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Course handouts

Course: Plant Ecotoxicology

(phytotoxicity and toxic substances effects)

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This document is intended for third-year students in Biotechnology and Plant Breeding. (3rd BPB)

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I. Course Information

- **Faculty: Natural and Life Sciences**
- **Department: Biotechnology**
- **Target audience: 3rd year Bachelor's degree in Biotechnology and plant improvement**
- **Course Title: Plant Ecotoxicology**
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- **Credit: 04**
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Chapter 1: Introduction to Plant Ecotoxicology

Introduction

The importance of studying the ecotoxicity of plants lies in protecting humans and animals that feed on them and raising awareness that their release into the environment follows the water cycle. Toxicity, therefore, is transmitted to us through inhalation of polluted air, consumption of contaminated water, or poisoned food from various sources. It is also crucial to recognize the disappearance of certain plant and animal species and the imbalances in several food chains caused by the accumulation of different pollutants in the same environment.

Natural climatic phenomena, to the same degree as anthropogenic stressors, affect species biodiversity and ecosystem balance. In polluted environments, organisms are exposed to a complex mixture of contaminants. This exposure sometimes causes toxic effects even if the pollutants are present individually and at concentrations much lower than the concentration determined without observable effects (Brian et *al.* , 2007). This phenomenon is defined as a combined effect, a mixed toxicity or cocktail effect. Chemical toxicity assessment is normally carried out substance by substance, neglecting the possibility of a combined effect. It is possible to underestimate the combined effects of contaminants with similar effects or different modes of action that interact and change the toxic effect of the two toxic substances tested separately. These combinations give rise to countless possibilities of additive, synergistic, or antagonistic combinations (Figure **I.1.a)**.

I.1. Definition of ecotoxicology

Etymologically, "Ecotoxicology" is Greek, it comes from the words: *logos* : knowledge, *toxicov* : poison, *oikos* : habitat; it is therefore the knowledge of

poisons in habitats (Van Coillie & Parent, 2011).

This science emerged and was first defined by Professor R. Truhaut (1977) as a discipline that deals with the impact of chemical compounds on ecosystems (Pelletier et *al.* , 2004). Ecotoxicology, according to Moriaty (1983), is the field of study that integrates the ecological and toxic effects of chemical pollutants on communities, populations, and ecosystems, as well as their fate (transformation, transfer, and degradation) in the environment.

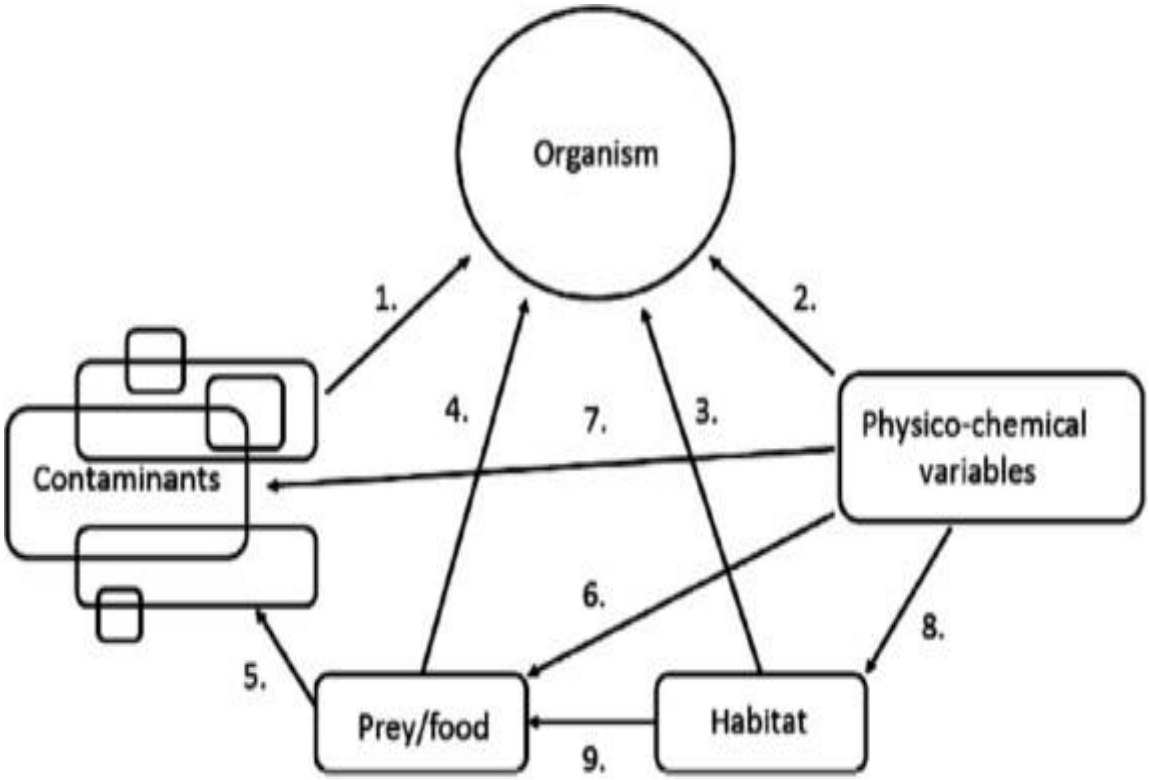


Figure I.1.a: Different stress factors affecting an organism.

1: **Exposure to and effects of contaminants (possibility of additive consequences/synergy/antagonism); 2: Physicochemical variability (climatic conditions); 3: Habitat change; 4: Availability, type, and nutritional value; 5: Food type influences the type and frequency of contaminant exposure; 6: Physical factors influence food availability (prey abundance); 7: Changes in environmental variables influence the biavailability of contaminants (transport/advection, diffusion, adsorption, etc.); 8: Changes in physicochemical factors affect organisms' habitats; 9: The habitat of predator and prey is the same, meaning that changes in physicochemical conditions also affect the amount of food available (Beyer et *al* ., 2013)** .

I.1.1. **History:**

Scientists from different disciplines have proposed definitions of ecotoxicology according to the experimental approach followed and/or the hypotheses imposed: starting with Truhaut who considered ecotoxicology as an extension of human toxicology to the environment, passing through Ramade who insisted on a holistic ecological approach to ecotoxicology and finally Butler and then Moriarty who describe it by a more ecological than toxicological approach (**Table I.1.).**

Table I.1. Some definitions of ecotoxicology (Van Coillie & Parent, 2011) .

Authors	**Proposed definitions**	**Remarks**
Truhaut, 1974 and 1977 (France)	A discipline that studies toxic substances that cause alterations or disruptions in the **functions of biological organisms,** leading to harmful effects.	**Truhaut** appears to have been the first to use the term **ecotoxicology,** starting in 1969. **A medical** toxicologist, he considered ecotoxicology as an extension of human toxicology to the environment and thus adopted a reductionist view of ecotoxicology.
Ramade, 1977, 1979, 1987 and 1992 (France)	Study of the ways in which **the environment is contaminated** by natural or artificial pollutants produced by human activity, as well as their mechanisms of action and effects on **living beings.**	Ramade is undoubtedly a leading figure in ecotoxicology. **An ecologist**, he insisted on a holistic ecological approach to ecotoxicology.
Butler, 1978 (Canada)	Study of the effects of toxic substances in **ecosystems**	Butler was a pioneer of ecotoxicology in Canada. **A chemist** , he described ecotoxicology with a greater emphasis on its ecological aspects.

Moriarty, 1983 and 1988 (England)	Ecotoxicology is concerned with the effects of pollutants in **ecosystems** , while toxicology studies these effects in individual biological organisms.	Moriarty, **an ecologist,** has treated ecotoxicology since 1983 with a more ecological than toxicological approach.
Cairns, 1988 and 1995 (United States)	Measuring toxic nuisances in **individual species** and at higher **levels of biological organization** to define minimum effects in ecosystems	Cairns, **an ecologist** , broadened and articulated the ecological vision of ecotoxicology.

I.1.2. **The relationship of ecotoxicology with other disciplines**

Ecotoxicology must integrate the problem of biological hierarchy and draw upon several disciplines. Ecotoxicologists have demonstrated that pollutants act at all levels of biological organization, but they still find it difficult to define the relationship between the effects observed at these different levels. (See Figure **I.1.b**) **(Forbes** & **Forbes** , 1997).

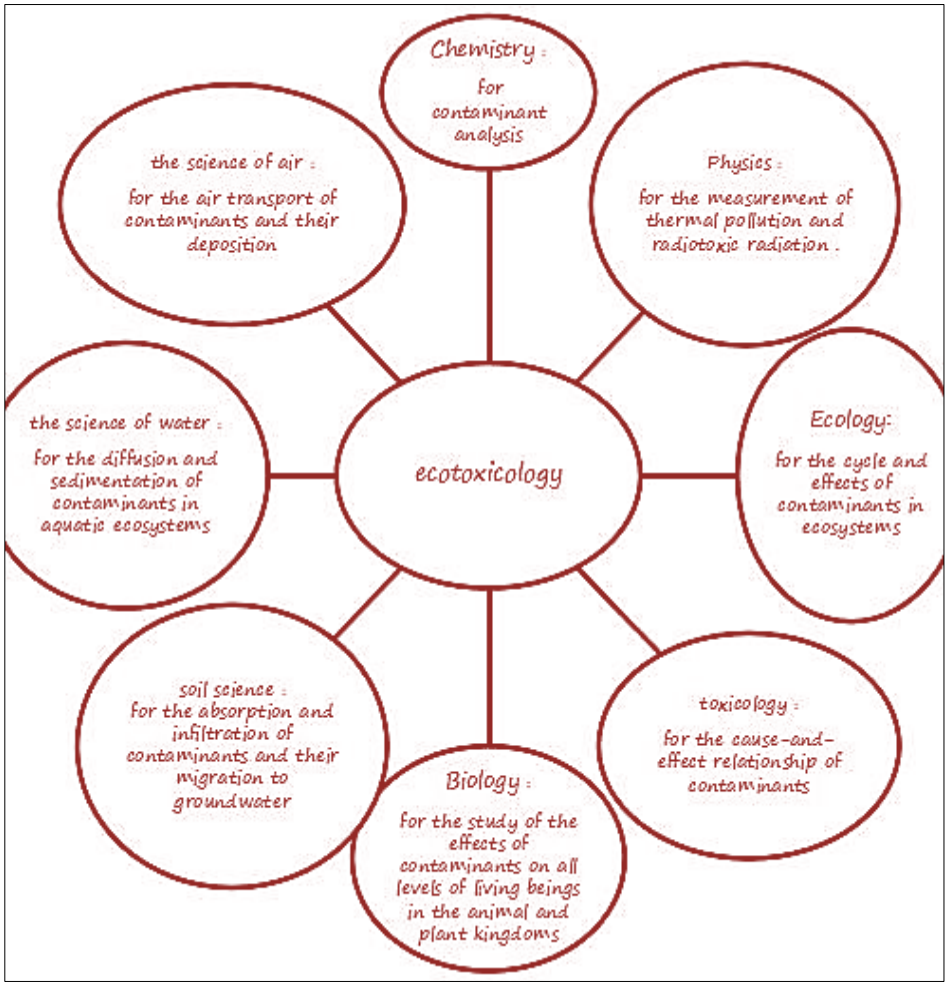


Figure .I. 1.b . : The disciplines involved in the interpretation of the toxicity phenomena of organisms for the benefit of ecotoxicity.

I.1.3. **Development of toxicology in the 20th century**

Several 20th-century works contributed to confirming toxicology as a scientific discipline: we will cite (Van Coillie & Parent, 2011) :

- Lewis, who studied the toxicokinetics of alcohols and published the first detailed treatise on toxicology in 1920-1929.
- Peters and his colleagues: developed, in 1945, the widely used antidote BAL (British Anti-Lewiste) to capture arsenic, mercury or lead.
- The mechanism of action of DDT (dichloro_dibenzo-trichloro-ethane), the first synthetic insecticide used from 1942, was discovered by Müller in 1946.
- Schrader in 1952: introduced and specified the toxicity of organophosphate pesticides.
- The central role of cytochromes P450 in the biotransformations of toxic substances and habituation to some of them was the result of the work of the team of Orrenius and Remmer (1964 and 1965).
- McClellan: evaluated, in 1979, the radiotoxic effects of radionuclides from the nuclear industry.
- Lauwerijs: explained, in 1990, occupational toxicology for, among other things, metals.

I.1.4. **Organizations and institutions contributing to the regulation/use/measurement of toxic products**

Regulations authorizing or prohibiting the use of toxic products in industry have developed under the responsibility of international organizations, following the dangers encountered in industry or in daily life (**Table I.2).**

Table I.2 : National and international organizations contributing to regulations limiting the use of toxic products (Van Coillie & Parent, 2011)

Organization	Year	assignment
FAO (Food Agricultural Organization). WHO (World Health Organization).	1962	Determination of acceptable daily intakes (ADIs) of pesticides and other toxic substances in food.
USNAS (United States National Academy of Science) USNRC (United States National Research Council).	1965	toxicological risk analyses.
ACGIH (American Conference of Governmental Industrial Hygienists) NIOSH (National Institute Of Safety and Health) ATSDR (Agency of Toxic Substances Disease Registry).	1968	Publication of toxicological profiles, toxicity measurements and exposure limits for numerous chemical substances
OSHA (Occupational Safety and Health Administration) USEPA (United States Environmental Protection Agency) USFDA (United States Food and Drug Administration)	1970	Regulation of toxicological evaluation and toxicovigilance procedures against toxic substances.
IARC (International Agency for research on Cancer)	1980	Classification and validation of carcinogenic agents
OECD (Organization of Economic Corporation and Development)	1981	Clarification of good laboratory practices for the use of toxic substances.

I.1.5. **Industrialization and environmental disasters**

Workplace poisonings necessitated the creation of powerful institutions that take charge of toxicology to reduce the risks of diseases related to the activity carried out: various cancers in petrochemical refineries, aluminum plants, and the organic chemical industry, miners' diseases such as silicosis, asbestosis and others, and hemorrhages linked to industrial solvents; on the other hand, the succession of a multitude of disasters in environmental and agri-food toxicology (**Figure I.2.a** and **b**) from 1950 (**Table I.3.)** .

Table I.3. Environmental and agri-food toxicology disasters since 1950 (Van Coillie & Parent, 2011).

Date and locations	Toxic substances	Adverse effects
1950-1960 Toyoma, Japan	**Cadmium** in **rice** contaminated by fumes from a metal smelter	Itae-Itae disease: more than 200 cases of kidney and bone damage
1965 Türkiye	**Hexachlorobenzene** in **wheat** as a neurotoxic fungicide: more than 4000 cases	Porphyria (due to excessive synthesis of a hemoglobin precursor)
1959 Morocco	**Tricresyl phosphate** in **adulterated** cooking oils	Neurotoxic effects: more than 10,000 cases
1962-1970 Vietnam	"Agent Orange," which was a **herbicide** composed of 2,4-D and 2,4,5-T (chlorophenoxyacetic acids) Contaminated with **chlorinated dioxins.**	Immunosuppression in American and Vietnamese soldiers: more than 3000 cases
1968 Fukuoka and Yusho, Japan	PCBs (polychlorinated biphenyls) and chlorinated furans in **rice oils**	Chlorotic acne on the skin: more than 1700 cases
1972 Iraq	**Mercury-phenyl** in Canadian and American wheat as a fungicide	Neurological damage: 500 deaths and more than 6000 non-fatal cases

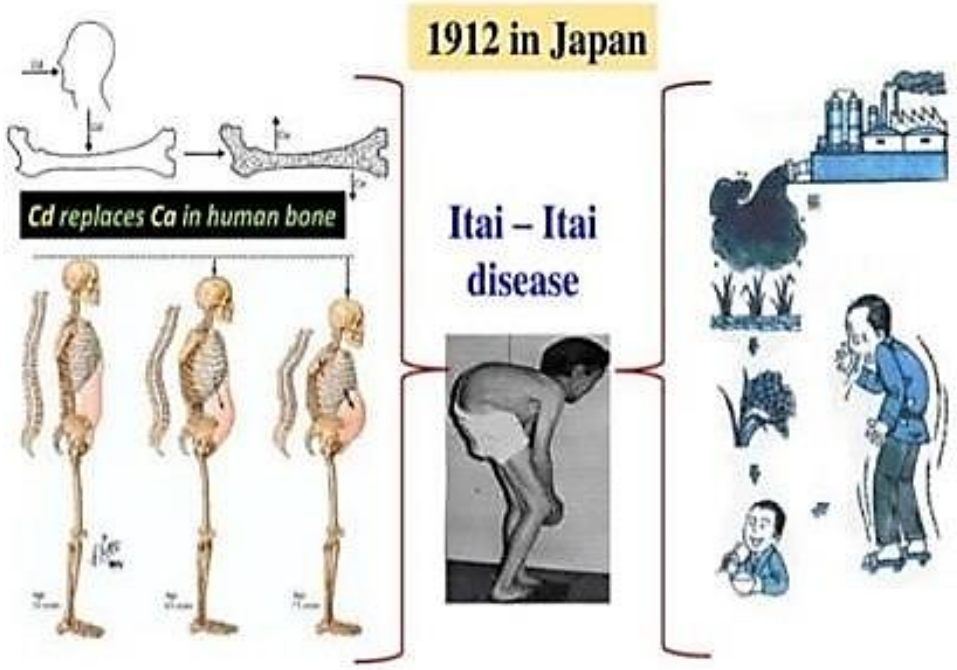


Figure I.2.a.: Consequences of Itai-Itai disease (Japan in 1912): kidney and bone damage due to the presence of Cadmium in rice (https://www.wikiwand.com/en/Itai-itai_disease).

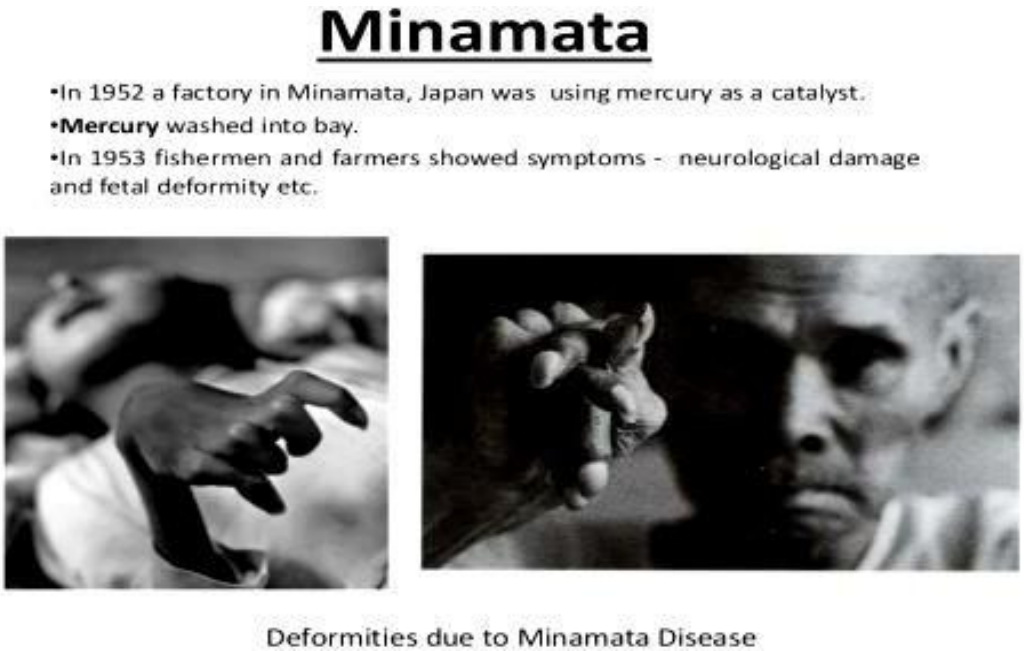


Figure I.2.b: Consequences of MInamata disease (Japan in 1952): neurological disorders and prenatal deformation due to the presence of Mercury in seawater and consumed fish (https://www.slideshare.net/PrakkanHillol/marine-water-pollution-and-diseases).

I.2. Toxicity

By definition, toxicity is " *the characteristic of being toxic"* (http://www.larouse.fr (2024)) or " *the set of adverse effects linked to the administration of a treatment. Toxicity is graded on a scale from 0 to 4* " (http://www.e-cacer.fr (2024)). Poisons are chemical substances that cause harmful effects in biological organisms: these effects constitute toxicities **(Forbes** & **Forbes** , 1997).

I.2.1. Distinction between toxic and toxin

According to Hayes (1991), a toxic substance is the general term for a poison, whereas a toxin originally referred to a labile protein-structured substance capable of generating specific immunity in humans and other mammals. Toxins are toxic agents secreted by certain animals and plants: venoms of snakes and scorpions, alkaloids from plants of the genus *Datura* with delirious effects, amatoxins from amanita mushrooms with a toxic effect on the liver, neurotoxins from the protozoan *Gonyaulax* in symbiosis with mussels during the summer season, etc. (Klaassen et *al.* , 1986).

Toxic substances can be grouped into three categories **(Forbes** & **Forbes** , 1997):

- natural elements in excessively high concentrations (heavy metals, sulfides, etc.),
- byproducts of incomplete combustion (polycyclic aromatic hydrocarbons (PAHs), chlorinated dioxins and furans, etc.)
- or human-made compounds (synthetic pesticides, plastics, etc.).

I.2.2. Pollutants

Moriarty (1983) classifies pollutants into two main categories:

a) effect pollutants **: (** toxins or toxicants) pollutants alter the structure and function at the physiological (or molecular or biochemical) level.

b) Pollutants with **indirect effects:** act mainly at the level of ecological structure and functioning.

I.2.3. Xenobiotics

Xenobiotics are considered **artificial** , although many natural disturbances

are equally artificial and unexpected from the perspective of the individual or the species.

New products – highly toxic – have been developed by living organisms during evolution; in fact, some of the most toxic molecules are of natural origin (Ray, 1991).

Xenobiotics are molecules, generally of organic origin, foreign to the body and therefore capable of causing biological alterations and toxic effects depending on their molecular nature, the duration of exposure, and the assimilated dose. Based on the nature of the substance and the adaptive strategy employed by the body to eliminate it, xenobiotics are classified into two groups:

1- Persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons, dioxins, and pesticides, are characterized by lipophilicity (allowing for their prolonged accumulation in fatty tissues) and resistance to xenobiotic metabolism (biotransformation: the process that reduces their toxicity by converting them into more hydrophilic metabolites that are easily eliminated). POPs such as polybrominated flame retardants, organochlorine pesticides, and dioxins accumulate in fatty tissues without biotransformation, leading to chronic exposure. Other POPs, such as benzo[a]pyrene (a polycyclic aromatic hydrocarbon), are rapidly biotransformed by the body, causing oxidative stress and/or producing toxic metabolites.

