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ENSE- 654

Ecophysiology and Ecotoxicology

ENSE-654

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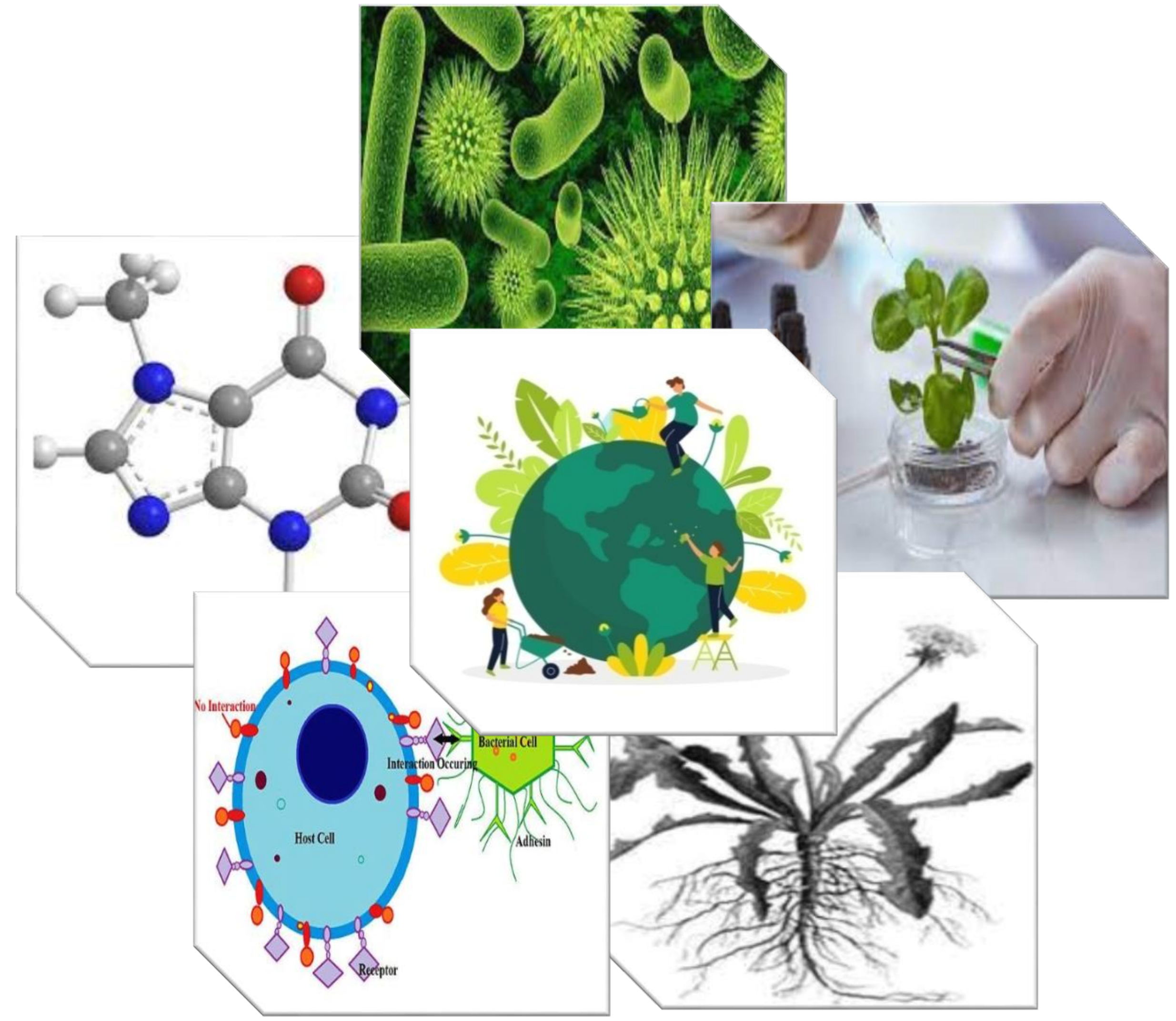

Department of Forestry and Environmental Science
School of Earth and Environmental Science

Uttarakhand Open University
Haldwani, Nainital (U.K.)

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Ecophysiology and Ecotoxicology

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Unit 1: Ecophysiology Introduction and Ecological Interactions

Unit Structure

1.0 Objectives

After reading this unit students should be able to:

- Understand about fundamentals of Ecology
- Understand about Ecological Interactions
- Describe the types of interactions
- Understand about the Mutualism and Co evolution
- Understand about the alleopathy

1.1 Introduction

Ecophysiology, also known as environmental physiology or physiological ecology, is a branch of biology that examines how the physiology of an organism reacts to its surroundings. It shares several similarities with evolutionary and comparative physiology. It's common to use the term "bionomy," which was coined by Ernst Haeckel.

The ecophysiology examines how organisms adapt to various ecological situations in order to operate properly and ensure the survival of the population. In essence, the abiotic environment and other organisms in its environment exist, are connected to, and influence the life of an organism. The environmental variables directly affect how an organism functions. We were tightly tied to non-living elements in our surroundings, such as temperature and other climatic elements, such as. The outside climate impacts how we live, therefore as humans, we dress accordingly—wearing cozy wool clothes in the winter and airy cotton clothing in the summer.

An experimental science called ecophysiology seeks to explain the physiological mechanisms underlying ecological observations. In other words, eco-physiologists, also referred to as physiological ecologists, research the interactions between plants and their physical, chemical, and biotic environments to learn how these interactions impact plant growth, reproduction, survival, abundance, and geographic distribution. By utilizing these ecophysiological patterns and mechanisms, we are able to appreciate the evolutionary history of certain plant traits as well as their functional significance. In its broadest definition, "ecology" refers to concerns from the environmental sciences, horticulture, forestry, and agriculture. These topics are addressed by ecophysiologists at a higher level of integration.

However, the mechanistic comprehension of the eco-physiological explanations frequently requires a lower level of integration (physiology, biochemistry, biophysics, molecular biology). Therefore, an understanding of ecological issues as well as biophysical, biochemical, and molecular techniques and processes is needed for an eco-physiologist. Additionally, an eco-physiological viewpoint is advantageous for a variety of societal challenges, frequently involving agriculture, environmental development, or nature preservation. Thus, a modern ecophysiologist needs to have a solid grasp of both the molecular elements of plant processes and the functioning of the complete pl**ant in its environmental perspective**.

1.2 Plant Physiology

The plant is the source of food, fiber, clothing, fuel for homes, medicine, and oxygen. These resources are all the outcomes of plant physiology. All of the aforementioned resources are created through photosynthesis, the fundamental and core mechanism in plants.

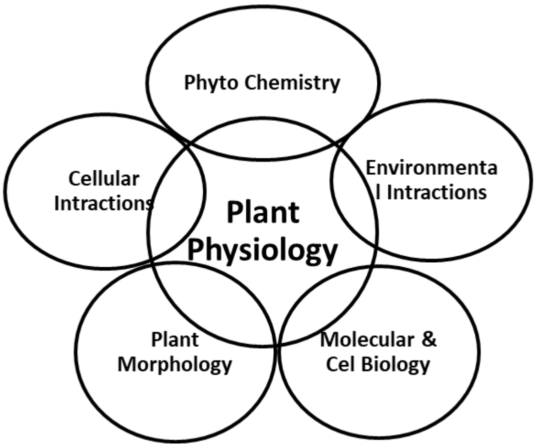


Figure 1: Different plant science areas studied under plant physiology.

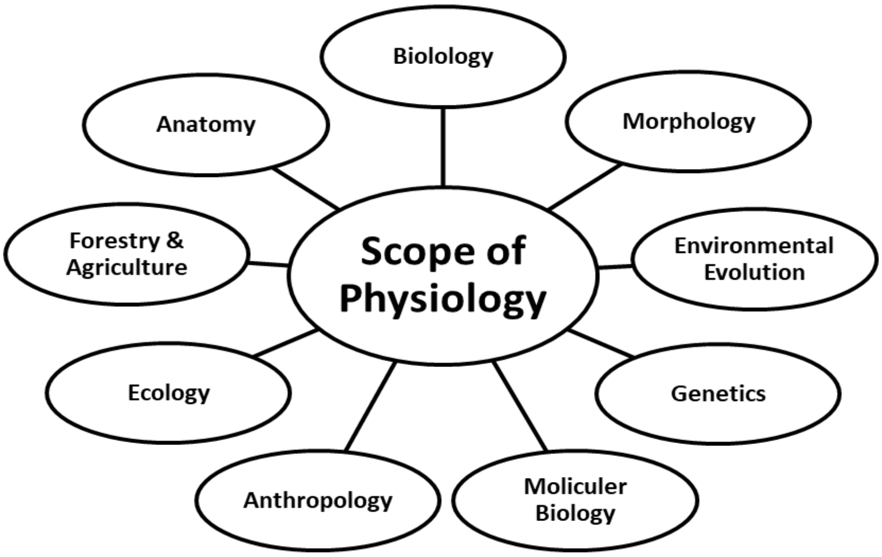
Plant physiology focuses on the various metabolic pathways and procedures that occur in plants. It serves as the hub of botany, the study of the different functions that plants do. It investigates the structure and operation of all the physiological, biochemical, and enzymatic processes of a plant.

1.3 The Scope and Importance of Physiology

- Physiological processes have a significant impact on a number of interactions between plants and animals. Whether on intention or by mistake, man changes his environment, and this change affects how plants behave physiologically.
- It helps to understanding different physiological process such as Seed germination, Growth and development, Photosynthesis, Absorption of water and minerals, Ascent of sap, Translocation of solutes, Transpiration, Photorespiration, Respiration, Photoperiodism, Vernalization, Flowering,

Ripening of fruits, Senescence and Death of plant gives huge knowledge and this knowledge finds wide application in every branch of botany.

- The knowledge of plant physiology will help in replicating several advancement in agriculture, horticulture, forestry, plant pathology, Human phsiology and other disciplines of biology.
- Physiological characteristics of genotypes or base material must be taken into account while producing new kinds and strains. Crop output has grown thanks to the management of soil fertility and the elimination of excessive salts in the soil via understanding of plant physiology.
- Understanding the fundamentals of plant metabolism can aid in improving the photosynthetic conversion of solar energy for the creation of human-useable food materials.



- Improved use of atmospheric nitrogen by various plant species will result from basic knowledge of nitrogen fixing. In the recent years, a number of tissue culture techniques have been created, helping to shorten the life cycles of various plant species, assisting in the growth of plants from seeds with reduced endosperm, and improving our knowledge of cell wall development and mineral intake.
- The understanding of plant hormones, their production, and their mechanism of action has made it much easier to use them to control water loss, manipulate the growth and development of some crops, and enhance the

quality of food ingredients. Weed growth in crop areas has been reduced thanks to the use of certain hormonal weedicides.

- Plant physiologists' roles are expanding as agriculture and agricultural products become more industrialised. Plant physiologists must be involved in all facets of environmental engineering, such as the use of unused land, the expansion of crop production, and the use of solar radiation by both naturally occurring and newly developed species.

1.4 Ecological Effects

The ecological consequences are a key component in the biotic community's survival and expansion in its surroundings. Light, temperature, humidity, precipitation, wind, fire, edaphic, topography, and biotic variables are examples of ecological factors that have an impact on vital physiological processes. Photosynthesis, transpiration, motility, flowering, and seed germination are just a few of the processes that light may impact. These functions are directly important to plant development and survival. Metabolism, flowering, growth and development, desiccation, chilling, and freezing are a few processes that could be impacted by temperature and have a direct impact on a plant's ability to grow and survive.

Environmental elements have an impact on fundamental physiological processes as photosynthesis, transpiration, motility, flowering, and seed germination. Plant structures that are primarily impacted or created by precipitation are ecological characteristics of the climatic environment. The following types of plants are spread throughout the area based on rainfall distribution: grassland, sclerophyllous woods, xerophytic vegetation, and evergreen forests. The most crucial element in edaphics is soil, which plays a huge part in plant growth because it gives plants their water, vital nutrients, and assistance to grow. Therefore, growing plants are divided into the following groups based on the type of soil they grow in: Oxylophytes (acidic soil), Halophytes (on salty/saline soil), Lithophytes (on rocks), Chasmophytes (in rock fissures), and Psammophytes (in sandy soil).

1.5 The physiological response (adaptation)

The xeric, ideal, and hydric conditions of soil are addressed in terms of the environmental gradient addressing the interaction of soil water and its availability to plant soil. The availability and lack of nutrients are intimately related to the xeric and hydric conditions. Furthermore, by causing the extinction of plants unable to withstand the circumstances of specialised microhabitats and by affecting the range of biotic interactions, these conditions lessen the biodiversity of the current region. Only those organisms that can sustain an environmental gradient will remain in the area. One species' decline from a tolerance range will make room for another species that will be able to adapt to the environmental variable. The organism may modify, lose, or acquire some characteristics, such as those associated with acclimatisation and adaptation.

1.6 Ecological Interactions

Organisms do not live alone, all the time they are influenced by their neighbors and show a response to this influence. In ecology this is known as species interaction and is defined as the relation between species that live together in a community. Interactive species have a tremendous influence on the size of each other' populations. The various mechanisms for these biotic influences are quite different from the way in which abiotic factors affect the size of populations.

Biotic factors regulate the size of populations more intensely. In an interactive community strong biotic interactions take place among species at the same trophic level within a local habitat (Cornell & Lawton, 1992) .Species interactions are basis for many features of community and ecosystem. The nature of these interactions can vary depending on the evolutionary context and environmental conditions in which they occur, as a result, ecological interactions between individual organisms and entire species are often difficult to define and measure and are frequently dependent on the scale and context of the interactions.

1.7 Direct and Indirect Interactions

Relationships between members of an ecological community can be classified within two broad categories, direct interactions and indirect interactions. Direct ones, deal with the direct impact of one individual on another and are not mediated or transmitted through a third individual while in the indirect interactions a species can ,through its direct interaction with some species ,indirectly influences the abundance of another species with which it is not interacting directly (Menge,1995).

1.7.1 Direct Interactions

The impact of direct interactions can be visualized very clearly, they can be positive (+), neutral (0) or negative (-):

i. Positive interactions are those through which at least one of the species obtains a benefit from another species without damaging to the second individual or altering the course of its life.

ii. Neutral interactions are those in which there is no direct damage or benefit from any of the interactive species. The damage or benefit if taken is always indirect.

iii. Negative interactions are those interactions by which one of the species obtains a benefit in detriment of the other species.

1.7.2 Indirect Interactions

The indirect effects are sequential processes in which direct effects are essentially involved, their effects are difficult to be detected as they are complicated by mixing of direct and indirect effects (Moon *et al*, 2010).Menge (1995) identified different pathways of indirect effects and reported that about 40–45% of all community changes in his study, resulted from indirect effects and suggested that the percentage could reachto 65% at other places. He emphasized that indirect effects are major source of bringing structural and dynamic complexity in communities.

1.8 Types of Direct interactions

There are eight main types of direct interactions which are classified by the net effect of the relationship on each interactor. Of these interactions mutualism and commensalism are positive interactions, there is no interaction effect in neutralism while the rest of the interactions are negative Commensalism is one sided interaction while mutualism is a cooperative interaction. Among negative interactions predation, parasitism and herbivory benefit one species and harm the other, while in competition the negative effect is on both the interactors. (Table 1).In nature all these interactions are prevalent and play a very important role in the ecological studies, the interactions are exhibited by a wide array of species and can be distinctly observed (Fig.1).

***Table 1:* Types of Species Interactions. The sign +, 0 & - represent the effect of one species on another and indicate positive, neutral or negative effect respectively.**

Effect onSpecies B	**Effect on Species A**		
	+	**0**	**-**
+	Mutualism	Commensalism	Predation Herbivory Parasitism
0		Neutralism	
-		Amensalism	Competition

Fig.1. Types of Species Interactions: a. Mutualism; b. Commensalism; c. Predation; d. Herbivory; e. Parasitism; f. Competition

(Source: a- www.sciencegeek.com; b- Planetearth-wiwkispaces.com; c- en.wikipedia.org;d- en.wikipedia.org; e- www.fcps.edu; f- www.kosm.com.my)

1.8.1 Positive Interactions

Positive interactions play a critical but underappreciated role in ecological communities by reducing physicalor biotic stresses in existing habitats and by creating new habitats on which many species depend(Stachowicz,2001).Positive interactions amongst different species in a community are broadly classified into four types(Table 2). The degree of benefit varies with each type.

Table 2. Types of positive Interactions

Interaction	**Effect on species A**	**Effect on species B**	**Nature of Interaction**
Commensalism	(+)	(0)	One is benefitted, other is unaffected

Scavenging	(+)	(+)	Beneficial to both
Facultative Mutualism	(+)	(+)	Beneficial to both, but not obligatory
Obligate Mutualism	(+)	(+)	Beneficial to both, obligatory

a) **Commensalism:** It is an interaction in which one individual benefits while the other is neither being helped nor harmed, the interaction is "+/0". In this positive interspecific interaction the smaller benefitted member is called 'commensal', while the larger member that is neither benefitted nor harmed is called 'host'. In nature, individuals of one species are often physically attached to members of another.

Ex. Epiphytes widely found in tropical rainforests grow on the branches of trees in order to access light, but the presence of those does not affect the trees the epiphytes absorb water and minerals from the atmosphere by their hygroscopic roots and prepare their own food. In general, the host plant is unharmed, while the epiphyte that grows on it gets benefit of occupying space. In tropical rain forests many species live on a tree together to form a mat .Commensalism can be difficult to identify because the individual that gets benefit may have indirect effects on the other individual that are not readily noticeable or detectable. Other examples of commensalism are several species of Barnacles (molluscs) which grow on the hard shell of Limulus polyphemus (horse shoe crab) and Entamoeba coli (protozoan) which lives as a commensal in the intestine of man.

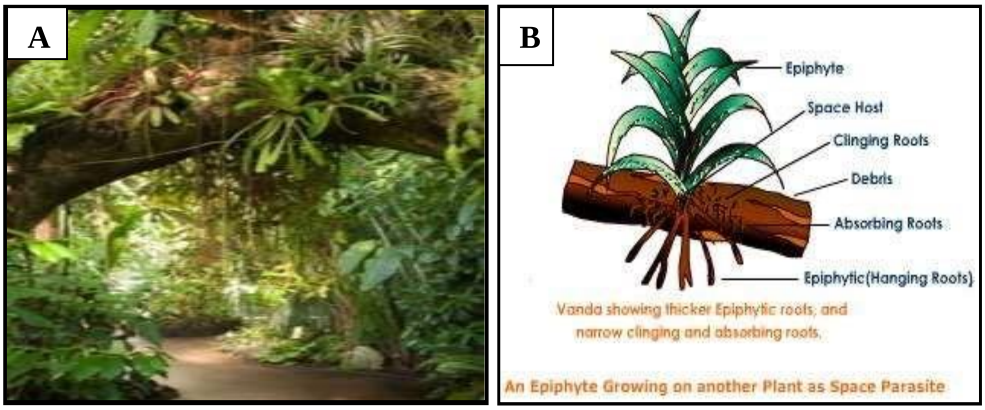


Fig.3 Many epiphytes growing on a tree trunk (A), parts of an epiphyte (B) (Source: A - Blogs.nybg.org, B- www.tutorvista.com)

b) **Scavenging:** It is a direct food related interspecific interaction in which one partner called scavenger or saprobiont eats the dead bodies of other animals which have died either naturally or were killed by some other animals. Scavengers clean the environment and the available food is ultimately disposed-off by which a major part of nutrients again enter for recycling. Animals such as foxes, hyenas, vultures etc. are examples of natural scavengers (Fig.3). Humans are much benefitted by activity of scavengers and have shown an interdependent relationship with them. This relationship has helped in shaping evolution of both, the humans and the scavengers (Moleon et. al., 2014). Decomposing microorganisms are also helping in nutrient cycling and are good scavengers.

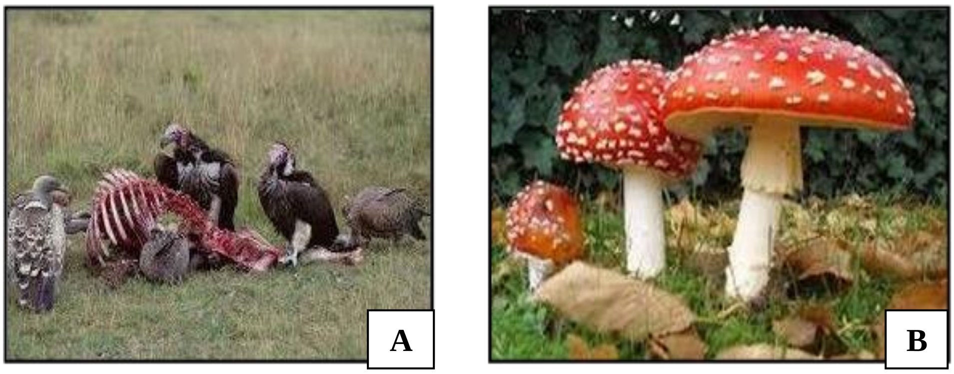


Fig.2 Scavengers: Vultures eating a dead animal, Fruiting bodies of a decomposing Fungus
(Source: A- www.ehow.com, B - www.imgarcade.com)

c) **Facultative Mutualism:** It is a positive inter specific interaction in which both the partners are mutually benefitted and increase the chance of their survival. However, the interaction is not obligatory for their survival as both can live without this interaction. The relationship is also termed as non-symbiotic mutualism or diffuse mutualism or protocooperation.

Ex. Protocooperation occurs between soil bacteria or fungi, and higher plants growing in the soil. Neither species is dependent on the association, but all microflora, higher plants, and soil fauna participate in determining soil composition and fertility. Soil bacteria and fungi interact with each other, forming nutrients required by plants. They obtain nutrients from root nodules and decaying organic matter. Plants benefit by getting necessary mineral nutrients.

Another excellent example is ants and aphids. Aphids are defenseless creatures and are easy catchy prey for most predators. The aphids feed on plant sap and have to process a significant amount of it to get their required nutrients. They excrete a lot of fluid, and some of the sugars still remain in the excreted fluid, which is called honeydew. The ants drink the honeydew and, in return, protect the aphids from an array of predators. The common pollinators and seed dispersers like birds, insects and mammals also show this relationship where they benefit many plant species by helping them in reproduction and propagation and they take advantage of gaining food and energy (Fig.4)

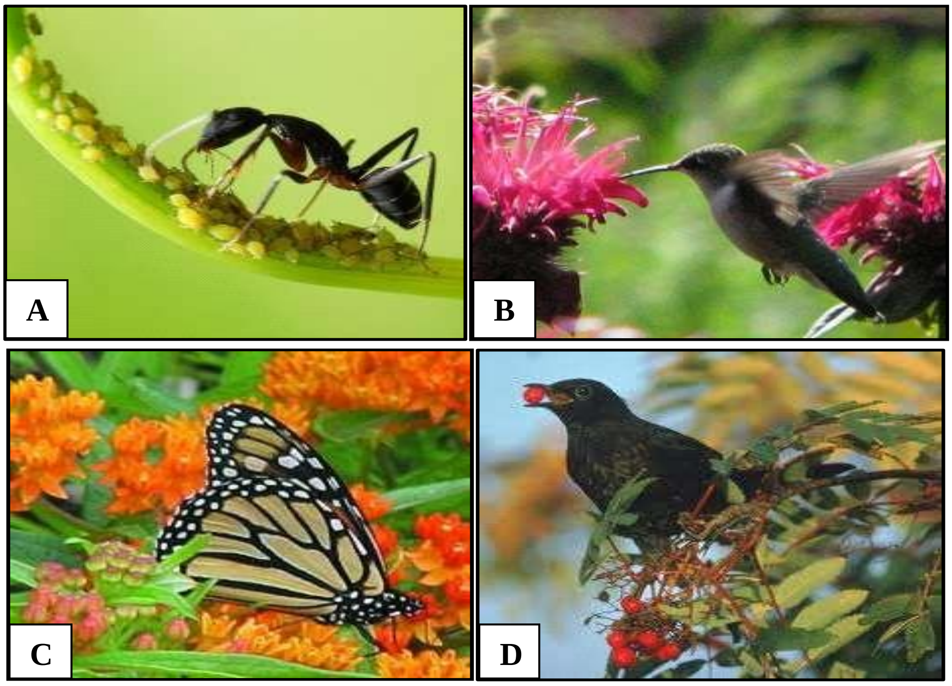
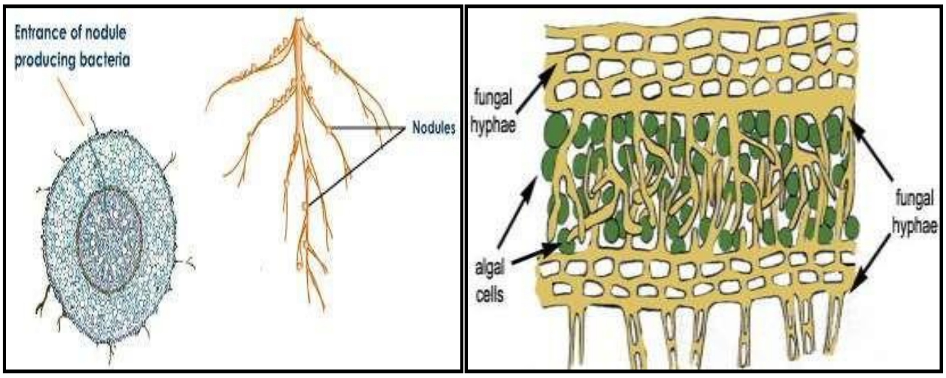


Fig.4 Protocooperation exhibited by: (A): Ants & aphids, (B & C): Pollinators and (D): seed disperser

(Source: www.lifescapes.org.in, www.blogpollinatorgardens.net, www.nativeplant center.net)

d) Obligate Mutualism: It is an interaction in which at least one species cannot survive without the presence of the other species. This is usually due to a long history of co-evolution, and involves direct interactions between the two species Obligate mutualism is also referred as ' symbiosis' (sym-together; bios-life) or 'symbiotic mutualism' and the partners are referred as 'symbionts'.

The excellent examples of obligate mutualism are lichens, mycorrhizae and the symbiosis between bacteria and legumes. Symbiotic nitrogen fixation by Rhizobium leguminosarum (bacteria) occurs in the nodules on the secondary roots of leguminous plants like pea, gram etc.The bacteria obtain carbohydrates and shelter from leguminous plants while in return they fix nitrogen as nitrites and nitrates for the plant which is required for their growth. Lichens are composed of a mixture of fungi and algae. In each "species" of lichen, the alga and fungus are so closely intertwined that whole lichens are classified as species, rather than the component fungus/alga. The type of fungus and alga are species-specific. The alga does photosynthesis and produces sugars for fuel for both. The fungus attaches the whole lichen to its substrate (tree, rock) and holds in water needed by the alga.



A B

Fig.5 Sections of a legume root (A) and a lichen (B)
(Source: A - www.bio.miami.edu; B - imagarcade.com)

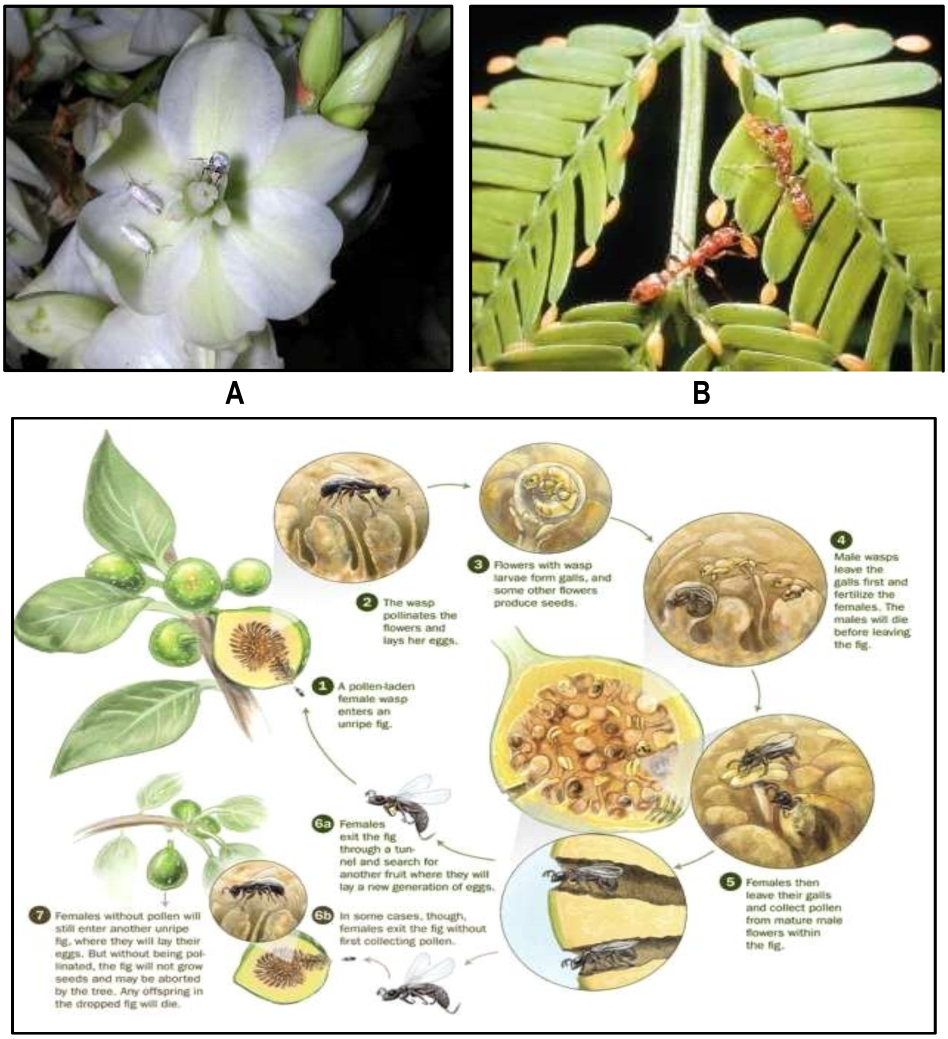
1.9 Mutualism and Co evolution

Co evolution is the mutual evolutionary influence between two species (the evolution of two species totally dependent on each other). Each of the species involved exerts selective pressure on the other, so they evolve together, they develop characters which are advantageous to them individually as well as synergistically. There are various examples where the non-obligate mutualism got turned into obligate mutualism through coevolution:

i) Yucca flowers have a typical shape so that only tiny moth can pollinate them. The moths lay their eggs in the yucca flowers and the larvae (caterpillars) live in the developing ovary and eat yucca seeds,

ii) Acacias are small, Central American trees in the Leguminosae. They have large, hollow thorns. The acacia ants live in the thorns. On the tips of its leaflets, the plant makes a substance used by the ants as food. The ants defend the tree from herbivores by attacking/stinging any animal that even accidentally brushes up against the plant. The ants also prune off seedlings of any other plants that sprout under “their” tree,

iii) iii) About 900 species of tiny wasps are responsible for pollinating the world’s 900 species of figs. Each species of wasp pollinates only one species of fig, and each fig species has its own wasp species to pollinate it. This extraordinary diversity of coevolution. Between figs and wasps has become so strong that neither organism can exist without the other.



C

Fig. 6.Examples of coevolution in mutualism :(A)Yucca moths on Yucca flower(B)Acacia ants on Acacia leaf (C)Association of Fig & Fig wasps

(Source: A-www.fs.fed.us; B - www.naturalist.com; C - www.shaysthoughtbox.blogspot.com)

1.10 Models of Mutualism

Mutualism is an interspecific interaction where two populations are interacting witheach other and are influencing each other's growth. Dynamics of these interacting populations are studied by many ecologists and various derivations have been proposed. Pianka (1994) modified the model proposed by Lotka-Volterra, in this model, the change in population density of the two mutualists is quantified as:

$$\frac{dN_1}{dt} - r_1 N_1 \left(\frac{X_1 - N_1 + \alpha_{21} N_2}{X_1}\right), \quad \frac{dN_2}{dt} - r_2 N_2 \left(\frac{X_2 - N_2 + \alpha_{12} N_1}{X_2}\right)$$

Where:

- X_1 and X_2 are the equilibrium density of species 1 and 2 in absence of the other species
- N_1 and N_2 are the population density of species 1and 2
- r_1 and r_2 are intrinsic growth rate of the population of species 1and 2
- $\dot{\alpha}_{12}$ is the beneficial effect of one individual of N_1 on N_2
- $\dot{\alpha}_{21}$ is the beneficial effect of one individual of N_2 on N_1 intrinsic growth rate of the population

The equations suggest that mutualism gives a positive feedback on population growth of both the species as population equilibrium density of each species is increased by the density of other species.

1.11 Importance of Positive Species Interactions

All types of species interactions influence the community / ecosystem in one or the other way. They have significant role in determining the species composition, species distribution, evolution and conservation while the negative interactions like competition may result in removal or exclusion of certain species the positive reaction s like symbiosis lead to co evolution of the interacting partners. Species interactions are part of the framework that forms the complexity of ecological communities. Species interactions are extremely important in shaping community dynamics.

The role of positive interactions in communities was not much appreciated by early ecologists because of prejudiced approach of contemporary community ecologists about competition. Lately attention has been given to these interactions by many ecologists and they have justified it. Positive interactions play a critical role in ecological communities by reducing physical or biological stress in existing habitats and by creating many habitats on which many species depend (Stacowicz, 2001). Positive interactions can be trophic and nontrophic, can act directly or indirectly (mediated by a third species) and can be symmetric (species have equal effects) or asymmetric (species have unequal effects). Positive species interactions are not static but vary in their outcome depending on the context under which they occur. They can act to promote species coexistence especially under harsh physical or biological stress and thus are important to species diversity, species invasions and the conservation and management of highly impacted systems. They are cooperative relationships among species and are important forces in shaping community structure. Positive interactions promote species coexistence and thus increase species diversity. Restoration of degraded communities can involve reintroducing dominant species that promote the colonization and maintenance of rare species by providing basic habitat and/or favorable physical modifications to the environment. (Filotas et al. 2010; Hacker & Gaines, 1997)

It was originally thought that competition was the driving force of community structure, but it is now understood that all types of interactions, along with their indirect effects and the variation of responses within and between species, define communities and ecosystems (Agrawal 2007). Understanding species interactions helps in conservation and management as this can be used to monitor changes in biological community structure, it also helps in predicting the impact of invasive species and human activities on native species.

