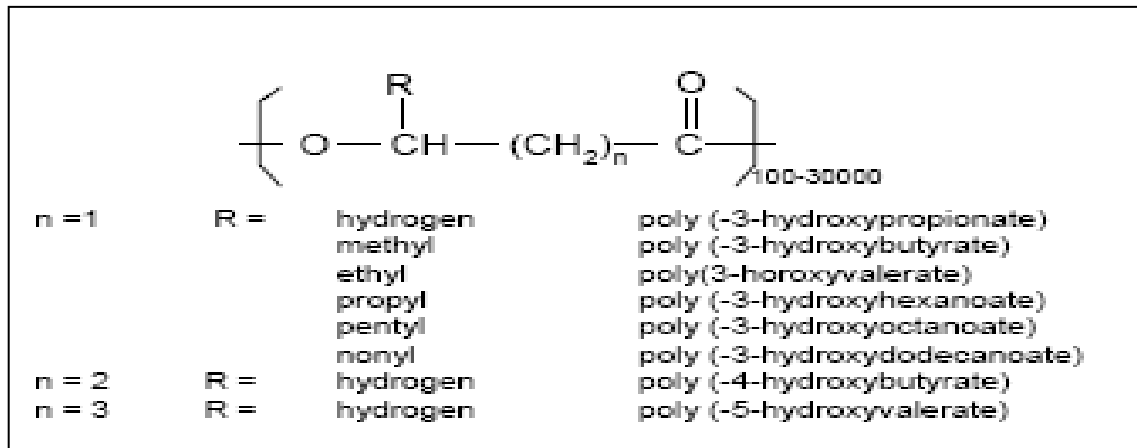
- ❖ Polyhydroxyalkanoates (PHAs) form a class of Bioplastics.
- ❖ Polyhydroxybutyrate (PHB) is most prevalent.
- ❖ Carbon Waste = FREE.
- ❖ Production costs typically decreased through the use of waste streams as carbon and nutrient sources.

Polyhydroxyalkanoates (PHAs)

- ❖ Produced under conditions of:
 - ❖ Low limiting nutrients (P, S, N, O)
 - ❖ Excess carbon
- ❖ 2 different types
 - ❖ Short-chain-length 3-5 carbons
 - ❖ Medium-chain-length 6-14 carbons
- ❖ ~ 250 different bacteria have been found to produce some form of PHAs.

Polyhydroxyalkanoates (PHAs)

❖ Polyesters accumulated inside microbial cells as carbon & energy source storage



Biodegradation of PHA

- ❖ Fastest in anaerobic sewage and slowest in seawater
- ❖ Depends on temperature, light, moisture, exposed surface area, pH and microbial activity
- ❖ Degrading microbes colonize polymer surfaces & secrete PHA depolymerase
- ❖ $\text{PHA} \rightarrow \text{CO}_2 + \text{H}_2\text{O}$ (aerobically)
- ❖ $\text{PHA} \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{CH}_4$ (anaerobically)

Anaerobic Degradation of Hydrocarbons

- ❖ Anaerobic degradation of hydrocarbons studies are recent.
- ❖ 10 years ago, toluene was reported to be degraded anaerobically by a microbe.
- ❖ A variety of denitrifying, sulfate-reducing, and iron-reducing were isolated that could anaerobically degrade aromatic carbons.

Alkane Metabolism

Alkane → alcohol
alcohol → aldehyde
aldehyde → acid
acid → beta oxidation
Krebs cycle

Alkane degradation in Gram
negative bacteria

AlkB: alkane hydroxylase

AlkF / AlkG: rubredoxins

AlkH: aldehyde dehydrogenase

AlkJ: alcohol dehydrogenase

AlkK: acyl-coA synthetase

AlkL: outer membrane protein

AlkT: rubredoxin reductase

AlkN: methyl-accepting transducer protein (chemotaxis)

AlkS: positive regulator of alkBFGHIJKL operon and alkS/alkT genes

Control Responses to Hydrocarbons

Membrane alteration, uptake, and efflux

1. change in membrane architecture
2. change in active uptake
3. change in efflux
4. change in chemotaxis

Hydrocarbons (lipophilic) → partitioning in a hydrophobic area in acyl chains of phospholipid (periplasmic space in g-)

- changing fluidity and protein conformation
- changing disruption of barrier
- changing energy transduction
- changing membrane-bound enzyme activity
- biofilm formation

Passive Bioremediation

- ❖ Hydrocarbon biodegradation by rhizospheric M.O (phytoremediation).
- ❖ Hydrocarbon uptake by plants and release to atmosphere without transformation (phytovolatilization).
- ❖ Wetland use for removal petroleum wastes depend on plant community, water depth and concentration of wastes.