2- Endocrine disruptors (EDs) are defined by the WHO (World Health Organization) as "a substance foreign to the organism that produces adverse effects on the organism or its offspring as a result of altering hormonal function" and by the EPA (US Environmental Protection Agency) as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural ligands responsible for maintaining homeostasis and regulating organismal development." The healthcare costs associated with

endocrine disruptors amount to more than €18 billion annually, linked to their contribution to the development of obesity and diabetes (**Baba, 2018)** . Studies implicating these substances have proven their involvement in the deregulation of the function of steroid receptors such as progesterone and estrogens and more specifically epigenetic deregulations transmissible to offspring in humans (Duarte-Hospital, 2019).

I.2.4. Interaction of organisms with regard to toxicity

There is nothing to prove that an organism is more predisposed to fight against natural toxins than against artificially produced toxins (Ray, 1991) example: The resistance of insects to insecticides (previously sensitive), because it seemed implausible that a natural population would contain genes of resistance to products to which it had never been exposed.

It has gradually become more and more evident that resistance results from selection acting on a natural variation already present in the population before exposure to the pesticide (Wood, 1981).

The founders of ecotoxicology explicitly included "natural" pollutants in the field of ecotoxicology in response to the fact that organisms have had to constantly adapt to the arrival of new "natural" products in order to survive; therefore, it is logical that the founders of ecotoxicology explicitly included "natural" pollutants in the field of ecotoxicology (Truhaut, 1977).

I.3. Mode of penetration of a toxic substance

Roots absorb toxins dissolved in the soil along with water. Meanwhile, toxic substances present in the air penetrate plant tissues primarily through the stomata.

I.3.1. Penetration at the root level

According to its chemical characteristics, the toxic substance penetrates more easily through the roots , dissolved in the water flow and transported in the xylem passively (**Site I.1)** .

Example : root-absorbed or root-absorbed herbicides (systemic pesticides) **(Site I.2)**

I.3.2. **Leaf penetration**

Penetration and retention depend on the nature of the leaf surface and the compatibility of the toxic agent with this surface (cuticle: waxy, thick, oily) However, some toxins (such as herbicides) must be combined with adjuvants to penetrate the cuticle and can either act locally or migrate throughout the plant (**Site I. 1**).

The choice of adjuvants for herbicides used depends on the characteristics of the cuticle. Attention is primarily focused on the differentiation between the aqueous and lipid phases (**Site I.2**).

Powdered herbicides are mixed with sticky agents to facilitate absorption. These deposition agents (adjuvants) are generally emulsifiable resins, emulsifiable mineral oils, emulsifiable vegetable oils, vegetable waxes (Witt 2001), guar gum (polysaccharide) (Bergeron et *al* ., 2000), water-soluble polymers, fatty acids (anionic surfactants), or any non-volatile fatty substance (Hazen, 2000). (Tu *et al* . 2003).

I.3.3. **Penetration into meristematic tissues**

This is the case with residual toxic substances, for example:

a) Germination-preventing herbicides applied to the soil to block seedlings that have not yet emerged (**Site I.2**).
b) growing plants (Gauvrit, 1996; Tissut *et al.* , 2006).

I.4. Mode of action of a toxic substance

Toxicodynamics explains why and, above all, how a toxic substance is harmful. It therefore allows us to understand the mechanisms of action of a toxic substance (Van Coillie & Parent 2011).

The mechanisms of action of toxic substances can be grouped into seven main types:

a) **Inhibition of an enzyme** or a biochemical function (lead (Pb) inhibits three enzymes in the synthesis of the central heme group of hemoglobin in red blood cells, which leads to anemia) (Leduc et *al.* , 1983).

b) **Competition between a toxic substance and a normal metabolite** , e.g., the binding of carbon monoxide (CO) to hemoglobin (Hb) at the expense of oxygen (O_2) in the blood, thus rapidly causing asphyxiation.

c) **General narcotic effect** (narcosis means a general physiological decline caused by an organic toxicant. This decline is manifested first by sublethal effects often reversible and then progresses towards death (weak organic acids and bases; esters, alcohols, phenols, PAHs…etc).

d) **Biochemical mimicry and related mechanisms** : Some toxins have the property of mimicking and taking the place of normal hormones and/or metabolites in their cellular receptors and/or their metabolic pathways (Clarkson, 1993). Example: endocrine disruptors (DDD and DDT) that mimic ACTH hormones

e) **Programmed cell death by apoptosis** : in the intracellular cytosol, a rapid and significant abnormal increase in the concentration of Ca^{2+} ions leads to cell death. This toxic mechanism of action, apoptosis, is a general cellular breakdown.

f) **Biochemical simulation and related mechanisms** : several toxins stimulate certain functions or metabolic reactions with adverse effects such as allergies;

g) **Genotoxic action** (affects DNA genes causing, among other things, damage to one or both DNA strands and mutations).

I.4.1. Mode of action of a toxic substance on a plant

The mode of action of a toxin on a plant manifests itself through physiological deterioration (necrosis, yellowing, wilting) [site 3] and takes into account both:

a) The physiological function disrupted: photosynthesis, pigment synthesis, amino acid synthesis, fatty acid synthesis, cell wall permeabilization …etc.

b) Auxin disruptors (the "hormones") act in all young parts of the plant by promoting disordered cell division and elongation. (ACTA Index; Heap, 2014; *Herbicide Resistance Action Committee*) Auxin disruptors are dicotyledonous weed control agents, as dicotyledonous weeds were the most abundant species in cereal crops in the mid-20th century .

c) The site of action of the active ingredient in the plant: inhibition of a protein or enzyme. For example, sulfonylureas, such as Cossak, inhibit ALS (acetolactate synthetase), which is essential for the production of amino acids. Without it, the plant stops growing rapidly.The site of cellular activity of the active substance: chloroplast, meristem; example: Most fungicides directly affect essential functions, such as respiration. This type of mode of action can lead, on the one hand, to risks for humans and non-target organisms and, on the other hand, to the development of resistant fungal strains **(Leroux, 2003)** .

I.5. Phases of action of a toxic substance

Toxicity depends on the plant's growth phase: a toxin that destroys the plant after emergence is sometimes completely inert during the germination phase (**Site I.2**):

- **Preventive treatments: concerns residual herbicides:** intended to keep the soil clean. Applied for pre-sowing treatment (pre-planting: eradication of weeds during non-cultivation periods) and herbicides applied for pre-emergence or post-sowing treatment to prevent the germination of weed seeds;

  Curative treatments : application of a herbicide for post-emergence treatment: this is the case of foliar herbicides applied after the emergence of

the plants in question, for example: herbicides used for potato haulm destruction.

Table I.5. Phases of elimination of toxic substances in different parts of the plant [Extract from the book (Van Coillie R. & Parent L. (2011)].

Toxic substance	Elimination phase, tissues	Half-life in hours, days or years	references
Organophosphates			
fenithrothion	Spruce: foliage	96 hours	NRC, 1976
	Fir tree: foliage	96 hours	NRC, 1976
	Pollen	48 hours	NRC, 1976
	Bees	12 hours	NRC, 1976
Glyphosate	Spruce: foliage	120 hours	MRN, 1994

II. The main toxic substances and their effects on plants

II.1. Pesticides

Pesticides are substances whose physical, chemical, and biological properties allow them to destroy or limit the development and growth of living organisms. Their use must necessarily be subject to strict rules **(Cavet** et *al.,* 2005).

This means that it faces several problems related to the technological aspects of the treatments, environmental pollution, handling safety and food safety.

There are two types of pesticides:

a) **Systemic or endotherapeutic pesticides** : must be absorbed by the roots and their activity depends on their mobility in the soil (**Figure II.1)** . They have the particularity of making the sap toxic to various pests of cultivated plants and phytopathogenic agents (Ramade, 2007).

b) **Non-systemic (direct-acting) pesticides** act directly on the target from either the gaseous phase of the soil or the liquid phase (**Figure II.1)** .

II.1.1. Toxicity assessment using biological tests

It is common practice to study the toxicity of various substances by establishing curves that link effects to doses. For example, a curve is established representing the effect of a herbicide on the growth of sensitive plants, on the production of a compound characteristic of the activity of the living organism used as a chlorophyll pigment, or on a physiological function such as respiration.

To be able to measure the amount of pesticide absorbed by a plant: molecules labeled with ^{14C} are used provided that the organism can absorb all the bioavailable amount before being destroyed **Cavet** et *al* ., (2005).

To solve the problem of weeds themselves being resistant to an active substance, mixtures of active substances (2, sometimes 3) have appeared, each with a different biochemical target (**Table II.1** .) and obviously the need for double, or even triple, resistance of the crop (Kilinc, 2010).

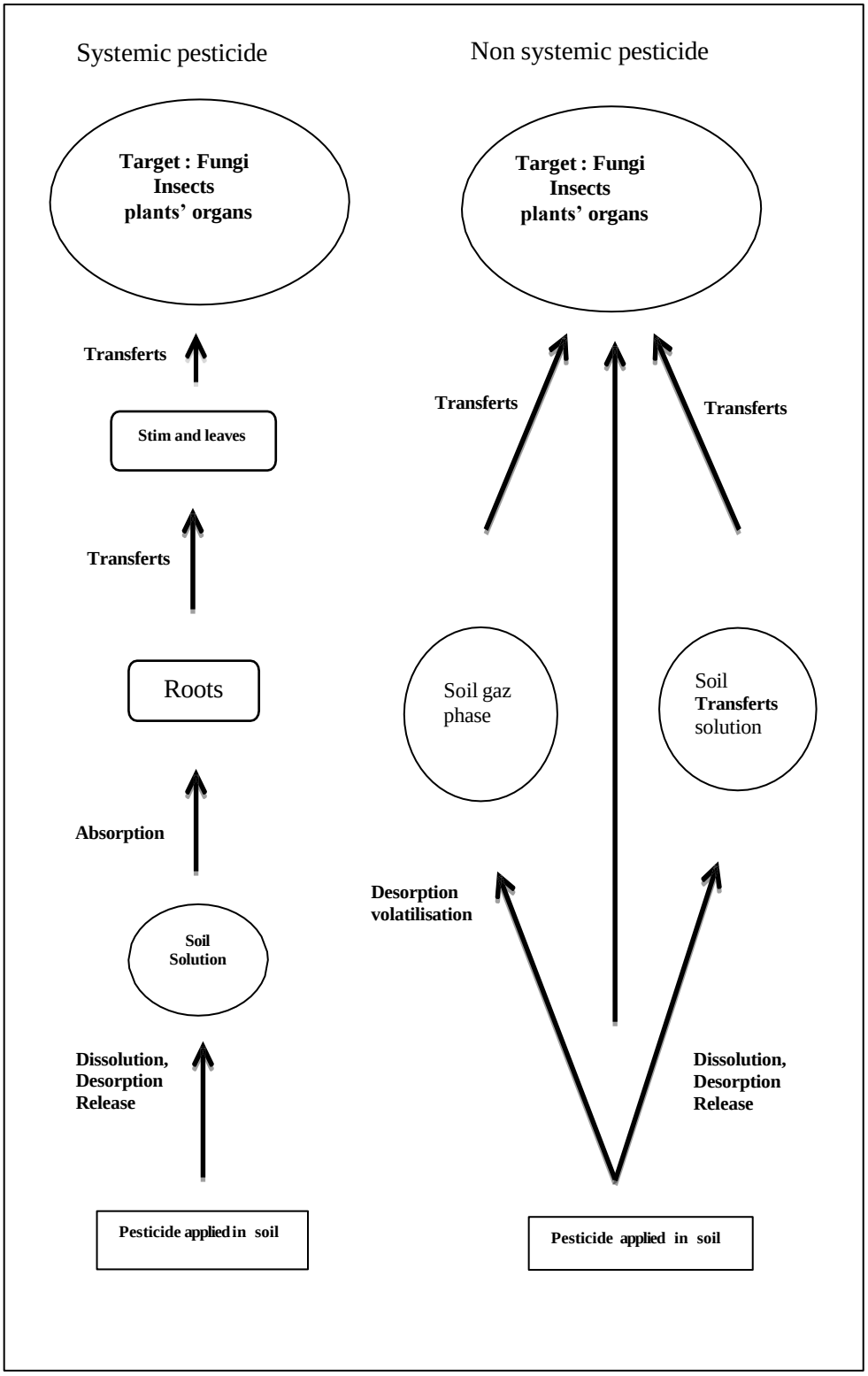


Figure II.1: Difference between systemic and non-systemic pesticides

Table II.1 . The following table groups together some examples of herbicides and their mode of action (Kilinc, 2010).

Herbicide class	molecules	target	references
inhibitors of amino acid biosynthesis pathways	glyphosate	aromatic amino acids	Amrhein et *al* ., 1980
	glufosinate	all amino acids	
	sulfonylureas	branched-chain amino acids	
Fatty acid synthesis inhibitors	thiocarbamates,	very active on many weedy grasses	Devine et *al* ., 1993; Böger and Sandmann, 1992
	FOP		
	SUN		
Pigment synthesis inhibitors	*diphenyl ethers* or DPE	The **synthesis of chlorophylls**	
	norflurazon, diflufenicanil, flurochloridone, aminotriazole	The **synthesis of carotenoids**	
inhibitors of a chloropaste stroma enzyme	**diphenyl ethers**	protoporphyrinogen oxidase	Martinge et *al* ., 1988

II.2. Heavy metals contaminating soils and terrestrial water

Metals are naturally present in the environment as normal constituents of the Earth's crust. They are also present in the tissues of living organisms, where some of them, called essential metals, perform functions indispensable to life (Cu, Fe, Zn, etc.), while others (non-essential metals such as Cd, Hg, Pb, etc.) have no known physiological function (Amiard et *al*., 2017). Over the course of evolution, organisms have therefore been able to develop strategies enabling them to cope with the presence of these elements in their environment. Organismal tolerance to metals can be

broken down into three aspects:

Limiting the entry of metal into the body;

- Detoxification of incorporated metal;
- Increased elimination.

II.2.1. Harmful effects of heavy metals

In the context of metal pollution, the biological effects of toxic metals present in the environment are triggered by the interaction of the metal with a biological receptor on the surface or inside the living organism. It is assumed that the metal concentrations required to trigger these responses are far lower than those that cause a crisis in the target organism or visible degradation of the ecosystem.

Thus, ideally, the detection of the quantification of molecular, biochemical or physiological responses within the organism to chronic exposure to metals could serve as an early, sensitive and specific indicator of environmental stress **(Pelletier** et *al.* , (2004).

Several metals exert their toxicity by competing **with Calcium** . This is particularly true for **Cadmium (Cd**). Cd is a contaminant whose presence in the environment is associated with industrial development.

Lead (Pb) and mercury (Hg) are metals that **interfere with many cellular functions** .

Some derivatives such as **methylmercury (MeHg)** and **methylmercury chloride** are **genotoxic** and lead **to the formation of DNA adducts (the attachment of a molecule to a nucleophilic site of DNA by covalent bonding). These DNA adducts can alter gene expression.**

II.2.2. Multiple interactions in ecotoxicology

The problem of the simultaneous presence of several pollutants in a given natural environment and their effects on the organisms that live and

reproduce there is as old as environmental toxicology itself. Among the attenuating interactions between xenobiotics is the interaction between selenium and mercury **(** Pelletier et *al.* , 2004). Research has demonstrated the presence of the Hg-Si complex in individuals who have absorbed a significant amount of mercury (Hg).

II.2.3. **Concentration of heavy metals in plants**

There has been considerable debate regarding the presence of heavy metals in soil, given their toxicity to plants, animals, and humans, and their low biodegradability. Heavy metal contamination of soil and crops is due to human activities (manure, fertilizers) (Sekomo et *al.* , 2011). Prolonged application of activated sludge promotes the accumulation of heavy metals in soil and crops (Bolan et *al.* , 2003). It is important to note that the capacity to accumulate heavy metals in vegetables is highly variable (Gomez-Ros et *al.,* 2013). However, heavy metal retention in plants remains low due to their limited migration capacity (Bian et *al* ., 2014).

Zhang et *al* . (2009) proved that the reed accumulated heavy metals in the following order (**Table II.2**):

Table II.2. Order of accumulation of heavy metals in reed

metal	Hg	Zn	As	Cu	Cr
Molecular weight ($g.mol^{-1}$)	200.6	65.4	74.9	63.5	52
Order of accumulation	Hg > Zn > As > Cu > Cr				

The concentration of heavy metals in the edible part of vegetables depends on their accumulation capacity and the different properties of the soil.

Plants that grow naturally in an environment contaminated with heavy metals adapt and accumulate high concentrations in their different parts (Kumar et *al.* , 2013).

Experiments prove that plants do not have the same capacity to absorb heavy metals and accumulate them in their different parts.

The order of absorption and accumulation of the tested heavy metals was as follows (Table II.3): Zn > Mn > Pb, Cr, Ni, Cu > As ≈ Cd ≈ Co

Table II.3 . Order of accumulation of heavy metals in reed (2nd test)

metal	Zn	Mn	Pb	Cr	Neither	Cu	As	CD	Co
Molecular weight (g.mol^{-1})	65.4	54.9	106.4	52	58.7	63.5	74.9	112.4	58.9
Order of accumulation	1	2	3	3	3	3	4 ≈	4 ≈	4 ≈
	Zn > Mn > Pb, Cr, Ni, Cu > As ≈ Cd ≈ Co								

Soil contamination by heavy metals reduces and slows the absorption and accumulation of mineral elements in plants. This phenomenon is due to combined pollution by heavy metals.

II.2.4. **Transfer of heavy metals from soil to plants** (Bian et *al* ., 2014)

Cd is strongly absorbed from the soil to plants. The availability of various heavy metals in the soil increases competition and slows absorption (Wang et *al.* , 2006), and therefore the concentration of these metals in plants remains relatively lower than predicted, except for Cd, which has a high

capacity for transfer from the soil to plants despite competition.

The accumulation of heavy metals (combined) in the tested plants (experiment by Bian et *al*., 2014) was in the following order:

Amaranth (herbaceous plant) > Water spinach > *Kalimerisindica* > Garlic ≈ Green onions > Green vegetable.

Amaranth quickly becomes enriched with heavy metals, unlike green vegetables which accumulate the lowest concentrations.

The capacity for transfer, accumulation and tolerance of heavy metals varies from one plant to another (Table II.4) :

On the one hand, this implies the exclusion of heavy metals by plants; in other words, heavy metals are eliminated from plants after absorption or are only minimally absorbed. On the other hand, some plants accumulate heavy metals to a significant extent and have detoxification capacity (Authur et *al*., 2000).

Table II.4. Concentration of heavy metals in vegetables (mg/kg) **(Bian et *al*., 2014)**

Concentrations of heavy metals in vegetables (mg kg^{-1}).

Concentrations (mg kg^{-1})	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb
Scallion	5.39 ± 0.91	21.03 ± 1.21	0.25 ± 0.09	4.17 ± 0.86	2.24 ± 0.64	48.91 ± 9.25	0.26 ± 0.09	0.17 ± 0.09	9.46 ± 1.03
Water Spinach	8.24 ± 0.65	27.35 ± 2.35	0.53 ± 0.10	5.73 ± 1.34	3.68 ± 0.86	53.84 ± 11.34	0.34 ± 0.13	0.52 ± 0.13	14.28 ± 2.10
Amaranth	7.74 ± 1.02	48.67 ± 6.23	0.66 ± 0.21	6.58 ± 1.46	4.46 ± 1.03	93.54 ± 24.36	0.56 ± 0.25	0.40 ± 0.13	16.03 ± 1.81
Garlic	3.59 ± 0.36	9.09 ± 0.94	0.18 ± 0.11	3.70 ± 0.65	2.34 ± 0.35	50.90 ± 9.54	0.31 ± 0.06	0.15 ± 0.10	2.39 ± 0.35
Kalimeris indica	7.23 ± 0.24	27.87 ± 1.25	0.51 ± 0.21	6.62 ± 1.06	4.74 ± 1.32	83.29 ± 10.35	0.43 ± 0.15	0.17 ± 0.03	5.08 ± 1.00
Greens	5.50 ± 0.46	17.28 ± 0.98	0.18 ± 0.09	3.06 ± 0.84	2.38 ± 0.87	40.39 ± 2.35	0.18 ± 0.08	0.09 ± 0.01	5.03 ± 0.39
Standards	0.5[1]	–	–	0.6[2]	10[3]	20[3]	0.5[1]	0.05[1]	0.2[1]

[1] Safety Qualification for Agricultural Product-safety Requirements for Non-environmental Pollution Vegetable (GB18406.1-2001).
[2] FAO/WHO Codex Alimentarius Commission Standards.
[3] Tolerance Limit of metals in Foods (GB2762-2005).

A study assessing heavy metal concentrations in commonly consumed vegetables in Colombia found that of eight heavy metals measured, arsenic (As), lead (Pb), and chromium (Cr) exceeded international standard limits. The distribution of heavy metal concentration in these vegetables was

directly proportional to the transfer of metal from the soil to the plant.

In parsley, for example, the transfer was relatively high due to the density of the foliage compared to other plants. It has been shown, based on correlation analyses of the physicochemical properties of heavy metals, that the transfer of certain heavy metals is conditioned by the presence of other heavy metals, as is the case for lead (Pb) and copper (Cu). The selection of plant species that accumulate and are tolerant to heavy metals is important for phytoremediation plans to restore degraded agricultural soils (Franco et *al* ., 2018).

The study by Shehu et *al* . (2016) on heavy metal concentrations in edible vegetables harvested from calcareous soil with a neutral pH (7.3–8.2) demonstrated the selectivity of heavy metal transfer in vegetables such as cabbage, which concentrates chromium (Cr) more than nickel (Ni), lead (Pb), and iron (Fe), and even small amounts of arsenic. In contrast to cabbage, and even with massive concentrations of heavy metals in the soil, pepper did not accumulate any of these elements.

II.2.5. **Harmful effects of heavy metals**

II.2.5. 1. Main physiotoxicological effects induced by exposure to pollutants :

The action of toxic substances on living beings can result in a wide variety of physiotoxicological consequences.

Somatic effects constitute a first group, and refer to those which affect one or more functions of vegetative life or of relation.