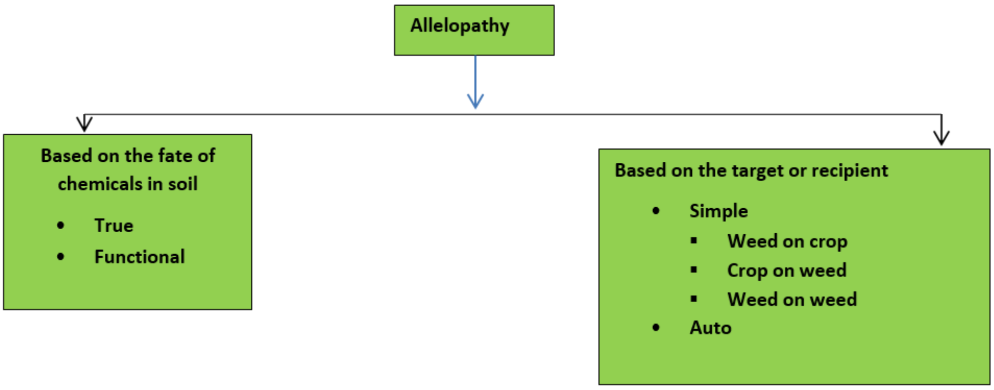
1.12 Allelopathy

Allelopathy is a combination of two words i.e., allelon which means "of each other", and pathos which means "to suffer". Simply, allelopathy refers to the chemical inhibition of one plant species by another plant species. The "inhibitory" chemicals are released into the environment and affect the development and growth of nearby plants. As per 'International Allelopathy Society' allelopathy is a process that involves the release of secondary metabolites produced by plants, algae, bacteria and fungi. These substances influence the growth and development of agricultural and biological systems. The allelopathic substance was first detected in black walnut tree by Davis in 1928. Allelopathic chemicals can be present in the leaves, flowers, roots, fruits, or stems and may be released from these parts of the plant. Allelochemicals are introduced by the plants into the environment via root exudation, foliar leaching, volatilization, residue decomposition. They can also be found inthe surrounding soil. Target species are affected by these toxins in many different ways. Thetoxic chemicals may reduce the plant growth by inhibiting the shoot/root growth, by blockingthe nutrient uptake, or by disturbing the naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient. Allelochemicals may be involved in plant- plant, plant herbivore or plant insect communication via chemicals.

1.12.1 History of Allelopathy

The term Allelopathy was first coined by Hans Molish in 1937. The term Allelopathy is relatively recent, but the concept of allelopathy is too old. For over 2000 years, phenomenon of allelopathy has been reported in the literature as plant interference. Initial observations of weed and crop allelopathy were made by Theophrastus (300 BC) and Pliny II (1 AD). Further in the 16th century, many botanists reported the irregular growth of some plants in the presence of others but no one found any experimental proof. In the seventeenth and eighteenth centuries, botanists relied strongly on a comparative approach and conducted some experiments on the plant growth and its relation to nutrition. A number of botanists, farmers, agronomists and gardeners conducted a series of experiments to describe allelopathic interactions among plants. However, intense interest in the field of allelopathy was revived in 20th century. Work of Schreiner and Reed (1907, 1908) and co-workers revived the interest of many scientists in this field.

1.12.2 Types of Allelopathy



Based on the fate of chemicals in soil:

a. **True Type:** The release of toxic compounds into the environment in their original form in which they are produced.

b. **Functional Type:** The release of a toxic chemical substance into the environment in modified form. The modification occurs due to the transformation by microorganisms.

Based on the target or recipient:

a. **Simple Allelopathy or Tele toxicity**: when target plants are other than donor plants.

i. **Weed on Crops:** When weeds like (Quack grass, wild oat, Bermuda grass etc) cause serious decrease in the yield of important edible and cash crops. The decrease in the yield is mainly due to the release of root exudates, residue remains and toxic allelopathic compounds.

ii. **Weed on Weeds:** When one weed inhibits the emergence of another weeds e.g. logon grass interfere the growth of button grass by releasing inhibitory substance.

iii. **Crop on Weed:** Some crops release the allelopathic chemicals which inhibits the growth of weeds. For e.g. oats pea suppress the growth of lambs quarter. The allelopathic effects of weed and crops on another weed may be applied for the development of natural herbicide.

b. **Autoallelopathy or Autotoxicity:** when target plants are same as donor plants.

For e.g., seed germination of horse nettle (Solanum carolinense L.) is inhibited by the material extracted from roots, stems and leaves of the same plant incorporated into thesoil.

1.12.3 Different types of allelopathic chemicals

In different plants and trees, a large number of chemical belonging to different class/naturehave been reported as allelopathic chemicals. The list of different types of chemical is as follow:

A. **Phenolic acids:** Phenolic compounds are the most common plant allelochemicals. They consist of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The phenolic compounds that exhibit the property of allelopathy are derived from coumarins, cinnamic and benzoic acids. The metabolic pathways responsible for the production of phenolic compounds in plants are shikimic acid and acetic acid pathways. They are included in the category of secondary metabolites. The presence of phenolic allelochemicals has been observed both in natural and man-made ecosystems. They cause various ecological and economic problems like replanting problems in orchards, reduction in crop yield, and regeneration failure of natural forests. They exhibit diverse modes of action and may be explored as lead compoundsfor the development of herbicides or pesticides.

The major function of phenolic compounds in plants is to provide scent and color to flower for attracting pollinators or to keep away herbivores or pathogens. The phenolic compounds like chlorogenic acid, protocatechuic acid (3, 4-dihydroxybenzoic acid), gallic acid, 3, 4-dihydroxybenzaldehyde, p-hydroxybenzoic acid, caffeic acid (3, 4-dihydroxycinnamic acid) and 3, 5-dinitrobenzoic acid, isolated from different plants have shown inhibitory effects on other plant species especially weeds.

B. Organic acids: Organic acids are organic compounds that possess acidic properties. The common examples of organic acids are carboxylic acid, tartaric acid, and citric acid. These acids are reported for their allelopathic nature. Citric, malic, oxalic, nd tartaric acids extracted from the leaf aqueous extract were reported for the allelopathic activity against the lettuce seedlings growth.

C. Flavonoids: Flavonoids are also categorized as secondary metabolites produced by plants. These are biologically active low molecular weight compounds. More than 10,000 structural variants of flavonoids have been reported till now. Flavonoids possess various physical and biochemical properties and therefore, they show interaction with different targets in sub-cellular locations. This interaction elicits several activities in microbes, plants, and animals. Flavonoids are known to play crucial roles in the development of roots and shoots, transport of growth hormones like auxin, and pollination. Flavonoids are also known to possess anticancer, antiviral, antibacterial, and antifungal activities. In plants, the transport of flavonoids occurs within and between plant tissues and cells. Thereafter, they are particularly released into therhizosphere.

In the rhizospheric region, they are involved in allelopathy or plant/plant interactions. However, the role of flavonoids is less characterized as compared to other secondary metabolites. The major flavonoids are kaempferol, quercetin, maackiain, coumestrol, luteolin etc. Methyltransferases and glycosyltransferases are responsible for the methylation and glycosylation of flavonoids. These modifications are responsible for the alteration of their reactivity, solubility, and stability in nature, flavonoids are present in the form of glycosides.

D. **Alkaloids:** Alkaloids are secondary metabolites that are widely distributed in plants. These compounds are mainly involved in defense mechanism of plants against insects, microorganisms and mammals. However, few of them have also been recognized as allelopathic compounds. Examples include paraxanthine, theobromine, mimosine, nupharolutine and caffeine. Alkaloids have also been employed in pharmacy, food preparation and as poisons. Recent investigations suggest that plant-produced alkaloids contribute to interference with otherplants and that primary allelopathic activity may disrupt a biochemical pathway in plants which is analogous to one more usually associated with animals.

E. **Terpenes:** Terpenes are wide-spread secondary plant metabolites. These are well known for their allelopathic nature. Greater part of plant Essential Oils (EOs) is constituted by Terpenes. Terpenes are further categorized as monoterpenes and sesquiterpenes with C_{10} and C_{15} atoms, respectively. The EOs can be extracted from leaves, roots, wood, bark, stem, flowers, and fruits, etc. The common examples are cineole's, *α* and *β*-pinenes, camphene, dipentene, eucalyptol, thyme, terpinen-4-ol, cerene, carvone, geraniol, limonene and *p*-cymene etc. The terpenes are known to inhibit seed germination in plants. These may also cause morphological and physiological changes in plant seedlings.

F. **Amino Acids:** Certain amino acids like lysine, methionine and tryptophan, produced by plants have also been reported to inhibit plant growth by inhibition of metabolic pathways inside plants. Apart from their action against weeds, the amino acids have also been reportedfor their inhibitory effects against some fungi.

1.12.4 Release of allelochemicals in nature

Allelopathy is about the production and release of allelochemicals in nature. The releasepathway varies for different species.

1) Exudation of volatile compounds and their deposition on the surface of leaves by rainfall.

2) Exudation of volatile compounds from the green parts of the plant.

3) Dead decaying organic matter of plant residues (e.g., litter fall or dead roots)

4) Root exudation

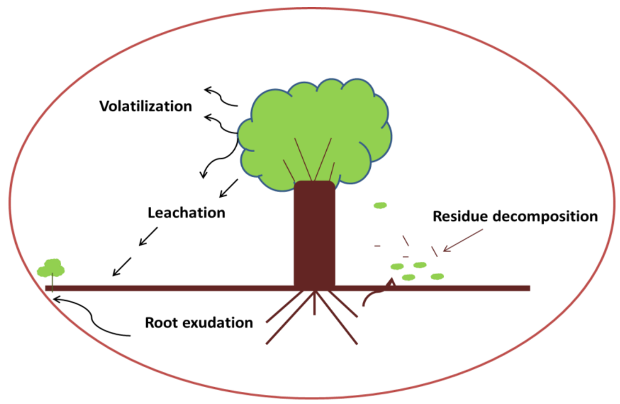


Fig.7: Mechanism of releasing of Allelopathic chemical

a) **Root exudation:** Root exudates are the chemicals secreted by roots into the surrounding environment of soil (i.e. the rhizosphere). Root exudates are the major repository of allelochemical input in the soil. Through the exudation, roots not only regulate microbial community in its close vicinity, but also change the chemical and physical properties of the soil for the regulation of the growth of neighboring plant species**.**

b) **Volatilization:** The essential oils (EOs) found in various plants are liberated through this mode. The EO escapes out through aerial parts of the plant. There are two way of recipient plant exposure to the essential oils. First, the recipient plant may be exposed to these oils directly through respiration or direct contact. Second, the EOs get absorbed on soil particles and further plants absorb them from the soil solution. These are complex mixtures of hydrocarbons and their oxygenated compounds, and thus impart characteristic odor or taste to the essential oil. Volatile oils after their release exhibit high phytotoxicity towards a number of plants**.**

c) **Leachation:** Leachation is the movement of large quantities of plant metabolites from aerial parts of the plants by the action of rain, dew, mist or fog in form of aqueous solutes**.** Leachation is an important mechanism of liberation of allelochemicals. It is as an effective way of releasing toxins by plants, thus avoiding autotoxicity and allelopathy to occur simultaneously. The materials that can be leached out include a diverse array of compounds, which may be organic or inorganic. Among these are amino acids, sugars, vitamins, phytohormones and allelochemicals such as phenolic acids, organic acids, alkaloids, terpenoids etc. The leachation of inorganic nutrients, sugars, phytohormones are beneficial for plant growth. However, the release of toxic substances has inhibitory effects on being absorbed. External factors including temperature, light, rain period and intensity etc. may also influence leachation.

d) **Residue Decomposition:** The old leaves, barks and branches of plant that have turned into the litter or residue are also responsible for the release of allelopathic compounds. The leaves and other plant parts fall onto the ground and are decomposed by weathering or soil microbes that release allelochemicals into the environment. The decomposing residues provide the largest quantity of allele-chemicals into the rhizosphere that can induce the growth inhibition of plant seedlings. Commonly in agriculture, the plant residues are incorporated into the soil to improve soil fertility. However, the growth and productivity may also be reduced by this incorporation.

1.12.4 Fate of allelochemicals in the soil

As soon as allelochemicals are released into the soil, they enter a complex plant-soil system. In this system, various factors affect their availability and effectiveness on target plants. Some processes are responsible for the addition of allelochemicals to the system whereas some processes like microbial breakdown, plant uptake and leaching are responsible for their reduction. Soil microbes take up the allelopathic compounds released from plants and are responsible for their degradation by secreting extra-cellular and intercellular microbial enzymes. Microbial transformations may lead to the production of more phytotoxic allelochemicals. Physiochemical degradation or oxidation of plant residues can also detoxify or produce additional allelochemicals.

1.12.5 Allelopathy in cropping systems

The phenomenon of allelopathy in crops has both positive and negative implications for cropping systems. Negative effects of allelopathy include changes in the distribution pattern of crops, reduction in yield and difficulty in replantation of crops. When a particular species releases allelochemicals that can cause damage to other plant species, this phenomenon is known as heterotoxicity. However, when a plant releases such type of allelochemicals that inhibit its own germination and development, this phenomenon is known as autotoxicity. The probable reason of autotoxicity is natural selection, where older plants avoid competition for light, water, nutrients etc. with younger individuals, by maintaining them at certain distance. For example, uniform spatial pattern can be seen in desert plant populations, where plants are spaced at even distance.

1.12.6 Techniques for allelopathy study

a) **Bioassay using Petridishes:** The most common technique for studying the allelopathy is bioassays with Petridishes. Bioassay studies involve the evaluation of plant extracts, essential oils, and isolated chemicals compounds on the germination and initial seedling growth of a target plant. The plant extracts can be made from any part of a plant like flowers, stems, roots, fruit and seeds.

Steps to carry out bioassay

i. Bottom of the Petridish is lined with filter paper.

ii. Moistened with distilled water or emulsion of allelopathic compound

iii. Seeds are lined on the filter paper so that they come in contact with tested compound.

iv. The Petridishes can be placed in growth chamber having a programmed photoperiod.

Germination percentage and seedling length are the most common parameters evaluated inthese bioassays.

b) **Competition:** Competition effects have been categorized into four types:

- Additive
- Substitutive
- Neighborhood
- Systematic

Among these, additive type can be used to identify allelopathic species. This type of identification involves a change in the density of one species while the density of other species remains constant. Once it gets confirmed that a particular species is showing allelopathic behavior, and then other types of competition experiments can be performed to select those cultivars that are having higher allelopathic potential.

c) **Residual toxicity in the soil:** Soil samples are collected from the rhizospheric region where allelopathic species are present. These samples are used as substrate for the germination of target species. Similarly, soil samples from nearby areas where allelopathic species are not present are used as control.

d) **Detoxification of the substrate:** Activated carbon has a peculiar property that when mixed into the soil, it can adsorb various organic compounds. This property can be used to evaluate allelopathic activity. On the basis of this property, it has been assumed that the allelopathic potential of a particular species can be diminished or eliminated by the presence of activated carbon. Activated carbon can be mixed with plant extracts, incorporated into the soil or hydroponic solution, or placed directly on the soil surface. To assess the allelopathic potential, a comparison is made between the treatments and control (without the addition of activated carbon).

e) **Hydroponic experiments:** Investigation of allelopathy via hydroponics is an interesting tool. A hydroponic apparatus provides a medium for the diffusion and delivery of allelo-chemicals to target plants.

f) **Amendment of plant residues:** In this method, plant materials are added in different amounts to the substrate to assess the allelopathic effect and the release of allelochemicals on the target species. This technique has been successfully used in greenhouse and field experiments.

g) **Plant box method:** This method is entirely based on the principle of dose response. This method establishes a direct links between growth inhibition and concentration of root exudates in the media. Agar is used as diffusion medium in this method, since agar allows the dispersion of allelochemicals from roots of the donor to the target plant.

Procedure

- Place the donor plant inside cellulose tube
- Place the tube in the corner of a plant box
- Keep the box in ice and fill it with cooled agar
- After the gelatinization of the agar, seeds of target plant are

placed concentrically nearthe donor plant

- Seal the container to avoid evaporation
- Place the boxes in pot so that the roots of the target seedlings get darken
- Keep it in a Biochemical Oxygen Demand (BOD) chamber
- Control consists of a plant box without the donor plant

1.12.7 Mode of action of Allelopathy Chemicals

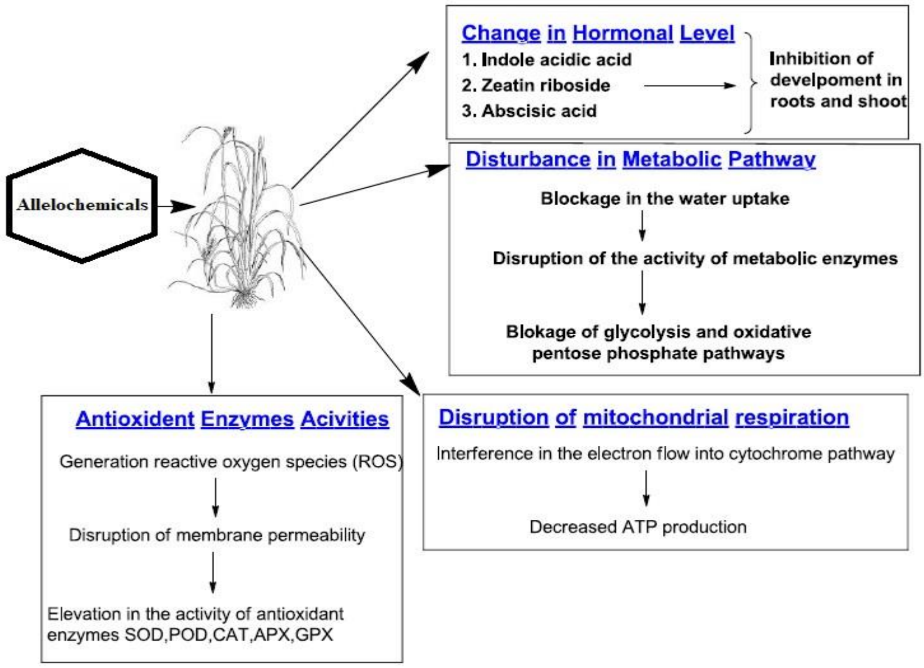


Fig 8: Mode of action of Allelopathy Chemicals

The morphological changes and restriction in the development of roots and shoots may arise due to the changes in the hormone levels. The concentration of plant hormone indole acidic acid and zeatinriboside which are responsible for plant growth (shoots and roots) decreased on treatment with allelopathic chemicals like essential oils (EOs) and phenols. The germination inhibition may be the consequence of blockage in the water uptake, or disruptionof the metabolic enzymes that are involved in the glycolysis and oxidative pentose phosphate pathways. Moreover, disruption of mitochondrial respiration system may be another mechanism for the inhibition of seed germination and radicle elongation, due to the interference of volatile essential oils. As a result, ATP production gets decreased. Allelopathic compounds are also known to inhibit the root growth by interfering with cell proliferation in root apical meristem and restricting the mitotic activity of cell. Allelopathic compounds cause generation of reactive oxygen species (ROS) like singlet oxygen andsuperoxide, hydroxyl as well as hydroperoxyl radicals. These ROS leads to disruption of membrane permeability, which cause damage to DNA, proteins and enhance the accumulation of lipid peroxide molecules.

Table 3. List of trees and shrubs showing allelopathic effects are as follow:

Trees		
Sugar Maple	Phenolics	Yellow Birch, White Spruce
Hackberry	Coumarins	Herbs, grasses
Eucalyptus	Phenolics	Shrubs, herbs, grasses
Black Walnut	Juglone (Quinone)	Pines (Austrian, Scots, red, white), Apple, Birch, Black Alder, Hackberry, Basswood, Azalea, et al.
Juniper	Phenolics	Grasses
Sycamore (Planetree)	Coumarins	Yellow Birch, herbs, grasses
Black Cherry	Cyanogenic glycosides	Red Maple, Red Pine
Oaks	Coumarins,	Herbs, grasses
Other phenolics		
Sassafras	Terpenoids	Elm, Silver Maple, Boxelder
Balsam Poplar		Green Alder
Southern Red Oak		Sweetgum
Shrubs:		
Laurel -- Kalmia angustifolia	Phenolics	Black Spruce
Manzanita	Coumarins,	Herbs, grasses
Other phenolics		
Bearberry	Phenolics	Pine, Spruce
Sumac	Phenolics, terpenoids	Douglas fir
Rhododendron	Phenolics	Douglas fir
Elderberry	Phenolics	Douglas fir

Source: http://www.extension.umn.edu/garden/landscaping/implement/trees_turf.html

Summary

The ecophysiology examines how organisms adapt to various ecological situations in order to operate properly and ensure the survival of the population. In essence, the abiotic environment and other organisms in its environment exist, are connected to,

and influence the life of an organism. The environmental variables directly affect how an organism functions. We were tightly tied to non-living elements in our surroundings, such as temperature and other climatic elements, such as. The outside climate impacts how we live, therefore as humans, we dress accordingly—wearing cozy wool clothes in the winter and airy cotton clothing in the summer.

An experimental science called ecophysiology seeks to explain the physiological mechanisms underlying ecological observations. In other words, eco-physiologists, also referred to as physiological ecologists, research the interactions between plants and their physical, chemical, and biotic environments to learn how these interactions impact plant growth, reproduction, survival, abundance, and geographic distribution. By utilizing these ecophysiological patterns and mechanisms, we are able to appreciate the evolutionary history of certain plant traits as well as their functional significance. In its broadest definition, "ecology" refers to concerns from the environmental sciences, horticulture, forestry, and agriculture. These topics are addressed by ecophysiologists at a higher level of integration.

Many aspects of ecology depend on the behavior of individuals within their own group and among differentgroups. Interactions among species living in an ecosystem lead to an ultimate picture of their existence.They can be direct and/or indirect. The direct interactions can be positive, neutral or negative .In positive interactions both or at least one interactor is benefitted like in mutualism and commensalism. In negative interactions harmful effects are imposed on either on both the interactors (competition) or at least on one interactor is harmed while the other is benefitted (predation ,parasitism and herbivory).Neutral interactions occur where there is neither benefit nor loss. Indirect interactions work via direct interactions, they also have great influence n community structure and properties. All these interactions decide species composition and species distribution in a community and also have long term impacts on species evolution and conservation.

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Unit 2: Ecological Significance of Physiological Processes

Unit Structure

2.0 Objectives

After reading this unit students should be able to:

- Understand about Carbon cycle
- Understand about Respiration
- Understand about the Photorespiration
- Understand about the CAM pathways

2.1 Introduction

The way we study, the external and internal morphology and physiology for understanding the structure and functioning of an organism and its life processes, similarly we can understand the ecosystem by studying the structural and functional attributes of all its living (biotic) and non-living (abiotic) components. Closely-linked structural and functional attributes are critical for the continued operation of an ecosystem and their integrated understanding provides a complete conceptualization of the ecosystem. To exemplify, the function of an ecosystem refers to processes and the causal relations that give rise to an ecological system, the role of organisms within it, the interactions between organisms of different species, overall processes that sustain it, long-term dynamics of different populations, overall biomass of the system, flow of energy and nutrients within it, and finally to the services it provides to human beings or other organisms.

2.2 Carbon cycle

Carbon cycle is one of the important atmospheric cycles. The cycle mainly portrays the circulation of carbon between the atmospheric gas carbon dioxide, assimilation of carbon as organic matter via photosynthesis and its subsequent release back into the atmosphere through respiration. Figure 1 depicts the carbon circulation through the carbon cycle. As we all know carbon is an important constituent of all life molecule. It is present in air as carbon dioxide (0.03%) and as carbonates and bicarbonates (CO_3^-, HCO_3^-) or molecular CO_2 (aq) in surface water and groundwater. It is also present in minerals associated mainly with magnesium and calcium as carbonates. Coal, lignite, petroleum and natural gas are the sources of carbon fixed due to high pressure and temperature deep below the earth's surface. The organic matter fixed as oil shale is termed as hydro carbonaceous kerogen and it contributes a major portion of the fixed carbon.

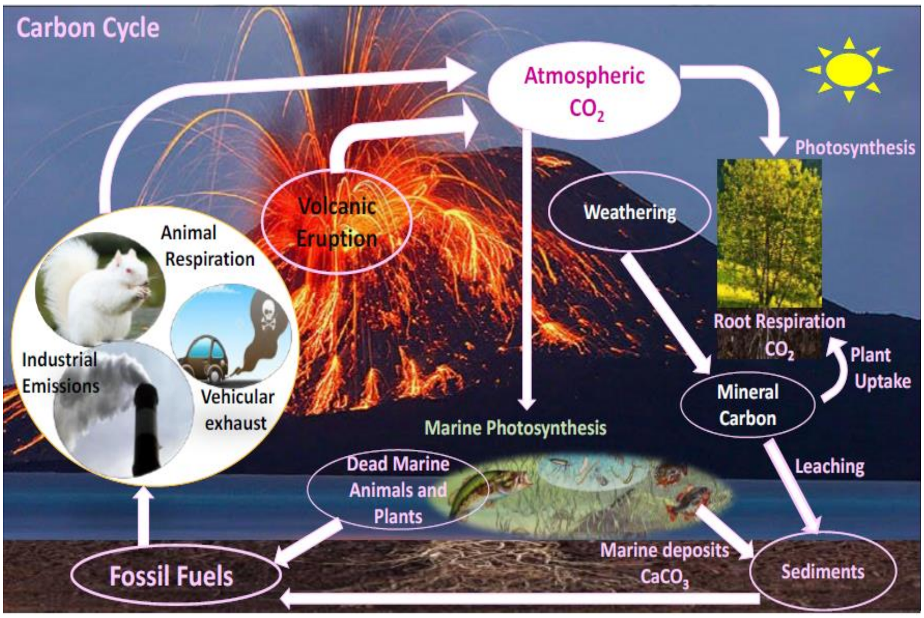


Figure 1 Global carbon cycle

Carbon is an element that cannot be broken down or converted into simpler ones. The amount of carbon in the earth is fixed but the dynamic nature of the earth moves it from one form to the other, between living and non-living. The carbon cycle occurs in a series of steps. It begins with the fixing of carbon in the biological systems by capturing solar energy via the process of photosynthesis. Plants utilize the carbon dioxide from the atmosphere and fix them as carbon in their various plant parts. The carbon is gradually transferred to herbivores, carnivores and other life forms through the food chain. The animals through the process of respiration inhales oxygen and release the carbon as carbon dioxide back to the atmosphere. The death and decay of plants and animals returns the carbon back to the soil by the action of decomposers such as bacteria, fungi etc. Microorganisms play a crucial role in carbon cycle by mediating a number of biochemical reactions. They degrade and fix organic carbon which later is transformed through various biochemical reactions into fossil fuels such as coal, lignite, peat, petroleum etc. Majority of the organic contaminants (xenobiotics) are degraded by the microorganismsto release the carbon back into the soil.

Carbon circulates within the ocean too where the photosynthetic algae fixes carbon dioxide and produce biomass. The carbon dioxide gas from the atmosphere dissolves in the water to form weak carbonic acid. The carbonic acid further dissociates into hydrogen ions and bicarbonate ions. The hydrogen ions react with the minerals and alters them resulting in clay and other ions such as sodium, potassium and calcium. Corals, a marine organism precipitates calcium and bicarbonates to form calcium carbonate. Calcium carbonate is stored in the sediments in the ocean floor as reserves. The calcium carbonates in the sediments are metamorphosed in deep subduction zones and orogenic belts. During volcanic eruptions, a huge amount of CO_2 is evolved from the mid oceanic ridges and hot spot volcanoes. The metamorphosed calcium carbonate is the source of CO_2 released from the ocean. The released carbon dioxide once again cycles back in three routes: dissolved in ocean water, some are bound as carbonate rocks and some bound as biomass.

Thus, carbon cycle is very vital because carbon is the backbone of life on earth. It has a lot of sources and sinks. The speed of carbon movement from source to sink is not same all the time and it varies for sources. Carbon cycle is altered by various anthropogenic emissions leading to impacts on globallevel. The impacts caused by anthropogenic activity are provided below.

- Burning fossil fuels leads to the release of huge amount of CO2
- As discussed earlier, trees sequester carbon dioxide during photosynthesis. Deforestation increases the level of in CO2 air
- Alteration in CO2 level due to man-made activities causes increase in temperature of the earth due to greenhouse effect
- The global warming results in destruction of the earth due to sea level rise, floods, drought, increased temperature and changing weather patterns.

2.3 Respiration

A continuous supply of energy is essential for each and every living organism. The energy is required to perform various necessary biochemical functions like maintenance of cellular organization, membrane permeability, transportation of metabolites etc. Respiration is defined as the process by which cells release energy from organic compounds to generate ATP through a series of chemical reactions involving the transfer of electrons and these ATP molecules act as energy resource for cell. A reduction in respiration cause decreased ATP production and consequently lesser energy is available for carrying biochemical processes associated with ripening, resulting in quality changes. Respiration is considered as a programmed continuation of both catabolic and anabolic processes. This process occurs in mitochondrion of the cell.There are mainly two types of respiration: aerobic respiration and anaerobic respiration, depending upon availability of oxygen. In aerobic respiration, oxygen (O_2) is the final electron acceptor while in anaerobic respiration, or fermentation, some other compound is the final electron acceptor.

2.3.1 Aerobic respiration

Aerobic respiration is the major biochemical process supplying energy. It involves the oxidation of certain organic compounds i.e. glucose, maleic acid, stored in the tissues. The compounds that are oxidised during this process are known as respiratory substrates. Aerobic respiration occurs in three phases: glycolysis or Embden-Meyerhoff-Parnas (EMP) pathway, the Krebs or Tri-carboxylic acid cycle and the electron transport chain.

In glycolysis, a molecule of the six-carbon sugar glucose is oxidized to two molecules of the three-carbon pyruvate. The Krebs cycle completes the oxidation of pyruvate to produce carbon dioxide (CO_2) and reduced electron carriers. In the electron transport chain, a proton (H^+) gradient drives the production of even more ATP and is coupled with the transfer of electrons to oxygen (O_2), producing water (H_2O). After the entire process of respiration is complete, much of the energy released from the glucose is recaptured in the production of ATP.

Glucose is the most favoured substrate for respiration. All carbohydrates other than glucose are converted into glucose first before they are used for respiration. Fats are metabolized into glycerol and fatty acids first and then to acetyl CoA and glyceraldehyde-3- phosphate, respectively. Proteins are degraded individual amino acids (after deamination) and then enter the respiratory pathway. The respiration is exothermic in nature and theoretically, 60% of the bond energy is lost as heat. However, calorimetry studies have shown that respiration in postharvest tissues often results in even more dissipation of energy as heat loss (90% or more) and less ATP synthesis. This heat contributes to an increase in the temperature of the commodity and is known as vital heat or heat of respiration. Heat of respiration is a primary consideration in designing the storage for horticultural crops.

2.3.2 Anaerobic respiration

Aerobic respiration is preferred energy producing pathway. But under the limiting O_2 conditions, fermentation becomes increasingly important. During this process, pyruvic acid produced during the glycolysis is converted to lactic acid, ethanol or acetaldehyde. Increase in fermentation helps the cell meet its ATP requirement under anaerobic conditions. Anaerobic respiration produces much less energy than aerobic pathway. Anoxia results in injuries to tissue. High concentrations of fermentative metabolites are also associated with various physiological disorders like necrosis, discoloured tissues, off- flavours, off-odours etc. The oxygen concentration at which anaerobic respiration starts is called extinction point. It varies between the tissue types and also species, cultivar, development stage, maturity etc.

Apart from aforementioned respiratory processes, two other forms, alternative and residual respiratory processes are also found.

Alternative respiration is an alternative pathway to cytochrome pathway of electron transport to O_2. This exothermic process results in production of CO_2 but no ATP generation occurs because the electron transfer chain is not coupled to oxidative phosphorylation. This respiration occurs when cytoplasm respiration is blocked. It is also known as thermogenic respiration or cyanide insensitive respiration. Alternative respiration causes a rapid loss of storage polysaccharides, electron transport without the loss of reducing power, and generation of heat.

2.4 Photorespiration

Photorespiration is important for energy dissipation from chloroplasts to prevent photoinhibition. Earlier it was thought that photorespiratory metabolism is a wasteful process which results from the inefficiency due to oxygenase activity associated with Rubisco. Photorespiratory metabolism involves the chloroplasts, peroxisomes and mitochondria. Glycolate is formed by the oxygenase activity of Rubisco. Transport of glycolate takes place from chloroplasts to peroxisomes, where it is converted to glyoxylate and further to glycine. Glycine is then converted to serine in mitochondria. Some of these reactions use the excess reducing equivalents generated in the cell. The phopshoglycerate formed in chloroplasts is used for the C3 cycle, and is reduced to form sugars.

Though photorespiration leads to a decrease in photosynthetic efficiency, it enables the utilization of the products of light reactions. Hence photorespiration effectively dissipates excess excitation energy from chloroplasts (1 ATP and 1 NADPH) and gives protection from photoinihibition (Figure 2).

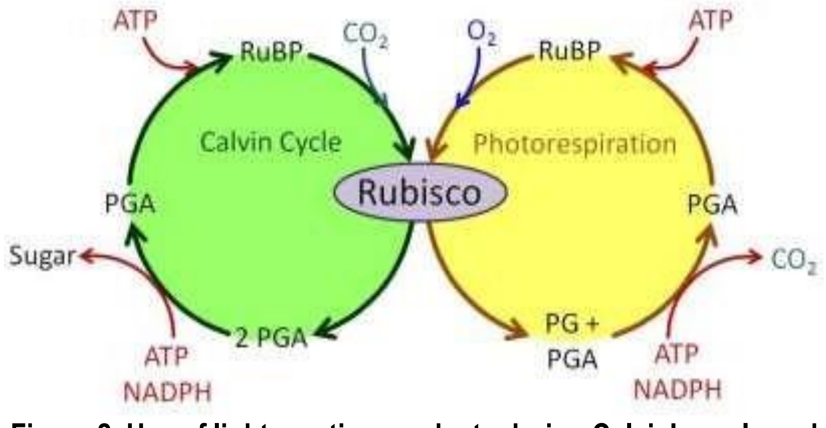


Figure 2. Use of light reaction products during Calvin's cycle and photorespiration.