Limitations:

1. Toxicity of contaminants
2. Availability of fertilizer and oxygen

Degradation of Halogenated compounds

- ❖ Biodegradation of halogenated compounds involves two distinct steps:
 - (i) elimination of the halogen groups, and
 - (ii) degradation of the non-halogenated product molecule.
- ❖ Removal of halogen molecule may occur either directly involving the removal of hydrogen halide (e.g., HCl), or it may involve the substitution of halogen by -H, -OH or a -thio group.
- ❖ The direct halogen removal produces a double bond and is relatively rare in nature. The mechanism involving halogen substitution, especially by -OH, is far more common particularly for fully reduced aliphatics or aromatics.

Degradation of Halogenated Compounds

The aerobic degradation of halogenated aromatic compounds usually involves the following steps:

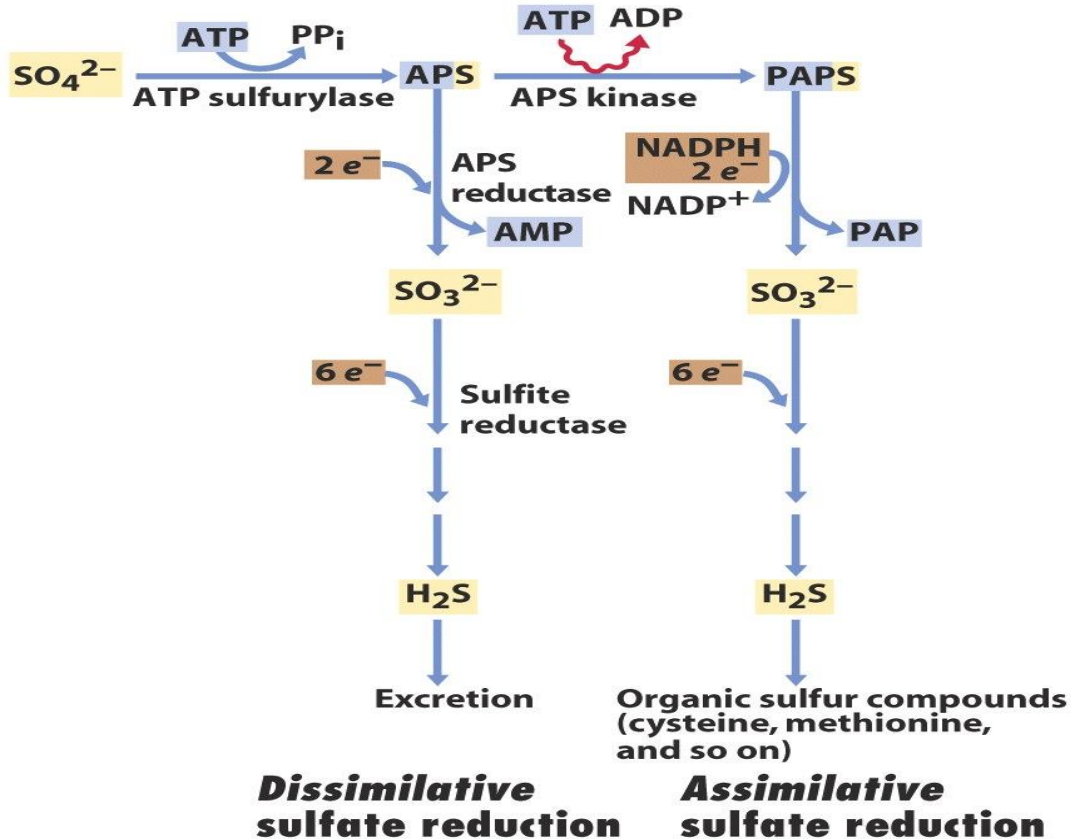
(i) addition of -OH group by a di-oxygenase to yield chlorinated catechols

(ii) cleavage of the ring by ortho or meta cleavage (meta cleavage often results in the halogen not being eliminated from the aliphatic product, which may lead to an accumulation of toxic metabolites)