Germinal effects concern any disruption of the reproductive functions of intoxicated individuals or any action affecting the physical integrity of offspring through mutagenic or teratogenic effects (Ramade, 2007) .

a) **Somatic alterations**

Exposure to toxic pollutants can cause disturbances and cellular damage which at the somatic level can be either common to all organisms, or specific depending on the case and will limit their action to autotrophic organisms (absorption of mineral nutrients, effects on the photosynthetic system).

b) **Effect on the root system** :

Many pollutants affect the root system by disrupting the absorption of water and nutrients and beyond, by inducing alterations or even necrotic lesions of the rootlets. The acidification of the soil solution by the strong acids contained in these rains dissolves the aluminum contained in the clays. This element, highly toxic, particularly to plants, causes necrosis of the root system, resulting in the dieback of woody vegetation (Ramade, 2007) .

c) **Effect on growth**

Many toxins affect meristem growth: herbicides derived from phenoxyacetic acid, known as auxinomimetics, cause plant death by stimulating and disrupting growth, thus exhausting the plants. When exposed to 2,4-D, vine tendrils begin to grow uncontrollably, exceeding one meter in length.

d) **Effects on germination**

In phanerogams, lower plants, and various microorganisms, there are particular forms of negative action characterized by the emission of toxic substances into the environment.

II.3.Allelopathy

In natural plant formations, the phenomenon that expresses the antagonism between plant species of the same phytocoenosis is reflected through the production of phytotoxic compounds by a given plant to eliminte competiting species that could grow near it (**FigureII.2)** .

This type of antagonistic relationship between different species is called

allelopathy when it occurs between higher plants (Ramade, 2007) .It is called antibiosis when it involves microorganisms.

Toxic interactions between vascular plants are also known as teletoxicity. In California, two shrubs, *Artemisia California* and *Salvia leucophylla,* invade natural grasslands by secreting volatile terpene substances that inhibit the germination of grass seeds and other annual plants.

Another classic example of teletoxicity is provided by walnut trees (genus *Juglans*), which secrete a phenolic substance, juglone, that inhibits the growth of herbaceous plants living near these trees. This compound is emitted by the above-ground parts and introduced into the soil through leaching by rain.

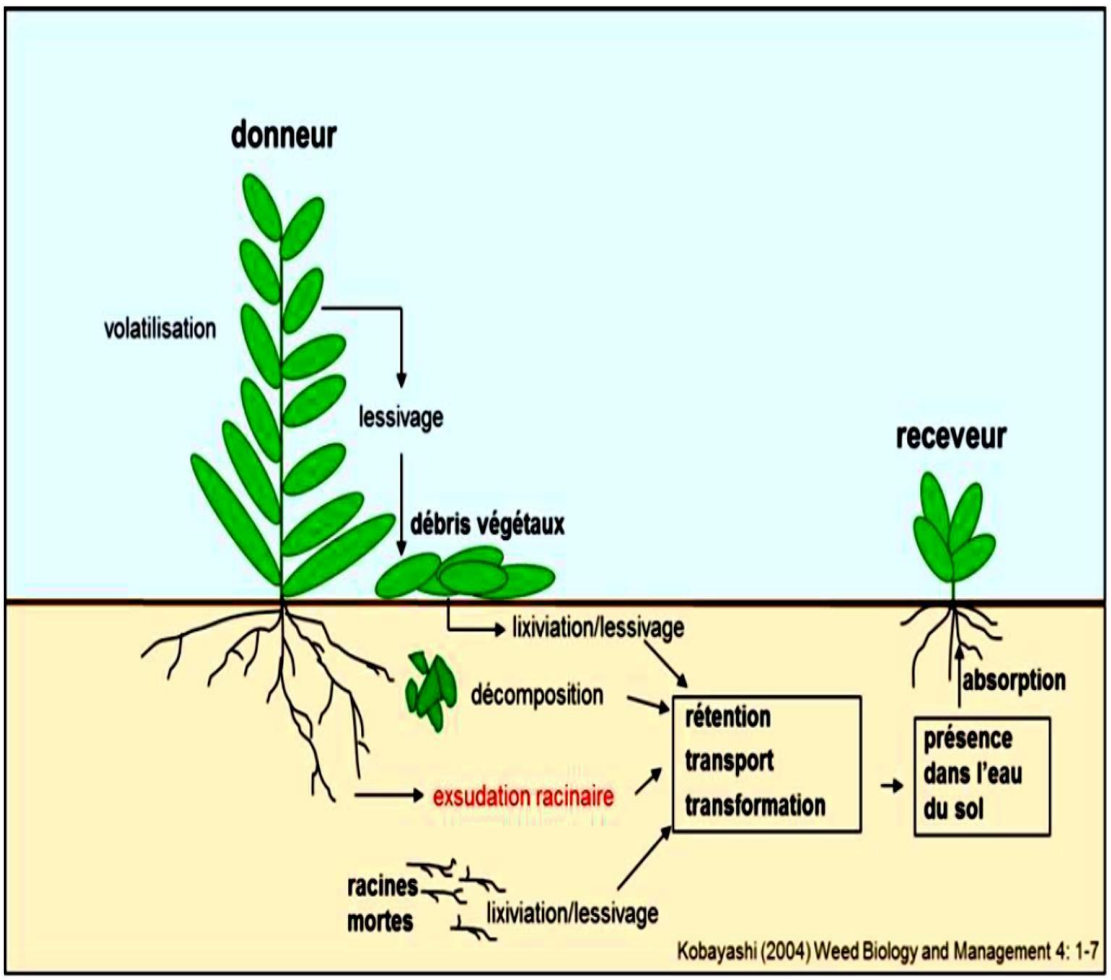


Figure II.2: Possible pathways for the release of allelochemicals into the environment by a donor plant according to Kobayashi (2004).

II.2.1. Conditions influencing the phenomenon of allelopathy:

In the laboratory, summer trials were conducted on the aerial parts (cress, lettuce) or roots, as well as on germinating seeds. Under natural conditions, biotic and abiotic soil interactions can influence the presence of allelopathic compounds, making the study more complex. Numerous factors, such as environmental conditions or the plant's health status, influence the synthesis and release of these molecules (**Figure II.3**). It is difficult to separate competition for resources from allelopathic effects, as allelopathy in the field is subtle and difficult to distinguish from competition (Duke, 2015).

phytotoxic molecules that exert their effects at low, constant quantities or increasing concentrations over long periods (Duke, 2015). The allelopathic effect is due to a single allelochemical compound or a mixture of molecules. Furthermore, these compounds can be transformed and degraded by soil microbes (Massalha et al., 2017).

Once released into the soil, the physical, chemical, and biological properties of allelochemicals change (Latif et *al* ., 2016). These compounds can be degraded by microorganisms.

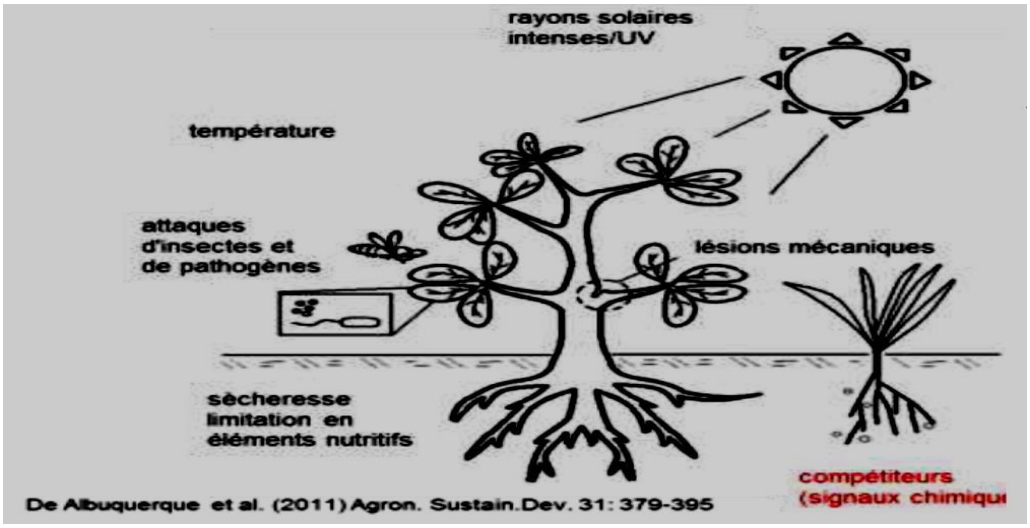


Figure II.3: Induction of allelotrophic production by biotic and abiotic factors according to Albuquerque et *al* . (2011)

II.2.2. Application of allelopathy in biological control

Applications of antibiosis to biological control are conceivable; thus, it has been possible to protect mustard plants against infection by Pythium by coating the seeds with spores of *Penicilium* and *Trichoderma Viride* (*In* Lemée 1978).

Various groups of synthetic mineral or organic substances, including many pollutants, inhibit plant germination. An intentional example is pre-emergence herbicides, such as those in the chloracetamide group, or diphenamide (Fedtke, 1882). The same is true of many other compounds, such as glycerol esters or benzyl ethers.

Table II.5 Plant Allelopathy properties applied in Agronomy

Species	Produced allelochemical	Effect	Targeted weeds	References
Maize	Benzoxazinoids, Phenolic compounds	defence against weed.	*Amaranthus viridis* & *Chenopodium album*	Jabran, K. (2017)
Sorghum	Sorgolene, which contains a range of allelochemicals, i.e. benzoic, ferulic, vanillic, chlorogenic, m-coumaric, dhurrin etc	As surface mulch, aqueous extracts spray, sorghum straws contains high C:N ratio which can be incorporated in soil, we can add sorghum in crop rotation, mix cropping etc.	. The toxicity of these chemicals can persist up to 22 to 28 weeks.	Hussain, (2021)
Sunflower	Chlorogenic acid and Isochlorogenic acid and from leaf leachate Alpha Napthol derivative and Sopolin.	bio herbicides	Many weeds like *Amaranthus retroflexus*, *Hordeum spontaneum* etc	Weed management Principles and practices (OP Gupta)
Plants of Lamiaceae family	extracts, especially essential oils (Eos), and volatile bioactive compounds.	herbicides or as insecticide		Islam,., (2022)
Parthenium hysterophorus (L.)	it secrets very toxic allelochemical	the extracts of leaf and root exudates can be applied to control weeds.	it causes drastic yield loss in various crops. it's not possible to include this weed in cropping system.	bashar. (2023).

III. Adaptive strategies of plants following the introduction of xenobiotics

Introduction

It has been proven that living beings possess natural means of defense and others that they develop, gradually, during repeated contact with a xenobiotic. Once introduced, the xenobiotic undergoes a biotransformation from an active form to an inert form that is easy to degrade and eliminate.

As a general rule, biotransformation is a process that leads to the production of metabolites that are more soluble than the initial molecule, which will facilitate their elimination (animals) or their segregation into vacuoles (plants) (Kilinc, 2010) .

III.1. Biotransformation

Biotransformation is a metabolic process leading to the modification and / or degradation of a xenobiotic by a living organism (**Site III.1.**).

Today, the number of xenobiotics is estimated at 70,000 synthetic organic compounds in addition to a considerable number of natural substances.

Xenobiotics are processed in living organisms through biotransformation reactions. The efficiency of biotransformation depends on the concentration, duration of presence, and form of the xenobiotics, whether inside or outside the organism. The biological effects following biotransformation can lead to accelerated biodegradation or resistance and can also influence the fate of xenobiotics in the environment—and the chemical quality of food (Mougin et *al.* , 2001).

III.2. Detoxification biomolecules in plants

Plant exposure to xenobiotics induces defense systems that are

essentially based on two types of responses (Amiard & Amiard-Triquet; 2017):

1- **bio-mining** of metals into non-toxic forms;

2- **Chelation** by various biological molecules, primarily:

- **glutathione** (a tripeptide composed of the condensation of glutamic acid, cysteine and glycine) (**Figure III.1.a**).

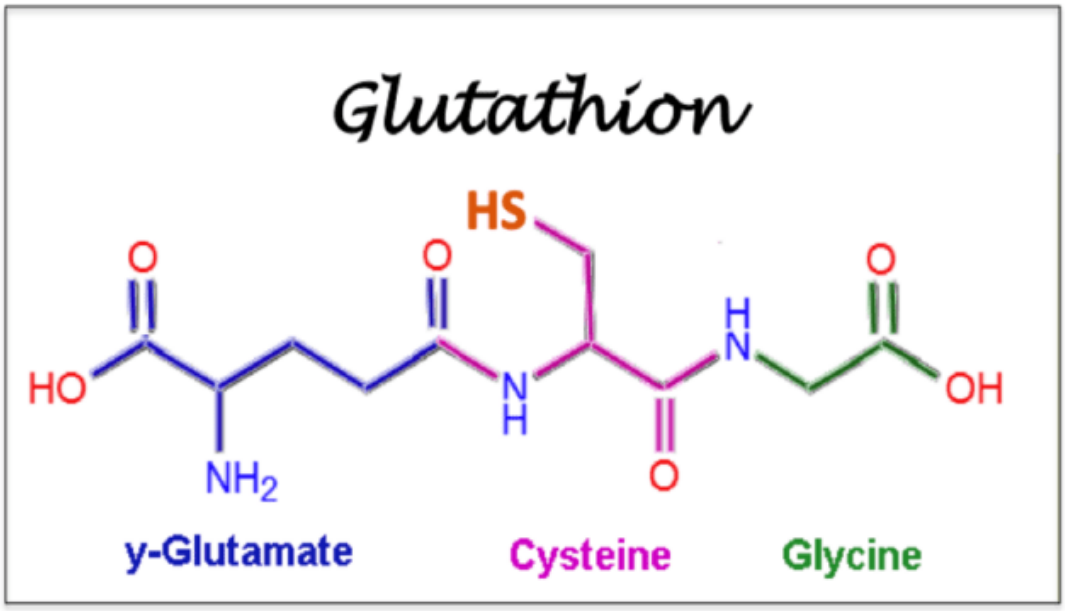


Figure III.1.a .: glutathione (a tripeptide): detoxification biomolecule in plants (Amiard & Amiard-Triquet; 2017).

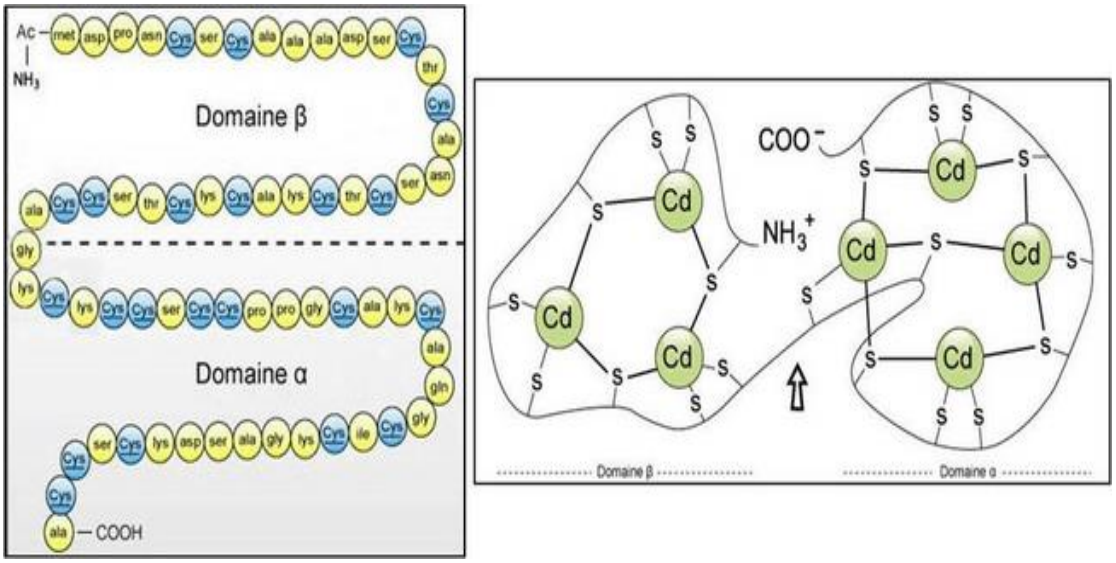


Figure III.1.b. Primary structure of metallothioneins (from http://www.cima.ualg.pt/piloto/UVED_Geochimie/UVED/site/html/2/2-3/2-3-3/2-3-3-5.html).

- **Metallothioneins (MT)** : These are proteins of low molecular weight (< 10 kDa), rich in cysteine (5 to 30%), thermostable, with high metal ontent, natively binding zinc or copper but having a strong affinity for non-essential metals such as cadmium (**Figure III.1.b)** and mercury.
- **Metallothioneins isoforms**
- **Phytochelatins (PCs)** : formerly known as class III MTs (Capdevila et *al.* , 2012), are a family of peptides with the general structure of (γ-glutamyl-cysteinyl-n-glycine (n=2-11). PCs are rich in cysteines, and their biosynthesis

  is stimulated, in particular, by cadmium (Cd) and arsenic (As) from the reduced form of glutathione (GSH) via PC synthase. In addition to these two elements, PCs can also bind metals such as copper (Cu), lead (Pb), zinc (Zn), and silver (Ag) via their sulfhydryl (-SH) and carboxyl (-COOH) groups.

  PCs play a fundamental role in intracellular detoxification and/or metal transport in various plants. This has been demonstrated using experiments targeting enzymes and genes involved in PC and GSH biosynthesis (Inouh et *al., In* : Gupta et *al.* , 2015). The role of PCs has also been highlighted in microalgae (*Phaeodactylum tricornutum, Chlorella sorokiniana*) in the management of intracellular mercury (Deng et *al* ., 2013; Gomez-Jacinto et al., 2015). (Amiard & Amiard-Triquet; 2017).

  Note: Phytochelatins and metallothioneins are present in plants (Amiard & Amiard-Triquet; 2017).

III.3. The use of PCs (Phyto-Chelatins) as biomarkers

The use of quantum dots (QDs) is less systematic than that of metals (MTs). Morelli's research group (2009) *in* (Amiard & Amiard-Triquet; 2017) developed a bioassay to evaluate the bioavailability of metals in marine

sediments. The study is based on determining QDs in diatoms exposed to sedimentary elutriates. Field studies implement this method due to its proven reliability. These authors also used QDs as biomarkers of cadmium bioavailability, released in soluble form in a medium contaminated with cadmium-seed quantum dots, with or without a protective zinc-sulfur (ZnS) layer (Morelli et *al.,* 2012, 2015) *in* (Amiard & Amiard-Triquet; 2017).

III.4. Biotransformation of xenobiotics by enzymatic mechanisms

The enzymatic mechanisms of action enabling the biotransformation of xenobiotics are mainly divided into three phases (Coleman et *al* ., 1997) *In* (Kilinc, 2010):

III.4.1. Phase I or functionalization reactions:

This involves a set of oxidation reactions (**Figure III.2**), reduction and hydrolysis, allowing the introduction or release of polar chemical functions on the xenobiotic, thus promoting the action of phase II enzymes. Cytochrome P450 monooxygenases are responsible for catalyzing oxidation reactions. Esterases are the main catalysts for hydrolysis (Siminszky, 2006) *In* (kilinc, 2010).

III.4.2. Phase II or conjugation reactions :

Conjugation reactions occur through the addition of a water-soluble endogenous molecule or one of the primary metabolites to the xenobiotic. These reactions require the intervention of enzymes specific to the endogenous substrate to be conjugated. In plants, the enzyme glutathione S-transferase conjugates glutathione (a tripeptide), but other enzymes may also be involved, such as glycotransferases that conjugate sugars (Öztetik, 2008) *In* (kilinc, 2010).

III.4.3. Phase III is the elimination phase of the products of phase II, releasing them into the external environment through an aqueous-phase

excretion process, simultaneously with the removal of degradation products from cellular components. It should be noted that plants do not possess an excretory function equivalent to that of animals (Kilinc, 2010).

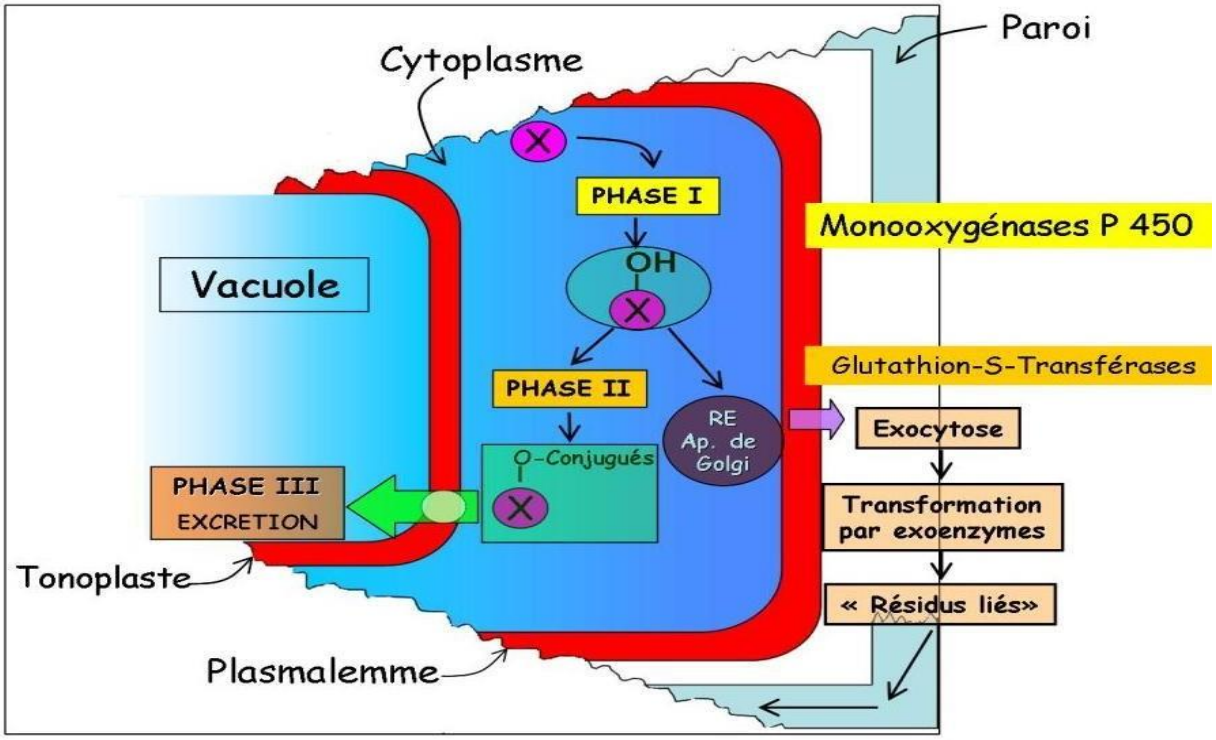


Figure III.2 : Phases of herbicide metabolism in plant cells (Kilinc, 2010).