2.5 Crassulacean Acid Metabolism (CAM) cycle or the dark fixation of CO2 in succulents

CAM is a cyclic reaction occurring in the dark phase of photosynthesis in the plants of Crassulaceae. It is a CO_2 fixation process wherein, the first product is malic acid. It is the third alternate pathway of Calvin cycle, occurring in mesophyll cells. The plants exhibiting CAM cycle are called CAM plants. Most of the CAM plants are succulents e.g., Bryophyllum, Kalanchoe, Crassula, Sedium, Kleinia etc. It is also seen in certain plants of Cactus e.g. Opuntia, Orchid and Pine apple families.

CAM plants are usually succulents and they grow under extremely xeric conditions.In these plants, the leaves are succulent or fleshy. The mesophyll cells have larger number of chloroplasts and the vascular bundles are not surrounded by well defined bundle sheath cells. In these plants, the stomata remain open during night and closed during day time. The CAM plants are adapted to photosynthesis and survival under adverse xeric conditions. CAM plants are not as efficient as C4 plants in photosynthesis. But they are better suited to conditions of extreme desiccation. CAM involves two steps:

A. Acidification

B. De-acidification

A. Acidification: In darkness, the stored carbohydrates are converted into phophoenol pyruvic acid by the process of Glycolysis. The stomata in CAM plants are open in dark and they allow free diffusion of CO_2 from the atmosphere into the leaf. Now, the phosphoenolpyruvic acid carboxylated by the enzyme phosphoenol pyruvic acid carboxylase and is converted in to oxalaoacetic acid.

$$\text{Phosphoenol Pyruvate} + CO_2 + H_2O \xrightarrow{\text{PEP carboxylase}} \text{Oxaloacetic acid} + H_3PO_4$$

The oxaloacetic acid is then reduced to malic acid in the presence of the enzyme malic dehydrogenase. The reaction requires NADPH2 produced in Glycolysis.

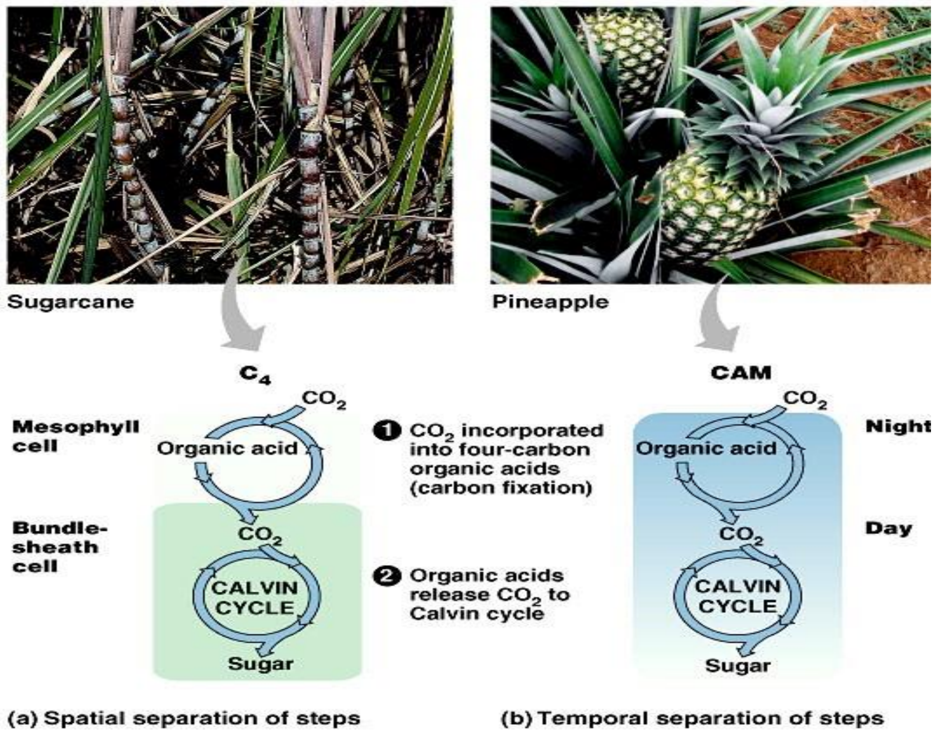
$$\text{Oxaloacetic acid} + NADPH_2 + \xrightarrow{\text{Malic dehydrogenase}} \text{Malic acid} + NADP^+$$

The malic acid produced in dark is stored in the vacuole. The malic acid increases the acidityof the tissues.

B. De-acidification: During day time, when the stomata are closed, the malic acid is decarboxylated to produce pyruvic acid and evolve carbon dioxide in the presence of the malic enzyme. When the malic acid is removed, the acidity decreases the cells. This is called deacidification. One molecule of $NADP^+$ is reduced in this reaction.

$$\text{Malic acid} + NADP^+ \xrightarrow{\text{Malic enzyme}} \text{Pyruvic acid} + NADPH_2 + CO_2$$

The pyruvic acid may be oxidized to CO2 by the pathway of Kreb's cycle or it may be reconverted to phosphoenol pyruvic acid and synthesize sugar by C3 cycle. The CO2 released by de-acidification of malic acid is accepted by ribulose diphosphate and is fixed to carbohydrate by C3 cycle.



CAM is a most significant pathway in succulent plants. The stomata are closed during day time to avoid transpiration loss of water. As the stomata are closed, CO_2 cannot enter intothe leaves from the atmosphere. However, they can carry out photosynthesis during the day time with the help of CO_2 released from organic acids. During night time, organic acids are synthesized in plenty with the help of CO_2 released in respiration and the CO_2 entering from the atmosphere through the open stomata. Thus, the CO_2 in dark acts as survival value tothese plants.

2.6 Comparison of the plants of C3 and C4 cycle

S.N.	C_3 Plant	C_4 Plant
1.	Only C_3 cycle is found.	Both C_4 and C_3 cycles are found.
2.	The efficiency of CO_2 absorption at low concentration is far less and hence, theyare less efficient.	The efficiency of CO_2 absorption at low concentration is quite high and hence, they are more efficient plants.
3.	The CO_2 acceptor is Ribulose-1, 5-diphosphate.	The CO_2 acceptor is phospho enol pyruvate.
4.	The first stable product is phospho glyceric acid (PGA).	Oxaloacetate (OAA) is the first stable product.
5.	Plants show one type of chloroplast (monomorphic type).	Plants show dimorphic type of chloroplast. The chloroplast of parenchymatous bundle sheath is different from that of mesophyll cells (dimorphic type). The chloroplasts in bundle sheath cell are centripetally arranged and lack grana. Leaves show *Kranz type* of anatomy.
6.	In each chloroplast, two pigment systems (Photosystem I and II) are present.	In the chloroplasts of bundle sheath cells, the photosystem II is absent. Therefore, these are dependent on mesophyll chloroplasts for the supply of NADPH + H+.
7.	The Calvin cycle enzymes are present in mesophyll chloroplast. Thus, the Calvin cycle occurs.	Calvin cycle enzymes are absent in mesophyll chloroplasts. The cycle occurs only in the chloroplasts of bundle sheath cells.
8.	The CO2 compensation point is 50-150 ppm CO2.	The CO2 compensation point is 0-10 ppm CO2.
9.	Photorespiration is present and easily detectable.	Photorespiration is present only to a slight degree or absent.
10.	The CO2 concentration inside leaf remains high (about 200 ppm).	The CO2 concentration inside the leaf remains low (about 100 ppm).
11.	The 13C/12C ratio in C-containing compounds remains relatively low (both 13CO2 and 12CO2 are present in air).	The ratio is relatively high, i.e. C4 plants are more enriched with 13C than C3 plants.
12.	Net rate of photosynthesis in full sunlight (10,000 – 12,000 ft. c.) is 15-25 mg. of CO2 per dm2 of leaf area per hour.	It is 40-80 mg. of CO2 per dm2 of leaf area per hour. That is, photosynthetic rate is quite high. The plants are efficient.
13.	The light saturation intensity reaches in the range of 1000-4000 ft. c.	It is difficult to reach saturation even in full sunlight.

14.	Bundle sheath cells are unspecialized.	The bundle sheath cells are highly developed with unusual construction of organelles.
15.	The optimum temperature for the process is 10-25°C.	In these plants, it is 30-45°C and hence, they are warm climate plants. At this temperature, the rate of photosynthesis is double than that is in C3 plants.
16.	18 ATPs are required to synthesize one glucose molecule.	30 ATPs are required to synthesize one glucose molecule.

Summary

References:

1. EPG Pathshala Subject: Environmental Science: Paper No: 8 Atmospheric Module: 31 Biogeochemical Processes Cycles
2. EPG Pathshala Paper No. 6: Technology of Fruits and Vegetables Module No. 6: Respiration of Fruits and Vegetables.
3. EPG Pathshala Subject: Botany: Paper No: 8 Plant Physiology II: Module: 31 Photoinhibition

Unit 3: Rhythms

Unit Structure

3.0 Objectives

After reading this unit students should be able to:

- Understand about the general introduction of Rhythms
- Describe about the Neural basis of Circadian Rhythms
- Understand Regulation of Seasonal Rhythms
- Understand about Biological Rhythms and Human Health

3.1 Introduction

Biological rhythms are cyclic changes in the way biological systems behave. These biological rhythms are cyclic behavior patterns of varying length displayed by animals, plants and even single celled organisms. Most of our behavior occurs on a rhythmic pattern. Because these rhythms last about a day, they are called circadian rhythms. Circadian rhythms refer to the 24 hours cycle which is endogenous. The word circadian comes from the Latin,' cicra' (about) plus 'dies'(a day).The two best known circadian rhythms are the sleep-wake- cycle and the body temperature cycle. Earlier research has indicated that humans may settle down into a cycle of roughly 25 hours when isolated from external stimuli like light and time-keeping. The biological rhythms are controlled by (i) internal biological clocks, called endogenous pace makers, and (ii) external cues from the environment, called endogenous zeitgebers. These include sunlight, food, noise, or social interaction.

The circadian rhythm is not the only biological rhythm. There are two other important rhythms, ultradian rhythm and infradian rhythm. The ultrdian rhythm spans less than a day. There is a 90 minute cycle of slow wave and REM sleep. The 90 minute sleep cycle is itself located within a 24 hour circadian cycle, so it would make sense to find that this 90 minute clock was also clicking throughout the day. It is called the basic rest activity cycle (BRAC). Kleitman (1982) found that eating, drinking, urination, heart rate, oxygen consumption, stomach motility and performance on various physical and mental tasks followed a 90 minutes cycle. A need to re-charge oneself was felt after each cycle. Even the infants demanded to be fed after every 90 minutes. This 90 minute cycle is also known as the ultrdian rhythm, and indicates heightened and lowered level of brain arousal. The infradian rhythm has a period of more than one day but less than one year, such as the menstrual cycle in women and seasonal affective disorder (SAD).

Some monthly and even yearly rhythms, particularly in the context of hormonal changes and reproductive behavior, have been observed. Passing of seasons, weather patterns, tidal waves, rising and setting of sun, menstruation in females, even rhythms of heart rate and respiration are examples of cycles governing nature as well as living organisms.

3.2 Zeitgebers

The internal clock which controls circadian rhythm is reset by light. The shift of activity producedby a synchronizing stimulus is referred to as a phase shift, and the process of shifting is called entrainment. Any cue that organism uses to synchronize its activity with the environment is called a zeitgeber (German for "time giver") . Light' is as an important and powerful zeitgeber , and it can be manipulated in the laboratory. In an experiment by Grobleuski, Nunz, and Gold (quoted in Carlson, 2001), the activity of rats was monitored by changing the level of illumination from normal day and night pastern to constant dim light. When the rats were kept in constant dim light, they exhibited a pattern of greater free running activity towards evening and midnight.

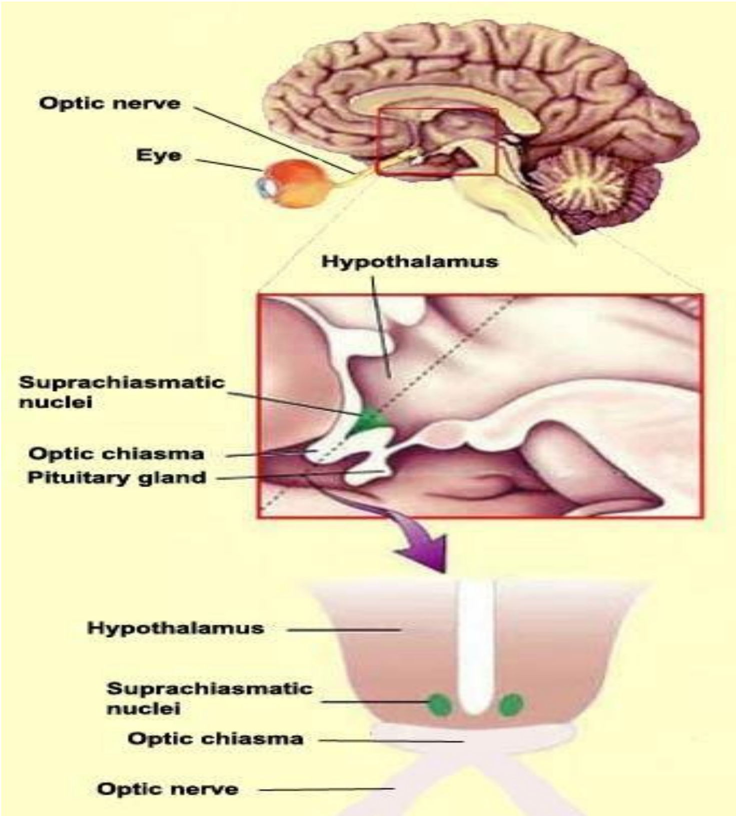
However, when the lights were on from 7:00PM to 7:00AM, the greater activity was observed in the morning and afternoon. Thus, the level of illumination, the zeitgeber, determined the cycle of rest and activity.

3.3 Neural basis of Circadian Rhythms

If the sleep-wake cycle is under the control of a circadian pacemaker, then where in the body does it exist? One of the first scientists to address this question was Carl Richter (1950, 1967), who found that large lesions of the hypothalamus interferes with the circadian rhythms. He made lesions to many different brain structures and examined their effects on circadian patterns of locomotor activity of rats. Only one structure appeared to disrupt circadian behavior- the ventral hypothalamus. But, partly because this region was so small, and anatomically complex, he was unable to go further with his investigation. It was subsequently discovered that a tiny part of the hypothalamus, the suprachiasmatic nucleus (SCN), named for its location above the optic chiasma, serves as a biological clock. Lesions confined to the SCN portion of the hypothalamus interfere with circadian rhythms of drinking and locomotion (Stephan and Zucker, 1972) and of hormone secretion (Moore and Eichler,1972). However, it was hypothesized that the SCN controlthe rhythm, but the time analysis for the activity is controlled by some other area of the brain.

3.3.1 Structure of the SCN

Suprachiasmatic nucleus (SCN**),** the main endogenous pacemaker, is a tiny cluster of nerve cells which lies in the hypothalamus. It is located just above the place where the optic nerves from each eye cross over (called the optic chiasm-thus ‘supra’, means above the chiasm) .In fact, SCN is a pair of structures, one in each hemisphere of the brain, and each of this is divided into a central and dorsal SCN. The ventral SCN is relatively quickly reset by external cues, whereas thedorsal SCN is much less affected by light. The latter is, therefore, more resistant to being reset (Albus et al, 2005). The structure of the SCN has been investigated in rat brains. It consists of approximately 10,000 neurons densely packed with each other. These neurons are extremely small in size. The unique feature of these neurons is that their dendrites synapse with each other.



This kind of connectivity is perhaps responsible for the maintenance of rhythms. The structure of SCN is shown in the following figure; Another special feature of these neurons is that they contain a large amount of RER (Rough Endoplasmic Reticulam) which helps in the production of proteins. They secrete neuro- modulators, and are therefore neuro-secretary cells. Such neuro-modulators form the chemical base for the control of biological rhythms. Now, an interesting question is 'How can light be a zeitgeber for setting or even re-setting the rhythm of rest and activity if the SCN is located so centrally in the brain?''How does the light reach here to serve as the stimulus for the cycle?'

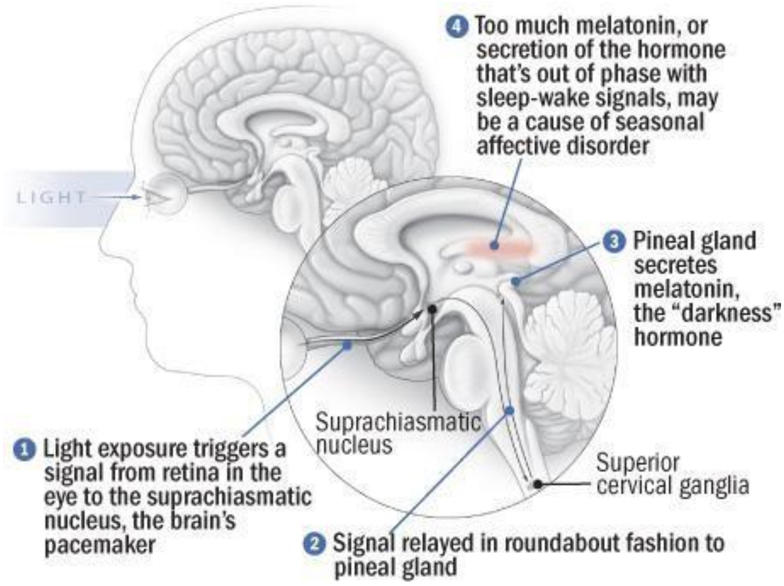
3.3.2 Pathways from the Visual System to the SCN

The studies have indicated that the light from the visual system does reach this small nucleus. There are two pathways that accomplish this. First, there is a direct projection of fibers from the retina to the SCN. It is known as the 'retinohypothalamic' pathway .Cells located on the ventral and the medial areas of SCN receive such an input from the retina. Further, investigations have revealed that rods and Cones, the normal receptors of light on the retina, are not involved in this process of sending information to the SCN. Even blind rats are found to be following the zeitgeber manipulated from outside. Freedman et al (1999) found that mutation of genes necessary for the production of rods and cones on retina did not destroy the circadian rhythms. Proventio et al (2000) discovered the photochemical and the receptive cells responsible for the rhythms. The chemical is known as melanopsin. The site of special cells projecting to SCN was found to be around amacrine and ganglion cells rather than rods and cones. It was, thus, found that there is a special mechanism for connection between light and the SCN.

Earlier studies of have shown that the Fos protein is produced in the cells when they are active. Experimental manipulation of the level of illumination was found to be related to the amount of Fos protein generated in SCN cells-showing that they do respond to light, and light does serve as the zeitgeber for the circadian rhythms. The neurotransmitter found in the SCN cells is Glutamate.

The second route of projection of light to SCN is the geniculohypothalamic Pathway. The lateral geniculate nucleus of thalamus, which receives optic information from the retina, projects information to the SCN. This is an equally important pathway that functions in co-ordination with the retinohypothalamic projection. The routes of the two pathways divert near the optic chiasma. Thus, even though SCN is not a part of the visual system, the light reaches it through two different pathways. Light is ,thus, a zeitgeber for maintenance of the circadian rhythms.

The connection between the visual system and SCN is shown in the following figure:



3.3.3 Mode of Functioning in the SCN

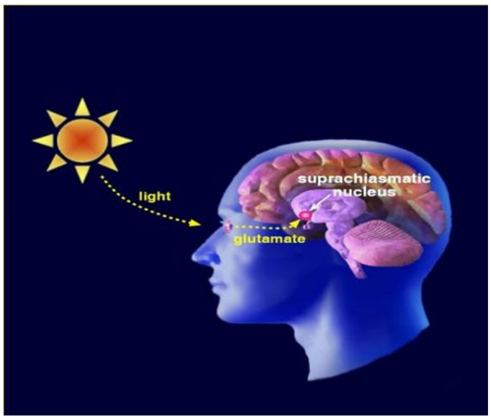
The clock like nature of SCN is also evident in its metabolic activity. If we take SCN cells out of the brain and put them in a dish, their electrical activity remains synchronized to the light dark cycle that the animal had previously experienced. This striking evidence supports the idea that the SCN contains an endogenous clock .But even stronger proof that the SCN generates a circadian rhythm comes from transplanting the SCN from one animal to another which suggests that the mode of functioning of the SCN neurons is chemical rather than electrical and axonal. In a study by Lehman et al (1987), SCN was first lesioned, and then another SCN from a donor animal was transplanted. It was found that the circadian rhythms which were destroyed by the lesion got re-established through the process of transplantation. The efferent connections were, however, not re-established.

In another investigation by Silver et al (1996), the SCN of Hamsters was destroyed which abolished their circadian rhythms. Then, small semi-permeable capsules were used to carry the SCN tissue taken from another animal. The capsules were transplanted into the ventricles of the experimental animals where no fiber connections could be established since ventricles contain only the cerebrospinal fluids. The nutrients reached the SCN tissue through the semi-permeable covering, but the axons could not connect with each other. The importance of the chemical base of functioning was evident through this investigation.

Loud noises and changes in temperature are other environmental factors that have been associated with circadian rhythms in terms of setting/re-setting the cyclic durations.

3.3.4 Radio-graphical Investigation of SCN

Schwartz and Gainer (1977) injected rats with radio-active 2-deoxyglucose (2-DG). This substance is similar to glucose but it can neither be utilized by the cells, nor can it leave the cell once it has penetrated them. So, it gets accumulated in the cells. When viewed under the microscope, it shines because it is radio-actively tagged.



For microscopic examination, the animal has to be killed and the slices of the brain prepared for examination. In the above mentioned study, the amount of radio-activity indicating greater/smaller activity of the SCN cells could be clearly differentiated for day and night activity for the rats. Greater metabolic activity was seen during the day.

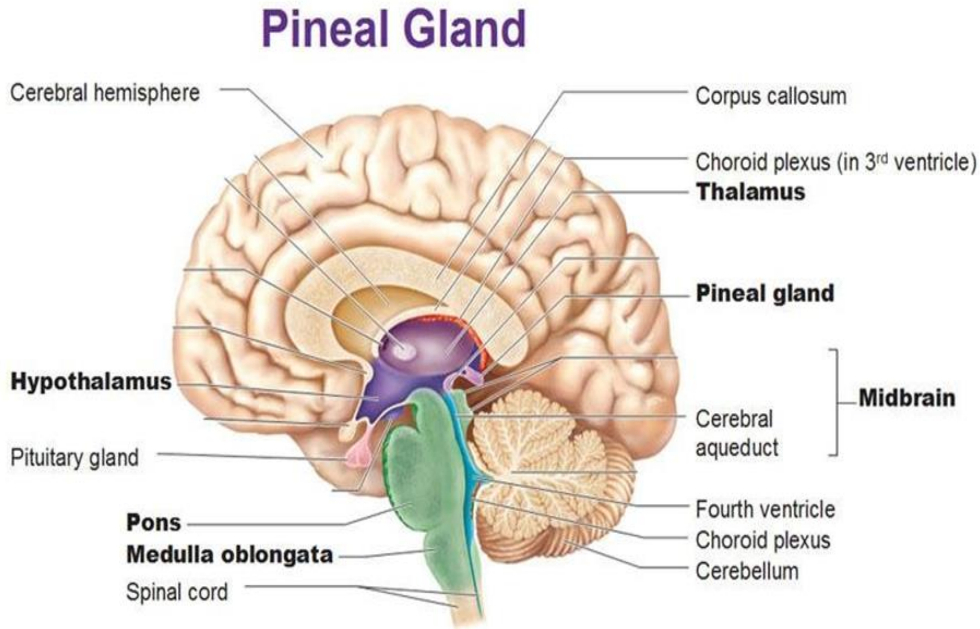
'How do cells in the SCN maintain the rhythm?' is the question that still needs to be answered. Inan innovative experiment, Welsh et al (1995) removed tissue from SCN of a rat. They put it into Papain, an enzyme which dissolved connections between the cells. Thereafter, they placed cells on an array of micro-electrodes so that their electrical activity could be measured. The cells independently became activated and showed circadian rhythms. The most striking finding was that peak activity occurred at different times in each cell. So, each cell maintains its own rhythms,and they do not fire in complete synchrony.

The question is' If cells do not have synaptic connections between them, and they do not even have a common circadian rhythm, what is the basis of a synchronized control of the rhythm?' What causes intra-cellular ticking, and timing? The obvious hypothesis is that some chemical is responsible for this cycle. Such mechanisms were studied in fruit fly by Hunterensor, Ousley, and Sehgal (1996), among others. They showed the activity of two genes 'PER (Periodic), and Tim (Timeless). At the beginning of the cycle, these genes became active and caused the production oftheir specific proteins. When the production of proteins reached a particular level, these genes turned off further production of their proteins whose level soon began to decline. When the level had declined to a particular level, the genes became active again, and initiated the next cycle. Ralph and Menaker (1988) showed that circadian abnormality was due to the mutation of a gene called TAU. Analysis of the molecular structure of this gene led to the discovery of circadian timing early in the history of evolution.

3.4 Regulation of Seasonal Rhythms

Cells in the suprachiasmatic nucleus seem to be involved in rhythms beyond the 24 hours cycle also. One of the interesting experiments in this context was done by Rusak and Morin (1976) on male hamsters. The hamsters show an annual rhythm of testosterone secretion and their reproductive season. Their breeding season coincides with the light present in the environment. The breeding season is active in summer and declines in winter when lesser amount of light is present. The results of the investigation indicated that .Lesion of SCN cells was linked with the loss of this annual rhythm, and the hamsters even started secreting testosterone all through the year.

One of the major centers involved with the control of seasonal rhythms is the pineal gland. Pineal gland is a small structure like a pine cone and is located on the epithalamus. It is located on the midline of the brain just ventral to the rear portion of the corpus callosum. The location of glandis shown in the following figure;



Pineal gland is a part of the Endocrine system and secretes a hormone called melatonin. Melatonin is synthesized from the neurotransmitter serotonin in the pineal gland. Secretion of melatonin is at the peak during the night and declines as the day advances. Melatonin has a role to play in sexual maturation in co-ordination with the light. It causes early maturation in the tropical regions with abundant light, and a much slower one in the cold countries. The chemical called melanin also causes darkening of the skin. Besides such endocrine functions, pineal gland has been found to be involved with the control of seasonal rhythms in animals. The SCN has an intricate circuit to reach the pineal gland. The SCN sends signals to the pineal gland , directing it to increase production of the hormone melatonin at night. Melatonin induces sleep by inhibiting the brain mechanisms that promote wakefulness.

Neurons in the SCN make synaptic connections with an area of the hypothalamus called the paraventricular nucleus (PVN). The neurons on PVN, then, extend excitation to the spinal cord and synapse with the pre-ganglionic fibers of the sympathetic nervous system. The excitation, then, travels through the post-ganglionic fibers and stimulates the pineal gland. The connections between SCN and the pineal gland are established through the synaptic connection. So, when SCN is destroyed and then transplanted again, the 24 hour cycle (the circadian rhythm) is re-established but the seasonal rhythms, which are mediated through the pineal gland, fail to re-appear. Several studies have shown that large doses of melatonin during the day, when levels of endogenous melatonin are low, lead to quicker and better sleep patterns. A dose of melatonin before dusk may help travelers suffering from jet lag while going towards east since melatonin is associated with darkness, and East experiences darkness much later than the West. Similar adjustments can be done for the travelers going towards the West.

3.5 Clocks Other than the Circadian Rhythms

Many organs and cells in the body maintain more or less independent Circadian Rhythms outside the SCN. It has been shown that other rhythms are retained whereas the circadian rhythms are abolished by the bi-lateral lesion of the SCN. There are other clocks to maintain them. These clocks, called the peripheral oscillators, are found in the adrenal gland, esophagus, lungs, liver, pancreas, spleen, thymus and the skin. Olfactory bulb and the prostate have also shown oscillations when cultured. Also, liver cells are shown to respond to feeding rather than light or any other Zeitgeber, such as temperature. Green and Menaker (2003) showed that even though most cells of the body contain a genetic Circadian Clock, they are normally entrained by neural and hormonal signals from the SCN.

3.6 Biological Rhythms and Human Health

Biological rhythms are driven by the endogenous oscillators (pacemakers). Some of these oscillators are easily reset by exogenous zeitgebers (such as day light, meal times and so on) whereas other oscillators are more resistant. The result is desynchronization. Health problems can result from a disturbance in the circadian rhythms. Disruption in the circadian rhythm is associated with obesity, diabetes, and other metabolic disorders. The two most common examples of the disruption of biological rhythms and the resultant desynchronization are shift work and and aeroplane travel –resulting in shift lag and jet lag. Shift work and frequent jet lag have profound effect on health. Levels of alertness also decline due to that. It poses a risk for the cardiac health also in terms of hypertension and chronic inflammatory diseases. Fatigue, disorientation and insomnia have been noticed in Pilots when they have long periods of awakening, and crossing of time zones. Thus, there are enormous health , safety and economic benefits to figuring out how the circadian clock works.

Summary

- An organism's life is governed by cycles related to most of the functions. Circadian rhythms maintain timing related to day and night. The circadian rhythm is controlled by the suprachiasmatic nucleus (SCN) located on the hyppothalamus. Electrical and chemical activity of cells on the SCN correlates with the activity levels during day and night in many organisms.
- The light reaching SCN via retinohypothalamic and the geniculohypothalamic pathway is the Zeitgeber for cells on the SCN. The major modeof functioning of the cells of SCN is chemical. Lesion of fibers connecting SCN to nearby areas does not disrupt the circadian rhythms. Individual neurons, rather than the circuits or groups of neurons are responsible for keeping the time. Genes called TIM and PER are involved in maintaining the circadian rhythms.
- Molecular analysis of the gene TAU has shown the presence of circadian rhythms in organisms very early in the history of evolution.
- Pineal gland, in coordination with SCN, controls seasonal rhythms. Hormone of the pineal gland, melatonin, can help people suffering from jet-lag or night shift work etc.
- It has been indicated in several studies that several organs of the body contain rhythm, and they oscillate at their own rates. Disruption in the circadian rhythms can lead to serious health problems.

Reference

EPG Pathshala: Subject: Psychology, Paper no.14: Neuropsychology, Module no.10: Circadian rhythms.

Unit 4: Physiological Stress

Unit Structure

4.0 Objectives

After reading this unit students should be able to:

- Understand about physiological stress
- Describe the mechanisms of physiological stress
- Understand about the tolerance by Plants
- Understand about stress effects.

4.1 Introduction

Crop plants are mostly affected by abiotic and biotic stresses; annually abiotic stresses cause more than 25% crop losses. Abiotic stresses, such as drought, extreme temperatures, heavy metal toxicity and high salinity *etc,* severely impair plant growth and productivity. In the present scenario of changing climate, reduced ground water table and increased un-productive lands for the production of crops have complicated the agrarian welfare worldwide. So there is an urgent need to establish ways and means to make an agrarian to be sustainable for his livelihood. In this regard now we will look into various aspects of abiotic stress tolerance in plants.

4.2 Mechanisms of water relations and abiotic stress tolerance in plants

When demand by the plants exceeds the actual supply then there is a situation of water deficit. There are two important parameters which help to know the water status of the plant: one is water potential and another is relative water content. Water potential is sum of turgor potential, osmotic potential and gravimetric potential (negligible). Water potential is nothing but, if water content of leaf is low (depends on surrounding atmosphere) and if water content of soil is high, water moves from soil to the aerial parts of the plant; means water moves from higher concentration to the lower concentrations along the gradient like that of electrical potential. Water potential of pure water is always equal to zero and osmotic potential is in general negative. Therefore only when osmotic potential is more negative and water potential less negative then turgor potential would be positive or if when they are equal then turgor potential is zero. If progressive loss of water from leaves and also situation of drying of soil persists then it is a water deficit condition where water potential of leaf reduces meanwhile soil water potential also reduces, so leaf turgor would be highly reduced. There will be closing of stomata to reduce transpiration, death of leaf cells occurs and leaf wilting will be take place. Then what are the ways out for the plant to survive? One way is that plant can accumulate salts and sugars to reduce its osmotic potential (means more negative) and it reduces leaf water potential; there by it creates a gradient between soil and plant so that plant can take up water even from drying soils. Otherwise leaf rolling and stomatal conductance would reduce, leaf temperature may increase as a result; photosynthesis would be hampered and ultimately crop yields are severely affected. Therefore under stress, if turgor potential and transpiration rates are maintained the crop yields could be sustainable. This can be achieved by osmotic adjustment; nothing but accumulation of solutes to create steeper gradient between soil and the leaf for the movement of water from the soil to the leaf. Extreme temperatures (heat stress), high wind velocity and high salt (osmotic stress) concentrations can be resulted in water deficit due to reduced soil water potential and elevated leaf water potential and during this situation it is hurdle for the plant roots to draw the water from the soil.