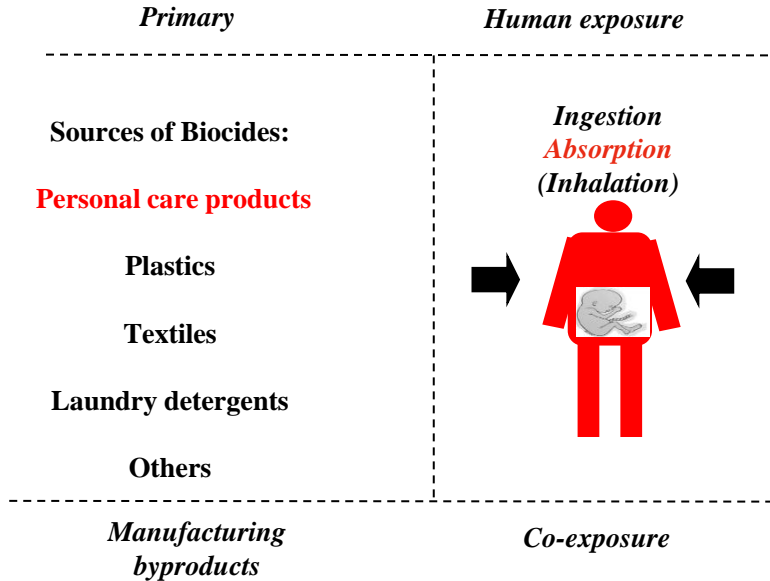
(iii) elimination of the halogen from the straight chain (aliphatic) product

(iv) degradation of the aliphatic hydrocarbon (non-halogenated) so produced. In the case of phenols (which already have one -OH group), the step 1 reaction is catalyzed by a hydroxylase, which adds another -OH group to yield the catechols.

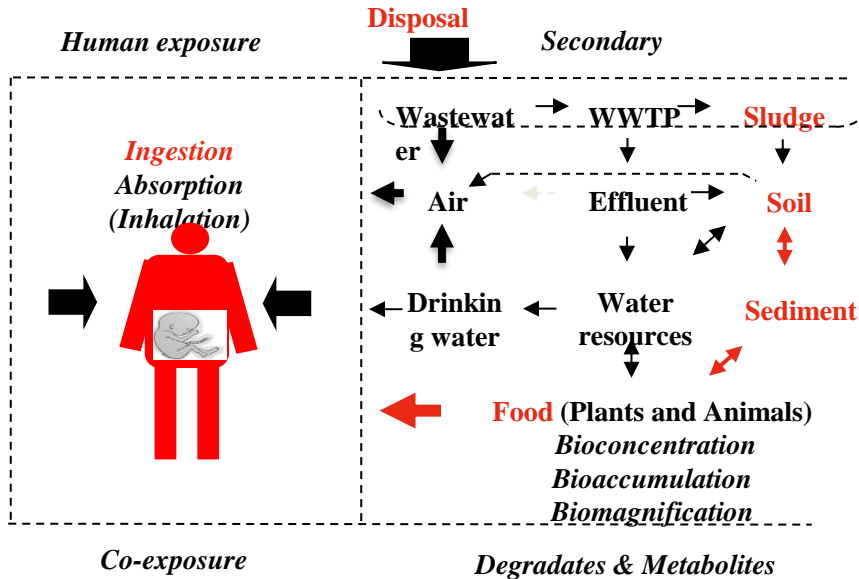
Degradation of Sulfonated Compounds



Routes of Primary Exposure in Biocides



Routes of Secondary Exposure in Biocides



Definition - EPA

“...a pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.”

“...a pest is any harmful, destructive, or trouble some animal, plant or microorganism.”

US Environmental Protection Agency (EPA)

Definition - FIFRA

“... any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insects, rodents, nematodes, fungi, or weeds or any other form of life declared to be pests. ... and any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant.”

Federal Insecticide, Fungicide, and Rodenticide Act
(FIFRA – 1947)

Problems

- ❖ Bioaccumulate – example DDT, PCB
- ❖ 35,000 or more commercial products that use pesticides
- ❖ Many pesticides are neurotoxic (affect the nervous system)
- ❖ Many kill desirable insects or plants
- ❖ Contaminate streams and lakes

Classes of Pesticides

- ❖ Insecticides (kill insects)
 - ❖ Organochlorines
 - ❖ Organophosphates
 - ❖ Carbamates
 - ❖ Synthetic Pyrethroids
- ❖ Herbicides (kill plants)
- ❖ Rodenticides (kill rodents)
- ❖ Fungicides (kill fungus)
- ❖ Fumigants (kill whatever)

Pesticide Definition

The function of a pesticide is to kill or harm some form of life.