III.6. Biotransformation of herbicides in plants

Following these reactions (phase I, II and III) herbicides, in most cases, lose their toxic power or their ability to migrate to target organs (leaves).

Most of the hydroxylation takes place at the root level, but there are also esterases and Glutathione-S-Transferases (GST) in the leaves whose role is to inactivate toxic substances like herbicides as they penetrate, making it impossible to block the target (kilinc, 2010).

The fate of herbicides within the plant, from absorption to elimination, is an interesting field to study and explore in depth to understand the detoxification and elimination mechanisms used to treat a toxic substance. The action of herbicides is essentially based on blocking one or more pre-

existing or induced enzymatic activities in the treated plant. Among the most surprising adaptive strategies, the classic example of the adaptation of resistant lines of lambs quarters or amaranth, whose populations exploded in maize crops following repeated atrazine treatments, is well explained (Gasquez, 1981; Heap, 2007).

Indeed, it is now common knowledge that the systemic application of plant protection products causes certain species to develop resistance through the appearance of mutant varieties that adapt well to the absorption and elimination of the plant protection product without the slightest apparent physiological or morphological deterioration (**Site III.2)** :

III.7. Pesticides and oxidative stress

Oxygen is vital for aerobic organisms; however, its toxicity has long been known. During endogenous metabolic processes, oxygen reacts with highly reactive derivatives such as free radicals. Numerous contaminants, including compounds with high redox potential, promote the formation of these oxidative radicals, which are controlled by enzymatic or non-enzymatic systems (Otto and Moon, 1996). *(* Pelletier et *al* ., 2004) Free radicals are characterized by an unstable electronic structure, as they possess an unpaired electron, which explains their high reactivity. The consequences of the formation of these compounds can lead, within the cell, to: membrane lipid peroxidation,
to the oxidation of thiol groups of certain enzymes and coenzymes,
and to the alteration of nucleic acids (adducts to DNA, carcinogenesis, apoptosis) (Gutteridge and Halliwell, 1990) *In* (Pelletier et *al* ., 2004).

III.8. Antioxidant defense systems

Plants confront oxidative stress by engaging antioxidant systems based on blocking the formation of reactive species and eliminating damaged cells (**Pelletier** et *al* ., 2004).

Organisms possess antioxidant action systems to process these highly reactive substances by:

1) elimination of reactive species and the catalysts for their formation,
2) induction of antioxidant synthesis,
3) Increased activity of repair/elimination systems for damaged molecules.

Three types of antioxidant enzymes are used to destroy reactive oxygen species:

1. Peroxidases
2. Superoxide dismutases
3. The Catalases.

The period 1950-1960 was very rich in the synthesis of new chemical structures with herbicidal potential and possible use in large-scale farming (Bourdin, 1983) *In* (Kilinc, 2010).

The period from 1950 to 1960 saw the synthesis and use of three major families of herbicides: **phenylureas, phenylcarbamates, and triazines** . These herbicides have different chemical structures but a single target (photosynthesis inhibitors): blocking the electron transport chain at a target protein involved in this process: the D1 protein. Blocking this transfer inhibits photosynthesis. Oxygen (O2) is then produced from cellular oxygen using electrons still generated by chlorophyll excitation. This root oxygen attacks the membrane systems, producing necrosis (Devine et al., 1993) *(* Kilinc, 2010).

Some species escape the described blockage for specific metabolic reasons Due to specific metabolic factors, some cultures escape the blockage described above. As with animals, plants possess a whole arsenal of enzymes capable of degrading xenobiotics (Edwards et *al*., 2000) (Kilinc, 2010).

This example particularly illustrates the link between the application of a toxic substance (the herbicide), its target at the plant level, and the

adaptation of certain crops to repeated contact with this toxic substance. (Kilinc, 2010).

III.9. Metalic toxicity effect and plant defense strategies

Understanding the molecular mechanisms of absorption, translocation, detoxification, and regulation of metals, trace metals (**Figure III.3a)**, and metalloids in plants is essential for addressing soil contamination problems. Soil contamination by metals can compromise food safety through phytotoxicity and yield losses (**Figure III.3b**), and also affect food safety through excessive accumulation in edible parts. (Tang et *al.*, 2023)

Future research should clarify hormonal regulation (ABA, JA, Eth etc.)-of metal stress in systematically defense signaling management. it's also Important to understand the mechanisms controlling the selectivity of metal transporters and subcellular compartmentalization (Kolhi et *al*., 2026)

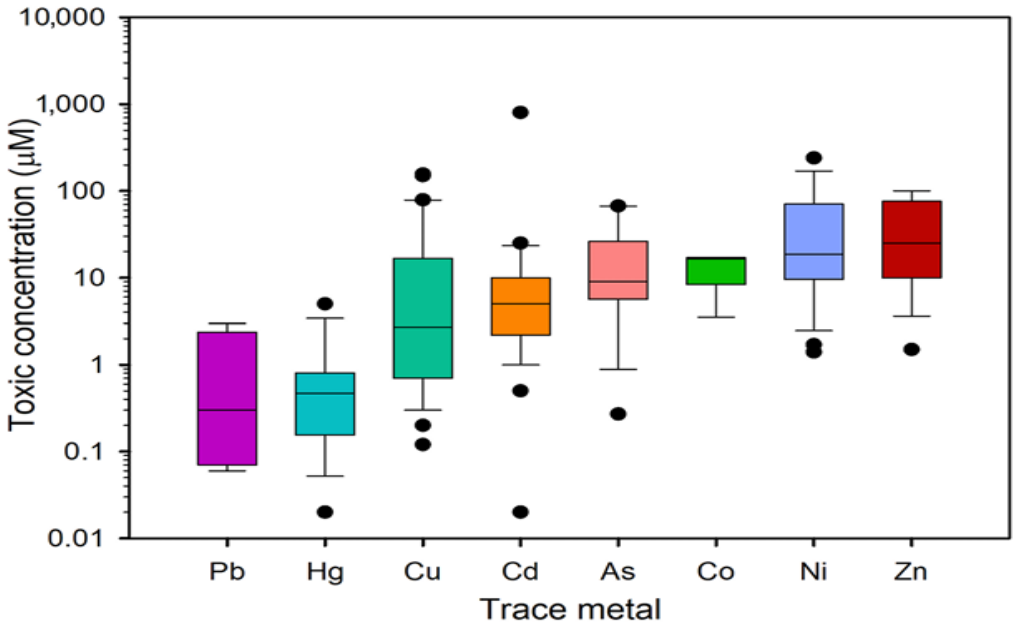


Figure III.3a: Boxplot of the toxic concentrations of eight trace metals/metaloids that reduced the growth of plants in solution culture (Tang et *al.*, 2023).

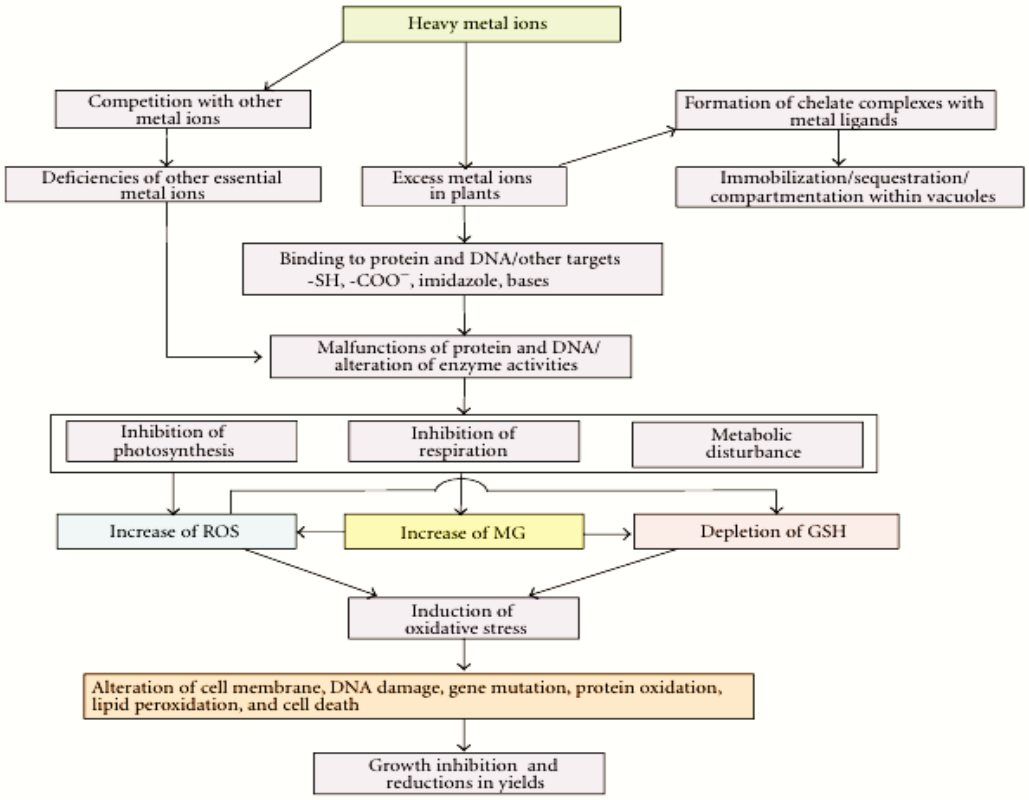


Figure III.3b : Possible Biochemical and molecular mechanisms of heavy metal mediated ROS induction and damage of the higher plants development (Hossain et *al*, 2012).

III.9.1. Heavy metal toxicity impact on different parts of plant (Kolhi et *al*., 2026)

a) Metal effect on seed germination

Seed germination is organized in distingt phases, such as imbibition, enzyme activation, reserve mobilization and cell division activities (**Figure III.4**) metals damage the lipidic membrane which affect membrane integrity and properties like permeability and water uptake leading to lack of hydratation or desiccation (Kumar et *al*., 2023). Furthermore, they inhibit alpha-amylase and protease (essential hydrolytic proteins) that play a crucial role in mobilizing starch and protein reserves from the endosperm (Li et *al*., 2021).

Hormones, such as gibberellic acid (GA), promote germination by activating genes and stimulating enzyme production, while abscisic acid (ABA) has the opposite effect, maintaining seed dormancy. These hormones are disrupted by metals during germination (Tabassum et *al*., 2021).

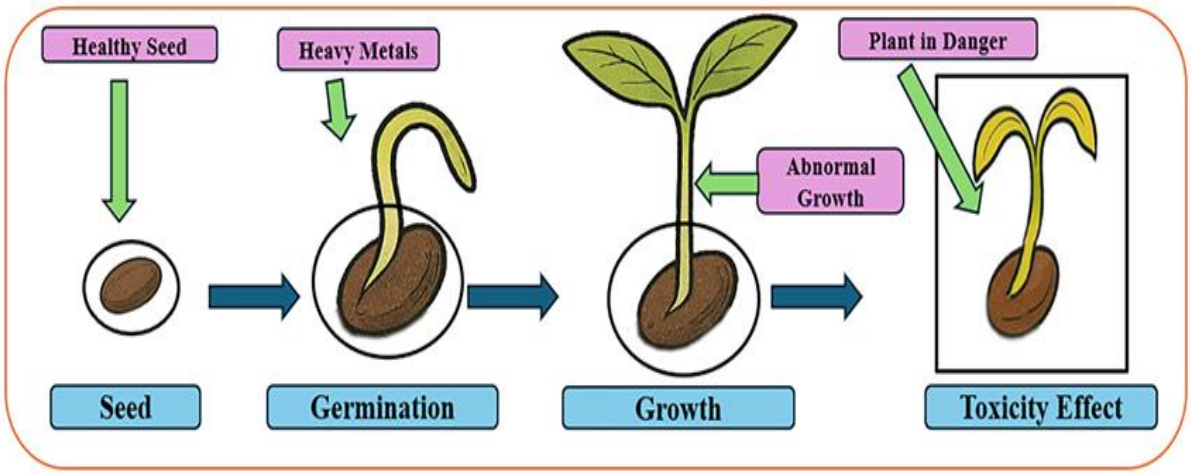


Figure III.4: Illustrates the metals toxicity on seed sprouting. Metals interfere with water obsorption, enzyme function and hormonal signaling, leading to oxidative stress damage (Kolhi et *al*., 2026).

b) Metals effect on root system architecture (RSA)

The root system architecture (RSA), constituting the primary interface for resource acquisition within the soil matrix, represents the initial site of exposure to toxins (Altaf et *al*., 2021; Xin et *al*., 2013). The role of roots is to proactively mitigate the bioavailability of metals through the exudation of low molecular weight organic acids (LMOAs), such as malate and citrate, which act as high-affinity ligands to sequester metal ions in stable, less bioavailable complexes. A polysaccharide-rich exudate, forming a mucilage, reinforces the chemical barrier and protects the sensitive root apical meristem (RAM) by immobilizing metals (Riyazuddin et *al*., 2021; Seregin and Kozhevnikova, 2024).

Root exudation activates rhizosphere microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, which enhance metal immobilization through biosorption and bioaccumulation and simultaneously induce systemic resistance. This constitutes a biological buffer that preserves the integrity of the root system (Barra Caracciolo and Terenzi, 2021).

Cadmium disrupts ionic homeostasis and cell signaling, processes essential for

root development, and interferes with Ca^{2+} and Zn^{2+} uptake pathways, as demonstrated in experiments conducted on Arabidopsis thaliana and Zea mays (Leonardo et *al*., 2021; Matayoshi et *al*., 2022).

c) Metals effect on plant physiological processes

Photosynthesis, a physiological process essential for plant development, is highly sensitive to metal stress. Recent studies reveal that metals significantly disrupt various processes, including the modulation of photosynthesis, pigment synthesis, alteration of photoprotection mechanisms, chloroplast ultrastructure, gas exchange, and electron transport, leading to decreased plant productivity (Hu et *al*., 2023). Experiments conducted by Kumar et *al*. (2023) demonstrate that metals such as cadmium (Cd), lead (Pb), and mercury (Hg) penetrate leaves via the xylem, altering their morphology, damaging roots, and disrupting cellular functions. Furthermore, cadmium and lead inhibit the action of enzymes essential for chlorophyll synthesis, particularly δ-aminolevulinate dehydratase (ALAD) and magnesium chelatase (Mg^{2+}) (Fusaro et *al*., 2025). The first indicator of impaired photosynthesis caused by metals is the immediate consequence of this inhibition, which is the onset of chlorosis, characterized by yellowing of the leaves (Fusaro et *al*., 2025).

Furthermore, the accumulation of reactive oxygen species (ROS) in the leaves causes oxidative damage to cell membranes, proteins, and nucleic acids **(Figure III.5 & 6 & 7).** This accumulation activates antioxidant enzymes, which are subsequently depleted through further interactions (Kumar et *al*., 2022).

Stem growth is inhibited by Cadmium (Cd) and lead (Pb), thus affecting numerous physiological processes such as transpiration and functions related to photosynthesis. This leads to an interruption of chlorophyll formation, a reduction in pigment concentration, and a decrease in the photosynthetic rate at the biochemical level (**Figure III.6**) (Kolhi et *al*., 2026).

Nitrogen alters leaf metabolism, inhibits nitrate reductase activity and glutamine synthetase formation, indicating nitrogen deficiency (Akhtar et *al*., 2024). Furthermore, the storage of moderate concentrations of toxic metals affects the formation of secondary metabolites such as flavonoids and phenolic compounds, which are responsible for reducing prolonged stress (Goncharuk and Zagoskina, 2023).

Aluminum (Al) toxicity is the main limiting factor for crop growth in acidic soils. Plants can resist this toxicity by regulating two mechanisms: external exclusion, which primarily reduces Al penetration into cells, and internal detoxification, which includes the antioxidant defense system, Al^{3+} ion redistribution (e.g., vacuolar compartmentalization), and the auxin response (Yan, 2021).

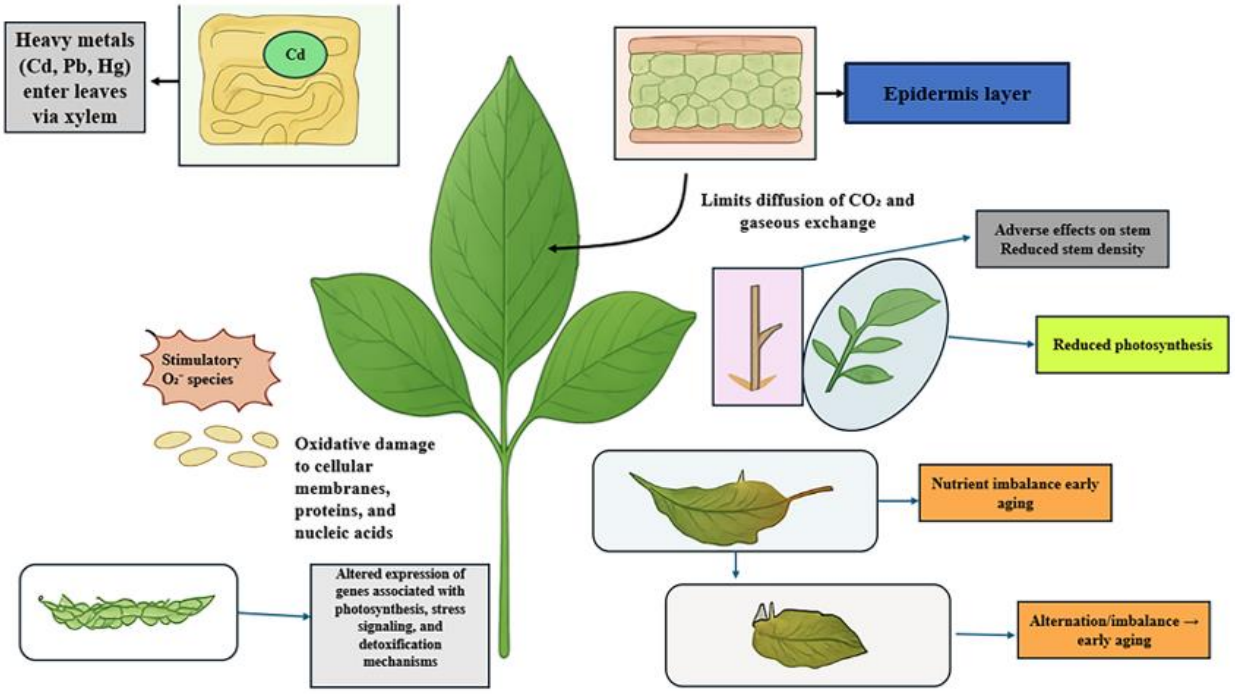


Figure III.5: Metal toxicity disrupts leaf structure and function, causing oxidative damage and stimulating ROS (Kolhi et *al*., 2026).

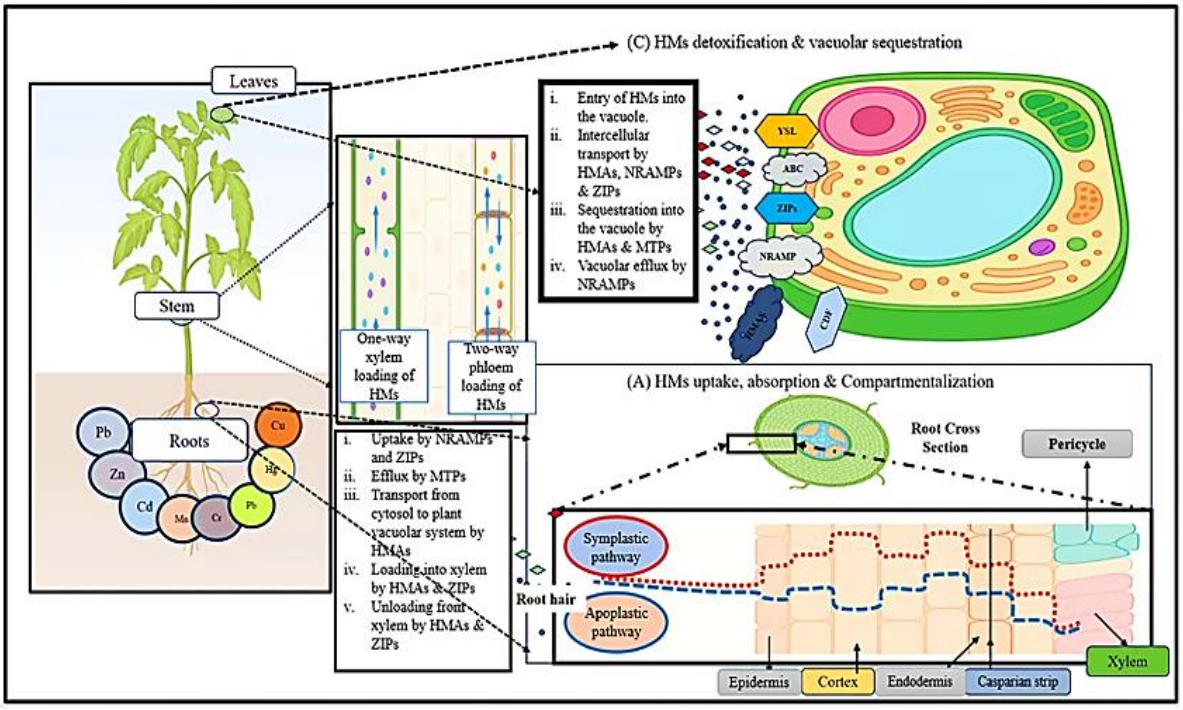


Figure III.6: Mechanisms of metal uptake, translocation, and detoxification in plants. (A) Root absorption and compartimentalization via NRAMPs and ZIPs through symplastic and apoplastic pathways (B) Systemic translocation involving one-way xylem and two ways phloem loading (C) Leaf-level detoxification and vacuolar sequestration mediated by HMA, ABC and MTP transporters (Kolhi et *al*., 2026).