4.3 Abiotic stress, its perception and signal transduction by the plants

Abiotic stress is a negative impact of non-living factors over the living entities of specific environment causing significant adverse effects on the physiology, biochemistry and molecular biology of an individual and/or population. Plants can perceive abiotic stress and elicit appropriate responses with altered metabolism, growth and development. Several factors determine, how plants respond to water deficit conditions viz., the genotype and developmental circumstances of the plant, the duration and severity of the stress, the number of times the plant is subjected to stress and any additive or synergistic effects of multiple stresses etc. Drought stress induces a range of physiological and biochemical responses in plants. These responses include stomatal closure, repression of cell growth and photosynthesis, and activation of respiration. Plants also respond and adapt to water deficit at both the cellular and molecular levels. An array of genes with diverse functions are induced or repressed which determine the adaption mechanisms of plant to moisture stress (Bartels and Sunkar, 2005). These include production of chaperones, late embryogenesis abundant (LEA) proteins, osmotin, mRNA-binding proteins, key enzymes for osmolyte biosynthesis, water channel proteins, sugar and proline transporters, detoxification enzymes, proteases, transcription factors, protein

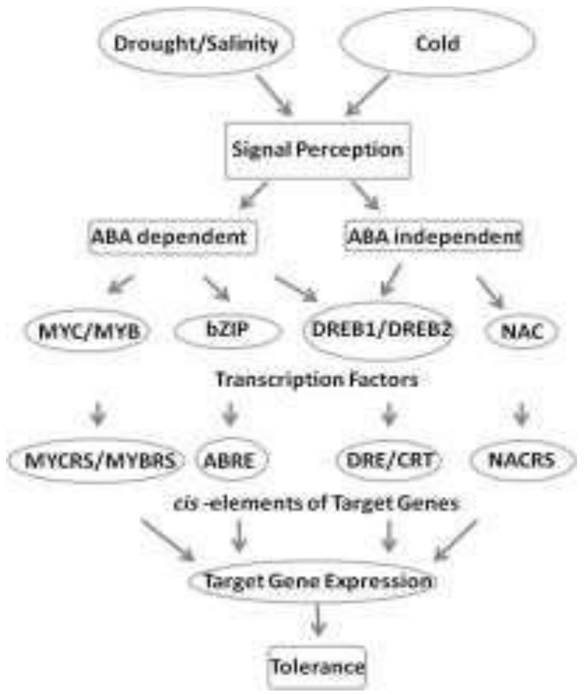


Figure 1. Schematic representation of ABA-dependent and independent pathways of stress signal perception and gene expression, at cellular level in plants (based on established

kinases, protein phosphatases, enzymes involved in phospholipid metabolism, and other signaling molecules such as calmodulin-binding protein *etc* (Xu *et al.,* 2009).

The differential stress tolerance of crop plants is infact not only due to differences in the perception of stress signals but also due to signal transduction cascades and gene expression profiles. The regulatory circuits include stress sensors, signaling pathways comprising network of protein-protein interactions, transcription factors and proteins or metabolites which imparts stress tolerance to plants. Abiotic stress signal transduction may takes place either by abscisic acid (ABA)-dependent or -independent pathways to regulate multiple stress responsive genes. Such genes can be grouped into functional genes and regulatory genes. Functional genes include those which are involved in synthesis of osmoprotectants and antioxidants. Whereas the regulatory genes include, transcription factors such as ABREB's, bZIP, DREB family, NAC family, MYB and MYC family. These regulatory genes exert their action due to presence of cis-elements at the promoter regions of abiotic stress responsive genes. The ABA-dependent pathways are under the control of an ABRE-element and b-ZIP transcription factors (TF's), whereas independent pathway is controlled by MYC and MYB family of transcription factors.

4.4 General strategies adapted by plants against abiotic stresses

Plants do not move from one place to the other and they also do not have an immune and blood circulatory system like that of animals. Therefore, they are forced to face several trajectories such as extreme temperatures, water supply and range of salinity profiles of soil in which they are being planted or grown (in general exposure to such extreme condition/s the term that is being called is "abiotic stress"). If this is the situation where; plants do not have an option of strategies executed by the animals to dissuade the effects of these physical fluctuations; then how do they survive? Plant resistance to abiotic stresses achieved by plant responses in terms of stress escape, avoidance and tolerance strategies:

(i).Escape: Stress escape strategies are nothing but completion of plant life cycle and/or stress sensitive life stages of plants in advance of the onset of the abiotic stress (drought, salinity, extreme temperatures etc). Plants over the years have evolved mechanisms broadly categorized as drought postponement and desiccation tolerance to combat water deficit. Most of the xerophytes adapt to this strategy.

(ii). Avoidance: Maintenance of plant water potential even under dry conditions is called as avoidance strategy. This helps the plants to survive under stress conditions and is achieved by various morphological and physiological changes. These includes; reduced leaf area, leaf rolling, cuticular wax, reduced transpiration, high water use efficiency (WUE), stomata closure, increased root length to the deeper layers of the soil *etc*.

(iii).Tolerance: It is an ability of plants to cope up under water stress for sustained cellular functionsfor its growth and development. This mechanism helps the plant cells to function even under lower water potential of plants. This is achieved by maintaining turgor and improved WUE through osmotic adjustment. This results from the synthesis of macromolecules and accumulating substances like osmolytes.

4.5 Biotechnological approaches for abiotic stress tolerance in plants:

4.5.1 Enhancement/incorporation of genes and/or biosynthetic pathways for the synthesis of osmoprotectants in crop plants: Osmoprotectants are also called as compatible solutes acting as osmolytes and are non-toxic molecules ranging from amino acids to cyclic alcohols, help in imparting osmotic stress tolerance to survive under drought, extreme temperatures and salinity conditions faced by the plants. Some of theosmoprotectants are Quaternary ammonium compound (glycine betaine), amino acid (Proline), sugar alcohols (Sorbitol, pinitol, mannitol, D-ononitoletc), and sugars(trehlose,fructans,octulose).

Table 1: Genes involved in plant responses to stress (modified from Pérez-Clemente *et al*., 2013, BioMed Research International Volume 2013, Article ID 654120, 10 pages).

Gene/s	Stress
14.3.3 gene family (GF14b, GF14c)	Salinity, drought, fungal
MAPK	Abiotic and biotic stresses
MEKK1 and ANP1	Environmental stress
MPK3, MPK4 and MPK6	Abiotic stress (pathogens) and oxidative stress

CBF/DREB families (CBF1, CBF2, DREB2A)	Drought, cold, salinity
HVA1	Salinity and drought
Glycerol-3-phosphate, acyltransferase gene	Cold
ICS	Pathogens, UV light
LOX	Wounding, drought, and pathogens
bZIPs family (e.g., ABF1, ABF2)	Drought, temperature, salinity
WRKY family (AtWRKY2, AtWRKY6, AtWRKY18)	Pathogens, wounding, salinity, temperature, drought, oxidative stress
ATAF	Wounding, drought, salinity, cold, pathogens

Table 2: Proteins and enzymes involved in plant responses to stress (modified from Pérez-Clemente *et al*., 2013, BioMed Research International Volume 2013, Article ID 654120, 10 pages).

Proteins and enzymes	Stress
ERF family	Cold, drought, pathogen infection, wounding, ET, SA
bZIPs family (e.g., ABF1, ABF2)	Drought, temperature, salt
WRKY	Pathogens, wounding, salinity, temperature, drought, oxidative stress
MYB family (AtMYB15, AtMYB30, AtMYB33, AtMYB60, AtMYB96, AtMYB101 AtMYB15, and AtMYB108)	Biotic and abiotic stress (pathogens, drought, cold)
ABF	Drought
NAC	Drought, salinity, cold
LEA family (PMA 80, PMA 1959)	Salinity and drought
Heat shock proteins	Temperatures
LOX family (e.g., LOX1)	Wounding, drought, and pathogens
Glutathione peroxidase, superoxide dismutase, ascorbate peroxidases, and glutathione reductases	Oxidative stress

Table 3: Metabolites and hormones involved in plant responses to stress (modified from Pérez- Clemente *et al*., 2013, BioMed Research International Volume 2013, Article ID 654120, 10 pages).

Metabolites and hormones	Stress
Abscisic acid, jasmonic acid, salicylic acid, polyamines and others	Drought, salinity, cold
Proline, glycine-betaine, and other compatible osmolytes	Environmental stresses: drought, salinity, osmotic
Phytoalexins	Microbial pathogens
Terpenes	Toxins and pathogens
Phenolic compounds (coumarin, lignin, flavonoids, tannins, isoflavonoids)	Pathogens, oxidative stress, UV light
Alkaloids	Pathogens (predators)
Unsaturated fatty acids	Environmental stresses
ROS, malondialdehyde	Biotic and abiotic stresses
Phytochelatins and metallothioneins	Heavy metal intoxication

4.5.2 Glycine betaine (GB): It is a quaternary ammonium compound, electrically neutral over wide range of p^H values, and is an excellent solvent in water. GB has three methyl groups with quaternary ammonium group enabling its interaction with hydrophobic as well as hydrophilic compounds to stabilize the membrane proteins.

Several speciesof plants, algae and microbes synthesize GB.The cultivated plants like spinach, wheat and sugar beet and other organisms such as halo-tolerantcyano bacteria, *E. coli*, *Arthrobacter globiformis* etc are known to synthesize GB. But several economically important crop plants such as tomato, rice and potatodo not synthesizes GB and are called as non-accumulators of GB. Let us look into its synthesis in different organisms and their prospects for improvement of non-accumulator plants.

Table 4. Crop plants and their respective cultivars/lines transformed with GB synthesizing genes (modified from Ahmad *et al*., 2013, *Plant Biotechnol. Rep.* 7:49–57)

Crop plant	Cultivar/line	Gene introduced	Enhanced tolerance	Growth stage
Brassica juncea	Pusa Jaikisan	codA	Salt	Germination
Brassica napus	Westar	COX	Salt, drought	Plantlets
Brassica oleracea	Capitata	betA	Salt	Plantlets

Diospyros kaki	Jiro	codA	Salt	Plantlets
Daucus carota	Half long	BADH	Salt	Plantlets
Gossypium hirsutum	Luyuan890	betA	Drought, osmotic	Seedlings, plants
Gossypium hirsutum	Simian 3	CMO	Salt	Plants
Oryzasativa	Nipponbare	coda	Salt, chilling	Plants, leaves
Oryzasativa	Pusa basmati 1	coda	Salt	Plants
Oryzasativa	TNG67	COX	Salt	Plants (24 days)
Oryzasativa	Sasanishiki	CMO	Salt	Seedlings
Solanum lycopersicum	Moneymaker	coda	Chilling	Seedlings, plants
Solanum lycopersicum	Bailichun	BADH	Salt	Plants
Solanum lycopersicum	Pusa ruby	coda	Salt, drought	Seedlings, plants
Solanum tuberosum	Superior	coda	Oxidative, salt, drought	Leaf disc, plantlet, plants
Solanum tuberosum	Gannongshu 2	BADH	Salt,	osmotic Plants
Triticum aestivum	Jinan 17	beta	Salt	Plants
Triticum aestivum	Shi4185	BADH	Drought, chilling	Seedling
Zea mays	DH4866	beta	Chilling	Germination, seedling
Zea mays	DH4866	beta	Drought	Plantlets, plants

Note: BADH: betaine aldehyde dehydrogenase; CMO: *Choline mono oxygenase*; choline oxidase catalyzes (COD encoded by *codA* gene); GSMT: Glycine sarcosine methyl transferase; SDMT: Sarcosine dimethylglycine methyl transferase.

As per the above biosynthetic pathways of GB, one can say that *Arthrobacter globiformis (AG)* and *Actinopoly sporahalophilia* possess a unique kind of GB synthesis. *AG* can synthesize GB with a single enzyme choline oxidase (COD/COX encoded by *codA* gene) utilizing choline as a substrate but in *AH* glycine is the initial substrate. As per the information available engineering of crop plants with AG codA gene could increase GB levels with enhanced stress tolerance. The AH enzymes GSMT and SDMT encoding genes can be engineered into crop plants to utilize readily available glycine as an initial substrate to synthesize GB to enhance stress tolerance. But engineering of plants with CMO/CDH, BADH could not increase GB quantities significantly. It may be due to lower production of intermediary betaine aldehyde in plants. As another approach can also go for gene stacking for the synthesis of GB with the all relevant genes in order to increase GB levels to provide abiotic stress tolerance.

4.5.3 Proline: Among all the protein-forming amino acids it is unique where, the amine nitrogen is bound to two alkyl groups but normally to one, so it is a secondary amine. It is the extensively studied osmolyte, proposed to function during drought and stressful situations of plants. Besides osmotic adjustment, as a sink of energy or reducing power, protector of plasma membrane, a source of carbon and nitrogen or hydroxyl radical scavenger (Hong *et al*., 2000). Proline is known to increase during abiotic stresses (drought, salinity etc) as compared to normal conditions. This is due to induction of proline biosynthetic genes (P5CS (Δ^1-Pyrroline-5-carboxylase synthase), P5CR (Reductase) etc., signaling molecules (ABA and calcium mediated Phospholipase D) and also presence of stress responsive cis-elements in proline biosynthetic pathway genes. On the other hand proline degrading enzymes would not be activated during stress situations and overall stress tolerance to the plants. Due to its immense roles during stress, metabolic engineering of rate limiting enzymes such as P5CS, Phospholipase D and other enzymes has been demonstrated in several crops. RNAi technology also has been employed to down-regulate proline degrading enzymes in some of the crops.

Table: 5. List of transgenic plants developed by enhancing proline accumulation and abiotic stress tolerance (modified from Khan et al., 2015, Electronic Journal of BiotechnologyVolume 18, Issue4, July 2015, Pages 257–266)

Target	Transgene	Stress tolerance
A. thaliana	Antisense ProDH cDNA	Freezing, salt
C. cajan	P5CSF129A	Salt
Daucuscarota	P5CS	Salt
N. tabacum	P5CS	Drought
Petunia	P5CS	Drought
N. tabacum	P5CSF129A cDNA	Drought

N. tabacum	OsP5CS1and OsP5CS2	Abiotic stress tolerance
O. sativa	P5CS	Salt
S. tuberosum	P5CS	Salt
Triticumaestivum	P5CS	Salt

4.6 Sugars and sugar alcohols

4.6.1 Mannitol: Transgenictobacco and Arabidopsis were developed by transforming the Mannitol-1- phosphate dehydrogenase from *E. coli*, which isencoded by *mt1D*. These plants do not produce mannitol but when this gene was transformed, there was substantial increase in the levels of mannitol expression and was found to confer water deficit tolerance (Salinity, osmotic stress,drought tolerance) in transgenics but not in non-transgenics. Mannitol is also known to fight back the effects of oxidative stress by providing plant system with an excellent ability to scavenge the hydroxyl radicals. However mannitol is also known to have side effects over plant growth and development. Hence it may not be a promising approach to develop abiotic stress tolerant transgenics by over-expressing mannitol.

4.6.2 Fructan: Fructan can be synthesized from fructose, Levansucrose generates fructose, scientists transformed tobacco plants with *sacB*gene (encoding for the synthesis of Levansucrose) from*Bacillus subtilis*. Compared with wild type plants *SacB* tobacco transgenics exhibited higher fresh weight(33%), growth rate(55%), dry weight (59%) and their performance was better than wild type plants during drought in a significant manner. Likewise transgenic beet plants producing fructan thrived very well compared with the non transgenics.

4.6.3 Pinitol and D-Ononitol: Myo-inositol forms ononitol and pinitol upon methylation and isomerization, these O-methyl inositols have got potential roles in abiotic stress tolerance. Myo-inositol 1-phosphate synthase (MIPS) and myo-inositol monophosphatase (IMP) converts glucose- 6-phosphate into Myo-inositol. Under saline conditions (High salt) themyo-inositol-O- methyltransferase (IMT) will be methylated and converts myo-inositol into D-ononitol followed by epimerization by D-ononitolepimerase (OEP) to D-pinitol. Transgenic tobacco plants over- expressing cDNA of IMT from ice plant (*Mesem bryanthemum crystallinum*) conferred drought and salinity stress tolerance due to accumulation of D-Ononitol (35 μmol g^{-1} fresh weight in the cytosol). Unlike mannitol, photosynthetic CO_2 fixation was less affected in transgenic plants during water deficit conditions when compared with wild type plants.

4.6.4 Sorbitol: Apple cDNA encoding sorbitol-6-phosphate dehydrogenase (S6PDH) has been expressed in Tobacco plants where it showed negative growth effects but when Japanese persimmon was transformed with the same gene conferred salt stress tolerance with better photosynthetic rates whencompared with the transgenic.

4.6.5 Trehalose: This is a disaccharide with an ability to prevent disruption of cellular components by forming gel phase upon water loss from the cell thereby it is an excellent agent for water deficit Yeasttrehalose-6-phosphate synthase (TPS1) was transformed into tobacco, even though there was a negative effect, drought tolerance was improved over wild tobacco plants. Like mannitol trehalose over accumulation also have negative effects over growth and development of plants. Therefore attention of researchers is invited to get insights into this phenomenon to rectify the facts to impart abiotic stress tolerance along with normal growth and development of plants.

4.7 Other approaches for salinity tolerance to withstand water deficit by the plants

Osmoprotectants can help to overcome osmotic stress due to salinity but protection against toxiceffects of some of the ions required additional approaches such as ion homeostasis. The glycophytic plants produces osmolytes in response to higher saline conditions for their protection but halophytes employ different kind of mechanisms to withstand saline conditions created by the toxic effects of Na+ and Cl- ions. Halophytes excrete higher salts through specialized glands present over their leafsurface as a phenomenon to regulate Na^+ concentration in the cytoplasm of their cells of non-vulnerable cells. In case of vulnerable cells, if vacuoles are small they actually transport it out oftheir cells and if vacuoles are large where they acts as a salt storage centers of the cells for the accumulation of excess sodium. Therefore special biosynthetic pathways and their rate limitingenzymes need to be uncovered for their utilization in crop improvement programs for abiotic stresstolerance .Influx of sodium ions results from the potassium ion influx process where transport proteins can't differentiate between these two ions and also the entered sodium ions compete forpotassium binding sites in the cell. Therefore sodium exerts its toxic effects over plant growth anddevelopment, however halophytes withstand/overcome the sodium effects but not by the glycophytes. Some of the potassium ion(K^+) carriers involved in the influx of Na^+, one such carrier is AtHKT1(selective Na+ transporter) and has got lesser affinity for K^+ transport. It has been provedthat influx of Na^+ into plant roots is regulated by the AtHKT1. Here in Arabidopsis, mutants of this transporter were developed (*hkt1*mutants) where Na^+ accumulation was suppressed.

Transgenic tomato plants were developed with AtNHX1 and were able to record 20 fold higher salt content than wild type plants when grown using 200mM NaCl. However sodium and chloride content of fruits were on par with wild type plants indicating vacuolar transport of salts (Sodium). NHX1 gene has been isolated from *Atriplexgemelini* (Halophyte) and has been transformed into rice, which showed improved salt stress tolerance. Another gene AtSOS1 encoding for plasma membrane antiporter when over-expressed in Arabidopsis, the callus growth was good in salt media along with reduced xylem NA^+ levels when compared with non-transformed callus cultures. The AVP1 Arabidopsis transgenics were raised to obtain cellular vacuoles with enhanced proton pumping potential. These transgenics not only conferred salt tolerance but also drought tolerance, by altering ion balance to retain water under stress.

4.8 Heat stress tolerance

In addition to compatible solutes, heat shock proteins are also known to have potential roles in response to extremely higher temperatures. These heat shock proteins (Hsp) essentially belong to five classes and are named according to their molecular weights *Viz*; Hsp100, Hsp90, Hsp70, Hsp60 and Hsp40. The members of these Hsp's play a vital role in protein refolding of affected proteins, proper protein synthesis and protection of proteins during severe heat stress and such kind of proteins are also called as molecular chaperones. Heat shock response in the tolerant plants is mediated by coordinated action of Hsp70 and HSF (Heat shock factor). When plants come across heat stress Hsp70 dissociates from HSF, otherwise HSF will be in the form of monomer and HSF assumes trimeric confirmation. The trimeric HSF have an ability to bind at HSE (heat shock element) regions of Hsp genes which actually impart heat stress tolerance by regulating the expression of other Hsp genes.*AtHSF1* encoding for transcription factor was over-expressed in *Arabidopsis* and was found to be inactive and these transgenics did not show heat tolerance. But then fusion of *AtHSF1* to N/C terminus of *gusA* could produce fusion protein and trimerized and transgenics were tolerant to heat. Rice plants were transformed with *Arabidopsis* Hsp101 inorder to study heat stress tolerance and they could obtain significant growth performance under stress.

4.9 Cold stress tolerance

Cold and freezing stress imposes differential effects over various plants growing across different climatic regimes, tropical plants has least tolerance whereas temperate species could withstand the cold. The process of acclimation that is prior exposure of the plants to cold and then to their normal climates would enhance cold tolerance to the plants. The plant breeding efforts could not make much improvement for cold stress tolerance. Therefore genetic engineering approach has been an attractive strategy to impart cold stress tolerance. When a particular plant under question subjected for cold/freezing stress, resistant plants are able to up- regulate cold response genes such as *COR* genes, *LEA* genes etc. The proteome part synthesized as a result of these genes speculated to impart cold/freezing stress by reducing cold imposed dehydration effects over the plants expressing them. Therefore identification of such regulons and associated genes for their over-expression in the susceptible plants would be better strategy to generate tolerant germplasm by genetic engineering.

4.10 Effects of reactive oxygen species (ROS) during abiotic stress and strategies for plant tolerance:

Like other stresses abiotic stresses also have secondary effects such as oxidative stress due to production of ROS *viz*; singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radical. It is known that chloroplast, peroxisomes and/or mitochondria are the main sites where ROS synthesis pathways are present and hence they exhibit intense rate of electron flow in the cells. These organellar membranes constitute PUFA (poly unsaturated fatty acids) and there is heavy oxidizing reactions and lipid peroxidation takes place as a result the plant cells face the problem of oxidative stress under stress conditions. In addition oxidative stress also damages proteins by amino acid modifications, peptide chain breakages, changes in electric potential of cells and makes the cells vulnerable to proteolysis. In case of DNA it causes sugar-base degradations, cross linkage with proteins and DNA strand breakages leading to heritable genetic effects would result. Therefore ROShave an ability to damage cellular macromolecules *viz* lipids, carbohydrates, proteins, nucleic acids *etc* and under severe circumstances death of plants may occur.

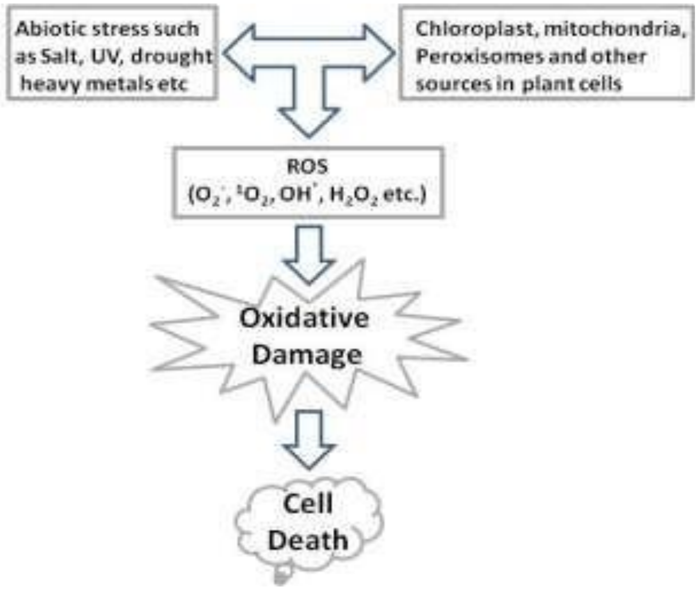


Fig. 2. Abiotic stress induced ROS production and cell death (Modified from Gill SS and Tuteja N. (2010) Plant Physiology and Biochemistry 48: 909-930).

4.11 Genetic engineering strategies to cope with the effects of ROS:

Plants can synthesize some special compounds called as antioxidants either by enzymatically or non-enzymatically which can be able to reduce or nullify the effects of ROS. Enzymatic antioxidants are: catalases, SOD (Super oxide dismutase), glutathione peroxidases, glutathione reductase etc. Non-enzymatic antioxidants are: carotenoids, flavonoids, glutathione, proline, α- tocopherols etc. As usual only few species of plants or any organism for that matter have such kind of strategies to cope with the adversities of their environment. Therefore identification of such species or living cells of any organisms and isolation and characterization of key enzymes and/metabolites is need of the hour. However substantial research has taken place in this regard but with limited commercial success. Engineering of the crop plants with such genes/regulatory molecules and even pyramiding of several of such genes would enhance the ability of crop plants toreduce toxic effects of oxidative stress.

Summary

Unlike biotic stress tolerance where it is generally governed by central switch called R-gene resistance but the abiotic stress tolerance is mediated by networks of switches (ex: TF's). However,further more understanding of molecular and genetic basis of stress tolerance needs to be made. This would help the scientific community to excel far beyond the available natural variation for stress tolerance through genetic engineering and other molecular tools and techniques. Molecular breeders need to identify true QTLs responsible for abiotic stress tolerance so that the biotechnologists can plan for cloning and successful incorporation of such true QTLs in crop plants. In this era of next generation sequencing there is a need to go for precise screening of the tolerant phenotypes of different plants to identify still more promising genes, TF's and regulons in response to abiotic stresses for improved productivity of crop plants. Another most important improvement needs to be taken care is that the development of effective inducible expression systems such as stress inducible promoters to increase the efficiency of various physiological parameters for the reduction of crop losses due to abiotic stresses.

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EPG Pathshala: Subject: Botany, Paper: Plant Genetic Engineering, Module: Strategies forAbiotic Stress Tolerance in Plants.

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Unit 5: Thermoreception and Thermoregulation

Unit Structure

5.0 Objectives

After reading this unit students should be able to:

- Understand Heat balance and homeothermy in humans
- Understand Thermoregulatory responses in humans
- Understand Adaptations taking place in hot and cold thermal environment.
- Understand Modes of heat transfer and thermogenesis
- Understand Thermoregulation and Adaptations taking
- Understand cold temperature stress in plants
- Understand high temperature stress in plants

5.1 Introduction

Humans are homeotherms -they have the ability to maintain a constant core temperature irrespectiveof changes in their ambient temperature.

5.2 Body Temperatures

Core temperature is defined as the temperature in the deep tissues, great vessels of the body. Clinically, temperatures measured in the mouth, axilla, tympanic membrane and rectum serve as measures of the core temperature. The normal core body temperature is 98.4°F (37°C) with a range of36.5-37.5°C. There is a diurnal variation of 0.5°C with the temperatures increasing towards the evenings. Maintenance of the core body temperature is a key homeostatic process as several metabolic, chemical and physical processes in the body are temperature dependent.

Skin temperatures can be measured at multiple sites - upper limb, lower limb, forehead, chest, back etc. The skin temperature changes with the environmental conditions. Clinically, a mean skin temperature is computed by taking a weighted sum of temperature recordings from multiple sites

Weighted T_{skin}(°C) = 0.25 Forehead T+ 0.25 chest + 0.25 back T+ 0.15 upper leg T+0.05upper armT+ 0.02 lower arm T+ 0.01 palm T+ 0.02 Foot T

5.3 Heat Balance

Heat balance represents the dynamic equilibrium between the heat production and heat dissipation in the human body. Heat is produced as a consequence of basal metabolism, digestion, physical activity. Heat is transferred to the environment by physical processes like conduction, convection, radiation and evaporation.

Heat Production = Metabolism+ Heat gain from environment-External work on environment

Heat Dissipation = Heat loss through (Radiation + Conduction+ Convection + Evaporation)

Heat Balance = Heat Production – Heat Dissipation

Positive heat balances over time would lead to increases in core temperature and a negative heatbalance over time would lead to a fall in core temperature.

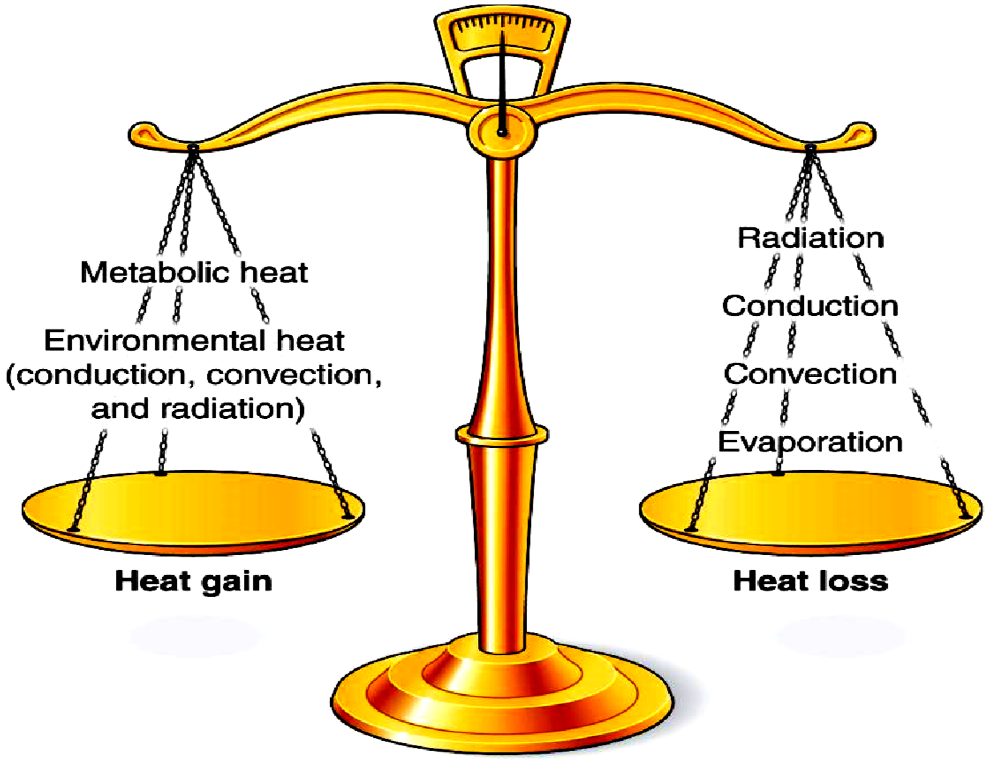

Fig 1: Heat balance

5.4 Heat production

The body produces energy through the oxidation of various substrates, some of this energy is released in the form of heat. Chemical processes taking place during metabolism, digestion, muscular contraction are associated with release of heat. Increases in the metabolic rate are associated with increased heat production. Shivering is a series of repeated alternating contractions and relaxations of muscles.

Shivering thermogenesis leads to additional heat production if and when the requirement arises. Non Shivering Thermogenesis takes place in certain tissues like brown adipose tissue through the uncoupling of oxidative phosphorylation leading to the entire energy produced by the oxidation ofrespiratory substrates being released as heat. The basal heat production in the body for an average person is approximately 70-75 kcal/hr. This can increase up to 800 kcal/hr based on the environment, physical activity, feeding.

5.5 Heat Transfer

The amount of heat transferred to depends on factors like temperature difference between the heat transfer surface and environment, surface area exposed to environment, humidity and wind flows.

5.5.1 Passive Heat Transfer

Conduction is heat transfer via thermal motion of molecules between surfaces in direct physical contact. In Convection, a bulk fluid flow serves as the mode of heat transfer. At conventional room temperatures, the body warms the air immediately overlying the skin and this warm air is gradually replaced by cool air due to warm air rising as a consequence of its lower density. Presence of a wind can lead to this warm air being replaced faster leading to a greater amount of heat loss through the process of forced convection. This is the basis of wind chill where faster heat loss takes place when the body is exposed to the wind. Usage of clothes can modify convective heat loss. Usage of loose fitting clothes favours convective heat loss to the environment in summers. Usage of tight fitting clothes, multiple layers of clothes helps to reduce convective heat loss in the winters. Radiation leadsto heat transfer by the emission of infrared rays. All physical objects emit and absorb radiation. The amount of radiant heat emitted is

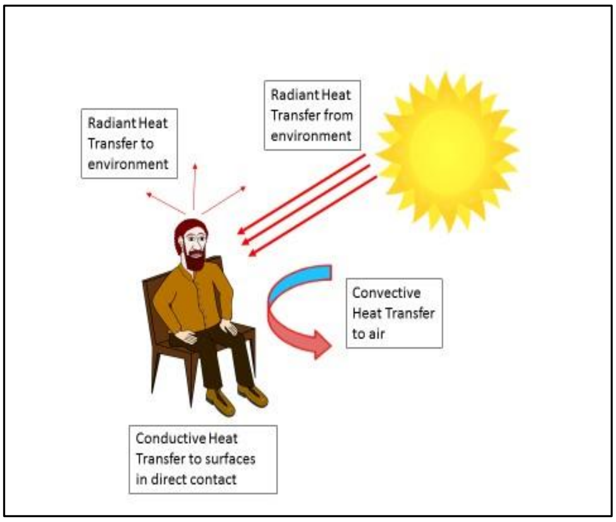

Fig 2: Modes of Passive Heat Transfer

directly proportional to the absolute temperature (Kelvin) of the body. In an indoor environment, radiation contributes about 60% of the total heat transferred. Conductive, convective and radiant heat transfer from the body surface add up to a net heat loss if thetemperature of body surface exceeds ambient temperature and to a net heat gain if the ambient temperature is warmer than the body surface.

5.5.2 Active Heat Loss

Evaporative heat loss takes place through the evaporation of sweat produced at the skin surface andrespiratory water vapour. The amount of evaporation depends on the difference between aqueous vapour pressure at the skin surface and environment. Thereby the amount of heat lost through evaporation is affected by the relative humidity of the environment. Heat loss through sweating andevaporation represents the only way the body can effectively lose heat to an environment with a higher ambient temperature than the body.

5.6 Sweat Glands

Sweat glands in the human body are of two types – Apocrine and eccrine aweat glands. Eccrine glands are distributed in the skin all over the body and are involved in thermoregulation. Sweat produced by eccrine glands consists primarily of water and NaCl. Water evaporation causes heat lossdue to the latent heat of vaporisation (0.54 kcal/g of water). Eccrine glands have a subdermal secretory coiled portion and a reabsorptive portion which opens into a pore at the skin surface. They are innervated by sympathetic nerves releasing acetylcholine at their terminals. Sympathetic stimulation increases the sweat production rate.