Insecticides

- ❖ Organochlorines
- ❖ Organophosphates
- ❖ Carbamates
- ❖ Synthetic Pyrethroids

Herbicides (Kill Plants)

- ❖ Silvex, 2,4-D, D,4,5-T
 - Most widely used
 - Possible carcinogen
 - Contaminated with TCDD (dioxin)
(older formulations)
- ❖ Paraquat & diquat
 - Serious toxicity following accumulation in lungs –
production of oxygen “free radicals” – often fatal once
started

Herbicide Decomposition/Fate

Pesticides are degraded into inactive substances (e.g., CO_2) or rendered inactive by several mechanisms:

- ❖ Adsorption to soil components
- ❖ Leaching out of plant available zone
- ❖ Volatility - escapes into air and degrades
- ❖ Photodecomposition - degraded by sunlight
- ❖ Chemical decomposition - broken down by reactions
- ❖ Microbial degradation - primary means

Rodenticides (kill rodents)

❖ Botanicals

Red squill – effects heart

Strychnine – blocks glycine receptors in spinal cord - convulsions

❖ Inorganics

Phosphorous – GI track

Thallium – hair loss, nervous system

Zinc phosphide – GI track

❖ Anticoagulants

Warfarin – inhibits blood clotting

Vacor – newer blood clot inhibitors

Fungicides (kill fungi/mold)

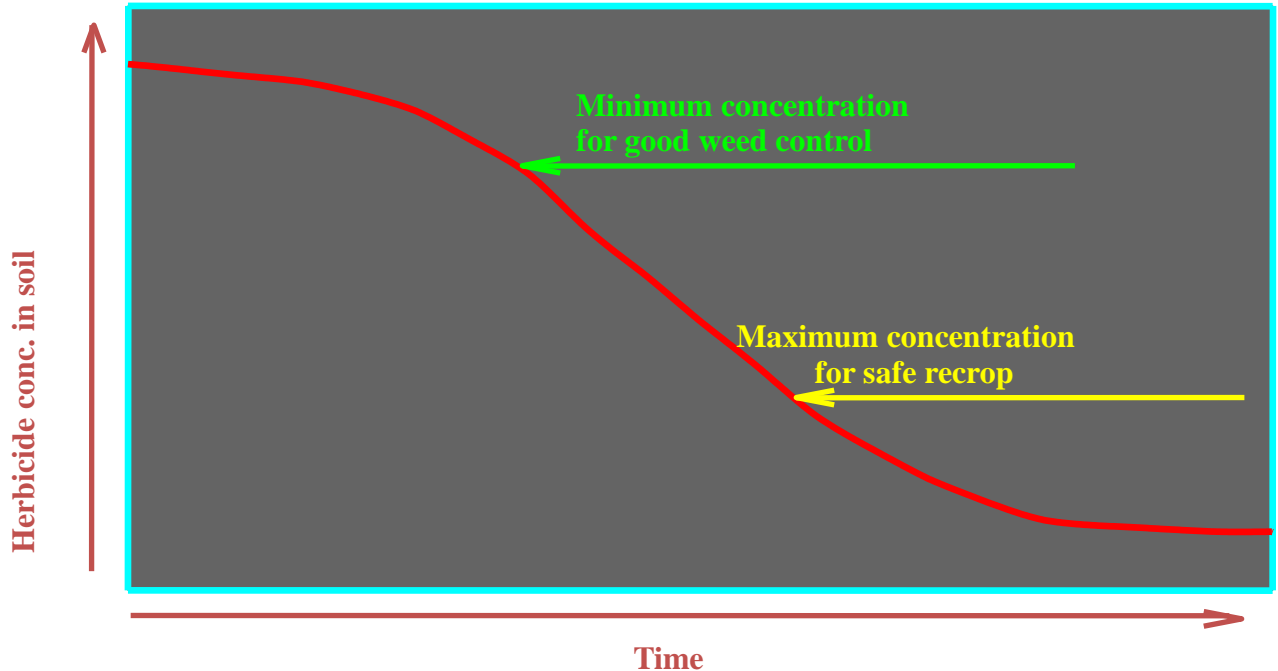
- ❖ Early Examples
 - ❖ Sulfur, copper sulfate
 - ❖ Mercury based compounds
- ❖ Hexachlorobenzene
- ❖ Pentachlorophenol
- ❖ Dithiocarbamates

Pesticide degradation

- ❖ Pesticide fate in the environment is affected by microbial activity.
- ❖ Some pesticides are readily degraded by microorganisms, others have proven to be recalcitrant.
- ❖ A diverse group of bacteria, including members of the genera *Alcaligenes*, *Flavobacterium*, *Pseudomonas* and *Rhodococcus*, metabolize pesticides.
- Microbial degradation depends on the presence of microbes with the appropriate degradative enzymes and a wide range of environmental parameters.

Pesticide degradation

Critical concentrations for soil-applied or residual herbicides



Pathways of Pesticide Movement

- ❖ Runoff
- ❖ Chemical degradation
- ❖ Volatilize (gas vapour)
- ❖ Leaching and breakdown in soil
- ❖ Leaching and degradation by microbes
- ❖ Photo degradation (sun)