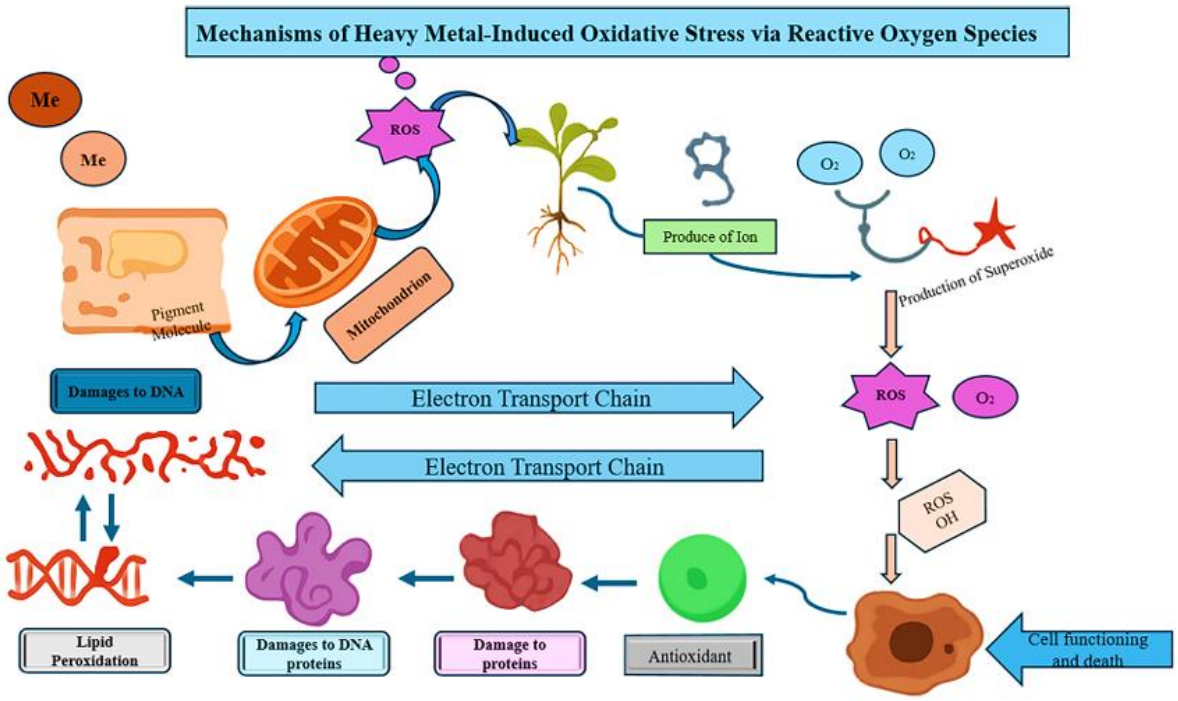


Figure III.7: Mechanisms of oxidative stress induced by metals through ROS (Kolhi et *al.*, 2026).

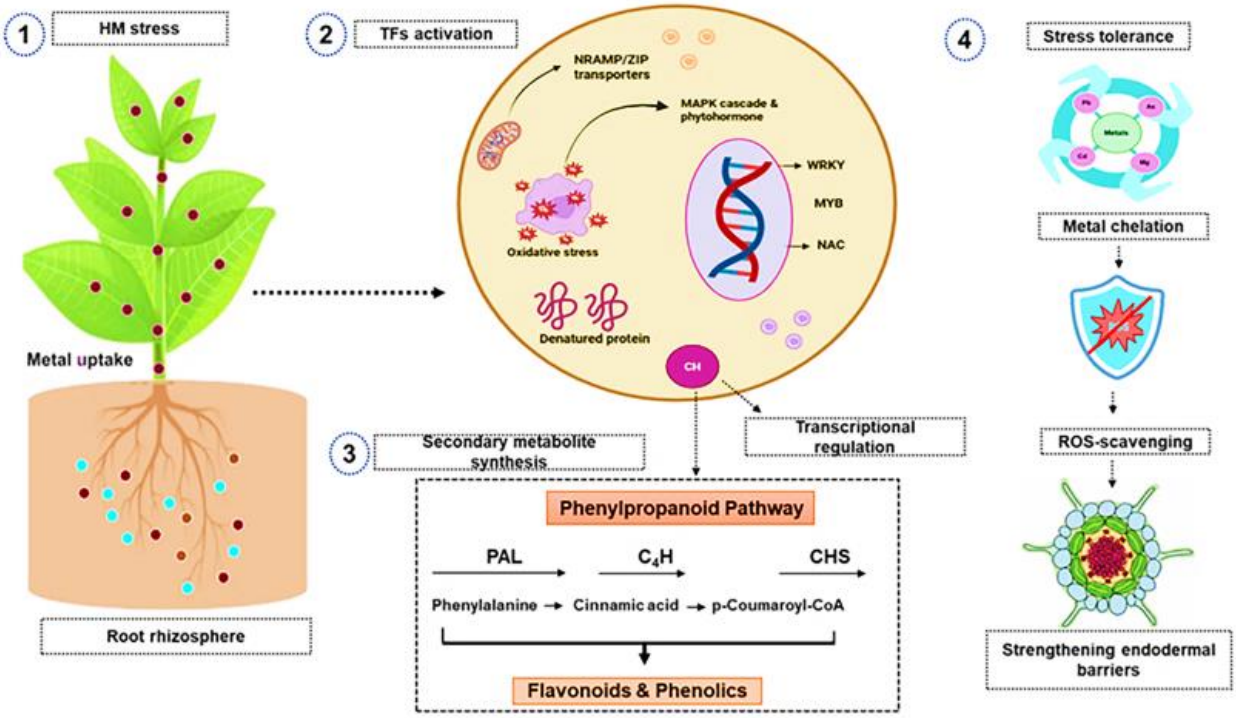


Figure III.8: Regulatory mechanisms of plant secondary metabolite biosynthesis under metal stress. (1) metal uptake from rhizosphere; (2) activation of signaling cascades and TFs (WRKY, MYB, NAC); (3) induction of the phenylpropanoid pathway for flavonoid and phenolic synthesis; (4) enhancement of stress tolerance via metal chelation, ROS scavenging, and structural barrier reinforcement.

III.9.2. Chelation and Sequestration of Heavy Metals

The main consequence of toxicity in plants is the alteration of gene expression involved in photosynthesis, stress signaling, and detoxification mechanisms at the root and leaf levels (Shehzad et *al*., 2023). These disruptions induce defense responses and reduce metal accumulation in plants (Chandrakar et *al*., 2020).

Numerous studies are currently focused on understanding the complex mechanisms of plant adaptation to metal toxicity, particularly chelation and sequestration (**Figure III.8)**. Accumulation is one of the defense strategies plants employ against metal toxicity. This is the case for hyperaccumulator species of Thlaspi caerulescens, which exhibit high uptake of Cd, Ni, and Zn (Figure III.5).

1. They facilitate the influx of metals into the roots through specialized transporters such as NRAMP and the activation of HMAs (Bhat et *al*., 2025).

2. For metal chelation, and after their absorption, plants utilize citrate, malate, and non-protein thiols from LMOA. Since stable nickel citrate and malate complexes are produced in (*Mesem bryanthemum crystallinum* and *Brassica juncea),* their transport into the xylem leads to cytoplasmic damage (Amari et *al*., 2016).

3. Similarly, phytochelatins (PCs) are produced enzymatically from glutathione and bind to Cadmium (Cd), Arsenic (As), and Mercury (Hg) in the cytosol. The resulting complex is transported into vacuoles by ABC transporters for the long-term sequestration of these metals (Bhat et *al*., 2025; Srivastava et *al*., 2024).

4- Metallotheonins (MTs) are cysteine-rich proteins that confer additional binding capacity to zinc (Zn) and reduce binding to copper (Cu). MTs decrease oxidative stress and DNA damage.

5- Chelated metals are sequestered in vacuoles, and cell walls are formed. This compartmentalization plays a crucial role in detoxification. Chemical chelation techniques are essential for improving metal mobility and extraction through the use of natural and synthetic ligands, particularly for soil remediation. Ethylenediaminetetraacetic acid (EDTA) remains widely used because of its high affinity for lead (Pb) and cadmium (Cd), but concerns about its persistence and environmental toxicity have prompted the search for greener alternatives.

IV. The phenomenon of stress and protective reactions

Introduction

This chapter deals in its entirety with air pollution through a description of the various air pollutants to which plants are exposed. Air pollutants are classified into two categories: primary and secondary pollutants.

IV.1. Primary and secondary pollutants

IV.1.1. Primary Pollutants

a) nitrogen oxides (NOx):

Nitrogen oxides (NOx) are formed as a result of combustion processes that initially release nitrogen present either in the fuel or in the combustion chamber. They primarily include nitrogen monoxide (NO), released by combustion, which rapidly oxidizes to nitrogen dioxide (NO_2) in the atmosphere. The nitrogen dioxide (NO_2) released into the air dissolves in water vapor to form nitrate particles and other compounds harmful to plants, animals, and the entire environment. Nitric acid (HNO_3) (one of the nitrogen-derived compounds) is responsible for the acidification of aquatic and terrestrial ecosystems. Nitrate (NO_3) deposition, combined with other compounds such as ammonia, leads to the secondary formation of particles smaller than 2.5 microns: PM2.5, which is inhalable and harmful to living organisms. Along with volatile organic compounds (VOCs), NO_2 is one of the two main pollutants that promote the formation of tropospheric ozone. (**Site IV.1**).

b) **Carbon monoxide (CO)** :

Complete combustion produces carbon dioxide (CO_2) and water (H_2O).

However, the combustion process is not always complete and non-fully oxidized carbon compounds are generated during combustion such as carbon monoxide (CO) and polycyclic aromatic hydrocarbons (PAHs) (Seigneur, 2013).

Carbon monoxide is a colorless and odorless flammable gas that forms as a result of the incomplete combustion of organic matter due to insufficient oxygen (gas, coal, fuel oil, wood, or other fuels) . Carbon monoxide is commonly produced when a car engine is idling or when a home heating appliance malfunctions.

Carbon monoxide contributes to the formation of tropospheric ozone (near the Earth). In the atmosphere, it is transformed into carbon dioxide (CO_2) and contributes to the greenhouse effect. **(Site IV.2** .).

c) Sulfur oxides (SO)

Sulfur monoxide belongs to the sulfur dioxide (SOx) family. At ordinary temperatures, it is a gas that produces a reddish-orange deposit when cooled to liquid air temperature. This liquid air is produced by passing an electrical discharge through a mixture of sulfur vapor and sulfur dioxide at low temperature (**Site IV.3**). This gas is formed during combustion and refining processes from sulfur present in the combustion atmosphere or in fuels such as coal, petroleum, and metal-containing ores. Once dissolved in atmospheric water vapor, SOx $_{\text{transforms}}$ into acids that interact with gases and particles in the air to form sulfate particles, becoming a source of acidification as they are deposited in aquatic and terrestrial ecosystems. Contact with compounds present in the atmosphere such as ammonia allows the secondary formation of P $_{2.5\ \text{particles}}$ (inhalable particles of less than 2.5 microns). These particles, harmful to the health of living beings, are the cause of dry haze and reduced visibility (**Site IV.4**).

d) Mercury

The presence of mercury (Hg) in the atmosphere is due to emissions from coal-fired power plants in two different forms: elemental mercury (poorly soluble) and oxidized mercury (soluble). There are currently devices that filter air containing mercury and transform elemental mercury into soluble mercury, which is then trapped in the particles of activated carbon (Seigneur, 2012).

Toxic metals, in general, contaminate soils and food and accumulate in living organisms, leading to disruptions in biological balance and mechanisms. The vulnerability of certain plant species, such as lichens and mosses, to toxic metals in the atmosphere is exploited by researchers who consider these species as bioindicators of air pollution (**Site IV.5**).

IV.1.2. Secondary pollutants

a) Ozone (O_3)

Tropospheric ozone is a colorless gas that forms in the lower atmosphere as a result of a complex series of chemical reactions primarily involving two precursor pollutants produced by industry and the transport sector: nitrogen oxides (NOx) and volatile organic compounds (VOCs). Ozone and these polluting gases can be transported over long distances (**Site IV.6).**

Tropospheric ozone forms just above the Earth's surface. This extremely irritating pollutant is considered "secondary" because it is created when two primary pollutants (NOx and VOCs, which are products of natural sources or human activity) react with sunlight and stagnant air . (**Site IV.7)** . In addition to the significant effects of this gas on human health, it can reduce vegetation productivity and deteriorate synthetic materials such as rubber and textiles. Ozone is the main component of smog. (**Site IV.8)** .

Polluting emissions from combustion lead to the formation of NOx or SOx

gases, which are responsible for smog formation. When smog formation coincides withsolar radiation, oxygen molecules oxidize, forming ozone. Exposure to ozone causes leaf discoloration followed by wilting and even premature leaf drop (**Site IV.8)**

Figure IV.1 : Deterioration of plant foliage following exposure to ozone (**Site IV.9)**

b) Sulfuric acid (H_2SO_4)

It is a colorless and odorless liquid composed of one sulfur molecule, two hydrogen molecules, and four oxygen molecules. It is a strong acid, formerly used as a herbicide in agricultural fields. It is the most manufactured chemical in the world due to its diverse and numerous industrial applications.

Most of the sulfuric acid recovered from hydrocarbon refining is transferred for processing and use in various industrial activities (**Site IV.10**)

- phosphate fertilizer production
- production of bleaching agents in pulp and paper manufacturing
- lead-acid battery production

- wastewater treatment in wastewater treatment plants
- potato harvest

Sulfuric acid is a highly corrosive substance harmful to the environment and human health. Droplets of sulfuric acid can dissolve in water vapor and fall to the ground as acid rain (wet deposition), in addition to dry deposition on soil surfaces (water, vegetation, buildings, etc.). Sulfuric acid is toxic to living organisms, but it is neither persistent nor bioaccumulative.

c) Nitric acid (HNO_3) :

Nitric acid (HNO_3) is also called **azotic acid** or **hydrogen nitrate and is widely used in the explosives industry, nitrate derivatives and metal processing, but also in the food industry for** the manufacture of nitrogen fertilizers.

Nitric acid (**HNO_3**) is a liquid that, under normal temperature and pressure conditions, is toxic by inhalation, has a **suffocating odor** detectable at extremely low concentrations, is colorless, and **corrosive** to the respiratory system. It can cause **severe chemical burns** as well as eye damage. Chronic exposure to this substance is strongly linked to the development of various forms of **cancer (Site IV.11).**

d) Peroxyacetyl nitrate (PAN)

Peroxyacetyl nitrate (PAN) is a type of air pollution. It is one of the gases that form smog. PAN can irritate the eyes and lungs of animals and humans. Damage related to PAN exposure observed in plants shows that it is 10 to 50 times more toxic than ozone (JETEC, 2014 & Kenneth, 2016).

PAN is formed by the reaction of acylate radicals with nitrogen trioxide under the action of sunlight (photochemical reaction) on secondary pollutants available in the air, such as ozone (O_3) , sulfuric acid, nitric acid, nitrogen dioxide (NO_2) , and peroxyacetyl nitrate (John, 2012). PAN forms

following the reduction of NO concentration to a very low level, a high concentration of NO_2, and the presence of ozone (Nwariaku, 1995). Laboratory tests have proven the toxicity of PANs by inhalation and even the risk of explosion due to condensation of volatile PANs (Abdo, 2013).

PAN (peroxyacetyl nitrate) acts on plants by inhibiting enzyme activity through attack on sulfhydryl groups. Damage caused by PANs is visible after four hours of plant exposure to a concentration of 14 ppb (Aslam, 2021).

e) Nitrogen oxides

Secondary pollutants follow a sometimes complex path to form from primary pollutants, particularly through the phenomenon of photochemistry, which is especially active at high temperatures (in summer). When the wind is weak, the ozone created stagnates above emission sources, leading to a pollution peak. When the ground is sufficiently heated by the sun, turbulent structures form and disperse nitrogen oxides (NOx). Simultaneously, the degradation of VOCs (Volatile Organic Compounds) produces radicals that promote the nitrogen oxide cycle. At nightfall and with the drop in temperature, ozone is completely consumed by these radicals, unlike NO and NO_2 . **(Site IV.12).**

f) PAHs (Polycyclic Aromatic Hydrocarbons)

PAHs can have two origins:

1- **Petrogenic** : present in fossil organic matter (coal, petroleum) formed during the geological transformation of organic matter (OM).

2- **Pyrolitics** : produced by the incomplete combustion or pyrolysis of any organic matter at high temperatures (Dupuy, 2014). They are formed during food cooking and contribute to air pollution. They affect humans through irritation upon inhalation and plants through dry deposition on seeds,

fruits, or vegetables that are subsequently consumed (WHO, 2000). They can cause systemic effects (hepatic, hematological, and immunological effects), as well as genotoxic and carcinogenic effects (Pichard, 2003) . The main source of PAHs in the environment is combustion. (Baek *et al.* 1991; Menzie *et al.* 1992) (Dupuy, 2014).

Polycyclic Aromatic Hydrocarbons (PAHs) are made up of carbon and hydrogen atoms and at least two benzene rings. (Dupuy, 2014).

PAHs are among the persistent organic pollutants (POPs), which tend to accumulate in the environment because of their:

i. slightly biodegradable
ii. poorly available and therefore tending to accumulate in compartments (*e.g.* sorption in the soil, bioaccumulation in the lipid fractions of organisms).

PAHs are hydrophobic to weakly soluble in water which tend to flee water by preferentially binding to materials of organic composition such as soil organic matter (Ouvrard, 2016).

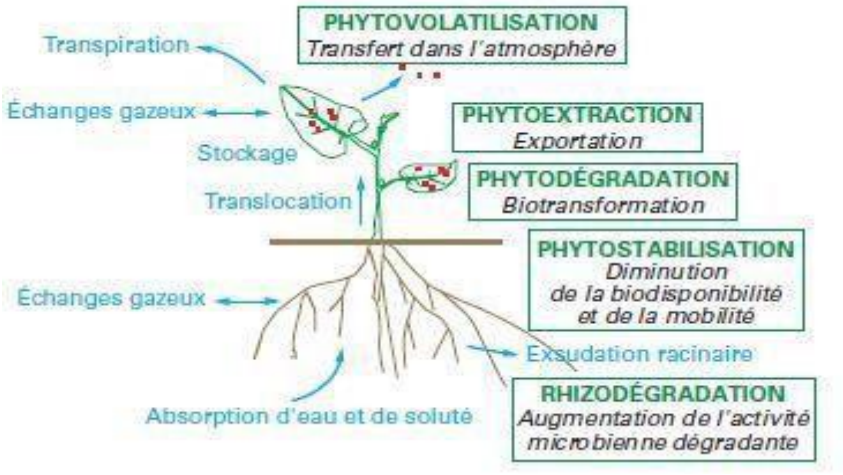


Figure IV.2 : The different processes of elimination of organic contaminants by the plant (Duby, 2014).

PAHs are persistent organic pollutants; and in the case of organic soil pollution, plants follow one of the following processes:

a) phytodegradation **:** is which consists of engaging certain organic compounds through the metabolic activity of the plant in order to degrade them (*e.g.* production of enzymes such as dehalogenase, oxygenase).
b) phytovolatilization **:** which allows the pollutant to be released into the atmosphere after its absorption from the soil and its metabolization into gaseous form at the plant level.
c) Rhizodegradation **:** at the root level the contaminant is treated by the microorganisms of the rhizosphere following the stimulation of the plant by the bacterial and fungal community (Sterckeman *et al.* 2012) (Dupuy, 2014).

g) SMOG

SMOG: The word smog comes from the words " *smoke* " and " *fog* ." In English, smog is a mixture of toxic gases and particles that saturates the air and forms a dry haze over the skies of megacities (large cities). Smog is a dense yellow or black haze of artificial origin (**Figure IV.3)** , unlike natural fog, which is due to the presence of water droplets in the atmosphere. Smog is dry and sober, laden with polluting particles and greenhouse gases, both toxic and non-toxic, particularly tropospheric ozone. The World Meteorological Organization, a specialized agency of the United Nations, defines it as follows: "The word smog is used to describe the simultaneous presence in the air of fog and high levels of air pollution."

The British capital is historically known for the Industrial Revolution, which intensified the use of coal in industry, transportation, and home heating. This megalopolis experienced a smog in 1952 that lasted four days, bringing transportation to a standstill; residents stayed indoors, and hospitals were

overwhelmed with thousands of sick people, resulting in 12,000 deaths. Exposure to smog is equivalent to exposure to and/or smoking from cigarettes (**Site IV.13)** .

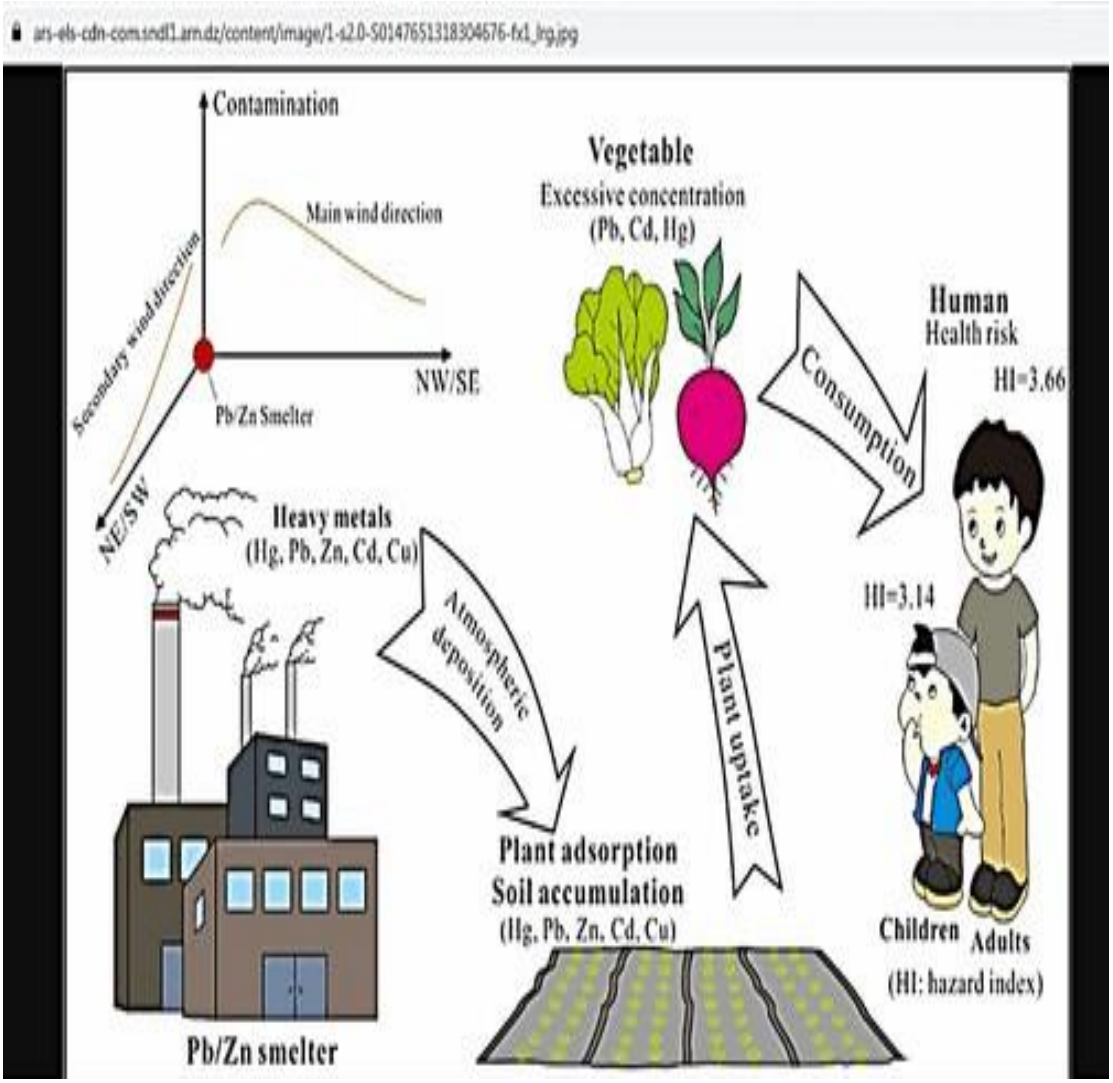


Figure IV.3: Direct and indirect exposure of living beings to toxic emissions.