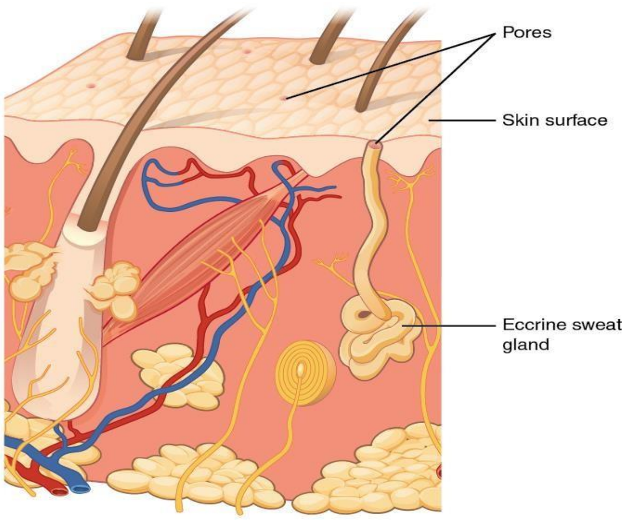

Fig 3: Eccrine Sweat Glands in skin

5.7 Thermoregulation

Thermoregulation refers to all the processes involved in maintenance of core temperature within a normal range. It involves sensing by the thermoreceptors, processing in neural pathways and effector responses regulating the production or dissipation of heat.

5.7.1Thermoreceptors

These are neurons which are responsive to changes in temperature. They are classified based on their location as central and peripheral thermoreceptors. Central thermoreceptors are found in brain, spinal cord, viscera and the great vessels. The preoptic area and anterior hypothalamus contain large numbers of temperature sensitive neurons. The hypothalamus is a key area for the sensing of changes in the core temperature. Peripheral thermoreceptors are present as free nerve endings in the skin distributed across various sites of the body. They are responsible for the sensing of ambient temperature. They can also be classified based on their temperature response. Warm thermoreceptors increase their firing rate at higher temperatures and cold thermoreceptors do so likewise at lower temperatures. Thermoreceptors serve as sensors for both the core and ambient temperatures. Neural pathways like the spinothalamic tract transmit information from peripheral thermoreceptors to the cerebral cortex for the conscious perception of external temperature. There is a parallel sensory pathway to the hypothalamus which serves as a neural integrating centre for coordinating the thermal sensory input and effector responses involved in thermoregulation.

5.7.2 Hypothalamic neural processing

The hypothalamus acts as a control system maintaining the core temperature at a set point. Preoptic area of basal forebrain and anterior hypothalamus are the key areas involved in thermoregulation. They integrates inputs from peripheral skin thermoreceptors and central thermoreceptors and initiates appropriate thermoregulatory responses. Changes in core temperature are sensed by central thermoreceptors and serve as feedback to the hypothalamic controller which initiates responses to correct the error in core temperature. Changes in ambient temperature are sensed by peripheral thermoreceptors which act in a feedforward manner on the hypothalamus to initiate thermoregulatorychanges before core temperature is affected

5.7.3 Thermal effectors

Hypothalamic output is received by medullary centres like the cardiac vasomotor centre (CVC) and the raphe pallidus. The medullary centres control the activity of the sympathetic system through the intermediolateral horn (IML) of the sympathetic system and the motor neurons innervating the muscles involved in shivering through premotor neurons arising from the shivering centre in the raphe pallidus. The Hypothalamus controls the following thermoregulatory effector responses

1. Vasomotor control
2. Shivering and thermogenesis response
3. Sudomotor response (sweat production).

Heat is transferred across the body surface. Regulation of cutaneous blood flow regulates the heat transferred from the body core to the surface. Skin blood flow is under the control of the autonomic nervous system. Increase in the sympathetic tone leads to a reduction in skin blood flow. Shivering and sweat production lead to active production and dissipation of heat respectively.

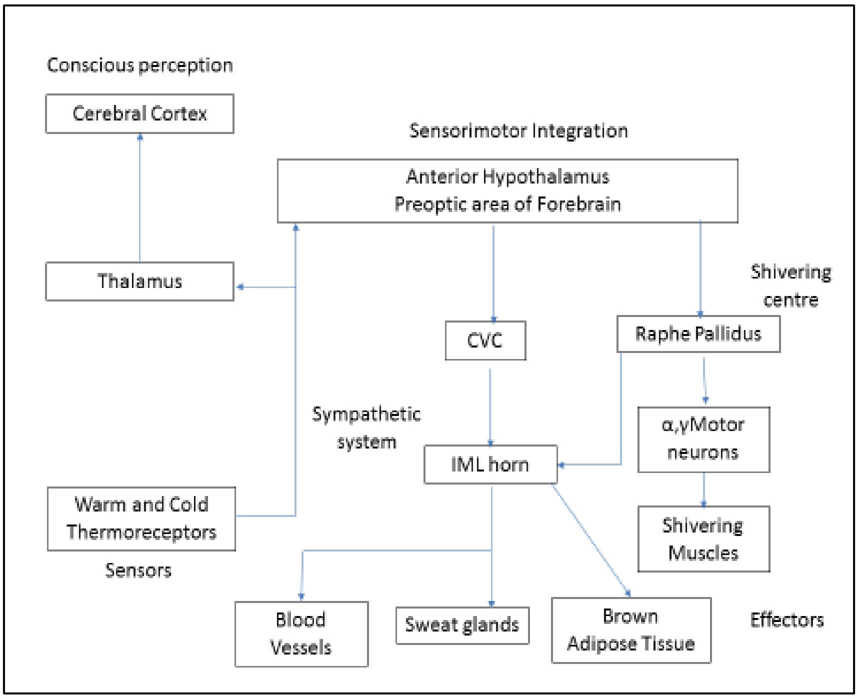

Fig 4: Central Thermoregulatory pathway

CVC – cardiac vasomotor centre, IML – Intermediolateral horn in spinal cord

In addition to the physiological thermoregulatory response, conscious awareness of ambient temperature can lead to behavioural responses like the selection of optimal clothing, controlling the environment through usage of cooling and heating devices, moving to more comfortable environments, indoor habitation etc. In human beings, behavioural responses enable the tolerance of awider range of temperatures than the one possible if thermoregulation was exclusively obtained through physiological means. Behavioural and short term responses enable thermoregulation on acute exposure to different thermal environments. Prolonged exposure to specific thermal environments lead to the development of long term changes which facilitate life in such conditions. Acclimatization refers to changes brought about by exposure to a new environment, which favour adjustment and survival in the new environment. Acclimation isa specific term referring to the responses brought about by a change in an experimental variable in a controlled environment. The term adaptation used in an evolutionary context refers to heritable traitswhich facilitate the survival and function in a population across multiple generations in challenging environments.

5.7.4 Thermoneutral Zone

This is a range of ambient temperatures where heat balance is obtained between heat produced during basal metabolism and heat transferred to environment. Thermoregulation is obtained just through vasomotor control without the need for additional thermogenesis or active heat dissipation through thermoregulatory sweating. The thermoneutral zone ranges from 25-30°C for a person in a neutral environment without clothes. The thermoneutral zone is modified by the usage of clothes and shifts toa lower range of temperatures based on the extent of insulation provided by clothing. At temperatures below the lower critical temperature (LCT) and above the higher critical temperature (HCT), active thermoregulation takes place leading to increased metabolic rates. The Thermoneutral zone is affected by:

1. Air Temperature (Dry-Bulb)
2. Relative Humidity
3. Air Velocity
4. Radiation (Mean Radiant Temperature)
5. Metabolic Rate
6. Clothing Insulation

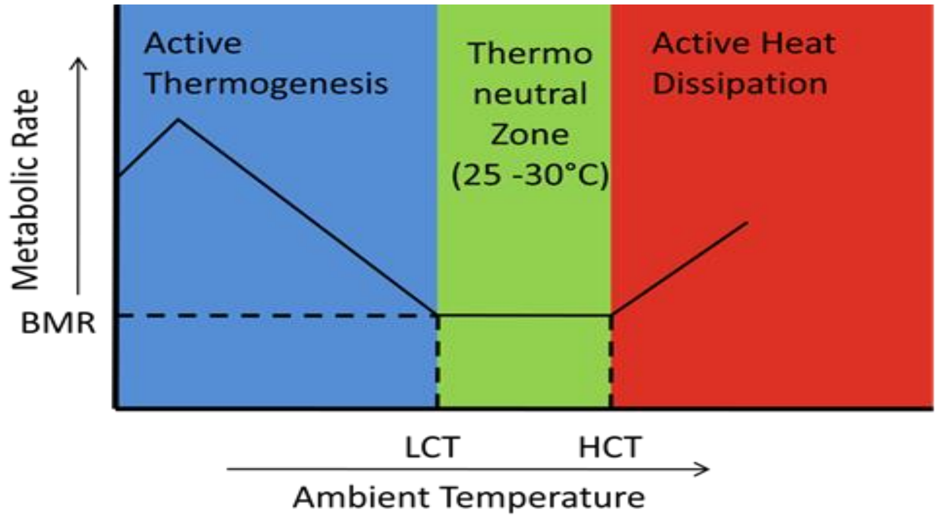

Fig 5: Thermoneutral Zone: The ambient temperature range associated with maximum thermal comfort. LCT – lower critical temperature, HCT – higher critical temperature.

5.8 Responses in a hot environment

On earth, a hot environment can be dry hot or hot-humid each presenting a different set of thermoregulatory challenges. Dry hot environments can be encountered in the interior continental summers or deserts where the ambient temperature can rise up to 45-50 °C and the ground surface temperatures can be even higher. At such high temperatures, there is cutaneous vasodilation. Sweat production and evaporation serves as the only means of heat dissipation. Excessive sweat production can lead to dehydration and volume depletion leading to heat syndromes like heat stroke etc. Behavioural responses like the usage of light coloured, loose fitting clothing, adequate water consumption, avoiding prolonged sun exposure, seeking cool environments contribute significantly to maintenance of the core temperature.

Hot, humid environments are encountered in tropical regions. In such environments, the increased atmospheric humidity prevents the evaporation of sweat. Thus sweat tends to drip but not evaporate leading to inadequate heat dissipation. This leads to increases in core body temperature. Thus the temperature perceived in a humid environment is higher than the one perceived in a dry environment with the same ambient temperature. The heat index is computed taking into account both air temperature and relative humidity.

Sudden exposure of unacclimatised individuals to hot environments leads to:

1. Rise in Core body temperature.
2. Delayed onset inefficient sweating.
3. Increased heart rate, stroke volume, cardiac output, blood pressure.
4. Volume depletion due to fluid and electrolyte losses during sweating. Increased plasma osmolality, increased thirst sensation, decreased plasma volume and total body water.

Table 1: Heat Index

Ambient Air temperature (°C)	21.1	23.9	26.7	29.4	32.2	35.0	37.8	40.6	43.3	46.1
Relative Humidity										
10%	18.3	21.1	23.9	26.7	29.4	32.2	35.0	37.8	40.6	43.9
20%	18.9	22.2	25.0	27.8	30.6	33.9	37.2	40.6	44.4	48.9
30%	19.4	22.8	25.6	28.9	32.2	35.6	40.0	45.0	50.6	57.2
40%	20.0	23.3	26.1	30.0	33.9	38.3	43.3	50.6	58.3	
50%	20.6	23.9	27.2	31.1	35.6	41.7	48.9	57.2	65.6	
60%	21.1	24.4	27.8	32.2	37.8	45.6	55.6	65.0		
70%	21.1	25.0	29.4	33.9	41.1	51.1	62.2			
80%	21.7	25.6	30.0	36.1	45.0	57.8	69.4			
90%	21.7	26.1	31.1	38.9	50.0	65.6	76.7			
100%	22.2	26.7	32.8	42.2	56.1	74.4				

Danger **Extreme Danger**

5.9 Heat Syndromes

The thermoregulatory capacity of the body can be overwhelmed by prolonged exposure to extremely hot environments. This can lead to heat syndromes of varying severity.

Heat Cramps- Cramps can be manifested in limb skeletal muscles following excessive fluid andelectrolyte depletion due to excessive sweating.

Heat Exhaustion – This resembles a peripheral circulatory shock due to widespread cutaneousvasodilation leading to hypotension,

Heat Stroke – Thermoregulatory failure leads to rise in core temperature to levels as high as 41°C -Hyperthermia. There is no sweating and systemic hypotension due to extensive vasodilation.

Dizziness, weakness and a transient loss of consciousness may take place due to reduction in cerebralblood flow. Hyperthermia is associated with tissue damage leading to rhabdomyolysis, disseminated intravascular coagulation, acute kidney injury, injury to brain parenchyma.

5.10 Acclimatization to hot environments

Continued exposure to hot environments lead to acclamatory changes which are initiated on days 4-6of exposure and peak around 2 weeks from the onset of exposure. Exercise and physical activity performed in hot environments have a synergistic effect on heat acclimatization. Acclimatization brings about changes in the onset thresholds and sensitivity of the thermoregulatory responses. This increases the range of the thermoneutral zone.

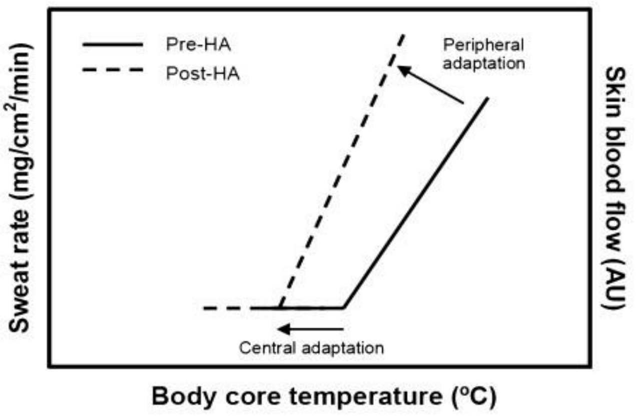

Fig 6: Effect of Heat Acclimatization (HA) on thermoregulatory responses

5.10.1 Efficient Sweating: Acclimated individuals exhibit enhanced sweating capacities, the threshold of ambient temperature for onset of sweating is shifted to lower temperatures thereby preventing increases in core temperature on exposure to warm environments. The volume of sweat produced is increased. Aldosterone secretion is also increased which causes increases reabsorption of salts in the sweat tubule thus minimising the loss of electrolytes during sweating. There is also improvement in the cutaneous blood flow, fluid balance and cardiovascular performance. Certain cytoprotective heat shock proteins are also expressed in cells which enable themto tolerate higher temperatures.

5.11 Responses in a cold environments

Cold environments are encountered at the higher latitudes and altitudes. The increased loss of heatdue to the body environment temperature difference presents a thermoregulatory challenge. Human beings are dependent on behavioural strategies like insulative clothing, warm shelters and artificial heating devices to control the microclimate around themselves. The first response is sympathetically mediated vasoconstriction which reduces blood flow and transfer of warmth to the periphery of the body. The arteries and veins are arranged in a counter current system which enables the transfer of heat from the warmer arterial circulation to the colder venous circulation favouring the retention of heat in the core of the human body. This causes the temperature of the exposed parts of the body to fall thereby minimizing heat loss to the environment. Piloerection (standing up of hairs) leading to the 'goosebumps' appearance is seen. In addition to the increased sympathetic output, there is also increased release of Thyroid stimulating hormone (TSH) and Adrenocorticotrophic hormone (ACTH) from the anterior pituitary. There is a compensatory increase in the metabolic rate and thermogenesis to prevent the fall of core temperature.

Thermogenesis is classified into shivering and non-shivering thermogenesis. During shivering, there is rhythmical activation of the motor neurons innervating the shivering muscles of limbs and trunk leading to an alternating contraction and relaxations in the muscles. This pattern of muscular activity does not perform external work against the environment and thus all the energy produced during the oxidation of metabolic substrates during muscle contraction is released as heat. The shivering response can be activated by a fall in core temperature and also by a fall in ambient temperature as sensed by peripheral thermoreceptors. Shivering can increase heat production to a level of about four times the basal metabolic rate

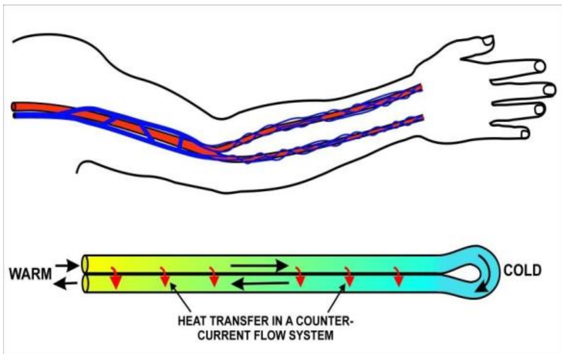

Fig 7: Counter-current arrangement of arteries and veins

Non shivering thermogenesis is observed in brown adipose tissue (BAT) in human infants. Human infants are prone to hypothermia due to increased heat losses due to a high surface to volume ratio. BAT is seen in sites like interscapular, substernal and paraspinal regions. BAT has dense sympathetic innervations and leads to production of heat through the process of uncoupling of ATP production from oxidation.

5.12 Adaptations to cold environment

Acclimatization to cold environments is rarely seen in humans as they are rarely exposed for prolonged durations to cold weather due to behavioural responses like clothing, sheltering and usage of artificial heating which help to regulate the microclimate the person is exposed to. Overall, the acclamatory responses to prolonged cold exposure in humans are of a lower magnitude than in animals. These are primarily of two types – Insulative and metabolic adaptations metabolic adaptations

There is an enhanced capacity of elevated thermogenesis during cold exposure. A reduction in basal metabolic rate (BMR) has been observed in Caucasian populations during sedentary, cold winters. However, in Korean women drivers (Amas), who are exposed to severe cold stress during open water diving in cold oceans, a reversible increase in BMR is observed during winters. There is also a decline in the shivering response to cold exposure. This is called habituation – blunting of a response on prolonged exposure to a stressor.

5.12.1 Insulative adaptations

Members of polar expeditions develop a thicker subcutaneous fat layer which reduces the heatconducted from the warm core to the cold exteriors of the body.

5.12.2 Other adaptations

The vasoconstrictor response is blunted in regional circulations of the hand and the leg which are subject to occupational cold exposure. This allows them to maintain a higher skin temperature than unacclimatised controls allowing them better functionality with their limbs.

5.13 Cold temperature stress in plants

Cold temperature stress is one of the common environmental factors which limits the geographical distribution and growth of many plant species. The low temperature limit at which plants can grow is about 5°C, independent of the growth form, life cycle or geographic location. Low temperature from 0 to 15^{o} C causes chilling stress and temperature less then 0^{o}C causes freezing stress. Plants which grow in tropical or subtropical areas are susceptible to low temperature whereas temperate plants survive even at-30^{0} C. Cold-adapted plants show genotypic changes that resist local low temperature extremes and enable their survival over generations. On the other hand plants showing cold acclimation are able to reversibly adjust their metabolism to cold temperature stress.

5.13.1 Low temperature induced damage

The potential cold stress symptoms include surface lesions on leaves, a water-soaked appearance of the tissue, discoloration, desiccation, embolisms in xylem vessels, frost-burn, tissue break down, accelerated senescence, delayed transition to flowering and pollen sterility. Freezing imposes dehydration stress due to reduced water uptake by roots and an inability to close stomata. Ice formation takes place outside cells, causing a decrease in water potential outside cells. This causes water from cell to flow outside, causing dehydration-induced damage to cells. Therefore, the main cause of frost damage to plants in nature is extracellular ice crystal formation that causes secondary water stress to the surrounding cells.

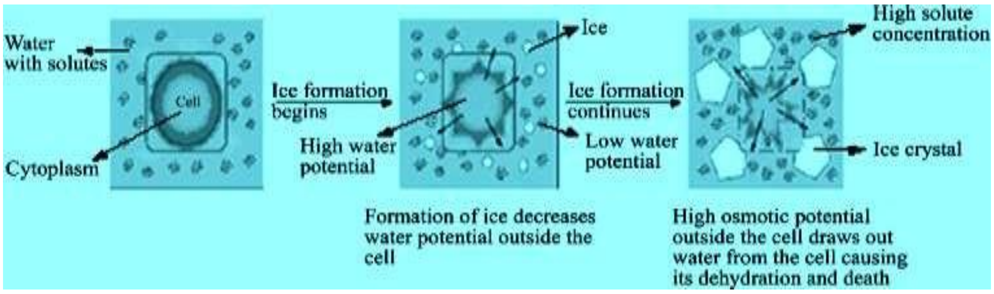

Fig 8. Ice formation outside cells in plants exposed to low temperature causes dehydration of cells.Metabolic activity is arrested or is reduced due to loss of enzyme activity at low temperature.

Photoinhibition, followed by photooxidative destruction of chlorophyll and ultimately cell death isseen in leaves of the chilling-sensitive plants like bean, cotton, maize, rice, and tomato. Decreased abundance and photodamage of the D1 protein has been reported in maize plants exposed to suboptimal temperatures. In addition to PSII photoinhibition, selective inhibition of PSI-related photochemical activities under low temperatures and either high light or moderate/weak illumination has been observed in chilling-sensitive plants.

Low temperature also decreases the fluidity of membrane lipids. Functional membranes are hydrated and in a liquid crystalline phase. On exposure to cold stress, dehydration of cells causes the membranes to go into a gel phase. On rehydration, the liquid crystalline phase is restored and the membrane becomes functional again. However, when rehydration occurs at low temperature, phase transition occurs, resulting in a leaky membrane. Such cold temperature induced phase transitions in membranes affect electron transport in chloroplasts and mitochondria. This causes a buildup of reduced electron transport components, which bring about the generation of reactiveoxygen species (ROS).

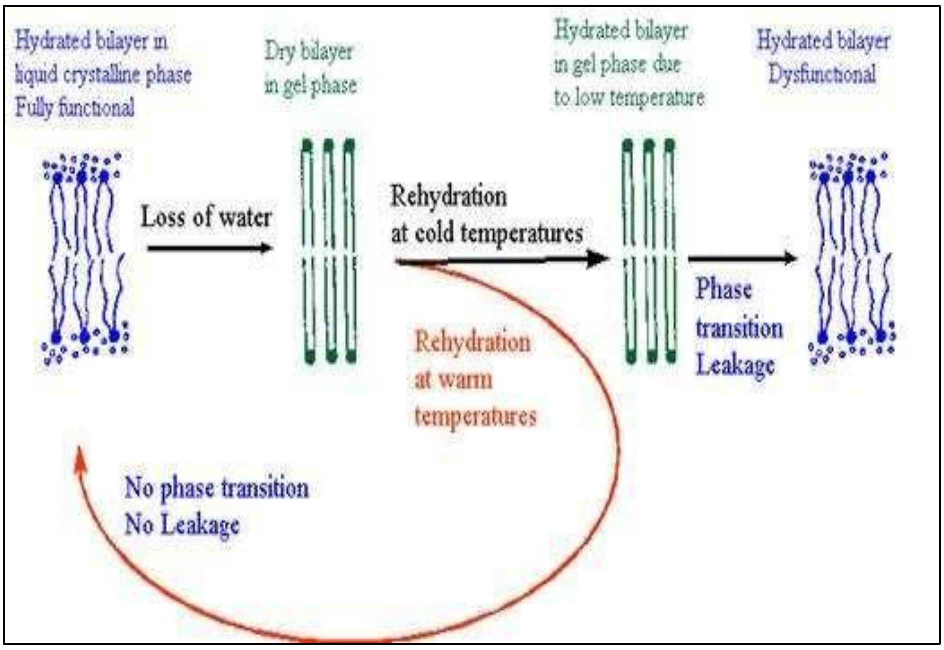

Fig 9. Phase transition in dehydrated membranes undergoing rehydration at cold temperature.This causes leakiness and loss of membrane function.

5.13.2 Adaptive mechanisms to low temperature stressMorphological and life cycle adaptations

In the evergreen trees of the temperate region the plants have to face freezing stress during most time of the year. Evergreen conifers and broad leafed evergreens have wax coated leaves so that they are protected in winter from desiccation. The conifers have small, narrow, needle-like leaves in spruces, pines, firs, or scaled leaves as in case of cedar and cypress, which reduces the surface area to reduce transpiration of water and the risk of freezing (Fig 10a). They also have fewer stomata in the needle leaves. They also show the presence of terpenoids and alcohol in the xylem sap, which prevent freezing of water. An important adaptation shown by plants growing in temperate regions is their ability to retain water within the plant tissues in a supercooled gel-like state, by which ice formation due to nucleation is prevented. Another adaptation involves the termination of growth activity during winter.

Fig 10. (a) Cold adaptation is shown by the needle like leaves of conifers. (b) Cold acclimation is shown by rye plants for survival in cold climate. (c) Leaf curling as an acclimation to cold stress shown by rhododendron leaves growing in extreme cold climate.

Cold acclimation is a plant's ability to reversibly adjust their metabolism to survive the changing weather. Annual plants like rye adjust their metabolism during the winter months to enable them to cope with cold stress (Fig 10b). In extreme cold, the leaves of many evergreen rhododendrons exhibit thermonastic leaf movements causing curling, thus minimizing exposure to freezing temperatures and also reducing winter-burn caused by winter sunshine (Fig 10c).

5.13.3 Physiological mechanisms for acclimation to low temperature

Low temperature causes dehydration stress as mentioned earlier. Some of the physiological processes that protect cells against dehydration stress, like osmotic adjustment and antioxidant metabolism have been covered in the module on dehydration and salinity.

Photo inhibition of photosynthesis takes place under low temperature because of low temperature induced desiccation. Conifers show acclimation to cold stress induced photo inhibition by reducing antenna size, a partial loss of photosystem II, and nonphotochemical quenching of absorbed light as heat. Winter rye plants also reduce sensitivity to photo inhibition, which they do by modulating their photosynthetic capacity to prevent low temperature induced photo oxidative damage. These mechanisms for protection against photo inhibition have been covered in the module on **photo**

inhibition. Plants acclimated to low temperature during winter also show changes in the photosynthetic apparatus. The size of the light absorbing chlorophyll antenna is reorganized by PsbS, an integral protein of PSII, to increase the capacity to dissipate absorbed light nonphotochemically (NPQ) as heat. The number of photosystem II (PSII) reaction center complexes are also reduced. Photosystem I (PSI) remains intact during winter and acts as an efficient non-radiative quencher or can drive cyclic electron transport supporting a pH gradient and ATP synthesis when the temperature increases. The plasto quinone (PQ) pool remains reduced during winter owing to the reduction power contributed by the stromal electron (e) pool and by cyclic electron transport.

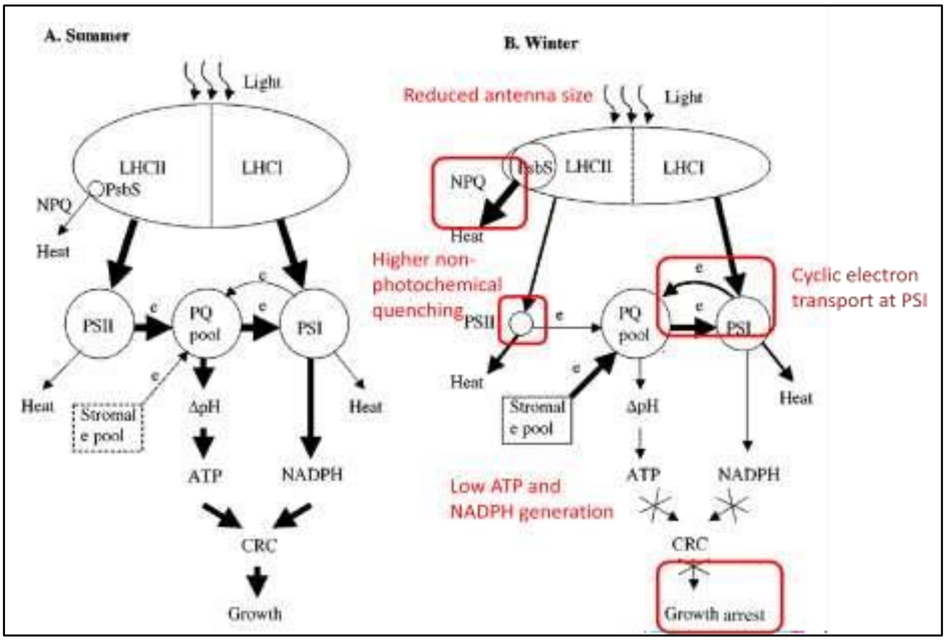

Fig 11. Changes in the photosynthetic apparatus during winter observed in cold acclimated plants.

Cold acclimation of plants is seen to improve when they are subjected to a brief exposure of low temperature, before coping with prolonged cold temperature stress. Such plants are said to be primed by the brief low temperature exposure for subsequent prolonged cold tolerance. For example wheat plants grown at normal warm temperatures are killed by freezing at about -5°C, but after a short period of cold acclimation, they survived freezing temperatures as low as -20°C. Activities of systems that scavenge oxygen species were seen to increase after cold acclimation. In chilling tolerant *Zea diploprennis* the activities of all reactive oxygen scavenging enzymes were higher than in *Zea mays* which is highly chilling sensitive. In the cold sensitive plant rice, some genotypes show tolerance to cold stress, like Jumli Marshi for example. This variety when compared with the cold sensitive genotype IR64, showed higher Fv/Fm ratios, indicating that PSII damage due to cold stress is much lower in Jumli Marshi as compared to IR64. The mechanism for better photosynthetic performance has been attributed to a better ROS scavenging system in JM.

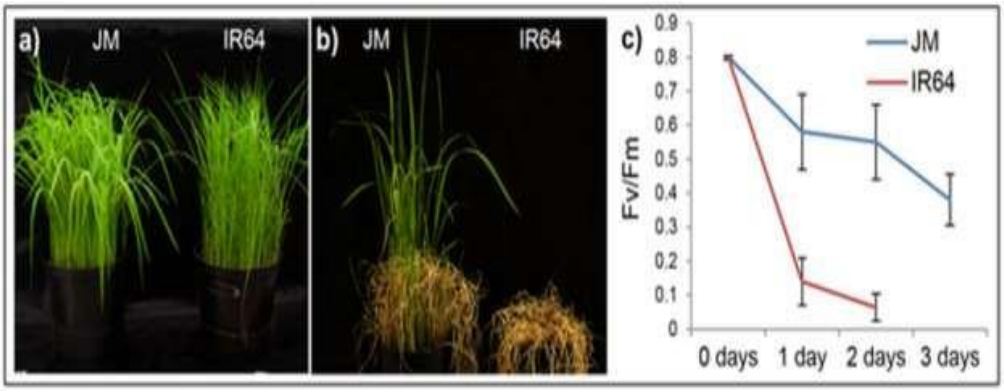

Fig 12. Cold stress acclimation in Jumli Marshi (JM) and IR64 genotypes of rice. Jumli Marshi (JM) and IR64 seedlings were grown for three weeks under regular growth conditions and then movedto +4°C (cold conditions). After three days in cold conditions, plants were moved back to regular growth conditions and allowed to recover for two weeks. (a) Plants just before cold exposure; (b) Cold treated plants after recovery for two weeks (c) Chlorophyll Fv/Fm ratios in JM and IR64 during cold stress exposure. From: Chawade A (2013)

Another cold acclimation strategy involves the maintenance of membrane fluidity at low temperature. This is achieved by qualitative as well as quantitative modifications in the membrane lipid composition. During cold acclimation there is increase in the unsaturated fatty acid content of phospholipids in membranes. An important role of unsaturated fatty acids in maintaining membrane stability during cold stress was confirmed by the increase in levels of linolenic acid and decrease in the levels of palmitic acid observed in the tolerant genotypes (deCruz et al

Fig 13. Snow drops emerging from snow. They synthesize antifreeze proteins, which are secreted into the apoplasm.

2010). Another mechanism used for cold acclimation is shown by Snowdrops (*Galanthus nivalis*) belonging to Amaryllidaceae family, which grows in temperate regions. The pure white blooms emerge from the snow and are a cheering sight in late winter, when the ground is blanketed by snow and nothing else grows (Fig 13). Snow drops synthesize anti-freeze proteins which provide them freezing tolerance. The narrow leaves are bluish-green in colour, and the leaf-tips are hardened to enable them to break through frozen ground.

Antifreeze proteins (AFPs), also known as ice binding proteins (IBPs), have evolved as an important adaptation in numerous organisms exposed to subzero temperatures Antifreeze proteins (AFPs) are secreted by overwintering plants and provide freezing tolerance. AFPs block ice-nucleation by binding to the small ice crystals and inhibiting ice growth and recrystallization in the intercellular spaces. AFPs have been purified from over 15 plants including gymnosperms, dicots and monocots. Fish and insects are also able to synthesize AFPs and they permit insects to remain unfrozen to temperatures of approximately −5 °C. AFP genes from fish and insects have been used to transform plants (Table 1).

Table 1. Transgenic plants expressing a gene encoding an antifreeze protein (From Griffith, M. & Yaish, 2004)

Gene origin	Plant	Phenotype of Transgenic lines
Fish Synthetic winter flounder (Pa) type I AFP fused with truncated staphylococcal protein A	Tomato (Lycopersicon esculentum)	Recombinant protein accumulated within cell and inhibited ice recrystallization
Insect Synthetic spruce budworm (Cf) AFP fused with the signal peptide from tobacco PR-1b	Tobacco (Nicotiana tabacum)	AFP accumulated in apoplast, inhibited ice recrystallization
Fire-colored beetle (Dec) AFP	Arabidopsis thaliana	AFP accumulated in apoplast and lowered freezing temperature of whole plants by 1.38oC with no effect on LT50
Carrot (Dac) AFP	Tobacco (Nicotiana tabacum)	AFP accumulated in apoplast, inhibited ice recrystallization

5.13.4 Cold stress signaling

The sensor for cold stress has not been identified, but membrane rigidification is thought to play a major role in cold perception. Membrane proteins are thought to respond to changes in membrane fluidity by activating a signaling cascade that leads to regulation of gene expression. A mutant strain of *Synechocystis* defective in a gene encoding fatty acid desaturase exhibited plasma membrane rigidification even at room temperature and was seen to bring about an increased expression of number of cold inducible genes (Inaba et al 2003). Some membrane located proteins are also thought to sense the transition of the physical phase from the liquid-crystalline to gelstate of membranes (see Fig 16).

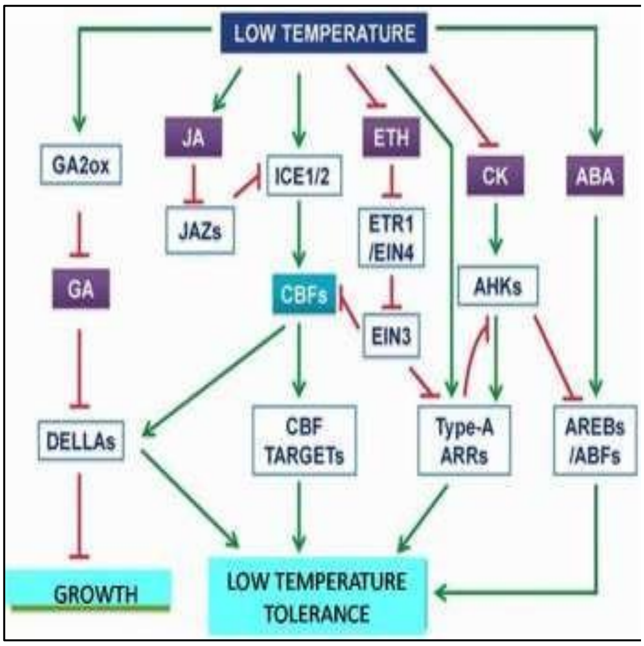

Fig 14. Hormonal signaling in low temperature tolerance. JA and ABA levels increase and GA, Ethylene and Cytokinin levels decrease in response to cold stress, whose signaling pathways interact to regulate growth and cold tolerance. From Shi et al. (2015).

Calcium is an important second messenger in the low temperature signal transduction pathway involved in regulating the cold-acclimation response and Ca^{2+} levels increase rapidly in response to low temperature. A cyclic nucleotide gated calcium (CNGC) channel has been identified as a low temperature activated Ca^{2+} channel. It has been shown that this increase in Ca^{2+} concentration is required for full expression of at least some cold-regulated genes, and for plants to cold temperature tolerance in plants. Besides Ca^{2+}, inositol polyphosphate (IP3) is also known to play a role in signaling for cold stress. Higher and sustained level of IP3 were seen to bring about enhanced induction of cold regulated (COR) genes. A mutation in IP3 phosphatase FRY1 (*hos2*), which dephosphorylated IP3, was seen to increase expression of COR genes too.

Hence IP3 mediated release of Ca^{2+} from vacuoles seems to be important for cold signaling. Reactive oxygen species also play a role in cold signaling by modifying the Ca^{2+} levels indirectly. Low temperature leads to growth arrest, which is brought about by a decrease in levels of GA and cytokinins.

Abscisic acid (ABA) and JA levels increase, leading to a higher expression of COR and ICE1/2 genes respectively. Ethylene signaling is also inhibited by low temperature, which prevents the repression of CBFs by the ethylene response transcription factor EIN3 (Fig 14). Hence hormones play an important role in fine tuning responses of plants to low temperature stress.

5.13.5 Cold-regulated gene expression

Transcription factors are regulatory proteins whose function is to activate transcription of DNA by binding to specific DNA sequences. They function as master switches and regulate a number of downstream genes. Low temperature stress is known to bring about regulation of gene expression by a signaling cascade composed of a hierarchy of transcription factors, called as a regulon (Fig 15). *ICE1* and *ICE2* (inducers of *CBF* expression), are MYC-type bHLH (basic helix-loop helix) transcription factors, which have been identified as upstream regulators of another class of transcription factors called *CBF3/DREB1A* (C-repeat

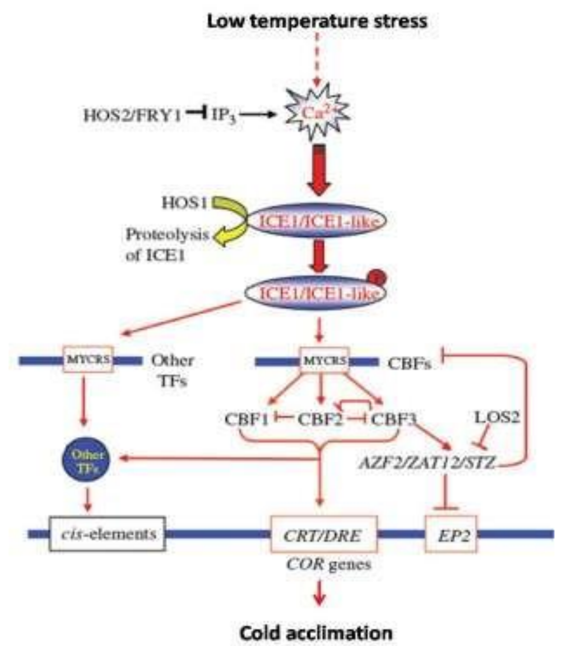

Fig 15. Low temperature signaling and expression of cold regulated (COR) genes. ICE, CBFs are major TFs in cold signaling pathway. HOS2/FRY1: IP3 phosphatase; HOS1: Ubiquitin E3 ligase protein; ICE1/ICE1-like: Inhibitor of CBF expression (TFs); MYCRS: MYC recognition sequence (*cis-* elements); CBFs: CRT binding factors (TFs); CRT /DRE: C-repeat / dehydration response elements (*cis-*elements); AZF2/ZAT12/STZ: Zn finger TFs (repressors); LOS2: Low expression of osmotically sensitive 2 (repressor); COR genes: cold regulated genes. From: Chinnuswamy et al. (2006).

binding/ dehydration response element binding) factors, which belong to the AP2 family. In *Arabidopsis*, three CBF/DREB1s are involved in the regulation of cold responsive genes. The *Arabidopsis ice1* mutant exhibited both chilling and freezing sensitivity and the over-expression of *ICE1* conferred increased freezing tolerance, thus confirming their role in regulating cold-responsive gene expression and subsequently cold tolerance. ICE1 was shown to be negatively regulated by ubiquitination mediated by the HOS1 (ring finger E3 ligase). Self-regulation of the ICE1-CBF pathway is mediated by some Zn finger proteins that are induced by CBFs, but act as negative regulators of CBFs. Some cold response genes are also induced by other transcription factors like HOS9 (homeodomain) and HOS10 (MYB) instead of CBFs.

The cold stress-activated transcription factors bind to the promoters of a variety of downstream genes (COR genes) which confer cold tolerance. The proteins coded for by the downstream genes include other transcription factors, dehydrins, late embryogenesis abundant (LEA) proteins, which protect other proteins against cold-induced damage, proteins coding for genes involved in the synthesis of osmoprotectants etc., all of which contribute to a cold tolerant phenotype.

5.14 High temperature stress on plants

High temperature is one of the major environmental stresses that affect plants' growth, metabolism, development and productivity. Unlike other environmental stresses, heat stress directly changes the physical properties of molecules hence their interaction which creates metabolic imbalance. For example, the stability of membranes, various proteins, RNA species and cytoskeleton structures, the efficiency of enzymatic reactions in the cell are affected by heat stress. Plants are static poikilotherms; therefore, they have the ability to adapt in variable temperature. Plants can avoid or tolerate heat stress by physical and metabolic changes.

5.14.1 Perception of Heat

Considering the large surface to volume ratio of the plant leaf, almost all macromolecules perceive the heat simultaneously. Therefore, all macromolecules might have role in sensing the heat.

However, who plays the role of primary sensor is needed to be identified. The primary sensor necessarily be able to perceive precisely and differentially react to diverse temperature rise and should also be able to differentially activate a unique signaling pathway that can specifically up-regulate heat shock responsive (HSR) genes.

In planta, heat stress can be sensed by different routes (Figure 1). Heat stress affects the sensor molecule either directly or indirectly. For example changes in the quaternary and tertiary structures of sensor molecule. Moreover, heat-induced changes in metabolic fluxes, ROS accumulation, reduced energy levels or release of ATP from cells could also activate the heat sensor molecule.

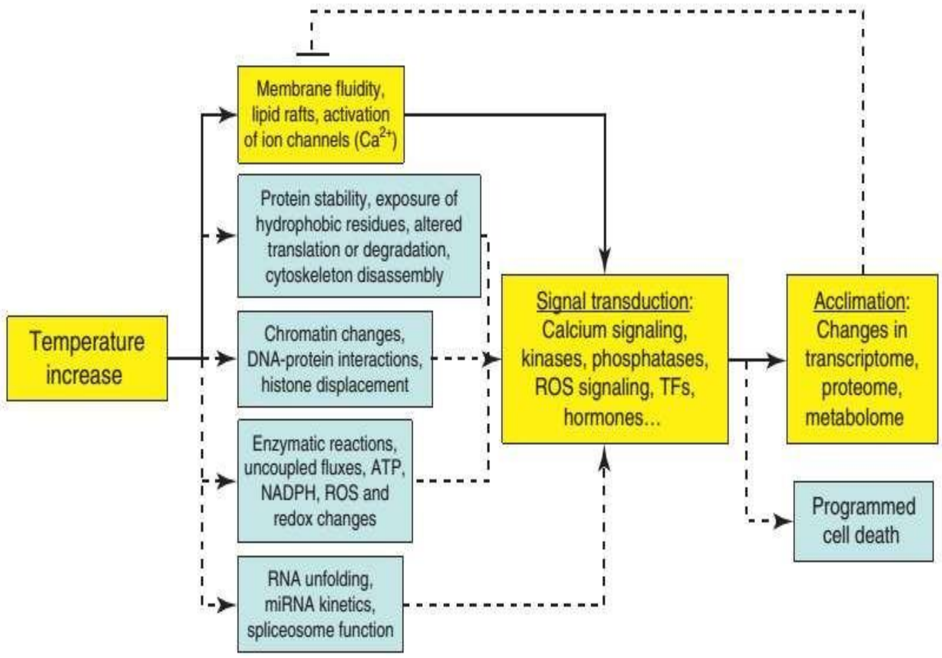

Figure 1: Schematic representation of the temperature sensing in plants. (Adapted from Mittler et.al(2012) Trends in Biochemical Sciences. 37 (2): 118-125

The classical model proposed for the heat stress perception is as follows: First, heat stress induces unfolding of proteins or RNAs. These unfolded proteins in the cell triggers the release of molecular chaperones from their constitutive inhibitory association with heat shock factor (HSF) monomers [38] and to bind the unfolded proteins. The free HSFs subunits trimerize, undergo phosphorylation, bind to heat shock response (HSR) promoters and activate the HSR. However, this model is not working in the temperature that does not unfold proteins. In the moss, *Physcomitrella patens* a slight increase in the temperature could be sensed by the plasma membrane. This opens a specific calcium channel that causes an influx of calcium into the cell and activates HSR (Figure 2).

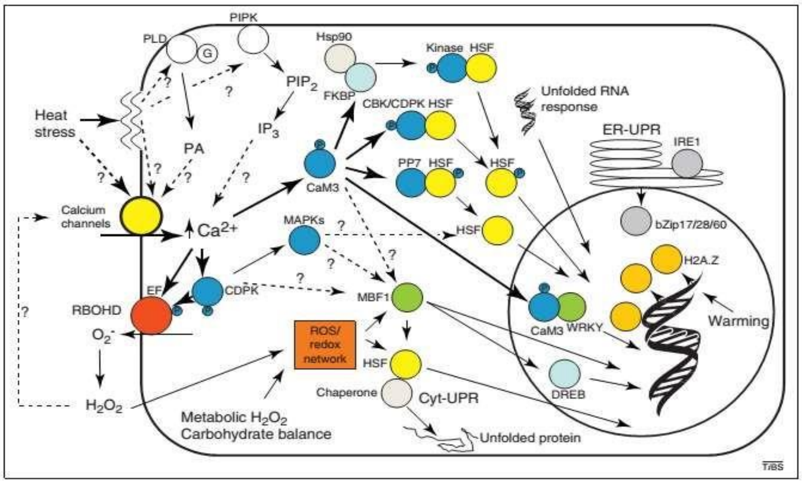

Figure 2: Heat stress induced signal transduction pathways.
(Adapted from Mittler et.al (2012)Trends in Biochemical Sciences. 37 (2): 118-125

5.14.2 Effect of high temperature on plant

Plants can operate most of the physiological processes normally in the temperature ranges from approximately 0°C to 40°C. Temperature higher than this causes injury to the plants. Therefore, plant's growth and development is relying on the temperature that the plant is exposed to (Figure 3). However, each species has a specific temperature range represented by a minimum, optimum and maximum (Hatfield et al., 2008, 2011).

Figure 3: Effect of heat stress on plant (Adapted from Mittler et.al (2012) Trends in BiochemicalSciences. 37 (2): 118-125

Moreover, plants responses to the high temperature are also dependent on the degree of temperature and the duration of the exposure. Heat stress affects germination, growth, development, reproduction and yield. High temperature stress also creates metabolic imbalance by impairing the stability of various proteins, membranes, RNA species, and cytoskeleton structures, and alters the efficiency of enzymatic reactions.

5.14.3 Effect of high temperature on photosynthesis and growth

Photosynthesis is one of the most important biochemical reaction by which plant assimilates carbon; however, this process is heat sensitive. More so, C3 plants are more susceptible to high temperature stress than C4 plants. High temperature causes injury to chloroplast by altering the structural organization of thylakoids, swelling of grana and impairing grana stacking ability. Moreover, the amount of photosynthetic pigments and the activity of photosystem II is also reduced under high temperature. In addition, high temperature also affects water content of the leaves, stomatal conductance, and intercellular CO_2 concentrations. Heat stress also declines the transcription and activity of two major photosynthetic enzymes namely RuBP Case and RuBP Case activate. Heat stress also reduces leaf water potential, leaf area and induces leaf senescence that negatively affects photosynthesis (Greer, 2012). Heat stress induced reduction in net assimilation rate is one of the main reasons for reduced plant growth. It has been shown in rice that height of plant, number of tillers and total biomass is reduced after heat stress (Mitra and Bhatia, 2008).

5.14.4 Effect of high temperature on plant development

Plant species are showing great variations in sensitivity to heat stress; however, during reproductive phase, a short duration of heat stress can significantly decreases production of flower buds, increases flower abortion and also impaired fruit and seed set. High temperature stress also impairs meiosis in both male and female organs, pollen germination and pollen tube growth, ovule viability, anomaly in stigmatic and style positions, reduced number of pollen grains retained by the stigma, disturbed fertilization processes etc. Together, all these abnormality contributes to increasing sterility. Heat stress increases the level of ethylene, a phytohormone that leads to the male sterility in rice plants.

5.14.5 Effect of high temperature on metabolism

Unlike other abiotic stresses, heat stress can directly modify the basic molecules like proteins and DNA. Therefore, heat stress damages many enzymes those are required for biochemical reaction in plants. The uncoupling of enzymes and metabolic pathways causes accumulation of reactive oxygen species (ROS) namely hydroxyl radical (OH•), singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) in plant. ROS can react with biomolecules and has the ability to oxidize protein, poly unsaturated fatty acids and DNA. Moreover, heat stress damages Photosystem I and II and causes peroxidation of membrane lipids. Extreme accumulation of ROS can also triggers programmed cell death. Though ROS exerts negative effect on plant metabolism, its role in signaling initiates heat shock responses that leads heat shock tolerance in plant.

5.14.6 Acclimation and tolerance to heat stress

Plants can survive under high temperature stress by implementing avoidance or acclimation- and tolerance- mechanism (Figure 4). Closure of stomata to reduce water loss is one of the prominent mechanism that provides short term avoidance or acclimation to heat stress. An Increase in stomatal and trachomatous densities and larger xylem vessels are also very common features observed in heat stressed plants. Some plants possess waxy cuticle and tomentose leaves that restrict the absorption of solar radiation. The strategies like paraheliotrophism and rolling of leaf blades are also used to reduce the solar radiation. Increase in transpiration rate helps plant to reduce the heat stress; however, this is possible only in well hydrated plants. As an alternative strategy, plants reduce their leaf size that causes smaller resistance to air boundary layer that helps the plant to maintain the ambient temperature. Many species have evolved strategies like abscission of leaves, production of heat resistant buds, and completion of the reproductive cycle during the cooler months to avoid heat stress.

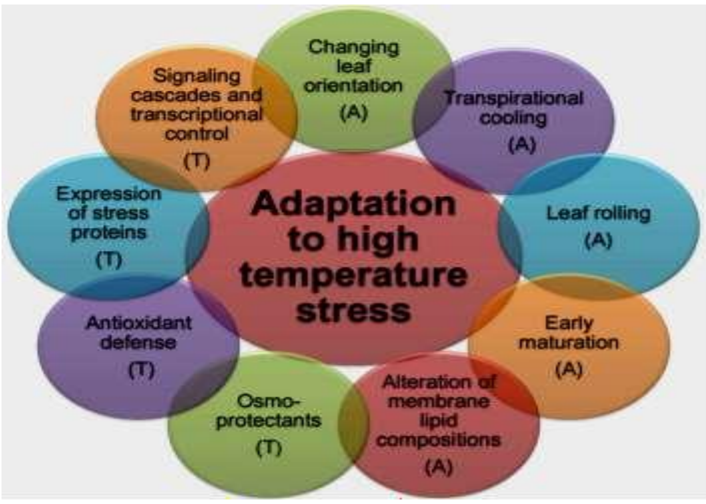

Figure 4: Schematic representation of avoidance or tolerance response in plants. A= avoidance; T=tolerance.

(Adapted from Mittler et.al (2012) Trends in Biochemical Sciences. 37 (2): 118-125

Besides avoidance, plants also develop tolerance mechanisms to counteract the heat stress. Generally, an increase in the antioxidative capacity is associated with enhanced heat tolerance. Therefore, the activities of ROS scavenging enzymes are increased in heat stressed plants.

However, the activities of different ROS scavenging enzymes are operated at different temperatures. In a study, it was evident that in tolerant varieties total antioxidant activity reaches the maxima at 35–40 °C but in susceptible varieties it is at 30 °C. The activities of catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) are increased up to 50^0C ; on the contrary, working of peroxidase (POX) and glutathione reductase (GR) activities are ranging fron 20- 50^0C. Plant also produces antioxidant metabolites like ascorbate, glutatione, tocopherol and carotene to protect themselves against heat induced oxidative stress. In addition to antioxidant enzymes and metabolites, heat stress also induces the expression of heat shock genes which encode heat shock proteins (HSPs) that work as a chaperon. Upon heat stress monomeric heat shock factors (HSFs) those present in the cytosol enter the nucleus. In the nucleus,HSF monomers are trimerized and form active trimer.

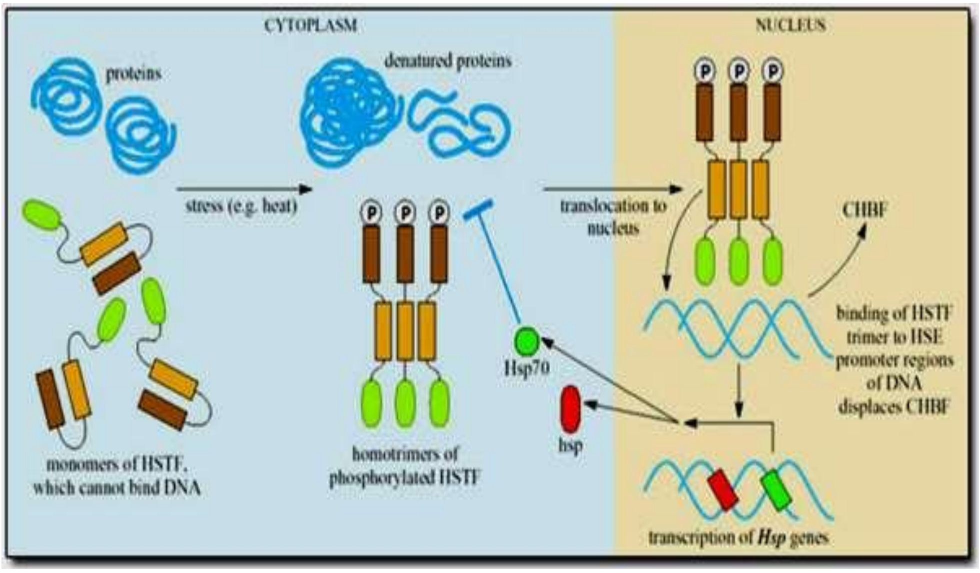

Figure 5. Mechanism of heat stress-induced production of heat shock proteins. (source: http://www.open.edu/openlearn/ocw/mod/oucontent/view.php?id=2477&extra=thumbnail_idp3634 848)

This trimer binds to the specific genomic region i.e. promoter or heat shock element (HSE) of the respective heat shock gene (HSG). After successful transcription, translation and post-translational modification functional HSPs are produced. HSP mediated protein folding protects the proteins from being denatured and restore their stability and function under heat stress. There are five different families of HSPs, namely HSP100 (or ClpB), HSP90, HSP70 (or DnaK), HSP60 (or GroE) and HSP 20 (or small HSP, sHSP). Among them HSP70 and HSP60 play a significant role in response to heat stress.

Summary

Human beings exhibit homeothermy- maintenance of constant core temperature irrespective of changes in the thermal environment. This is obtained by the maintenance of a balance between heat production and heat loss from the body. Thermoregulatory responses elicited during acute exposure to thermal environments are responsible for homeothermy. Prolonged exposure to hot or cold environments lead to long term adaptations which optimize life and survival in such conditions.

Low temperature limits the geographical distribution and growth of many plant species. This stress shares several features with other abiotic stresses like dehydration and salinity, since cold stress leads to dehydration of cells. It also affects membrane fluidity and affects electron transport processes. Photoinhibition and oxidative damage are seen in response to low temperature. Plants show various features to acclimate to cold stress and are equipped with an elaborate cold signaling pathway which confers tolerance to plants. An understanding of how plants sense and respond to freezing stress has practical applications in evolving new strategies for the improvement of freezing tolerance of agronomical important plants and in expanding the geographical distribution of a plant species.

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Unit 6: High Altitude and Human Beings

Unit Structure

6.0 Objectives

After reading this unit students should be able to:

- Explain about the relationship between high altitude and human beings
- summarize the ecological rules
- describe standard temperature trials and acclimatization to temperature
- describe adaptation to hypoxia and organ-system adaptations
- describe the various facets of individual adaptation to hypoxia
- explain population adaptation to hypoxia

6.1 Introduction

In this module, we will focus only on those aspect of adaptation that is comprises genetic adaptation that are characteristic of groups of people some of whose phenotypes better suited to environment were produced as a matter of natural selection. Restriction of this module only to heat and altitude stress would be arbitrary since most of the population undergoes cold stress on a seasonal or diurnal basis.

The primary problem for man in hot environment is heat dissipation and there are no major racial differences in the physiological mechanisms involved in dissipating heat. Man relies on convection and radiation forms of heat loss upto the point when the surrounding air and objects become hotter than the skin. Adjustment of heat loss is accomplished by altering the flow of blood through the capillary bed of the skin by vasolidation (widening of the blood vessels to increase the blood flow and result in the loss of heat) and vasoconstriction (narrowing of the blood vessels and reduction in the flow of blood which results in decrease in the loss of heat). While exercising, heat is loss by thermal sweating (evaporative heat loss) and this evaporation is the only way to lose heat when air temperature exceeds the temperature of the skin.

The assessment of adaptation of man to high altitudes presents an interesting paradigm of dual problem-delineating the actual pattern of structural, functional or behavioral response and evaluation ofthe adaptive value to a response based on the benefit of that response to the individual or population. The most apparent stresses for man at high altitude include hypoxia, high solar radiation, cold, aridity, high winds, limited nutritional base and rough terrain. Of these, hypoxia is of predominant interest because the other stresses are present equally or in greater degrees in other geographical zones, but hypoxia resulting from reduced barometric pressure at altitude stands alone. Barometric pressure and the degree of hypoxia varies in different geographical locations at elevations that are comparable (Moore, 1968). Hypoxia is interesting even though it affects only a small segment of the people. It is due to the fact that hypoxia is an ever-present stress which can be ameliorated by altering the behavioral patterns. It also affects all organ systems and physiological functions of the human body. Even though hypoxia is present only at high altitude, it is encountered commonly at sea level. Evaluation of genetic basis for population adaptation to environmental stress is another reason for altitude effect studies on human populations.

6.2 Ecological rules

Bergmann's rule: Bergmann (1847) observed that the body size increases with decrease in temperature of the habitat within a single, polytypic, warm-blooded species.

Allen's rule (1877): Allen observed that mammals living in cold climates have smaller extremities and appendages than those who stay in warmer climates.

The aforesaid rules are applicable only when the species are subjected to wide range of climatic extremes and the species have not differentiated to the point where apparent thermoregulatory responseis seen.

6.3 Standard temperature trials

To explore differences in group of people to heat adaptation, they are subjected to heat stress and their responses are compared which is termed as the "experimental approach" as coined by Henschel in 1967. Skin temperature and some measures of internal temperature, rate of sweating, heart rate and oxygen consumption are used as measure of metabolic response in such studies. A combination ofwork and heat is utilized in most of these studies because this shortens the time period to achieve high level of protective response (production of sweat) since it imposes both an external and internal heat load. There are many factors that influence the performance of an individual in combination of heatand exercise, some of which are body size, state of heat acclimatization, state of physical fitness, nutrition, state of health, etc.

Temperature of skin is not a vital measure in these experiments although it is recorded. A subsequent rise of skin temperature above 36 ˚ C indicates sweating and is interpreted as a sign of danger. Measurement and interpretation of evaporation is essential as it is the only way of heat loss.

Sweat production ranges from almost none to 2 liters per hour and it may decrease from early peak levels if the exposure is prolonged. Dripping sweat refers to the marked capacity of man to produce sweat more than can be evaporated on the skin. It is a maladaptive phenomenon unique to humans.

Exposure to heat causes a slight increase in heart rate which compensates for the increased vascularbed resulting from peripheral vasodilation. However exercise with exposure to heat also increases heartrate to fulfill the greater metabolic demand and the skeletal musculature.

When exercise and heat are combined, there is a dramatic increase in heart rate at the initial stage followed by a slow increase subsequently. Any rise or fall in heart rate indicates collapse. Accomplishing a given workload with least increase in heart rate is an efficient response when there is adequate transport of heat due to the fact that the volume per beat i.e., stroke volume is relatively fixed. Physical training increases stroke volume through repeated exercise and allows the workload to be performed with small increase in heart rate. Extensive exposure to heat without exercise (passive heat acclimatization) has no effect on heart rate hence this kind of measurement is only indicative of physical fitness.

6.4 Acclimatization to temperature

Artificial heat acclimatization is a set of changes in response to controlled exposures of heat in extentof sweat production, changes in heart rate, and changes in endocrine function brought about by loss of sodium contained in the sweat. These changes can be observed in healthy subjects exposed to a heat work situation after 5-10 days. Natural heat acclimatization is a thermoregulatory adaptation which requires frequent stimulation for full maintenance.

Internal temperature does not show as much initial increase and level off or rise very slowly with exposure. Sweat is produced sooner and reaches a high point earlier and is maintained at a constant rate for an extended period of time. Heart rate is not much increased as indicated earlier that it is primarily a function of exercise. Temperatures of skin were reported to be lower in acclimatization involving dry temperature/heat (Lee, 1964) and higher in humid heat (Ladel, 1964). An additional change that occurs in heat acclimatization is the reduced concentration of salts, particularly sodium chloride in each unit of sweat .But this is not of importance for assessing the effects of short term exposure to heat because the higher production of sweat balance the decreased concentration of the salts. In 1966, Wyndham observed acclimatized Negros having lower hourly sweat rates than acclimatized Europeans but when the group averages were corrected for body weight or surface area, the differences diminished.

Sweating is an efficient mechanism to maintain internal temperature only when the sweat can be totallyevaporated as fast as it is produced. But this is quite impossible in humid heat, thus man's tolerance is reduced in such environmental conditions. It would be beneficial if man regulates sweat production to fit the evaporation rate but he does not do so. Regardless of how much is evaporated and how much drips to the ground, greater sweating is promoted by an increase in internal temperature because sweating is regulated mainly by internal temperature than by skin temperature except for special circumstances as high input or application of hot water to the skin.

This module has emphasized the uncertainties and the complexities involved in comparing different groups of people with respect to heat adaptation. A given population may have ignored or buffered the heat stress for generations or may have exploited maximum biological potential. One way in which we can contribute to the understanding of temperature adaptation is by trying to identify the interface between what people can do and what they actually do starting with the assumption that these parameters are not identical.

6.5 Adaptation to hypoxia

6.5.1 Assessing adaptation to hypoxia

The basis for assessing human adaptation to hypoxia comprises of evaluation of relative benefit or degree of necessity to environmental stress (Mazess, 1975). Adaptive implies selective advantage or fitness.

The adaptive abilities of highlanders to lowlanders are enhanced ventilator efficiency or an increased work capacity. Adaptation at the individual level is assesses at adaptive domains which include physical performance (exercise and motor abilities/skils), functioning of nervous system (sensory, motor and neural functions), growth and development (progression in rate and attainment), nutrition (requirements, utilization, efficiency), reproduction (survival, reproductive advantage), health (morbidity, mortality, resistance to diseases), cross-tolerance and resistance (stress resistance), affective functioning (happiness, tolerance, sexuality) and intellectual ability (learning, expression).

Adaptation at the infra-individual level implies adaptation that is of benefit or necessity to the individual. This benefit or necessity are translated as homeostasis and resistance/tolerance. At high altitude, hypoxia produces stress at the beginning of oxygen transport chain. The responses at infra- individual level are by increasing oxygen transport at any level between the inspired air and tissue or by allowing function to continue despite lower oxygen tension. A cellular change that maintains homeostasis is an infra-individual adaptation but if it benefitted the individual or population then it is termed as individual or population adaptation.

6.5.2 Problems in assessing adaptation to altitude

Assessment needs to examine relative benefit or necessity in an adaptive domain. It is not only sufficient to show that a response occurred or that individuals and populations differed in theirresponses but rather some benefit has given. Large chest or high hematocrits in native highlanders are viewed as distinctive characteristics which are beneficial (Monge, 1948). For instance, hyperventilation i.e., increased oxygen transport might be beneficial to individual or populations without demonstrating advantage in any of the adaptive domains.

Secondly, all phenotypic stable adaptive (aptitudes) characteristics are not genetic adaptations. Acclimatization responses are far more vital than aptitudes in altitude hypoxia. The only common example of a hypoxic aptitude mistakenly considered as genetic adaptation is large size of chest and large lungs. However, large chest and large lungs are mere ventilator acclimatization as hyperventilation.

It is also essential to note that the adaptive benefit maybe temporal, spatial and population specific. For instance, those characteristics that occur with long term exposure may not be beneficial in short term exposure and may be also harmful. In the first few days to months at altitude exposure, increased hypoxic sensitivity and hyperventilation is acclimatizational. A diminished hypoxic ventilator defect occurs during developmental period in long term exposure. Hyperventilation would be advantageous to a population with a rightward shift on the hemoglobin-dissociation curve where arterial saturation is maximized by high alveolar oxygen tension than to a population with leftward shift.

6.6 Organ-system adaptations

A variety of responses enhance the oxygen transport or increase the resistance to hypoxia at organ system level. The only major aptitude considered important at organ system level in altitude hypoxia is a large chest and a large lung. Hurtado (1964) was of the view that it is not certain if this larger chest is directly associated with larger lungs or whether larger lung volume would be functionally important because the Andean highlanders differ from lowlanders only in their larger lung residual volume. Therefore, a large chest cannot be assumed to be an adaptive aptitude because it does not have a direct relation to increased transport of oxygen.

Following are the infra-individual adaptations to hypoxia which are acclimatizational:

a) Red blood cells: Increase in blood viscosity may affect the flow of blood and circulation.

b) Hemoglobin-Oxygen dissociation curve: This curve shifts towards decreased affinity leading to enhanced release of oxygen to the tissue. This shift is more advantageous to the newcomers than the residents.

c) Circulation: There is increase in cardiac output at rest during first days of exposure and then return to normal levels. Such circulatory alterations such as reduced flow to kidneys and heart occur with greater flow to muscle and brain.

d) Capillarity: Increase in capillarity aids the process of diffusion in the tissue.

e) Ventilation: Among native residents and long sojourns there is diminished hypoxic sensitivity and hyperventilation.

f) Cellular metabolism: There is higher concentration of muscle myoglobin and phosphates, increase activity of glycolytic and oxidative enzymes and enhanced aerobic glycolysis in the tissues of highlanders. These changes lead to increase in resistance to tissues to low oxygen tensions.

6.7 Individual adaptation

Here the effects of individual adaptive domain are described:

6.7.1 Physical performance

Exposure to high altitude impairs physical abilities. The maximal work capacity of newcomers shows a decreasing trend and submaximal work becomes more stressful leading to increase in heart rates and rapid breathing decreasing the endurance time. Decrease in aerobic capacity is directly proportional to the training extent: athletes show greatest decrease, sedentary individuals the least and active individuals the intermediate. Submaximal work at high altitude use a greater percentage of reduced capacity of total work load which is known as relative work load.

Highlanders have a slightly increased aerobic capacity at sea level indicating that they have slight reduction in maximal performance at high altitude. The high endurance of submaximal work in them reveals their maximal capacity and the lower relative work at a fixed oxygen intake. This may be due totheir small body build, and their consequent usual activities involving moving of body weight and their endurance ability to work due to their vigorous activity level.

6.7.2 Nervous system: Sensory and psychological performance

Nervous tissue is susceptible to oxygen deprivation and functioning of sensory and motor system is not possible at altitude extremes. Development of central nervous system is delayed at high altitude. Brief anoxia in fetal stage causes damage and retardation of brain with late appearance, eg: Andean Quechua infants. Even at moderate altitude, visual and colour sensitivity decreases.

6.7.3 Growth and development

Organs such as lung, heart and spleen gets reduced in weight although not proportional to body weight while thymus and kidney appear to be so much reduced. The reduction in hypoxia is due to reduction in number of cells with normal or large size of cells. Growth of genetically similar people is advanced in mountainous regions than the disease prone low -landers. There is relative increase in rate of growth of the chest in highlanders without functional importance to ventilator function. The reduced size of the body of the highlanders may be beneficial during chronic food shortage, famine and/or energetics of work.

The overall diminution of growth and development that occurs in hypoxia indicates that adjustment even with chronic exposure is not complete. Results of alterations in growth that occur in hypoxia maybe beneficial (increased diffusion capacity of lungs), harmful (reduced ventilator drive) or uncertain (alteration of pulmonary circulation).