V- The putative reasons for forest decline

V.1. Definition of Forest Decline

Decline is an overall deterioration in the health of an individual or a population that can lead to death (Manion, 1981).

Forest decline corresponds to all kinds of agents that negatively affect the state, vitality and biodiversity of forests **(** Requardt et *al.* , 2007).

V.2. Causes of forest decline

Significant mortality, loss of vitality, productivity, or value of trees and other components of the forest ecosystem are grouped under the term forest decline. These are a consequence of the action of biotic and abiotic agents, or a combination of both (UNECE, 2000). They can be caused by purely natural factors, human factors, or a combination of both (Requardt et *al.* , 2007).

This phenomenon is the result of a succession of interacting factors occurring in a certain way, leading to a general deterioration of the tree, weakening it or causing its death.

V.3. Factors causing the decline

The main factors causing the decline are grouped into three categories:

Predisposing, triggering and aggravating factors, which can be of biotic or abiotic origin (Manion, 1981).

A tree exhibiting a factor from each of these three categories is considered to be declining (Manion, 1981).

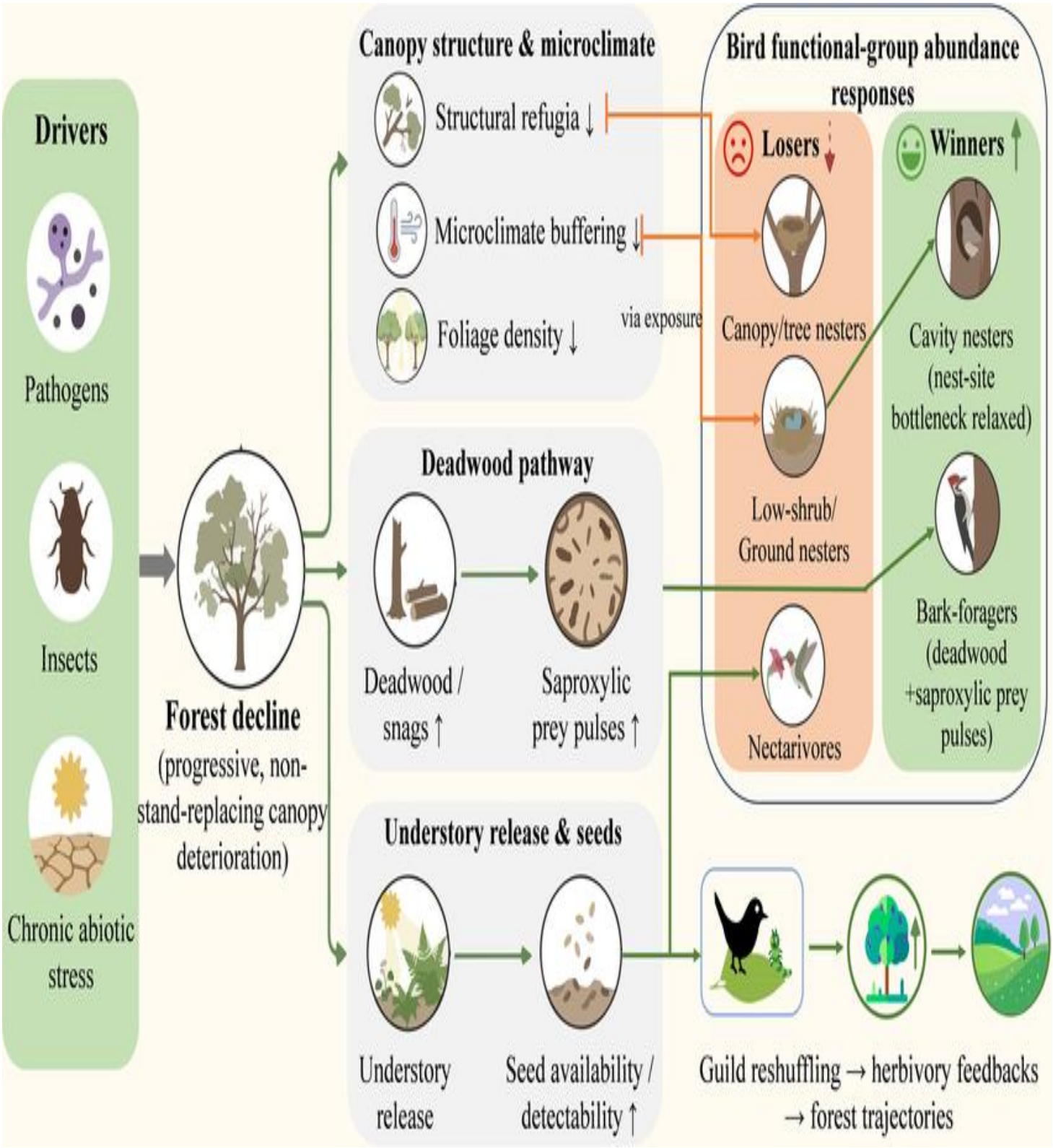


Figure V.1 : Interacting factors in decline (source: Li , et *al*. 2026).

V.4. Main causes of forest decline

Disturbances that arise in forest ecosystems, even in the form of natural disasters, essentially occur in the development cycle. They are at the origin of a local dynamic that allows for the modification of the stand and even the microclimate (Table V. 1.). Disturbances affecting individual trees or groups of trees in a stand, on a small scale, can have a positive or negative effect:

Table V.1 : Assumed effects of storms on ecosystems (Requardt et *al.* ,2007)

Positive effect	**Negative effect**
increase in the quantity of dead wood	Deterioration of vegetation cover,
Restructuring resulting in a diverse forest stand	Affection for the quality of wild habitats
Positive effect on disturbed areas due to increased diversity in fauna and flora	destabilize the timber market
Triggers the process of biological regeneration and adaptation.	Destruction of livelihoods bases and private forest properties.
Promotes diversity	Increased likelihood of insect pest infestations in the years following storms
	Amplification of the impact of storm damage caused by monoculture plantations or poor species selection (Requardt et *al.* , 2007).

The risks of forest fires are often favored and facilitated by the following causes (Table V.2.) (Requardt et *al* ., 2007):

Table V.2: Causes and damage caused by fires **(Requardt et *al.* , 2007)**

Causes of the fires	**Damage recorded after the fires**
Use of the forest for recreation (uncontrolled man-made fires or disposal of heat-absorbing objects) often leads to considerable economic damage and even loss of life.	Impact on the ecological aspect
reforestation and, in part, the spontaneous revegetation of abandoned land	Huge impacts on livelihoods and infrastructure
A rural exodus leading to agricultural decline	
the abandonment of livestock farming and forestry activities and changes in land use.	
Climate change	
Accumulation of piles of combustible materials and/or dense brush susceptible to fire	

V.4.1. Climatic causes of the dieback

According to the European Environment Agency (EEA, 2005), variations in environmental conditions caused by climate change increase the vulnerability of forests and trigger adaptation processes.

The increasing frequency and extent of climatic events (drought, heavy precipitation, changes in wind patterns) affect forests and are followed by other disturbances such as fires, storms, insects and diseases…etc.

The effects of global warming in the Mediterranean region are mainly prolonged drought which plays a role in defoliation, increases the danger of forest fires and contributes to accelerating desertification (Requardt et *al.* , 2007).

Future risks to the health and vitality of the forest ecosystem caused by global warming are largely overlooked in forest management programs. Future instruments must therefore more strongly incorporate mitigation and control measures in addition to existing prevention activities such as

To address the consequences of global warming, it is important to support tree species adapted to the location and to strengthen the forest stand. Control measures may include improving the means of monitoring, assessing and predicting forest fires (Requardt et *al.* , 2007).

V.4.2. Forest Decline due to Insects and Diseases

The health and vitality of forest ecosystems are constantly threatened by massive attacks of insects and phytopathogens (bacteria, viruses, fungi), leading to considerable losses, especially for forest owners. The condition of the tree is damaged by these biotic attacks during the year of onset and even in subsequent years.

The potential target of these parasites is trees already weakened by adverse abiotic conditions such as drought, frost, hail, storms, or fire. This relationship means that these diseases are part of the natural dynamics of forests and can lead to significant destabilization of the stand (Requardt et *al* ., 2007).

Parasitic diseases caused by insects can be favored by extreme weather conditions such as the massive spread of bark beetles in Europe following the drought during the summer of 2003 (IPC Forest, 2004). Specific problems caused by invasive species

International trade is a means of introducing and spreading different animal and plant species beyond their natural zone, space or region (CMPFE, 2003).

A foreign species represents an invasive species whose introduction and spread threaten the habitat, the species and even the ecosystem with damage not only on the environmental level but also socio-cultural, economic and/or damage to human health (CMPFE 2002b).

Experience has shown that the threat posed by non-native tree species to forest ecosystems and their biodiversity, as well as to human interests, is rather limited. It should be emphasized that the introduction of non-native species is carried out primarily for the following reasons:

- for an aesthetically pleasing and functional landscaping design,
- as cover for native species to allow their natural regeneration instead of serving as prey for invasive aggressors (Requardt et *al.* , 2007).

V.4.2.2. Forest Decline due to Wildlife and Livestock

Forest wildlife consumed at least 5% of the forest flora according to the CMPFE (2007), not counting livestock since no information on cattle browsing is currently available (Requardt et *al.* , 2007). *Browsing* " is the consumption of native forage from pasture or grassland by livestock or wildlife," while *nibbling* " is the feeding of livestock or wildlife on buds, shoots, and leaves of shrubs and trees."

V.4.2.3. Damage directly caused by human beings

According to the CMPFE (2007), human activities can affect the ecosystem by:

The decline in wood quality, decomposition, rot, destruction of natural regeneration and soil degradation in addition to damage due to air pollution, traffic, livestock farming; soil erosion and/or hydrological imbalance (Requardt et *al.* , 2007).

V.5. Symptoms of forest decline

Decline is measured either by quantifying the evolution of the growth rate of already weakened trees (Cailleret et *al* ., 2017) or by detailed observation of the crown of an individual (Vuillermet, 2019).

The major symptoms of wasting

Severe magnesium (Mg) and calcium (Ca) deficiency in acidic mountains causes yellowing of trees in acidic mountains (Landmann et al., 1987),

Critical defoliation, collapse of phloem bundles, needles and twigs (Fink, 1983; Parameswaran et *al.* , 1985).

The phenomenon of decline is manifested by the symptoms grouped in the following table (Table V.3.):

Table V.3.: Robust indicators of the level of decline of an individual and observable effects (Vuillermet, 2019).

robust indicators of an individual's level of decline	Observable effects
(1) modification of the canopy architecture.	summit descent, reduction in the length of annual shoots reduction in the connection rate.
(2) reduction of leaf mass.	reduction in the size and number of leaves produced, falling leaves.
3) degradation of their color	browning, blushing
(4) the presence of dead twigs or branches	
(5) in conifers resin flows are observed at the level of the bark (Nageleisen, 1993).	

Several works and studies have established the link between periods of drought (increased temperatures and decreased rainfall) and a

decrease in the radial growth of

forest stands manifested by increased mortality and defoliation rates (Guit et *al*., 2016).

The symptoms of wasting are therefore easily identifiable, and two robust indicators of the level of wasting are generally observable in an individual:

1- Foliage deficit: is considered significant if it exceeds 25%. An individual is considered to be declining when it shows more than 50% defoliation.

the presence of dead branches in the functional crown (Figure V.2): An individual is considered to be declining when it has more than 50% dead branches (Figure V.3) in the functional crown (Guit et *al* ., 2016).

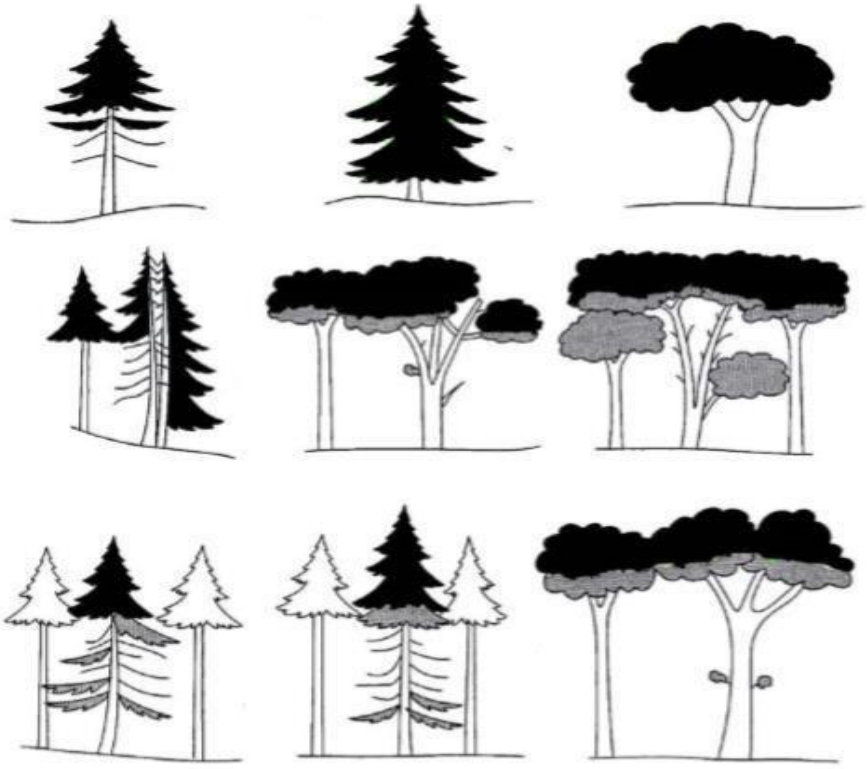
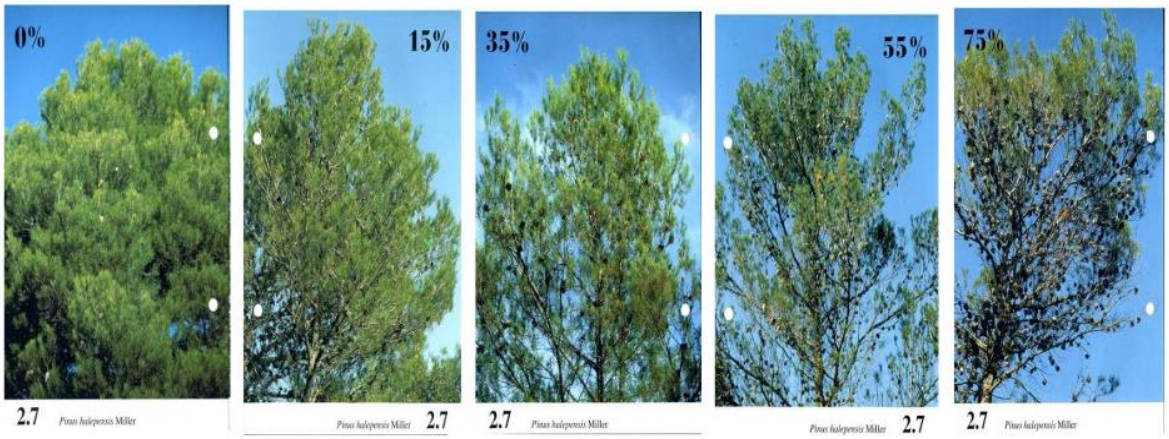


Figure V.2 : Characteristic of the notable (black) crown **(**Vuillermet, 2019)



FigureV.3: Photographs of the leaf deficit estimation for Aleppo pine **(**Vuillermet ,2019).

V.5.1. Climate change

Definition of climate change (Vuillermet, 2019): These **are naturally occurring variations** that unfold over thousands of years **and have been occurring on Earth for millennia, depending on location and time period** . These changes occur under the influence of certain parameters on the climate system, such as volcanic activity and variations in the amount of solar energy received by the Earth. Current climate changes are primarily anthropogenic and occur rapidly, on a timescale of decades (Puget et al., 2010).

Current climate changes worldwide **are resulting in numerous** observable cases of vegetation decline. Mediterranean forest ecosystems, once well-adapted to periods of drought, are suffering considerably from the increased frequency and intensity of these events.

Small trees located in favorable climatic, topographical, and pedological conditions (cool and humid) are the most susceptible to dieback and mortality. The accumulation of dieback cases is followed by the phenomenon of necromass (or sapromass, defined as the mass of dead organic matter present in a given plot, volume, or ecosystem) (Romaric

Forêt, Dico de Bio, De Boeck Supérieur, 2012, p. 854). Necromass significantly increases the risk of fire outbreak and spread, a characteristic of Mediterranean forests (Vuillermet, 2019).

V.5.2. The Ozone Hole and Global Warming

The reduction in the protective effectiveness of the ozone layer is assumed to be the cause of the disruption of development and even the existence or non-existence of the first links in the food chain (Mascle, 1997).

The existence of ozone is essential for maintaining life on Earth and the planet's energy balance, supporting all living beings, whether human, animal, or plant (Seinfeld; Pandis, 2016). The existence of ozone gas was discovered in 1840, and studies have shown it to be the most important trace gas supporting life on Earth due to its ability to absorb ultraviolet (UV) radiation entering the atmosphere. Water vapor (H_2O), carbon dioxide (CO_2) , and oxygen (O_2) are essential to the planet's energy balance (Dobson et al., 1968; Salby et al., 1996). In 1930, Chapman discovered the formation of ozone (O_2) through various processes. Ozone is found in high concentration (about 90% of total ozone) in the stratosphere, at about 15 to 35 km altitude in a region known as the "Ozone Layer" (London et *al* .,1985). Bittencourt et al. (2018) analyzed an extreme ozone depletion event in mid-latitude regions such as the South Pole (Antarctica). This depletion is accompanied by an increase in solar ultraviolet (UV) radiation reaching the Earth's surface (Guarnieri et *al* ., 2004; Laat et *al* ., 2010) . The highest concentration of stratopheric ozone *is* found at the equator, where temperatures are stable throughout the year, meaning that the consequences of a disturbance are only observable at other latitudes, such as mid-latitudes like Antarctica (Bittencourt, 2022).

Ozone is a molecule composed of three oxygen atoms that has the ability to absorb ultraviolet radiation and release energy in the form of heat. Thus, in

the stratosphere, the temperature increases with altitude or its concentration is highest.

Ozone is found primarily in two important regions of the atmosphere:

1. The troposphere, a layer that contains 10% of the ozone and extends from the surface up to about 10-15 km altitude depending on the region observed, up to the tropopause region, O_3 is formed by a different set of chemical reactions involving natural gases and gases from atmospheric pollution. (Fishman et *al* ., 1990). The presence of ozone in this layer can have a negative impact on several aspects, being toxic to human health, causing considerable damage to agriculture and ecosystems, in addition to being an important greenhouse gas on the planet (Bittencourt, 2022).

2. The stratosphere, this layer, contains the majority of the ozone, located between 15 and 50 km altitude. As latitude increases, the temperature rises due to the absorption of ultraviolet radiation by O_3 molecules and the release of ozone. Heat reduces the stratospheric ozone content by at least 1%; in this case, the incidence of UVB increases by up to 2%, further increasing the risks to living beings, including diseases such as skin cancer. Skin due to overexposure to the sun (Seinfeld; Pandis, 2016).

Mario Molina and F. Sherwood Roland discovered the release of human-caused chlorofluorocarbons (CFCs), which are believed to be the main source of ozone-depleting chlorine in the stratosphere (Molina; Rowland et *al* ., 1974). This discovery was awarded the Nobel Prize in Chemistry in 1974. (Bittencourt, 2022).

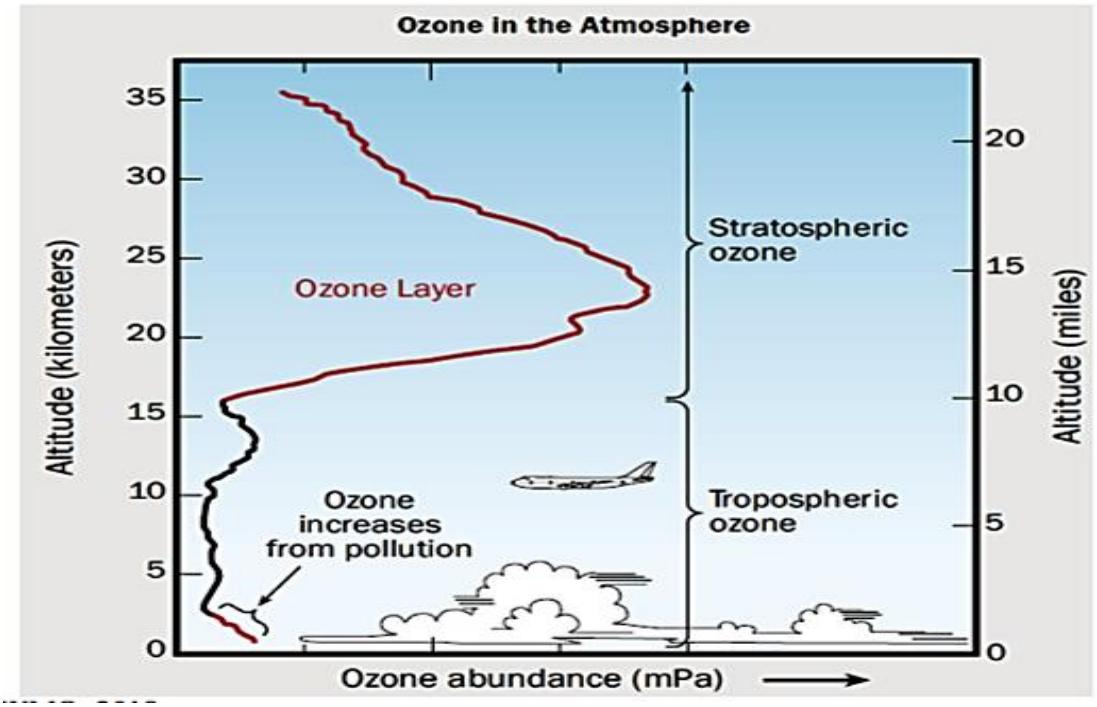


Figure V.4.a.: Vertical profile of ozone in the atmosphere (WMO, 2018).