6.7.4 Nutrition

People living in high altitudes are subjected to seasonally deficient calorie and nutrient intake than the lowlanders. Acute exposure to hypoxia is associated with loss in body weight, including loss of both water and tissues. Hypohydration and anorexia occurs at varying degrees. We see negative balances of nitrogen and calories and other nutrients in response to extreme exposures leading to reduced efficiency of food utilization. There is exposure of fat mobilization from the body leading to increased serum free fatty acids. There is alteration of carbohydrate and protein metabolism as well.

6.7.5 Reproduction

There is effect of moderate altitude on reproduction during the first days or weeks of exposure. There is marked effects on litter size, birth weights, lactation, gonadal function and success of pregnancy due to exposure to extreme altitudes. Adaptation to high altitude among neonates include increased placental size and vascularity and increased oxygen capacity of the fetal blood due to increase in mass of hemoglobin. Better care of neonates had led to lower mortality in the US.

6.7.6 Morbidity and mortality

Lack of medical facilities in high altitude regions has caused two problems: lack of adequate data on incidence of disease and cause of death and the statistics that exists are biased by this lack of medical care in the region. Infectious disease seem be higher among the highlanders than the lowlanders. Altitude also has debilitating effects on the lungs thereby exacerbating pulmonary disease. There is low incidence of chronic diseases and hypertension of some altitude residents which could be primarily due to genetic and dietary factors rather than of hypoxia. Varicose veins occurs at high altitudes in relation with higher hematocrits and greater blood viscosity. Acute mountain sickness that includes symptoms such as dizziness, shortness of breath, stomach upset is most widely prevalent. Pulmonary edema is the most severe of altitude sickness which is characterized with impaired exchange of pulmonary oxygen and pulmonary hypertension. Congenital abnormalities of different kinds appears to be more common at high altitudes since these are marked by fetal anoxia.

6.7.7 Cross tolerance and resistance

Humans exposed to hypoxia continuously or for a short period of time show enhanced responses and survive in more severe stress of hypoxia. Cross tolerance and resistance that occur in hypoxia are results of specific hypoxic responses rather than from “general adaptation syndrome”. In chronically exposed individuals to severe stresses such as mountain climbing, adrenal activity and catecholamine appear normal. Increased vascularity affecting both capillary and larger vessels occur in hypoxia enhancing diffusion of oxygen to the cells.

6.8 Population adaptation

Population adaptation is assessed in association to demographic optimality, spatial-temporal spread, ecological efficiency and reproductive success. Infra-individual adaptations demonstrated among natives have made investigators view altitude native as an adapted individual and consider the population as also adapted. Humans have mildly impaired fertility in hypoxia which is associated with increase in perinatal mortality but there is no such mortality at moderate elevations. Census data show reduced reproductive success of native highlanders but enhanced reproductive success of the former with sojourners indicate selective advantage of the former group.

Altitude population show high proportion of males except where there is selective out migration of males and relative deficit of older people which may be beneficial in a nutritionally marginalized society. There is evidence of temporal permanence of native highlanders. Density of altitude populations are less as compared to lowlanders which reflects the technological and cultural patterns.

There is little or no benefit at altitude for any population. Some population may in fact be in disadvantage. High altitude populations have different demographic structures but their significance is unknown. Highlanders have survived of their own but have shown no relative benefits/advantage.

Summary

The primary problem for man in hot environment is heat dissipation and there are no major racial differences in the physiological mechanisms involved in dissipating heat. Man relies on convection andradiation forms of heat loss upto the point when the surrounding air and objects become hotter than the skin. Adjustment of heat loss is accomplished by altering the flow of blood through the capillary bed of the skin by vasodilation and vasoconstriction. While exercising, heat is loss by thermal sweating and this evaporation is the only way to lose heat when air temperature exceeds the temperature of the skin. Bergmann (1847) observed that the body size increases with decrease in temperature of the habitatwithin a single, polytypic, warm-blooded species. Allen observed that mammals living in cold climateshave smaller extremities and appendages than those who stay in warmer climates.

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A given population may have ignored or buffered the heat stress for generations or may have exploited maximum biological potential. One way in which we can contribute to the understanding of temperature adaptation is by trying to identify the interface between what people can do and what they actually do starting with the assumption that these parameters are not identical.

The assessment of adaptation of man to high altitudes presents an interesting paradigm of dual problem-delineating the actual pattern of structural, functional or behavioral response and evaluation ofthe adaptive value to a response based on the benefit of that response to the individual or population.

The most apparent stresses for man at high altitude include hypoxia, high solar radiation, cold, aridity, high winds, limited nutritional base and rough terrain. Of these, hypoxia is of predominant interest because the other stresses are present equally or in greater degrees in other geographical zones, but hypoxia resulting from reduced barometric pressure at altitude stands alone.

The basis for assessing human adaptation to hypoxia comprises of evaluation of relative benefit or degree of necessity to environmental stress (Mazess, 1975). Adaptation is examined at different levels of hierarchy, namely, organ system, individual and population. In infra-individual level, many adjustments maintain homeostasis or increase hypoxia tolerance. Some abnormalities in reproduction occurs and physical performance, growth and development and nervous system deteriorates in extreme altitudes. The only advantage of hypoxia happens to be enhancement of resistance and cross-tolerance produced by increased cellular metabolism and oxygen transport. The adjustments that occur at the infra-individual level doesn't provide full adaptation to the individual.

There is little or no benefit at altitude for any population. Some population may in fact be in disadvantage. High altitude populations have different demographic structures but their significance is unknown. Highlanders have survived of their own but have shown no relative benefits/advantage. Hypoxia is one of the environmental variables and both the pattern and adaptive value of responses is modified by interaction of many environmental and cultural variables of the population.

References:

EPG Pathshala, Subject: Anthropology, Paper No.: 11 Ecological Anthropology: Cultural and Biological Dimensions, Module: 29 Adaptation to ecological stress-temperature, latitude and altitude by: Ms. Imkongtenla Pongen & Dr. Meenal Dhall Department of Anthropology, University of Delhi

Unit 7: Allelopathy

Unit Structure

7.0 Objectives

After reading this unit students should be able to:

- Define allelopathy
- Describe the historical background of this discipline.
- Understand about the allelochemicals
- Describe about the mechanism of action allelochemicals

7.1 Introduction

"Allelopathy can be defined as an important mechanism of plant interference mediated by the addition of plant produced secondary products to the soil rhizosphere" (Weston 2005). These products are typically showed into the soil profile and impact the development of plants that are growing near the allelopathic species. Allelopathic species produce these compounds to reduce populations of other plant species that are directly competing for similar resources such as nutrients, sunlight, or water. Allelopathic compounds can show selectivity towards different plant species and can alter the composition of plant populations. Allelochemicals can be move in the soil by decomposition, exudation from root, and volatilization of compounds by the allelopathic species. Allelopathy could be potentially used for weed control by producing and releasing allelochemicals from flowers, leaves, seeds, roots, and stems of decomposing or living plant materials.

Allelopathic species can also suppress weed or crop growth by direct competition for limited resources, thus cover crops are normally killed off before crop establishment to avoid competition with the crop. Recently agricultural research has looked at weed control using allelopathic crop residues. There is also interest in developing cultivars that produce higher amounts of these toxic compounds that suppress weed growth. Allelopathic cover crops would provide other benefits besides weed control in minimum or no tillage practices such as erosion prevention, moisture conservation (mulch), and improving soil productivity.

Using a living allelopathic crop that is incorporated into crop rotations, and also using it as mulch have looked at allelopathic weed suppression. Studies have shown that when compared to other cover crops that are leguminous such as peas or hairy vetch, rye provides much better weed suppression which suggests that it is caused by a chemical interaction and is not solely due to direct competition. Allelopathic species weed suppression could provide us with another possible approach to integrated weed management especially in organic farming or minor use crops. Although, allelopathic species provide weed suppression, replacing herbicide use with allelopathic species is not a feasible option for complete weed control in large-scale operations. If allelopathic species could be incorporated in some cropping structures to provide weed suppression, this could reduce dependency on synthetic herbicides that are possibly hazardous to our environment.

7.2 Historical Background of Allelopathy

The word “allelopathy” was first coined by Molisch (1937), using Greek words: allelon (meaning ‘of each other’) and pathos (meaning ‘to suffer’). Allelopathy or allelobiogenesis is the outcome of the combination of abiotic and biotic stresses triggered by donor plants on the recipient plants. As per many literatures, allelopathy is characterized as an organic chemical mediated amid microorganisms or plants through its indirect or direct impact. Allelopathy existed in the world from 2000 years and may be many more. During the recent decades there has been many scientific works which have recorded allelopathy and has acknowledged its existence in the world. Theophrastus, ‘the father of Botany’, in late 300 B.C. wrote how the chickpea plants wrecked weeds and exhausted the soil. The attention was then captured by Willis (1985) and the deadly impact of cabbage on a vine due to the effective odour. During

234–140 B.C. Cato the Elder in his book mentioned the harmful effect of barley and chickpea on the corn land, and also revealed the negative effects of walnut trees on different plant species. The historical framework can be divided into major phase or periods:

1. De Candolle Phase (between 1785 and 1845)
2. Pre-Molisch Phase (extending from 1900 to 1920)
3. Post-Molisch Phase (1937 to 1960)

Chemicals found to constrain the growth of a species at some concentration may help to arouse the growth of the similar species or another at a lesser concentration. Aldrich (1984) designates two kinds of allelopathy:

- **True type:** - The disperse into the environment of compounds that are poisonous in the form in which they are produced.
- **Functional Type:** - Toxic substance that goes into the environment as a result of transformation by microorganisms.

Allelopathic compounds when come in contact with neighbouring plants, which can then stimulate, or more typically negatively influence the growth of these neighbouring plants. Although these compounds are not restricted to plant-plant interactions, they can also interact with other organisms. Lovett looked at allelopathy as the composite of elusive communications between plants and, also between plants and other organisms. Allelopathic species can indirectly affect their growth rate by manipulating microbes in their surrounding rhizosphere, and altering the plant-microbe interaction. Biorational alternatives are taking much attention for weed control because of problems related to pesticide usage and diminishing numbers of labeled products, particularly for negligeable usable crops. In certain cropping situations allelopathy may have the potential to be integrated into a weed management plan in order to lessen the use of artificial herbicides, as well as provide other added benefits from the allelopathic crop. Allelopathic crops can be incorporated into weed management plans as crop rotations, living mulches, or as residue cover crop. Genetic engineering could be a useful tool in order to create cultivars that produce greater amounts of allelopathic compounds for weed suppression in agriculture. Pliny stated the nature of some plants though not actually deadly is injurious owing to its blend of scents or of juice. This statement shows that Pliny hypothesized how plants were releasing chemicals that resulted in this interference. Although allelopathy does not always cause negative effects to neighbouring plants, throughout history it was probably easier to observe negative plant responses. De Candolle in the 1800's was one of the first to perform research on toxic root exudates from allelopathic species. Examples of allelopathy have shown up throughout agricultural history, mainly by farmers that have observed the abnormal negative impacts on crops that are grown with other certain species. The Romans described allelopathy as a process resulting in the "Sickening" of the soil.

7.3 Proof of Allelopathy

Many field studies implicate allelopathy, but isolation and identification of the chemical agents require a rigorous laboratory effort. It is extremely difficult to prove that any deleterious effect is due to allelopathy rather than to competition for essential products. Numerous research studies have provided proof, but rarely have a specific decorum to be followed for achieve convincing evidence. Many authors have pointed out those shortcomings of discipline which make it difficult to distinguish between competition and allelopathy. These shortcomings comprise: -

- Lack of nomenclature to sufficiently designate the plant responses that occur in this manner.
- Scarcity of techniques to distinct competition from allelopathic interactions.
- Prove the presence of direct influences compared with indirect influences via other micro environmental / organism's modification.
- A substantial body of information has gathered linking allelopathy as an imperative form of plant interference.

According to Willis (1985), Putnam and Tang (1986) and Cheng (1992) the methodology decrees firm points for allelopathic research to be recognized to suggest that is functioning. A design of inhibition one species by the other must be made known using appropriate controls, recounting the symptoms and measurable growth reductions; The supposed aggressor plant must produce a poison; There must be an approach of poison release from the target plan;

Mode of toxin transference or accumulation in the environment must be obvious; the distressed plants must have some passage of toxin uptake that should be exposed to the chemical in adequate quantities and time to create damage, and to notice analogous symptoms. The detected pattern of inhibition should not be merely by physical factors or other biotic factors, especially competition. It is imperative to stress that the overhead points do not demonstrate that allelopathy is operative, only that it proposes the most sensible explanation for the detected pattern. According to Cheng (1992), as soon as the chemical go in the environment, a number of interrelating processes will take place. These processes have been identified as:

- **Retention:** The associated movement of the chemical from one site to another, through soil, water.
- **Transformation:** The variation in structure or form of the chemical, causing fractional change or total disintegration of the molecule;
- **Transport:** Describes how the chemicals move in the environment.

Cheng (1992) also suggested that these progressions are affected by the organism present, nature of the chemical, environment conditions and the properties of the soil. The fate of the chemicals depends on the kinetics and interactions of individual processes with time, at a particular site under a particular condition.

7.4 Allelochemicals

Allelochemicals can be divided on the basis of their structural properties- (1) straight-chain alcohols, water soluble organic acids, ketones, and aliphatic aldehydes; (2) long-chain fatty acids and polyacetylenes; (3) simple unsaturated lactones; (4) cinnamic acid and its derivatives; (5) quinines (complex quinines, benzoquinone, and anthraquinone); (6) flavonoids; (7) tannins; (8) phenolics; (9) coumarins; (10) terpenoids (diterpenes, triterpenoids, sesquiterpene lactones, and) and steroids.

In the creation of allelochemicals environmental conditions also play a significant role in the, some of which are mentioned as follows:

1. Mineral deficiency: It causes more production of allelochemicals.

2. Light: Some allelochemicals are affected by the duration, amount, and intensity of light.

3. The age and type of plant tissue: Between species and within species as well, the production of allelochemicals differs. Therefore, it gains its importance during the process of extraction as a result of the irregular distribution of compounds in plants.

4. Temperature: Production allelochemicals is favorable at cooler temperatures

5. Drought stress: Allelochemicals production is enhanced under such conditions.

6. Shade, plant diseases, and herbicides can also impact allelopathy.

According to Putnam and Tang (1986) all suspected cases of allelopathy that have been considered for studies seem to encompass a complex of chemicals. No solo phytotoxin was merely accountable for or produced as a consequence of intrusion by a neighbouring plant, Rizvi *et al.* (1992) jagged out that the subject not only pacts with the gross biochemical relations and their possessions on the physiological developments but also with the mechanism of action of allelochemicals at specific sites of action at the molecular level.

Limited studies on allelopathy, focus on the mechanism and processes, related in the production of allelochemicals. Putnam and Tang (1986) and Einhellig (1987), raised query whether assumed biochemical agents were in adequate concentrations and with sufficient persistence in the environment to influence a neighbouring plant. These chemicals could be altered during the progression of extraction. According to Cheng (1992), allelopathic signs may not be established at the time or site of where plants injury has actually occurred. Allelopathy also may be one of numerous attributes, which allow a plant to generate in a new ecosystem.

From the discharge of allelochemicals, plants can control the soil microbial community in their immediate area, reassure valuable symbiosis, influence herbivory, variation in the chemical and physical properties of the adjacent environment, and directly constrain the growth of rival plant species. Allelopathic compounds play vital roles in the fortitude of dominance, plant diversity, succession, in the plant productivity of agro ecosystems and in climax of natural vegetation.

7.5 Sources of allelochemicals

Allelopathic potential have been recognized in many natural products, these have been classified into the following groups: (a) organic acids, (b) cytotoxic gases, (c) coumarins, (d) simple unsaturated lactones, (e) aromatic acids, (f) flavonoids, (g) quinones, (h) terpenoids and steroids (i) alkaloids, and (j) tannins. According to Aldrich (1984), allelochemicals must be focused in the stem, leaves, or roots than in the flowers or fruit. If it is focused in these organs it is probable that it could be accessible in time to interfering with neighbouring plants. Rodosevich and Holt (1984) specified that the key effect of allelopathy appears to result from an association with plant litter in or on the soil. Rice (1984) and Putnam (1985) stated that allelochemicals are existing in almost all plant tissue, i.e., fruit, stems, leaves, and roots. Leaves may be the greatest constant source, while roots are known to contain lesser potent toxins. According to them, there are four ways in which the chemicals are released: -

a) Volatilization: - Release into the atmosphere, only noteworthy under arid or semi-arid conditions. The compounds may be captivated in vapour by adjacent plants, be absorbed from dew or might spread the soil and be picked up by the roots. Kanchan (1975) described that *Parthenium hysterophorus* through volatiles released allelochemicals. The genera, which release volatiles, are *Artemisia, Parthenium, Salvia,* and *Eucalyptus*.

b) Leaching: Dew, rainfall, or irrigation may discharge out allelochemicals of plants that are afterward deposited on other plants; also, soil leaching also might happen via plant residues. Their solubility will influence their movement in soil water.

c) Root exudation: In the soil environment through plant roots. Whether these compounds are vigorously leaked, exuded, or arise from the cells exuviating off the roots is not evidently understood at this time.

d) Decomposition of plant residues: It is tough to determine whether toxic substance is confined in residues and basically released upon decomposition, or produced instead by microorganisms utilizing the residues.

7.6 Mechanism of Action of Allelochemicals

Allelopathy could be considered as “an imperative component of plant impedance interceded by the addition of secondary metabolites produced by plants into the soil rhizosphere” (Weston, 2005). These secondary metabolites are classically dispersed into the soil rhizosphere and disrupt the growth of plants that are growing in the vicinity of allelopathic plants. Chemical compounds that perpetrate allelopathic effects are called allelochemicals or allelochemics, which are by immensely considered to be those chemical groups, for example, flavonoids, glucosinolates, alkaloids, terpenoids and phenolics. Allelopathy has been well known for many countless years, however, the knowledge of the allelochemical’s mechanism about the mode of action of stays darken. Many biosynthetic pathways are accountable for the production of the numerous classes of these chemical compounds, though they are not essential for primary processes of development and reproduction for the allelopathic species. However, these compounds can impact plant growth indirectly by altering the interspecific competition for the plants in association. A extensive array of these compounds are recognized today, however, just a restricted number has been documented as allelochemicals. Allelochemicals are chiefly present all over the plant including stems, leaves, roots, inflorescence, rhizomes, fruits, pollen, and seeds. The production of allelochemicals in a plant species may differ spatially and over time scale; Singh, Jhaldiyal, and Kumar (2009) found leaf litter and foliar leachates of Eucalyptus species more fatal than its bark leachates to few commercial crops. Allelopathic intrusions frequently are a result of the combined activity of different compounds. No sole phytotoxin was wholly in charge of or distributed allelopathy because of interference by a neighboring plant. Allelochemicals found to hamper the development of a species at a specific concentration may improve the growth of the same species or another at a different concentration. The response of different species to different allelochemicals is concentration-dependent, the degree of inhibition rises with the growing concentration. The selective action of tree allelochemicals on different plants and crops has been testified as well. Leachates of the elder tree can obstruct the growth of pangola grass however augment the growth of bluestem. As indicated by Cheng (1992) once the allelochemicals are released by the donor plant into the environment, several connecting processes will occur. These processes have been distinguished as;

(a) Retention, the obstructed movement of the substance through soil, water and air from one area to another;

(b) Transformation, the modification in form or structure of the compound, instigation to its fractional change or total decomposition and,

(c) Transport, designates how the compounds transfer in the environment. Soil properties, environmental factors, species and nature of the compound involved in the interaction together influence these processes.

Moreover, the destiny of the allelochemicals hang on the contacts and kinetics of individual processes spatially, at a exact site under a specific set of natural condition. The mode of activity of a substance could expansively be partitioned into direct and indirect action. Influences through the modification of nutritional status, soil properties, and a transformed population or role of nematodes and microorganisms prompt indirect action. The direct action includes the biochemical/physiological effects of allelochemicals on different vital processes of plant metabolism. Procedures affected by allelochemicals include:

1. Mineral absorption: allelochemicals can alter the rate of ion absorption by plants. Phenolic acids decrease the acceptance of both macro and micronutrients.

2. Cytology and ultrastructure: collection of allelochemicals have been seemed to suppress mitosis in plant roots.

3. Phytohormones: the plant growth hormones, IAA (Indole acetic acid) and GA (gibberellins) regulate cell enlargement in plants. IAA is accessible in both active and inactive forms (inactivated by IAA-oxidase). IAA-oxidase is suppressed by diverse allelochemicals. Ethylene and ABA (abscisic acid) production enlarged upon allelopathy stress.

4. Membrane permeability: Many organic compounds apply their effect through the alteration of membrane permeability. Exudation of chemicals from roots on root cuts has been used as a penetrability index subsequently plant membranes are tough to study.

5. Photosynthesis: benzoic and cinnamic acid reduced chlorophyll content in soyabean thus repressed photosynthesis. Photosynthetic inhibitors may be electron inhibitors or uncouplers, energy-exchange inhibitors electron acceptors or a mixture of the above.

6. Respiration: allelochemicals can strengthen or repress respiration, both of which can be injurious to the vivacity of the energy-producing system.

7. Protein synthesis: Studies using amino acids or radio-marked C14 sugars, and traced their amalgamation into protein, stated that allelochemicals hinder protein synthesis.

8. Enzyme activity: Rice (1984) gave an interpretation of numerous allelochemicals that confine the precise enzyme activity in the plants. N. plumbaginifolia allelochemicals stimulated the catalase (CAT) and superoxide dismutase (SOD) activity.

9. Proline content: Accumulation of proline in plants exposed to any stress.

13. Growth and improvement: Upon introduction to allelochemicals, the growth and development of plants are impacted. The closely visible impacts comprise repressed or impeded germination rate; reduced extension of root or radicle and shoot or coleoptile; seeds obscured and swollen; twisting of the root axis; swelling or rotting of root tips; absence of root hairs, discoloration; rise in number of seminal roots; reduced reproductive potential; and diminished dry biomass accumulation. These net morphological influences may be auxiliary indexes of important processes, caused by a mixture of additional specific influences acting at the cellular or less or more at the molecular level in the recipient plants. Effects of allelochemicals on seed germination seemingly intercede through an interruption of normal cellular processes as an alternative of injury through organelles. Reserve mobilization, a mechanism that usually occurs quickly within early phases of seed germination seems to be by all accounts deferred or reduced under allelopathic stress. The biological responses of recipient plants to allelochemicals display a threshold response and are identified to be concentration. The responses are, classically, incitement or attraction at little concentrations of allelochemicals, and suppression or repellence at moderately higher concentrations. These prodigies have been roughly reported in allelochemicals from living plants, in allelopathic influences from plant residues undergoing decomposition, and from the extensive range of morphology to biochemical activity, including other growth-regulating chemicals and synthetic herbicides. As recommended by Einhellig (2002), a vital impact of phenolic acids is on the plasma membrane, and this alteration in the permeability barrier adds to numerous physiological modifications hindering growth and development.

Summary

Terminal Questions

1. What is allelopathy? What is its significance in the environment?
2. Describe the historical background of allelopathy.
3. What are the environmental conditions which help in the creation of allelochemicals?
4. What are the various sources of allelochemicals?
5. Which are the biochemical/physiological mechanisms procedures affected by allelochemicals?

Unit 8: Environmental Factors and Eco Physiological Responses

Unit Structure

8.0 Objectives

After reading this unit you will be able to:

- Define about environmental factors
- Describe the eco-physiological responses
- Understand biological management of environmental factors

8.1 Introduction

Efficiency of crop production, refer to production per unit of land area (yield) or per unit of time or per unit of inputs such as fertilizer, water, labor etc. In near future, in many parts of the world burgeoning population will create the need of vast increment in crop production efficiency due to great variations in consumption patterns and requirements of agricultural products. In addition, there will be decrease in the available arable land area suitable for crop production. The unavailability of arable land resources and presence of degraded land will be the major challenge in fulfilling the future global food demands. In the prevailing scenario, the land use paradigm shifts, socio-economic based advancements and irrelevant extensive farming techniques are the major driver in degrading the available arable land resources worldwide.

In an agroecosystem, growing plants or crops requires a healthy environment and soil-plant relation to achieve optimal yield. However, the presence of plethora of environmental stresses or factors (origin is either natural or man-made) abrupt the growth and development of plants resulting in decreased yield and other constraints of plant. An understanding of plants or crop responses towards environmental factors or stresses will help in developing methods and strategies that can increase agricultural production efficiency. Plant responses to environment are determined by the adaptation of plants and influence the improvement of cropping systems that can be achieved through changes in management practices and plant breeding. Like other existing organisms, the plants are exposed to different environmental factors or stresses, such as waterlogging, drought, cold, heat, soil salinity and acidity, and air pollution etc. The concept of stress is associated with stress tolerance. Degree of tolerance differs within different plant species. According to Levitt (1972), “Stress is any change in environmental conditions that might reduce or adversely change plant’s growth and development”. According to Jones (1989) “Adverse influence or force that tends to hinder normal systems from functioning”. Different environmental factors or stresses are broadly classified in two types i.e., biotic and abiotic as discussed further (Figure. 1).

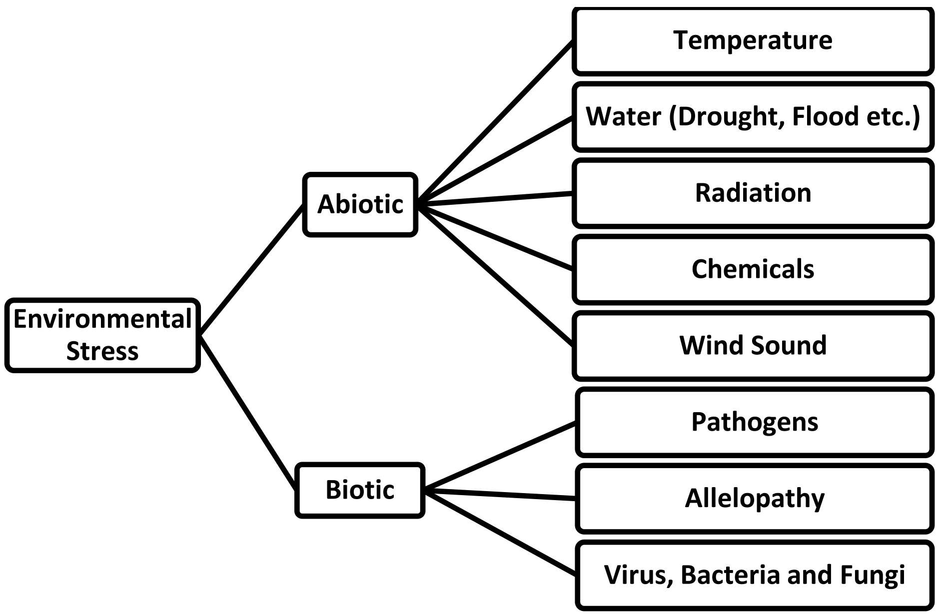

Figure 1: An overview of different environmental stresses

In accordance with the fact that higher proportion of population living in developing countries, it become necessary to enhance the agricultural production to meet out the escalating food demand.

The needed increases in yield of major food crops have been estimated by researchers. Demand for wheat has been projected to increase by 1.3% per year on a worldwide basis and 1.8% in developing countries. Meeting projected world demands for rice has been estimated to require increases in the average yield of Asia's irrigated rice land from 5.0 to 8.5-ton ha^{-1} in the 30-year period from 1995 to 2025, which will require an increase of 1.8% per year.

Note: **One metric ton = 1000 kg = 2205 lb, and one hectare = 10,000 m^{-2} = 2.47 acre.**

Demand for maize has been projected to increase by about 1.5% per year on a worldwide basis up to 2020. Substantial improvements in crop cultivars and management methods will be needed if the increases in yield of wheat, rice, and maize are to even approach these projected increases in demand of 1.3% to 1.8% per year. For example, the worldwide increase in maize yield from 1982 to 1994 was only 1.2% per year, and achieving increases of 1.5 to 1.8% would require 25 to 50% greater gains from systems that are becoming harder to improve. Duvick and Cassman (1999) pointed out that, even with a substantial investment in research on maize breeding, there is little compelling evidence that the yield potential of maize hybrids adapted to the north-central United States has increased during the past 25 years. Consequently, for maize in the United States, the main opportunities for increasing yield may involve breeding to enhance stress tolerance or the development of improved management methods. Increases in average grain yield of maize in major production zones of the United States between 1966 and 1997 were only 1% per year, using 1997 levels as a baseline. Major cultivars of rice released during the period from 1966 to 1995 by the International Rice Research Institute in the Philippines were compared in field studies in 1996 and 1998. They were shown to have exhibited a 1% genetic gain in yield per year for the period from 1966 to 1995. The authors point out, however, that the increases may not represent genetic gain in yield potential in that old cultivars had lower yields in recent years than they had in the past. Instead, new biotic and/or abiotic constraints may have arisen, and the more recent rice cultivars could be better adapted to these changes. Simply maintaining yields at current levels often requires new cultivars and management methods, since pests and diseases continue to evolve, and aspects of the chemical, physical, and social environment can change over several decades. Higher agricultural production without compromising sustainability is required in all countries to maintain profitability, enhance the sustainability of agricultural enterprises, and also to maintain good environmental health.

8.2 Abiotic Factors

Abiotic environmental factors can be of natural or man-made origin. There are a number of naturally present environmental variables, such as sunlight, temperature (heat and cold), magnetic field, rainfall, wind exposure, nutrient availability and atmospheric gases that make life possible on Earth. Man-made abiotic factors include pesticides, heavy metal, wastes, atmospheric pollution, poor air quality, oil spillages, etc. which contaminate the environment. Environmental factors like sunlight, water, air, soil, nutrient availability, etc. plays an important role in maintaining ecological functioning. Abiotic factors are non-living parts of the ecological environment. They can often influence growth and development of different living organisms in different ways. There are some of the common abiotic factors which are atmosphere, water, sunlight, temperature, chemical substances, and wind. The abiotic factors can also be influenced by the other biotic and abiotic factors. These abiotic environmental factors are essential for establishment of an ecosystem. These factors can control a wide range of environmental conditions in a specific area or region. As abiotic factors change across space and time, no two distinct locations on Earth surface share the similar set of environmental conditions. Due to the different level of tolerance or resistance of living organisms towards the environmental abiotic factors, the species richness and composition of animals, plants and all other living organisms, like algae, fungi and bacteria, are varies worldwide.

Among the environmental factors affecting growth and development of plants, light and temperature are the most temporally variable; even during a single day plants get exposed to rapid fluctuations due these factors. Plants are very sensitive towards the most heterogeneous factor i.e. light, they get affected by minor fluctuations in intensity of light. Light can be a limiting factor at lower intensities and a stress factor at higher intensity. Seed germination, which is the most critical stage in establishment of plant or crop. Plants can adapt to the changing factors, because they exhibit a notable ability to modify their morphology and physiology according to the prevailing environmental conditions.

This article gives seven main environmental factors that affect plant growth. The factors are:

- Light
- Temperature
- Gravitation and magnetic field
- Water
- Salt
- Nutrients (nitrogen and phosphorous)
- Fire and grazing

8.2.1 Light

The eco-physiological responses of light which affects plants in various ways are as follows:

A. Photosynthesis: The plant shoots are adapted to receive and absorb sunlight. Most leaves get saturated with only about 20% of full sunlight. Of this quantity about 20% is stored in sugar molecules produced therefore the efficiency is 4%. The dry matter content of the plant represents only 1-3% of the light supply that was available. Leaves in shade produce little sugar since they receive limited energy supply. When, a plant is not carrying on photosynthesis. Its dry weight decreases as a result of respiration. The amount of light essential for photosynthesis to equal the respiratory use of carbon compounds is called the GHT compensation point. This value is always higher than the absolute minimum for photosynthesis, ranging from about 27 to 4200 L in higher plants.

B. Transpiration: High light intensity promotes transpiration. Light stimulates the guard cells to open and also increase the permeability of the plasma membranes. Of the total light energy reaching leaves about one-third is reflected back and about two-thirds is absorbed, and only small percentage is utilized photosynthesis. it is changed into heat energy and then lost by radiation or used up the vaporization of water.

C. Seed Germination: The seeds of most plants become sensitive to light when wetted. In some cases, they germinate whereas in other cases the germination is retarded. *Lactuca sativa* (salad) seed will not germinate without light stimulation (photoblastic) and *Daucus carota* (carrot) seeds germinate better with exposure to light. In contrast many Cucurbitaceae germinate better in darkness.

D. Photoperiodism: The length of duration of light (photoperiod) is important to most plants especially for Inducing flowering. This response is called photoperiodism. Twelve to fourteen hours of daylight is a critical duration for most plants. The plants may be long day or short-day plants according to their requirement of photoperiod. The stimulus is received by a pigment called phytochrome in the buds or leaves and then transmitted to other parts of the plant. In some cases, a single cycle of appropriate photoperiods enough to start flowering. Photoperiodism help plants to detect seasons. It also affects the distribution of plants. Similarly, it enhances the economic value of the plants, for example at high altitudes beets change to annual mode of life and become useless economically. Photoperiodism helps to check such transformations.

E. Reproduction and Growth

Insufficient light suppresses flowering and the vascular plants may remain vegetative indefinitely. Fungi require light for production of spores. The crops grown for vegetative parts are favored by cloudy climates whereas fruits, grains and seeds are favored by bright sun.

F. Effect on structure of Plant

The amount of light available to a plant exerts a notable influence on the structure and function of the organs. The process of differentiation is governed by blue and violet wavelengths of light. Plants growing in full sunlight have thick stems, profusely branched, less leaf area per plant and smaller cells in leaf blades, thicker cuticle, fewer and smaller chloroplasts, better developed palisade, longer and more branched roots, etc. Their photosynthetic rate is higher, transpire rapidly, show greater vigor of flowering and fruiting, produce seeds with more calories per gram dry weight, and are more resistant to temperature injury, drought and parasites.