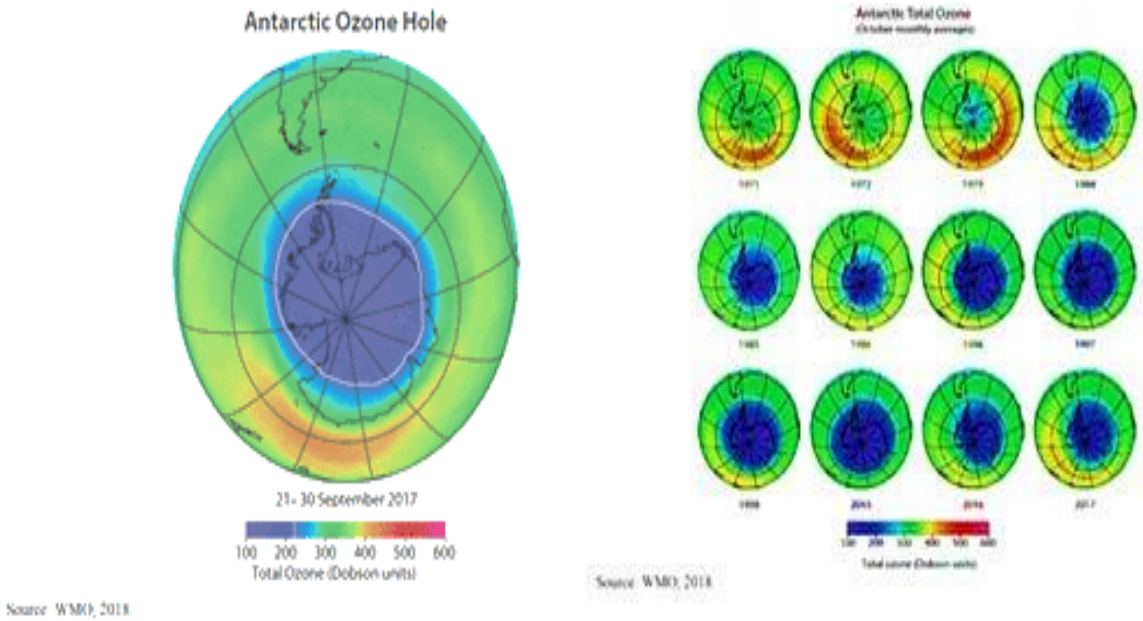


Figure V.4.b.: Variation of total ozone quantities over Antarctica between 1971 and 2017 (Monthly average of October) (WMO, 2018).

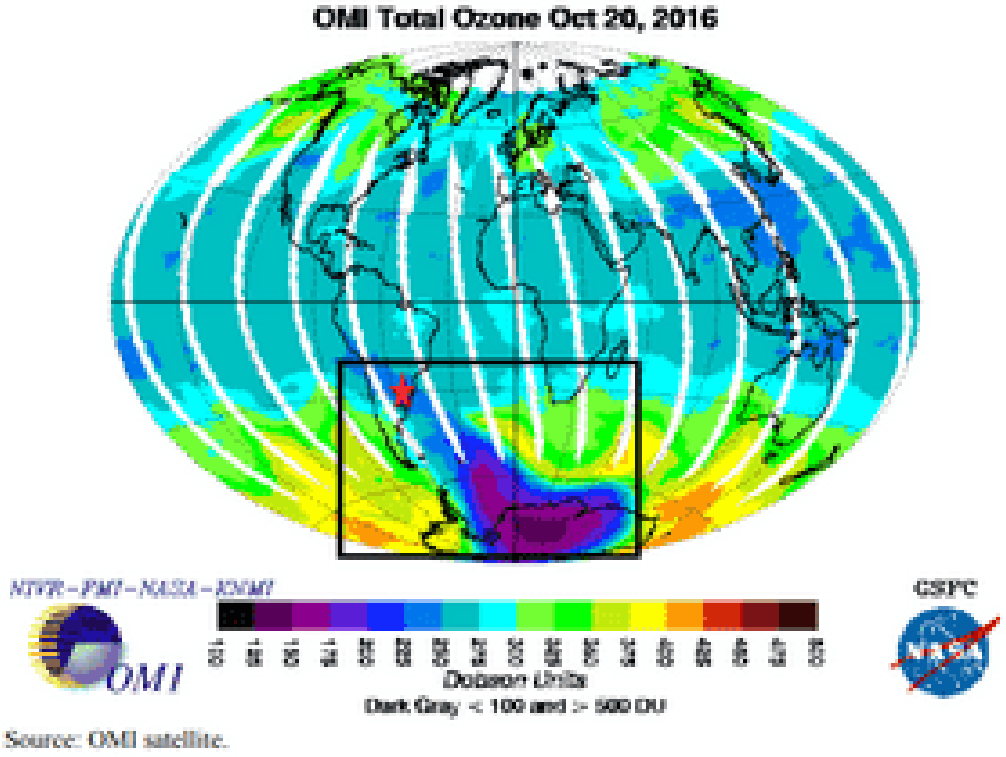


Figure V.4.c.: Distribution of ozone gas throughout the world (Bittencourt, 2022)

V.5.3. Greenhouse effect:

The greenhouse effect is a natural phenomenon that causes an increase in the temperature at the Earth's surface. When a portion of solar radiation, approximately 28.3%, reaches the atmosphere and is directly reflected by clouds, aerosols, the air, and even the Earth's surface, this is referred to as the albedo phenomenon (Vuillermet, 2019).

Most of the incident radiation will be absorbed by the Earth's surface, and approximately 20.7% of it in the atmosphere will be assimilated by

greenhouse gases. The portion of radiation absorbed by the Earth's surface will later be re-emitted as infrared radiation, which will combine with the greenhouse gases and then disperse in all directions, notably towards the Earth's surface, thus creating the greenhouse effect by adding extra heat to the Earth's surface. This allows for an average temperature of 15°C; otherwise, the average temperature would drop to -18°C (**Figure V.5)**. This explains why the greenhouse effect is a natural phenomenon essential for the survival of living beings (Vuillermet, 2019).

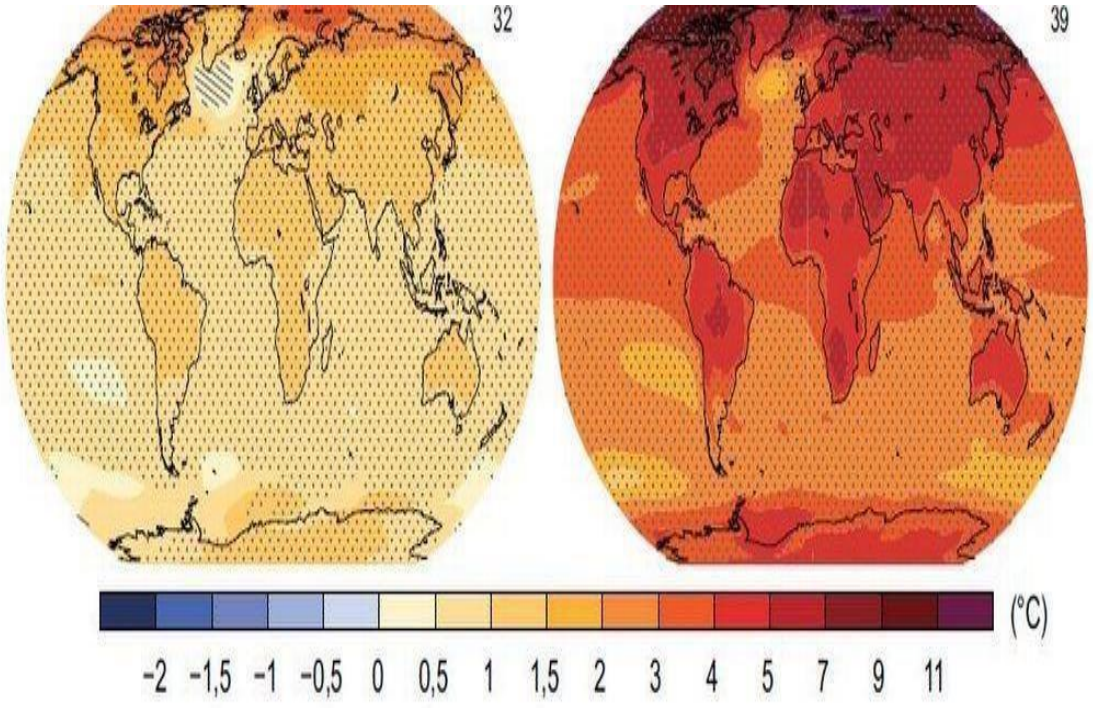


Figure V.5: Evolution of average surface temperature (between 1986-2005 and 2081-2100), according to the RCP 2.6 (left) and RCP 8.5 (right) scenarios.
Source: IPCC, 2014

V.6. Responses of forest ecosystems to climate change

Climate change causes stress in species, which react in various ways to survive, adapt as best as possible, and continue to develop. The ability to adapt is a guarantee of survival for species in changing environments (Bonfils et *al* ., 2015). By definition, adaptation, in general, is the ability of species " *to live and reproduce in a given environment* " (Lefèvre et *al* ., 2015). Ecological flexibility is a characteristic found in all species, reflected in an ability to accommodate environmental situations, accompanied by a capacity for genetic adaptation (Ronce et *al* ., 2015).

The responses of forest ecosystems to climate change are reflected in the reaction of species (Figure V.7.) and vary from one species to another within the same ecosystem.

The different responses of forest ecosystems to climate change can be summarized by the following effects:

1. <u>genetic adaptation</u>:

- A very slow and long process (age and maturity) that can exceed several decades (Verdu, 2002).
- Genetic diversity varies between the same species developing in different ecosystems as well as between individuals within the same population.

2. <u>Accommodation</u>:

- morphological and physiological modifications employed by an organism to cope with a change in its environment (phenotypic plasticity (Dupouey, 2013)

<u>3. Migration</u>:

- the expansion of species' range in response to climate change.
- The slow and unpredictable migrations of trees can be explained by the difficulty that seeds have in being dispersed over long distances and then by the difficulty in colonizing new environments, competing with seeds and plants already present.

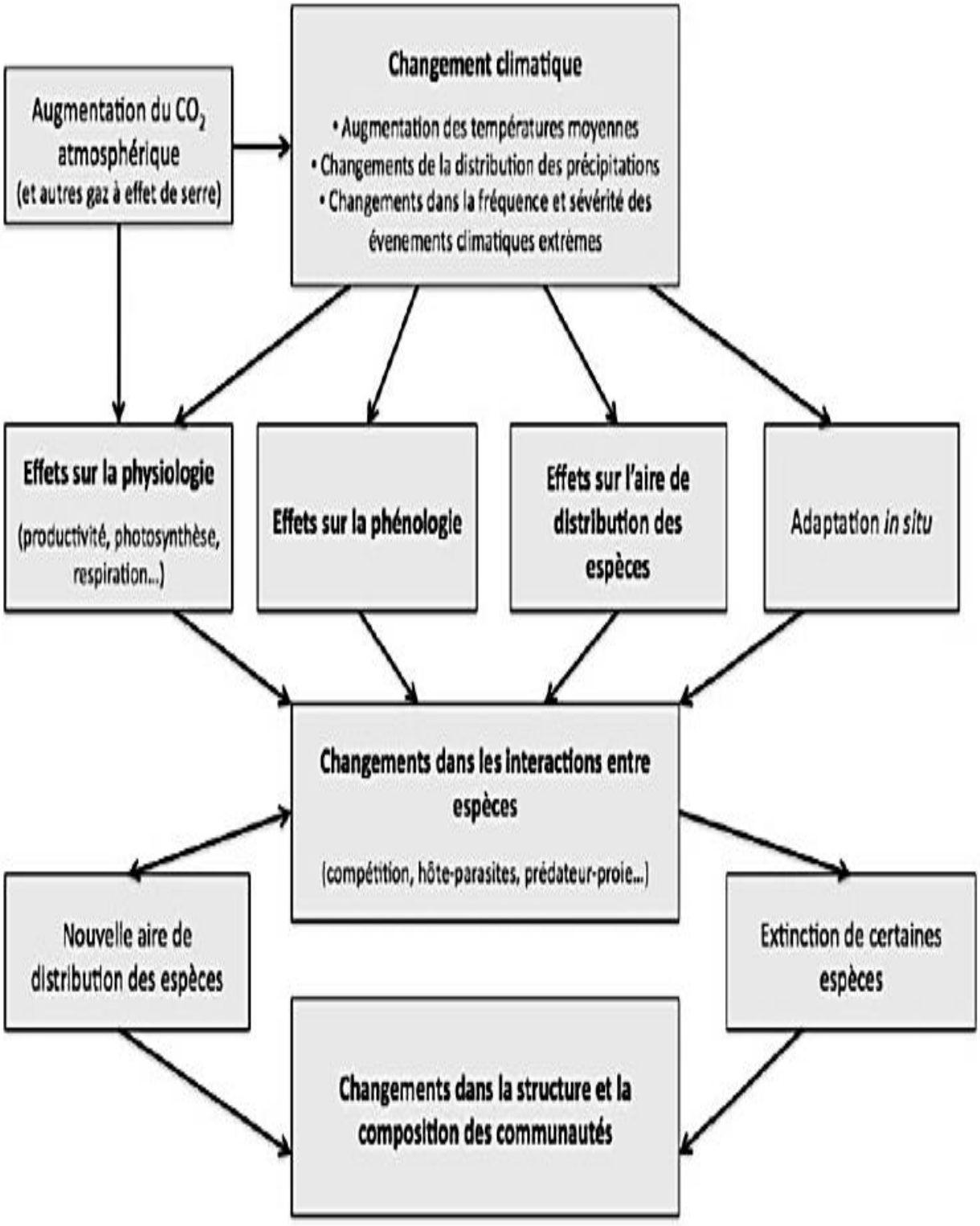


Figure V.7 : Potential pathways of community change due to climate change (source: Hughes, 2000 *in* Vuillermet, 2019)

4.Mortality:

- When individuals' tolerance limits and their capacity to accommodate are exceeded, they will gradually deteriorate and then die.
- Species migrations to the limits of their ranges are generally accompanied by mortality of the species in environments that no longer offer optimal conditions for its development (Dupouey, 2013).

The degree of adaptation to stresses and disturbances therefore depends on the ecological, physiological, or behavioral characteristics of each species relative to others (Ronce et al., 2015). The intensity, frequency, and type of pressure influence and condition the adaptive potential of ecosystems and individuals (Lefèvre et *al.* , 2015; Ronce et *al* ., 2015).

V.6.1. Climate change and its interaction with metal stress responses (example of abiotic stress effect)

Together metal toxicity and climate changes (the levels of climatic temperatures, CO_2 levels in the atmosphere, rain patterns, and extreme weather conditions) damage complex molecular processes in plants, excessive production of ROS, antioxidant defense responses, hormonal change and the induction of stress responses linked to heat shock proteins (Kolhi et *al*., 2026).

High CO_2 , although it may occasionally enhance plant growth, can lead to changes in plant metabolism and exudation patterns of roots, and the rhizosphere microbial community and metal-chelation processes, causing an increase in metal concentrations (Barra Caracciolo and Terenzi, 2021). In addition, climate-induced drought causes soil water deficiency, making metal ions more concentrated in the soil solution and more toxic to the roots, whereas flooding causes anoxia of the soil, altering the redox state and releasing metals ions into soil available forms (Yuan et *al.*, 2024).

A better comprehension of these interactions is essential to develop modified cultivars and implement new agronomic strategies that induce metal stress tolerance in plants (Sytar et *al.*, 2021; Zhang et *al.*, 2024b).

Citographic references :

site I.1:https://mots-agronomie.inra.fr/index.php/Herbicides_agricoles_:_modes_%27 action et_devenir

Site I.2: http://www.fourragesmieux.be/prairie_desherbage_herbicide.html Site**1** : https://www.aquaportail.com/dictionnaire/definition/4850/biotransformation.

Site IV.1.: https://www.canada.ca/fr/environnement-changement-climatique/services/pollution-atmospherique/polluants/principaux-contaminants/oxydes-azote.html

Site IV.2.: https://atmo-reunion.net/Le-monoxyde-de-carbone-CO

Site IV.3.: http://www.eionet.europa.eu/gemet/concept/11734

Site IV.4.: https://www.canada.ca/fr/environnement-changement-climatique/services/pollution-atmospherique/polluants/principaux-contaminants/oxydes-soufre.html

Site IV.5.: https://atmo-reunion.net/Les-metaux-toxiques

SiteIV.6.:https://www.canada.ca/fr/environnement-changement-climatique/services/gestion-substances-toxiques/liste-loi-canadienne-protection-environnement/ozone.html

SiteIV.7.https://www.canada.ca/fr/environnement-changement-climatique/services/pollution-atmospherique/polluants/principaux-contaminants/ozone-tropospherique.html

SiteIV.8.:https://www.canada.ca/fr/environnement-changement-climatique/services/pollution-atmospherique/polluants/principaux-contaminants/ozone-tropospherique.html

Site IV.9.: ecotoxicologie.fr/notions-essentielles

Site IV.10.: https://www.canada.ca/fr/environnement-changement-climatique/rechercher.html?q=acide+sulfurique&wb-srch-sub=

Site IV.11.: https://www.gazdetect.com/informations-gaz/detecteur-gaz-acide-nitrique-hno3/

Site IV.12.: http://www.ssents.uvsq.fr/spip.php?article1157

Site IV.13.: https://www.geo.fr/environnement/quest-ce-que-le-smog-205973

References

Abdo A, Jani N, Cunningham SC (2013) Pancreatic duct disruption and non-operative management: The SEALANTS approach. HepatobiliaryPancreat Dis Int 3:239-43.

Akhtar, K., Ain, N. U., Prasad, P. V., Naz, M., Aslam, M. M., Djalovic, I., ... & Wen, R. (2024). Physiological, molecular, and environmental insights into plant nitrogen uptake, and metabolism under abiotic stresses. *The plant genome*, *17*(2), e20461.

Altaf, M. A., Shahid, R., Ren, M. X., Altaf, M. M., Jahan, M. S., & Khan, L. U. (2021). Melatonin mitigates nickel toxicity by improving nutrient uptake fluxes, root architecture system, photosynthesis, and antioxidant potential in tomato seedling. *Journal of Soil Science and Plant Nutrition*, *21*(3), 1842-1855.

Amari, T., Lutts, S., Taamali, M., Lucchini, G., Sacchi, G. A., Abdelly, C., & Ghnaya, T. (2016). Implication of citrate, malate and histidine in the accumulation and transport of nickel in Mesembryanthemum crystallinum and Brassica juncea. *Ecotoxicology and Environmental Safety*, *126*, 122-128.

Amiard J-C., Amiard-Triquet C. (2017) Biomarqueurs en écotoxicologie aquatique. 2e Edition lavoisier.

Arthur, E., Crews, H., & Morgan, C. (2000). Optimizing plant genetic strategies for minimizing environmental contamination in the food chain: report on the MAFF funded joint JIC/CSL Workshop held at the John Innes Centre, October 21-23, 1998. *International journal of phytoremediation*, *2*(1), 1-21.

Aslam F (2021) Toxicity of Peroxyacetyl Nitrate on Human. Glob J Res Rev Vol.8 No.2: 60 (https://www.imedpub.com/articles/toxicity-of-peroxyacetyl-nitrate-on-human.php?aid=35918)

Aslam F (2021). Glob J Res Rev Vol.8 No.2: 60.Nitrate on Human Strategy of pancreatic injury. Japan Expert Trauma Evaluation and Care. Tokyo: The Herusu Press 100-3.

Asse D. (2012) dépérissement forestier et changement climatique : que nous enseignent les observations récents de la forêt française. Université Paris sud France.

Baba T., Ito S., Yuasa M., Yoshioka E., Miyashita C., Araki A., Sasaki S., Kobayashi S., Kajiwara J., Hori T., Kato S., Kishi R..(2018) Association of prenatal exposure to PCDD/Fs and PCBs with maternal and infant thyroid hormones: The Hokkaido Study on Environment and Children's Health, Science of The Total Environment, Volume 615, 2018, Pages 1239-1246, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2017.09.038.

Barra Caracciolo, A., & Terenzi, V. (2021). Rhizosphere microbial communities and heavy metals. *Microorganisms*, *9*(7), 1462.

Barra Caracciolo, A., & Terenzi, V. (2021). Rhizosphere microbial communities and heavy metals. *Microorganisms*, *9*(7), 1462.

bashar hmk, juraimi as, ahmad-hamdani ms, uddin m.k, asib n, anwar m.p, et al. (2023) evaluation of allelopathic effects of *parthenium hysterophorus* l. methanolic extracts on some selected plants and weeds. *plos one* 18(1): e0280159.

Bergeron, V., Bonn, D., Martin, J. Y., & Vovelle, L. (2000). Controlling droplet deposition with polymer additives. *Nature*, *405*(6788), 772-775.

Beyer J., Petersen K., Song Y., Ruus A., Grung Merete, Bakke T., Tollefsen K. E. (2014) *Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper* Marine Environmental Research 96 (2014) 81e91

Bhat, B. A., Rather, M. A., Bilal, T., Nazir, R., Qadir, R. U., & Mir, R. A. (2025). Plant hyperaccumulators: a state-of-the-art review on mechanism of heavy metal transport and sequestration. *Frontiers in Plant Science*, *16*, 1631378.

Bian B., Lv L., Yang D., Zhou L ;. (2014) Migration of heavy metals in vegetable farmlands amended with biogas slurry in the Taihu Basin, China. Ecological Engineering 71 (2014) 380–383. http://dx.doi.org/10.1016/j.ecoleng.2014.07.026 0925-8574/Crown Copyright © 2014 Published by Elsevier B.V. All rights reserved.

Bittencourt G. D. (2022) Influence du trou d'ozone antarctique et la dynamique atmosphérique sur l'ozone dans le sud du Brésil. Océan, Atmosphère. Université de la Réunion; Universidade federal de Santa Maria (Brésil), 2022. Français. ffNNT : 2022LARE0018ff. fftel-03783737f

Bolan, N. S., Khan, M. A., Donaldson, J., Adriano, D. C., & Matthew, C. (2003). Distribution and bioavailability of copper in farm effluent. *Science of the Total Environment*, *309*(1-3), 225-236.

Bonfils, P., Rigling, A., Brändli, U. B., Brang, P., Forster, B., Engesser, R., ... & Günthardt-Goerg, M. S. (2015). Le chêne face aux changements climatiques. *Perspectives d'avenir d'une essence. Not. prat*, *55*, 12.

Bonneau M. (1989) Que sait-on maintenant des causes du " dépérissement " des forêts ?. Revue. forestière française, AgroParisTech, 1989, 41 (5), pp.367-386. 10.4267/2042/25988. hal-03425171

Brian, J. V., Harris, C. A., Scholze, M., Kortenkamp, A., Booy, P., Lamoree, M., ... & Sumpter, J. P. (2007). Evidence of estrogenic mixture effects on the reproductive performance of fish. *Environmental science & technology*, *41*(1), 337-344.

Cailleret, M., Jansen, S., Robert, E. M., Desoto, L., Aakala, T., Antos, J. A., ... & Martínez-Vilalta, J. (2017). A synthesis of radial growth patterns preceding tree mortality. *Global change biology*, *23*(4), 1675-1690.

Cavet R., Barriuso E., Bedos C., Benoit P., Charnay M.-P., Coquet Y.(2005) les pestticides dans le sol : conséquences agronomiques et environnementaleseditions

France Agricole.
Chandrakar, V., Yadu, B., Xalxo, R., Kumar, M., & Keshavkant, S. (2020). Mechanisms of plant adaptation and tolerance to metal/metalloid toxicity. In *Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives II: Mechanisms of Adaptation and Stress Amelioration* (pp. 107-135). Singapore: Springer Singapore.
Clarkson T.W. (1993). *Molecular and ionic mimicry of toxic metals.Annualreview of pharmacology and toxicology,* 33, 545-573.
CMPFE, 2002b: Bollmann, K., & Braunisch, V. 1.1 Systèmes intégratifs ou ségrégatifs: trouver un équilibre entre production de biens et conservation de la biodiversité dans les forêts européennes. *Les approches intégratives en tant qu 'opportunité de conservation de la biodiversité forestière*, 18.
CMPFE, 2003, State of Europe's forests 2003, The CMPFE Report on Sustainable Forest Management in Europe, 114 p.
CMPFE, 2007: State of Europe's Forests 2007, The CMPFE report on sustainable forest management in Europe, Jointly prepared by the CMPFE Liaison Unit Warsaw, UNECE and FAO, Warsaw 2007, 263 p
Depledge, M. H., & Fossi, M. C. (1994). The role of biomarkers in environmental assessment (2). Invertebrates. *Ecotoxicology*, *3*(3), 161-172.
Dobson, G. M. (1968). Forty years' research on atmospheric ozone at Oxford: a history. *Applied Optics*, *7*(3), 387-405.
Duarte-Hospital, C., Huc, L., Bortoli, S., & Coumoul, X. (2019). Les xénobiotiques, quel impact sur les maladies métaboliques?. *Cahiers de Nutrition et de Diététique*, *54*(5), 286-293.
Dupouey, J. L. (2013). Forêts et changement climatique, quelques éléments issus de la recherche. *Forêt Entreprise*, (211), 24-29.
Dupuy, J. (2014). *Interactions entre les hydrocarbures aromatiques polycycliques et les plantes supérieures: prélèvement et réponses toxiques* (Doctoral dissertation, Université de Lorraine).
EEA, 2005: Vulnerability and adaptation to climate change in Europe. European Environmental Agency, Technical Report 7/2005. 79 p.
Fishman, J., Watson, C. E., Larsen, J. C., & Logan, J. A. (1990). Distribution of tropospheric ozone determined from satellite data. *Journal of Geophysical Research: Atmospheres*, *95*(D4), 3599-3617.
Forbes V.E. et Forbes T.L. (1997) ecotoxicologie théorie et applications. INRA France Ed. P21, P31, p40, 78
Forests, I. C. P. (2007). The conditions of forests in Europe–2004 Executive report. *Federal Research Centre for Forestry and Forest Products: Hamburg. 36p*.
Franco, H., Celis, C., Forero, S., Pombo, L. M., & Rodríguez, O. E. (2018).

Phytoremediating activity of Baccharis latifolia in soils contaminated with heavy metals. *Int. J. Curr. Pharmaceut. Rev. Res*, *9*(4), 38-43.

Fusaro, L., Lanni, F., Varone, L., Falasca, G., Serranti, S., Gasbarrone, R., ... & Manes, F. (2025). Functional traits as indicators of maize (*Zea mays* L.) strategies to cope with Zn, Pb and Cr heavy metal-induced stress. *Chemical and Biological Technologies in Agriculture*, *12*(1), 82.

Gasquez J., (1981) Evolution de la résistance aux triazines chez les espèces annuelles: la résistance chloroplastique. Compte Rendu 11ème Conférence COLUMA, Tome IV, 981-1008

Gauvrit, C. (1996). Efficacité et sélectivité des herbicides.

Gomez-Ros, J. M., Garcia, G., & Peñas, J. M. (2013). Assessment of restoration success of former metal mining areas after 30 years in a highly polluted Mediterranean mining area: Cartagena-La Unión. *Ecological engineering*, *57*, 393-402.

Goncharuk, E. A., & Zagoskina, N. V. (2023). Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium. *Molecules*, *28*(9), 3921.

Guit, B., Nedjimi, B., Chakali, G., & Guibal, F. (2016). État sanitaire des peuplements de Pin d'Alep (Pinus halepensis Mill.) dans le massif forestier de Senalba (Djelfa, Algérie). *Revue d'écologie*, *71*(2), 156-167.

Hayes W.J., Jrand Laws E. R. (1991) Handbook of pesticides toxicology. Academic Press, San Diego, CA.

Hazen, J. L. (2000). Adjuvants—terminology, classification, and chemistry. *Weed technology*, *14*(4), 773-784.

Heap I., (2007) - The International Survey of Herbicide Resistant Weeds. Online. Internet.

Hossain, M. A., Piyatida, P., da Silva, J. A. T., & Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of botany*, *2012*(1), 872875.

Hu, Z., Zhao, C., Li, Q., Feng, Y., Zhang, X., Lu, Y., ... & Ji, W. (2023). Heavy metals can affect plant morphology and limit plant growth and photosynthesis processes. *Agronomy*, *13*(10), 2601.J Trauma 72:100-5.

Hussain, M.I.; Danish, S.; Sánchez-Moreiras, A.M.; Vicente, Ó.; Jabran, K.; Chaudhry, U.K.; Branca, F.; Reigosa, M.J. (2021) Unraveling *Sorghum* Allelopathy in Agriculture: Concepts and Implications. Plants, 10,1795.

Islam, A.K.M.M., Suttiyut, T., Anwar, M.P., Juraimi, A.S., Kato-Noguchi, H. (2022) Allelopathic Properties of *Lamiaceae* Species: Prospects and Challenges to Use in Agriculture. Plants, 11, 1478.

Jabran, K. (2017). Maize allelopathy for weed control. In: K. Jabran, Manipulation of Allelopathic Crops for Weed Control. Springer Briefs in Plant Science, Springer

International. Publishing AG, Switzerland. pp. 29-34.
JETEC: Japan Expert Trauma Evaluation and Care (2014) Toxicity of Peroxyacetyl.
John PS, Louis JM, Jordan AW, Ben LZ, Stickley SM, et al. (2012) Impact of a defined management algorithm on outcome after traumatic pancreatic injury.
Kenneth DB (2016) Pancreas the Japanese society for the acute care surgery, The Japanese Association for The Surgery of Trauma. DSTC. Tokyo: IgakushoinPress 151-61.
Kilinc O. (2010) Etude du mode d'action et du devenir d'un herbicide : l'aclonifen. Biologie végétale. Université Joseph-Fourier - Grenoble I. Français. tel-00554905. https://tel.archives-ouvertes.fr/tel-00554905 Submitted on 11 Jan 2011.
Klaassen C.D. and Eaton, D.L. (1986).Principle of toxicology. In: Casarett and Doull's Toxicology: The basic Science of poisons, 4thed (Eds M.O. Amdur, J. Doull and C.D. Klaassen), pp. 12-49. Pergamon Press, New York.
Kolhi, M. L., Hussain, A., Junejo, A. R., Zhang, L., Gu, Y., Zhu, Y., ... & Liu, H. (2026). Mechanistic insights into heavy metals detoxification and signaling in plants: Molecular coordination and stress tolerance an updated overview. *Plant Stress*, 101284.
Krige JEJ, Kotze UK, Setshedi M, Nicol AJ, Navsaria PH (2015) Prognostic factors, morbidity and mortality in pancreatic trauma: A critical appraisal of 432 consecutive patients treated at a Level 1 Trauma center. Injury 46:830–36.
Kumar, K., Shinde, A., Aeron, V., Verma, A., & Arif, N. S. (2023). Genetic engineering of plants for phytoremediation: advances and challenges. *Journal of Plant Biochemistry and Biotechnology*, *32*(1), 12-30.
Kumar, N., Bauddh, K., Kumar, S., Dwivedi, N., Singh, D. P., & Barman, S. C. (2013). Accumulation of metals in weed species grown on the soil contaminated with industrial waste and their phytoremediation potential. *Ecological engineering*, *61*, 491-495.
Kumar, P., Goud, E. L., Devi, P., Dey, S. R., & Dwivedi, P. (2022). Heavy metals: transport in plants and their physiological and toxicological effects. In *Plant metal and metalloid transporters* (pp. 23-54). Singapore: Springer Nature Singapore.
Landmann, G. (1994). Concepts, définitions et caractéristiques générales des dépérissements forestiers. *Revue forestière française*, *46*(5), 405-415.
Landmann, G., Bonneau, M., & Adrian, M. (1987). Le dépérissement du Sapin pectiné et de l'Epicéa commun dans le massif vosgien est-il en relation avec l'état nutritionnel des peuplements?. *Revue forestière française*, *39*(1), 5-11.
Le Meignen, P., & Micas, L. (2008). Bilan des dépérissements forestiers dans les Alpes-de-Haute-Provence. *Forêt méditerranéenne*, *29*(2), 177-182.
Lefèvre, F., Fady, B., Jean, F., Davi, H., Pichot, C., & Oddou-Muratorio, S. (2015). Les processus biologiques de réponse des arbres et forêts au changement climatique:

adaptation et plasticité phénotypique. *Innovations Agronomiques (47), 63-79*.(2015).

Leonardo, B., Emanuela, T., Letizia, M. M., Antonella, M., Marco, M., Fabrizio, A., ... & Adriana, C. (2021). Cadmium affects cell niches maintenance in Arabidopsis thaliana post-embryonic shoot and root apical meristem by altering the expression of WUS/WOX homolog genes and cytokinin accumulation. *Plant Physiology and Biochemistry*, *167*, 785-794.

Leroux P., C. R. Biologies 326 (2003).*Published by Académie des sciences/Éditions scientifiques et médicales Elsevier SAS.*

Li, J., Dong, R., Jia, Y., Huang, J., Zou, X., An, N., ... & Chen, Z. (2021). Characterization of metal tolerance proteins and functional analysis of GmMTP8. 1 involved in manganese tolerance in soybean. *Frontiers in Plant Science*, *12*, 683813.

Li, L., Zhang, X., Guo, X., Jiang, J., Li, Y., Maqsood, I., & Rong, K. (2026). Forest Decline Differentially Affects Bird Abundance Across Functional Groups: A Meta-Analysis. *Animal Conservation*.

London, J. Observed distribution of atmospheric ozone and its variations. In: Whitten, R. C., & Prasad, S. S. (1985). Ozone in the free atmosphere. *New York*.

Manion P.D. (1981) *Tree disease concepts,* Englewood Cliffs N.J., Prentice Hall, 1981, 324 p.

Mascle, G. (1997). Crise de la biosphère et trou d'ozone: une hypothèse nouvelle. *Geobios*, *30*, 373-378.

Matayoshi, C. L., Pena, L. B., Arbona, V., Gomez-Cadenas, A., & Gallego, S. M. (2022). Biochemical and hormonal changes associated with root growth restriction under cadmium stress during maize (Zea mays L.) pre-emergence. *Plant Growth Regulation*, *96*(2), 269-281.

Molina, M. J., & Rowland, F. S. (1974). Stratospheric sink for chlorofluoromethanes: chlorine atom-catalysed destruction of ozone. *Nature*, *249*(5460), 810-812.

Montoya Oliver, J. M., & Garcia, M. L. M. (1995). Le dépérissement des chênes méditerranéens en Espagne. *Revue forestière française*, *67*(4), 401-402.

Moriarty, F. (1983) Ecotoxicology : The Study of Polluants in Ecosystems, Academic Press, London.

Mougin C., Jolivalt C., Chaplain V.. (2001) Biotransformation fongique des xénobiotiques : du sol au gène. Rencontres des microbiologistes de l'INRA, May 2001, Dourdan, France. ffhal-01600764f

Nageleisen, L. M. (1994). Dépérissement actuel des chênes. *Revue forestière française*, *46*(5), 504-511.

Nihlhgard (B .) . — The ammoniom hypothesis . An additionnai explanation to the forest dieback in Europe . — Ambio, vol . XIV, n° 1, 1985, pp . 2-8. *In* Bonneau, M. (1989). Que sait-on maintenant des causes du «dépérissement» des forêts?. *Revue forestière française*, *41*(5), 367-386.

Nwariaku FE, Terracina A, Mileski WJ, Minei JP, Carrico CJ (1995) Is octreotide

beneficial following pancreatic injury? Am J Surg 170:582-5
Ouvrard S.(2016) Phytoremédiation des hydrocarbures aromatiques polycycliques (HAP) dans les sols industriels : de la rhizodégradation à la rhizoatténuation. Ingénierie de l'environnement. Université de Lorraine, 2016. tel-02445869].
Parameswaran, N., Fink, S., & Liese, W. (1985). Feinstrukturelle Untersuchungen an Nadeln geschädigter Tannen und Fichten aus Waldschadensgebieten im Schwarzwald. *European journal of forest pathology*, *15*(3), 168-182.
Pelletier E ; Campbell P. G.C., Denizeau F. (2004) Ecotoxicologie moléculaire : principes fondamentaux et perspectives de développement. Presses de l'université du Québec .p181.
Pichard A. **(2003)** Hydrocarbures Aromatiques Polycycliques (HAPs) Évaluation de la relation dose-réponse pour des effets cancérigènes : Approche substance par substance (facteurs d'équivalence toxique - FET) et approche par mélanges Évaluation de la relation dose-réponse pour des effets non cancérigènes : Valeurs Toxicologiques de Référence (VTR) Rapport Final INERIS
Pichard, B. D. A., & des Risques Chroniques, D. (2003). Hydrocarbures Aromatiques Polycycliques (HAPs).
Puget, J. L., Blanchet, R., Salençon, J., & Carpentier, A. (2010). Le changement climatique. Académie des sciences. Synthèse des débats sur le climat, 20.
Ramade F. (2007) introduction à l'écotoxicologie fondement et application. Edition TEC & DOC. Lavoisier.
Ray, D.R. (1991) Pesticide derived from plants and other organisms. In: Handbook of pesticide toxicology, Vol.2 (Eds W.J. Hayes Jr and E. R. Laws Jr.), pp. 585-636. Academic Press, San Diego, CA.
Ray, D.R. (1991) Pesticide derived from plants and other organisms. In: Handbook of pesticide toxicology, Vol.2 (Eds W.J. Hayes Jr and E. R. Laws Jr.), pp. 585-636. Academic Press, San Diego, CA.
Requardt A.;Poker J.; KöhlM.(2007) Étude de faisabilité sur les moyens de lutte contre le dépérissement forestier dans l'Union Européenne Rapport Technique BFH, EFI 2007
Roelofs, J. G. M., & Van Dijk, H. F. G. (1986). The effect of airborne ammonium deposition on canopy ion-exchange in coniferous trees. *Direct effects of dry and wet deposition on forest ecosystems—in particular canopy interactions. Air pollution research report*, (4), 24-29.
Ronce, O., Boyen, C., Caillon, S., Charmantier, A., Cheptou, P. O., Chuine, I., ... & Weill, M. (2015). Réponses et adaptations aux changements globaux: quels enjeux pour la recherche sur la biodiversité? Prospective de recherche.
Ronce, O., Boyen, C., Caillon, S., Charmantier, A., Cheptou, P. O., Chuine, I., ... & Weill,

M. (2015). Réponses et adaptations aux changements globaux: quels enjeux pour la recherche sur la biodiversité? Prospective de recherche.

Salby, M. L. (1996). Fund, Atmos. Phys. *International geophysics series, Academic Press*, *61*, 29-54.

Seigneur C. Émissions des polluants atmosphériques. Laboratoire commun École des Ponts ParisTech. http://cerea.enpc.fr/fich/support_cours/POLU1_2012-2013/Emissions.pdf

Seinfeld, J. H., & Pandis, S. N. (2016). *Atmospheric chemistry and physics: from air pollution to climate change*. John Wiley & Sons.

Sekomo, C. B., Nkuranga, E., Rousseau, D. P., & Lens, P. N. (2011). Fate of heavy metals in an urban natural wetland: the Nyabugogo Swamp (Rwanda). *Water, Air, & Soil Pollution*, *214*(1), 321-333.

Seregin, I. V., & Kozhevnikova, A. D. (2024). The role of low-molecular-weight organic acids in metal homeostasis in plants. *International journal of molecular sciences*, *25*(17), 9542.

Shehu I., Bajraktari N., Demaku S., Bekolli A., Malsiu A. (2016) The Study of Absorption of Heavy Metals from the Soil at Some Vegetables in Anadrinia Region in Kosovo. Int. J. Pharm. Sci. Rev. Res., 40(1), September – October 2016; Article No. 59, Pages: 324-329 ISSN 0976 – 044X

Shehzad, J., Khan, I., Zaheer, S., Farooq, A., Chaudhari, S. K., & Mustafa, G. (2023). Insights into heavy metal tolerance mechanisms of Brassica species: physiological, biochemical, and molecular interventions. *Environmental Science and Pollution Research*, *30*(50), 108448-108476.

Srivastava, R., Singh, Y., White, J. C., & Dhankher, O. P. (2024). Mitigating toxic metals contamination in foods: bridging knowledge gaps for addressing food safety. *Trends in food science & technology*, *153*, 104725.

Sytar, O., Ghosh, S., Malinska, H., Zivcak, M., & Brestic, M. (2021). Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants. *Physiologia plantarum*, *173*(1), 148-166.

Tabassum, Jeena, A. S., & Pandey, D. (2021). Metal induced genotoxicity and oxidative stress in plants, assessment methods, and role of various factors in genotoxicity regulation. In *Induced genotoxicity and oxidative stress in plants* (pp. 133-149). https:// doi.org/10.1007/978-981-16-2074-4-5..

Tang, Z., Wang, H. Q., Chen, J., Chang, J. D., & Zhao, F. J. (2023). Molecular mechanisms underlying the toxicity and detoxification of trace metals and metalloids in plants. *Journal of Integrative Plant Biology*, *65*(2), 570-593.

Tissut, M., Delval, J. M., & Ravanel, P. (2006). Plantes herbicides et désherbage ACTA Paris France pp 635.

Truhaut R. (1977) Ecotoxicology :*Objectives, principles and perspectives. Ecotoxicol. Env. Safety*, 1, 151-73.

USEPA, 2000.Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. US Environmental Protection Agency, Washington DC, p. 209.

Van Coillie R. & Parent L. (2011) Ecotoxicologie générale et appliquée Ed. Télé-université (TELUQ) (p229). p 194- 229.

Van Coillie, 2004b *In* Van Coillie R. & Parent L. (2011) Ecotoxicologie générale et appliquée. Ed. Télé-université (TELUQ) (p229) vol . 15, 1985, pp . 168-182.

Verdú, M. (2002). Age at maturity and diversification in woody angiosperms. *Evolution*, *56*(7), 1352-1361.

Vuillermet F. (2019) Dépérissement de la végétation et production de biomasse morte des forêts méditer- ranéennes françaises : rôle des conditions topographiques et climatiques. Sciences de l'environnement. 2019. hal-02609690 p (13-59).

Wang, G., Su, M. Y., Chen, Y. H., Lin, F. F., Luo, D., & Gao, S. F. (2006). Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. *Environmental pollution*, *144*(1), 127-135.

Weed management Principles and practices (OP Gupta)

WMO. *World Meteorological Organization: Scientific Assessment of Ozone Depletion. Estados Unidos da América*, 2018.

Wood R. J. (1981) *Insecticide resistance : Genes and mechanismes. In: Genetic Consequences of Man Made Change* (Eds J. A. Bishop and L.M. Cook), pp. 97-128. Academic Press, London.

Xin, J., Huang, B., Yang, Z., Yuan, J., & Zhang, Y. (2013). Comparison of cadmium subcellular distribution in different organs of two water spinach (Ipomoea aquatica Forsk.) cultivars. *Plant and soil*, *372*(1), 431-444.

Yan, L., Riaz, M., Liu, J., Yu, M., & Cuncang, J. (2022). The aluminum tolerance and detoxification mechanisms in plants; recent advances and prospects. *Critical Reviews in Environmental Science and Technology*, *52*(9), 1491-1527.

Yuan, Z., Cai, S., Yan, C., Rao, S., Cheng, S., Xu, F., & Liu, X. (2024). Research progress on the physiological mechanism by which selenium alleviates heavy metal stress in plants: a review. *Agronomy*, *14*(8), 1787.

Zhang, B., Li, Z., Feng, Y., Qaharaduqin, S., Liu, W., & Yan, Y. (2024). Impact of Cd and Pb on the photosynthetic and antioxidant systems of Hemerocallis citrina Baroni as revealed by physiological and transcriptomic analyses. *Plant Cell Reports*, *43*(9), 226.

Zhang, M., Cui, L., Sheng, L., & Wang, Y. (2009). Distribution and enrichment of heavy metals among sediments, water body and plants in Hengshuihu Wetland of Northern China. *Ecological engineering*, *35*(4), 563-569.