G. Ecological Classification

According to comparative requirements of light (Sun energy) or shade the plants may classified ecologically as followings:

- **Heliophytes:** The plants that produce best in full sunlight are called heliophytes

- **Sciophytes:** The plants that raise best at low light intensity are known as sciophytes.
- **Facultative sciophytes:** Some heliophytes can grow fairly well under shade. They are known as facultative sciophytes.
- **Obligative heliophytes:** Similarly, some sciophytes can nurture better in full sunlight, these are known as obligative heitophytes.

8.2.2 Temperature

Temperature is a degree of the intensity of heat the bound of survival of living organisms has generally been described to be between -35°C and 75°C. The series of growth for maximum agricultural crops is between 15°C and 40°C. At temperatures much below or above these limits' growth decreases rapidly. Optimum temperatures for plant growth are dynamic since they variation with the varieties and species, period of exposure, stage of development, age of the plant, etc. The vital plant metabolic processes like respiration, photosynthesis, evapo-transpiration etc. are affected by the temperature. Besides these, temperature impacts the absorption of nutrients and water, and also influences the microbial activity which eventually affects the plant growth.

8.2.3 Gravitation and magnetic field

A magnetic field (MF) is an inevitable environmental factor for plants on the Earth. During the evolution progression, all living organisms experienced the action of the Earth's MF (geomagnetic, GMF), which is a natural component of their environment. GMF is progressively acting on living systems, and is known to affect many biological processes. There are substantial local alterations in the strength and direction of the earth's magnetic (geomagnetic) field. At the surface of the earth, the vertical component is maximal at the magnetic pole, amounting to about 67 μT and is zero at the magnetic equator. The horizontal component is maximal at the magnetic equator, about 33 μT, and is zero at the magnetic poles. The literature unfolds the properties of weak MFs on living systems contains an excess of contradictory reports, limited successful independent replication studies and a lack of reasonable biophysical interaction mechanisms. Utmost such investigations have been haphazard, lacking of testable theoretical forecasts and, ultimately, unimpressive. The development and status of research on the outcome of MF on plant life have been reviewed in the past years.

Krylov and Tarakonova (1960) were amongst the first to account on MF effects on plants. They projected an auxin-like upshot of the MF on germinating seeds, effect known as magneto-tropism. The auxin-like result of MF was also recommended to elucidate ripening of tomato fruits. There was indication that the root-growth response was not directly heliotropic but rather geo-magnetotropic or magnetotropic. Observation of the roots of a number of additional plants recommended that some inherent factor within a species or even within a variety of a species could also be essential before the tropism became apparent.

8.2.4 Water

Water is one of the utmost significant factors that control the distribution of species around the globe. Insufficient rainfall and soil bound water leads to water shortage in crops. Water stress is known to be the most significant restrictive factor regulatory primary production in terrestrial environments. Water stress is referred to as a limited water supply to plant roots, which decreases the rate of transpiration in plants. It is chiefly caused by water deficit as an outcome of soil salinity or drought conditions. Water stress marks in disturbance of agriculture, hence it disturbs food production in the world, resulting in famine. Water deficit stress is one of the furthermost common environmental factors that affect plant growth. Abundant changes have been detected in plants as a result of water stress. These variations depend on duration, severity, and time course of water stress. Water is important for growth and development of plants. Permanent or temporary water deficit limits the growth and distribution of natural vegetation and also the performance of cultivated plants. Water stress brings metabolic changes in plants accompanied with reduction in growth and photosynthesis.

8.2.5 Salt

Salt stress is triggered by the occurrence of high concentrations of salt ions-mainly sodium and chloride. High salt ion concentrations aggravate not only a chemical (ionic) imbalance in plant cells, but also tempt a drought stress by raising the water potential (Ψ) of the soil. Major resistance mechanisms to salt stress are related with transporter-mediated export/sequestration of Na^+ ions across the plasma membrane and tono-plast, respectively. Numerous physiological processes such as seed germination and plant development are affected by salt stress and thus bound the generation potential of the plant. It was projected that tolerance to salt stress is a stage-specific governing phenomenon, as it is not essential that tolerance at one stage of plant development also delivers tolerance at some other stage. The biochemical and physiological function of the plant cell is changed due to salt stress, as it leads to water loss which causes drought stress and it also leads to ionic toxicity. The main target of salt stress is photosynthetic sites and its components. Two primary effects are forced on crop plants by salt stress; osmotic stress and ion toxicity. The osmotic pressure under salinity stress in the soil solution surpasses the osmotic pressure in plant cells due to the occurrence of more salt, and thus, bounds the ability of plants to take up water and minerals like K^+ and Ca^{2+}. These primary effects of salinity stress cause some secondary effects like reduced cell expansion, assimilate production, and membrane function as well as diminished cytosolic metabolism.

8.2.6 Nutrients

Nitrogen is the mineral nutrient that plants entail in highest quantity and that most often limits development in both agricultural and natural systems. The carbon consumed in acquiring nitrogen can make up a momentous, if not predominant, fraction of the total energy a plant consumes. Plants have established numerous approaches to nitrogen acquisition, together with such diverse phenomena as mycorrhizal associations, fixation of atmospheric nitrogen, and carnivory. So far, most plants attain their nitrogen completely through root absorption of the inorganic ions ammonium and nitrate. The carbon costs for these several methods differ. We foresee that the chosen method depends in large part on the carbon and nitrogen status of the plant and on the comparative availability of energy and nitrogen in the environment. Nitrogen acquisition contains of numerous processes: transporting nitrogen from the environment into the plant; absorption, translocation, assimilation, moving inorganic nitrogen within the plant, and converting nitrogen from inorganic to organic forms. P deficiency decreases plant growth which is credited to either reduction in photosynthesis or increase in energy outlay. Its restriction negatively effects crop yield and quality. It has been projected that P deficiency lessens the crop yields on 30–40% of the world's arable land. This executes the use of an enormous amount of phosphatic fertilizers to overcome its deficiency. The phosphorus use efficiency (PUE) is 15–20% in agricultural fields representing that utmost of the soil-applied P remains unavailable to plant and leaches into ground and surface water leading to eutrophication. Phosphorus plays a significant role in an array of cellular processes, together with maintenance of membrane structures, synthesis of biomolecules and formation of high-energy molecules. It also supports in enzyme activation/inactivation, cell division, and carbohydrate metabolism. At whole plant level, it stimulates flower and seed formation; development of roots, stalk and stem strength; seed germination; crop yield; and quality. In addition, availability of P rises the N-fixing capacity of leguminous plants. Hence, P is vital at all developmental stages, right from germination till maturity.

8.2.7 Fire and Grazing

Prescribed burning (PB) is one of the furthermost debated land management techniques, mainly in relation to the qualitative profits of PB for wildfire hazard abatement, increased likelihood of asset protection and ecological impact on forest stands. Although a number of studies have evaluated PB effects on diverse forest ecosystem components, several ecological concerns persist. Prescribed burning has imperative impacts on forest structure, forest soil and nutrients, understory vegetation, seed banks, and overstory trees mainly in relations of productivity. However, little is identified about the probable impacts of PB on xylem hydraulic efficiency/safety, water transport, tree water use efficiency (WUE), and overall tree vulnerability to drought. Heat stress produced by fire can potentially affect stem hydraulics by means of deformation of xylem tissue and/or by inducing embolism in xylem conduits. High temperature plumes during the fire can significantly upsurge xylem water tension, therefore causing 'air seeding' and embolism possibly triggering strong falls of root-to-leaf water transport capacity. When embolism-induced damage of hydraulic conductivity exceeds a critical threshold, trees can undergo crown desiccation and even death.

Moreover, heat and drought stresses coupled to xylem embolism can cause extended stomata closure and abridged photosynthetic rates. Eventual heat-induced deformations of xylem conduit walls may also cause increase in the xylem vulnerability to embolism construction, thus increasing the vulnerability of surviving trees to successive drought events. All these physiological effects have potentially vital long-term consequences for surviving trees, since droughts are expected to increase in frequency, severity and duration with potential negative upshots for many forest areas.

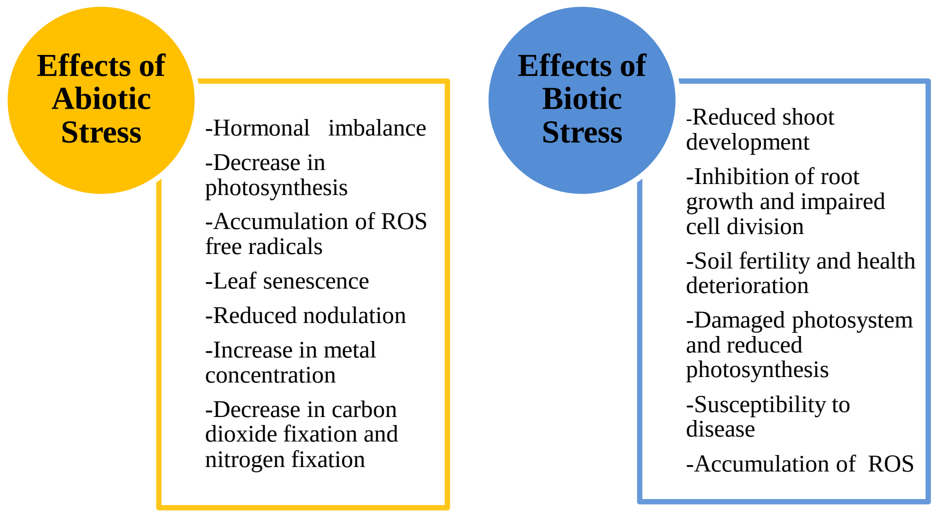

Figure 2. Effects of different abiotic and biotic stresses on morphological, biochemical and physiological attributes of plants

8.3 Biotic Factors

The biotic environment comprises all living things and their actions as well as reactions. From practice and observations, growth capabilities and ecological amplitudes of several plant species are known. Each plant species has its own specific environmental requisites for growth and reproduction. As conditions differ from the ideal, the proficiency of metabolic processes declines, growth diminishes, and vigor drops, sometimes consistently. Persistent extreme variances produce a stress condition in which one or more types of diseases, injuries, or disorders may appear, e.g., cankers, visible leaf spots, yellowing or blight. Attacks by several pathogens such as fungi, bacteria, oomycetes, nematodes and herbivores are involved in biotic stresses. Biotic stress in plants is produced by living organisms, especially viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds. The agents producing biotic stress directly deprive their host of its nutrients can lead to great losses in yield or death of plants. Biotic stress can become chief because of pre- and postharvest losses. On a global scale, these biotic stresses are responsible for 20% to 40% of reduction in production of major crops which costs nearly US$ 300 billion annually. In India, 15% to 20% of cash crops and principal major food is lost apparently due to biotic stress. Despite of lacking the adaptive immune system plants can respond biotic stresses by developing themselves to some specific sophisticated strategies. The defense mechanisms which act against these stresses are regulated genetically by plant's genetic code stored in them. The resistant genes against these biotic stresses present in plant genome are encoded in hundreds. The biotic stress is totally different from abiotic stress, which is imposed on plants by non-living factors such as sunlight, salinity, cold, temperature, drought and floods having adverse impact on crop plants. It is the climate in which the crop lives that elects what type of biotic stress may be levied on crop plants and also the ability of the crop species to counterattack that particular type of stress. Many biotic stresses affect photosynthesis, as chewing insects lessen leaf area and virus infections reduce the rate of photosynthesis per leaf area.

Plants respond to biotic stress through a defense system. The defense mechanism is classified as a systemic and innate response. After infection, reactive oxygen species (ROS) are generated and oxidative bursts bound pathogen spread. Also, in response to pathogen attack, plants increase cell lignifications. This mechanism restricts invasion of parasites and lessens host susceptibility. The defenses to biotic stress include structural and morphological barriers, chemical compounds, enzymes and proteins. These consult resistances to biotic stresses by defensive products and by giving them rigidity and strength. The resistance to biotic stress can be induced through specific chemical compounds such as benzothiadiazole (BTH) or β-aminobutyric acid (BABA). Plant hormones, jasmonic acid (JA), salicylic acid (SA), and ethylene play important roles in biotic stress signaling. Several transcription factors (TFs) are mediators in multiple hormones signaling. Plant ramparts against biotic stresses involve several signal transduction pathways.

8.4 Biological Management of Environmental Factors for Plant Growth and Development

Some of the environmental factors or stresses can be managed and mitigated by applying different biological techniques. The application of some beneficial microorganisms is proven to be an effective tool in protecting soil-plant system from different biotic and abiotic factors. These beneficial microbes include various species of bacteria, algae, fungi, actinomycetes, protozoa, nematodes etc. In agro ecosystem, the entire soil-plant system is populated by a variety of beneficial and harmful microorganisms. Microbes resides in plants as endophyte or exophyte, some of them live in making associations with plants and some resides free-living either in soil or on different plant parts. Many of these microbes performs vital mechanisms such as bioremediation of contaminants from soil, organic matter enhancement, mineralization and solubilization of nutrients, facilitates nutrient uptake, produces phytohormones, production of important plant growth promoting traits (PGP-traits), and several antioxidant enzyme productions. These mechanisms directly or indirectly provide tolerance to the plant and promote growth and development in stressful conditions.

8.4.1 Bacteria

The soil micro flora is dominated by bacterial communities, which interacts with plants either symbiotically or non-symbiotically. In comparison to exophytic living bacteria, the endophytes feature more abilities for stress tolerance and effectively alleviate different environmental stress in plants. Rhizobia are endophytic bacteria, belongs to a group of nitrogen fixing symbiotic bacteria which comes under family *Rhizobiaceae*. As a result of symbiosis between bacteria of *Rhizobiaceae* and leguminous plants, agricultural farms get around 80% of total symbiotically fixed nitrogen. There are many rhizobia identified which are capable of tolerating stress conditions like soil salinity, high temperature, metal toxicity, drought, and soil alkalinity and acidity. Some rhizobia provide tolerance to the plants in stressful conditions by nitrogen fixation, nutrient solubilization, phytohormones and vitamins production. They also produce bioactive molecules which induce systemic tolerance in plants and inhibit pathogens. Some of the rhizobial species like *Rhizobium* sp., *Mesorhizobium* sp. are known to fix nitrogen at higher temperatures (ranging from 25 to 65 ºC). Plant growth promoting rhizobacteria (PGPR) are a proven to be effective in mitigating abiotic and biotic stress in soil-plant system. According to the name rhizobacteria resides adhered to plant roots and known to provides systemic tolerance and performs many vital tasks. They enable growth and development of plants in stressful conditions by performing an array of mechanisms which includes modulation in stress enzyme activities (like reduces stress ethylene, increases proline), phytohormones production (gibberellins, Indole acetic acid, cytokinin, etc.), production of osmolytes, siderophore, antioxidants and mineral solubilization. Some of the species of PGPR like *Enterobacter* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus* sp., and *Stenotrophomonas* sp. are identified for providing heavy metal tolerance to plants and promoting growth and development. These species can tolerate and remove higher concentrations of heavy metals such as, Cd, Pb, Cu, and Ni from contaminated soils. The heavy metal tolerance mechanisms of bacteria mainly involve exopolysaccharides and biosurfactants production, enzymatic detoxification, metal complexation or chelation, and efflux mechanism.

8.4.2 Fungi

Many free living and endophytic fungi are identified which are capable of alleviating negative effects of different abiotic and biotic stress on plants. Fungal species such as, *Aspergillus sp.*, *Acaulospora sp.*, *Aurobassium sp.*, *Dothideomycetes sp.*, *Penicillium sp.*, *Funneliformis sp.*, *Glomus sp.*, *Rhizophagus sp.*, *Scutellospora sp.*, *Rhizopus sp.*, and *Serratia sp.* are known to improve plant growth in drought stress, salt stress, soil acidity, heat stress, and heavy metal stress. Fungus are highly suitable for removal of heavy metals from contaminated soils especially because of their mycelial attributes and capabilities to accumulate a variety of metals. They perform several mechanisms such as chelation, vacuole formation, metal exclusion, and production of metal binding compounds. Symbiosis of plant roots and arbuscular mycorrhizal fungi (AMF) is believed to prevent plants from different environmental factors. AMF inoculation in acidic soils is suggested as a best way to alleviate deleterious effects of acidic soils (lower pH) and nutrient deficiency. Some strains of AMF also help in protecting plants from drought stress, they modulate plant's aquaporin genes. Aquaporin genes are responsible for the symbiotic exchange of water and vital nutrients between host plant roots and AMF. AMF also protects plants from oxidative stress by producing antioxidant enzymes such as, superoxide dismutase, guaiacol peroxidase, catalase, and peroxidase.

8.4.3 Algae

Algae are a large and diverse group of photosynthetic aquatic organisms. Many species of micro and macro algae are found locally and known to produce bio-stimulants. Bio-stimulants are organic compounds which are very useful in crop or plant improvement when applied in limit. The application of products, extracts and bio-stimulants of the algal species such as, *Sargassum sp.*, *Ascophylum sp.*, *Arthrospira* (*Spirulina*) sp., *Botryococcus sp.*, *Chlamydomonas sp.*, *Durvillea sp.*, *Dunaliella sp.*, *Fucus sp.*, *Isochrysis sp.*, *Ecklonia sp.*, *Kappaphycus sp., Laminaria sp.*, *Lessonia sp., Macrocystis sp.*, *Porphyridium sp.,* and *Himanthalia sp.* are known to improve plant growth and provide tolerance towards different biotic and abiotic stress. The bio-stimulants and extracts derived from these algal species constitutes of amino acids, phytohormones, phenolics, betains, carbohydrates, carotenoids, flavonoids, polysaccharides and some essential micro and macro nutrients. Polysaccharides derived from brown algae (*Lessonia nigrescens*) are known to enhance wheat plant growth in drought and salt stress. The polysaccharides decreased membrane peroxidation and increased chlorophyll, antioxidants activities, compartmentalization and exclusion of intracellular ions. The algal extract supplemented plants uptake more nutrients such as, N, Mg, P, K, Mn, Cu, Fe, Ca and Zn. Nowadays, extracts and products derived from these beneficial algal species commercially provided as bio-fertilizer, animal feeding material, stress alleviators and much more.

Summary

Plant establishment and crop efficiency mainly depends upon the prevailing environmental conditions, i.e. environmental factors or stresses. The environmental factors can be of natural or man-made origin, but one thing is common that they influence growth and development of living things at large. These factors can control a wide range of environmental conditions in a specific area or region. Changes in prevalence and intensity of these environmental factors act as a stress for living organisms. Different living organisms have different level of tolerance towards these environmental factors. According to the level of tolerance plants, animals and other living organisms adapt or resist the prevailing environmental conditions. Apparently, spatial and temporal variations in species abundance, richness and compositions of living creatures depend upon their degree of tolerance or adaptations towards environmental factors. These environmental factors broadly classified into two types i.e., abiotic and biotic factors. Abiotic factors are non-living parts of the ecological environment. Some of the common abiotic factors which are water, salts, sunlight, nutrients, temperature (heat and cold), chemical substances (fertilizers, pesticides, heavy metals etc.) and wind can also be influenced by the other biotic and abiotic factors. These factors impose detrimental effects on plant growth and development, due to which the plants or crop fails to produce optimal yield. Due to these environmental factors world faces enormous agricultural yield reduction annually. Some of these factors or stresses can be managed, reduced and mitigated through application of biological techniques. The biological techniques include application of different bio-fertilizers, extracts, beneficial products derived from microorganisms.

Microbes perform vital mechanisms such as, symbiosis, detoxification of contaminated sites, phytohormones production, nutrient solubilization and their mobilization. These mechanisms provide multiple benefits and impart tolerance to the plants growing in stressful conditions.

Terminal Questions

1. Define biotic stresses with examples.
2. Define abiotic stresses with examples.
3. Differentiate between abiotic and biotic stresses.
4. Write about essentiality of environmental factors. How they influence living organisms?
5. How a change in environmental factors does causes loss in agricultural productivity?
6. How do the biotic and abiotic factors can be managed?
7. Write about the role of bacteria in alleviating different abiotic factors?

Unit 9: Ecotoxicology

Unit Structure

9.0 Objectives

After reading this unit students should be able to:

- Define ecotoxicology and differentiate it from classical toxicology.
- Describe the historical background of this discipline.
- Understand commonly used terms in this field.
- Identify the consequences of human actions on the ecosystem functions and services.
- Understand effect of ecotoxicants across all levels of organization.
- Understand the interdisciplinary significance of ecotoxicology.

9.1 Introduction

Ecotoxicology is the science dealing with the study of the adverse effects of chemicals on biological organisms especially at the population, community, ecosystem, and biosphere level. It is a multidisciplinary field which integrates toxicology and ecology. It is a discipline, just developed recently during the last five decades, directly associated to the need of identification, prediction, control and minimization of the negative consequences of the human activities on the environment. Ecotoxicology involves the application of the principles of toxicology to the environment, focusing on human activities resulting in the release of pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorofluorocarbons (CFCs), pesticides, heavy metals, radio-nucleotides and gaseous pollutants into the environment. Ecotoxicology integrates environmental science, chemistry, biology, molecular biology, physiology, environmental law and various other fields. It is considered as a critical and revolutionary field. The objective of ecotoxicology is to identify and predict the effects of pollutants with consideration of other ecological factors. Using this knowledge the most efficient and effective measure can be employed to prevent or remediate any negative effect. In already damaged ecosystems, ecotoxicological studies can help to choose actions needed to revive ecosystem and restore its services, structures and functions. On the basis of environmental spheres, ecotoxicology can be divided generally into aquatic, terrestrial, soil, and sediment ecotoxicology.

In this unit we will learn about basic concept of ecotoxicology. We will study various toxic chemicals and their effects on the individuals, population, community and ecosystem. We will have some idea of how ecotoxicological studies are conducted using various tests. Also we will learn about the interdisciplinary sciences applied in ecotoxicology and their significance.

9.2 Origin and History

Ecotoxicology is a relatively new science that was introduced in the 1970s in the broader field of environmental sciences. Its methodology, derived from toxicology, is widened to cover the natural environment and the biosphere in general. Ecotoxicology evolved after pollution events that occurred during and after World War II that raised awareness towards the effects of toxic chemicals on humans and the environment. In 1962, Rachel Carson's published her book, "Silent Spring" which triggered the separation of environmental toxicology from typical toxicology. This separation carried on to ecotoxicology, the offshoot of environmental toxicology. The distinctive feature of Carson's book was its extension of toxic effects from individual organism to effects at the level of ecosystem and on the "balance of nature". Ecotoxicology is different from environmental toxicology in the respect that it incorporates the effects of toxic chemicals across all levels of ecological organization from the organism to the communities to the ecosystems, whereas environmental toxicology involves toxicological studies at the level of organism and below. The term “Ecotoxicology” was used for the first time by René Truhaut, a French toxicologist, at an environmental conference held in Stockholm in 1969. He defined ecotoxicology as “a branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems, animal (including human), vegetable (sic!), and microbial, in an integral context”. As a result, he was recognized as the founder of this discipline. But the actual pioneer in this discipline was Jean-Michel Jouany, Truhaut’s assistant, who established this discipline and defined its objectives. In Jouany’s idea, ecotoxicology is mainly linked to ecology as its goal is to study the impact that environmental contaminants can exert on relationships existing between organisms and their environment. Jean-Michel Jouany was a young and brilliant alumnus of René Truhaut, who at the time allowed Truhaut to publicize the evolving discipline proposed by him at the international level. He and his colleague, Jean-Marie Pelt later established the teaching and research principles for ecotoxicology. Two French universities (Metz and Paris-Sud) markedly contributed to expand this rapidly growing discipline during the 1980s and 1990s. Many institutes followed this pursuit. Institutes like CEMAGREF (now IRSTEA), INERIS, IFREMER, CNRS and other French universities created research centers in ecotoxicology. Gaining full recognition by institutions, ecotoxicology continues to advance.

9.3 Ecotoxicants

There are various toxic agents in the environment capable of adversely affecting the organisms. These toxics agents are called ecotoxicants and some common classes of such toxic agents are as following.

Pesticides and their residues- Pesticides are widely used to prevent, destroy or repel organisms harmful to crops and livestock. However, only a small portion of applied dose reaches the target pests and rest find its way into non-target organisms.

Heavy metals- Metals which have a density of more than 5g/cm^3 are called as heavy metals. Some heavy metals are essential nutrients like iron, cobalt and zinc but in context of toxicology heavy metals refer to poisonous metals like cadmium, mercury, arsenic, chromium and lead.

Plasticizers- A plasticizer is a substance added to polymer to increase its durability, flexibility, strength, resistance to water/oil/fire or to facilitate its handling. Plasticizers like phthalates have been classified as potentially harmful to humans and animals.

Volatile organic compounds (VOCs)- These are organic chemicals having high vapour pressure at room temperature. Most of the anthropogenic VOCs have various health implications and are thus regulated by law. Common sources include solvents, disinfectants, cleansers etc.

Dioxins and dioxin-like substances- Dioxins are a group of highly toxic chemical formed by combustion processes like incinerating waste and burning of fuels like coal and oil. Compound like polychlorinated biphenyls (PCBs) are also included in this group. These are persistent organic pollutants and exhibit biomagnification.

Mycotoxins- These are toxic secondary metabolites of fungi which have the potential to cause disease and death in humans and animals. Some common mycotoxins are aflotoxin, citrinin, fumonisins etc.

9.4 Effects of Ecotoxicants

The toxicants can affect an organism by one or by combination of two or more of the following ways:

1. **Interference with Enzyme Activity-** Enzymes are proteins which act as a biocatalyst to speed up the chemical reactions taking place in the body of an organism, required to occur at rates fast enough to sustain life. Every metabolic reaction has its own enzyme which operates by "lock and key mechanism". An environmental toxicant can interfere with the enzymatic activity in following ways.

    - **Chelation-** In this process a metal binds with the enzyme, alters its shape and prevents its ability to carry out the reaction.
    - **Receptor Binding-** There are certain molecules situated on the surface of a cell waiting for a signal to come by. These molecules are called receptors and the signal is provided by enzymes, hormones or other proteins. Contaminants may bind with these receptors and affect their functioning by stimulating them, inhibiting them, or blocking them from reacting to the signal.
    - **Neurotransmitter Interference-** Many pesticides react with acetylcholinesterase (AChE), an enzyme which inhibits neurotransmitter

acetylcholine (ACh), and block its ability to breakdown ACh leading to erratic and uncontrolled muscle contraction and the animal dies from asphyxiation. Similarly, some organochlorine pesticides interfere with gamma aminobutyric acid (GABA), another inhibitory neurotransmitter in the central nervous system of mammals which prevent hyperexcitation.

2. **Impairment of Immune System-** An animal's immune system is very complex. It has two components, nonspecific and specific that protect against any foreign invader. The first line of defense is the skin, followed by stomach secretions, and mucous of epithelial tissues. Also included in this nonspecific category are white blood cells or leukocytes in the circulatory system that ingest bacteria and small parasitic organisms. Following this protection, the body has an adaptive, specific system of protection, which operates by learning to recognize and attacking foreign antigens. All foreign organisms, including viruses, have proteins on their surfaces; these proteins can serve as antigens. When foreign agents such as toxins, bacteria, or viruses enter another organism, lymphocytes in the host become sensitized. Two types of lymphocytes, B-cells and T-cells, exist, with B-cells coming from the bone marrow (in mammals) or liver and T cells from the thymus. It takes some time for these lymphocytes to produce their specificity, so the first encounter with foreign antibodies may result in a slow response. However, once the system has been activated, sensitized lymphocytes are continually produced so that if subsequent exposures occur, the host organism can respond more quickly. When activated, B cells form antibodies, which are small proteins that attack the antigens. Antibodies can neutralize some toxins, deactivate viruses, and activate cell killing proteins that work with the antibodies to destroy cells, or attract leukocytes that engulf the foreign agents. Activated T-cells will attack a foreign substance with their entire bodies or make substances called lymphokines that signal to leukocytes to go to the site of infection.

   Studies have shown that contaminants can cause cellular damage in organs responsible for immune function, such as the thymus. Exposures to pesticides and other chemicals reduce lymphocyte numbers and induce structural changes in T-cells. Because contaminants interfere with enzymes in many ways, they could also affect enzymes that are involved with immune functions. This could interfere with the signaling that goes on between T-cells and leukocytes. Heavy metals, particularly cadmium, lead, and mercury, also negatively impact immune functions in laboratory animals. Exposure to environmental concentrations similar to those found in fish from the Great Lakes showed that these metals lead to increased susceptibility to infections, autoimmune diseases, and allergic manifestations.

3. **Endocrine Disruption-** The endocrine system consists of several glands that do not have direct connection to the circulatory system and secrete hormones. These hormones control many processes in an organism, including metabolism, reproduction, responses to stress and functioning of the nervous system. Any alteration in the normal operation of the endocrine system can be called endocrine disruption.

Contaminants can exert endocrine disruption by:

- Mimicking natural hormones in the body, like estrogens, androgens, and thyroid hormones, causing overstimulation.
- Binding to cellular receptors and blocking the naturally occurring hormones from binding which interrupts the normal signal and the body is not able to respond properly.
- Interfering or blocking the pathways natural hormones or their receptors are synthesized.

Some organic contaminants that cause endocrine disruption include phthalates, bisphenol A, dioxins, furans, pharmaceuticals, dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and some pesticides.

4. **Genotoxicity-** Any alternation in the genetic makeup of an organism caused by contaminants or other factors such as radiation constitutes genotoxicity. These alterations can include simple mutations in a sequence of nucleotides making up a strand of DNA such as substitutions of one nucleotide by another, insertions of one or more new nucleotides, reversals in a segment of a strand, or deletions in which one or more nucleotide is removed from the sequence. The alteration can also include chromosomal damage such as breakage. Genotoxicity can occur in a wide range of species, both plant and animal, and with many different contaminants including heavy metals, PAHs, organochlorine pesticides, and PCBs. Fortunately,

most of the research has shown that genotoxicity occurs mostly in somatic cells- that is, body cells such as liver, kidney, and skin. These mutations might impact physiological processes in an organism and even lead to cancer, but they cannot be transmitted to the next generation. Substantially fewer studies have shown mutations in germ cells- sperm or eggs. These might be passed on to subsequent generations. Certain contaminants such as benzo[a]pyrene, a PAH and radionuclides are well known for their ability to induce mutations in spermatocytes of laboratory rodents.

9.4.1 Impacts on Individuals

- **Lethal Effects-** Most easily understandable effect of contaminants is death of organisms. This effect can be assessed by using the acute toxicity test and determining the median lethal dose (LD50) or median lethal concentration (LC50). It is the estimated dose or concentration at which 50% of the test population is expected to die in a test specified period. For acute toxicity it is 24, 48, or 96 hour exposure. LD50 is used for test populations in which concentration of ingested toxicant is unknown whereas LC50 is used when concentration of ingested toxicant is known.
- **Sublethal Effects-** When the toxicant does not produce significant mortality but causes illness and behavioral changes in the organisms, then these are called sublethal effects. General signs include lethargy, lack of appetite, hiding, reduced growth, or diminished vigor.
- **Genetic Effects-** The genes of the organism may be affected as the toxicants can have direct effect on the DNA which if are not repaired can cause mutations.
- **Teratogenic Effects-** Malformations may occur due to toxicant exposure at embryonic stages which are called as teratogenic effects.
- **Reduced Fecundity-** The reproduction rate of organisms is directly affected by significant exposure to these contaminants. The litter size is reduced considerably and number of viable offsprings is reduced.
- **Addition to Existing Stressors-** As already discussed that the environmental contaminants can impair the immune system of an animal, thus any exposed individual will be more susceptible to diseases. Also we know that they can

diminish the vigor of an individual, so it will be more prone to stress induced by food and water shortage as well as predation.

9.4.2 Impact on Population, Community and Ecosystem

Effects on Population- A population is defined as a group of interbreeding organisms of same species occupying same habitat or area. In order study an individual we consider birth, death, sex and age, but a population is defined by birth rate, death rate, sex ratio and age structure. The contaminants affect the following characteristics of population.

- **Growth-** Population growth is the increase in the number of individual of same species in the area. The growth of population can be represented by the following equation.

  Growth = Natality rate - Mortality rate + Immigration rate - Emigration rate

  As we already know that the toxicants have direct affect on the ability of an individual to reproduce the impact will translate at population level as well.

- **Density-** Population density is the number of individuals present per unit area. Higher mortality rate caused by exposure to lethal concentration of contaminants will negatively impact the population density.

- **Sex Ratio-** The male to female ratio at birth is called the primary sex ratio. However in various species the sexes have different vulnerability to predation, sensitivity to toxins or other factors which lead to a different sex ratio at the time of maturity. Likewise some species are polygamous in which one strongest male monopolizes several females. Thus the sex ratio between actually breeding individuals is called operational sex ratio. As already mentioned one sex may have different sensitivity to toxins thus exposure to certain contaminants can affect the sex ratio of a population.

- **Age Structure-** The distribution of different age groups in the population is called as age structure. In various studies the younger individuals are found to be more sensitive to contaminants thus leading to higher mortality among them. On the other hand older individuals had a longer time in bioaccumulation of contaminants in their body. This can significantly change the age structure of the population depending upon the nature of the contaminant.

- **Effects on Community-** A community is a group of species living and interacting or potentially interacting within an area. These species include all the microbes, fungi, plants, and animals in an area, which might consist of a wetland, a terrestrial landscape, or even an ocean. The aspects of community ecology affected by ecotoxicants are:
- **Species Richness and Diversity-** Species richness is simply the number of species occupying a habitat. Species diversity on the other hand takes into account that how equal the species are in number, also called species evenness. Many studies support the fact that species richness and diversity are reduced due to the influence of contaminants. This occurs due to variability of sensitivity among the species.
- **Food Chains and Food Webs-** The sequence of transfer of energy and matter in the form of food from organism to organism in community is called a food chain and the interlinking of different food chains form a food web. Food webs are more complex and reflect the actual conditions of an environment. Contaminants are well documented to bioconcentrate in a food chain/web from lower trophic level to higher trophic level. This process is called as biomagnification. By this process the contaminants can reach a concentration high enough to impose detrimental impact on a sensitive species. Removal of sensitive keystone species (species having higher ecological significance) from the food web through contaminant toxicity will disrupt the whole food web.

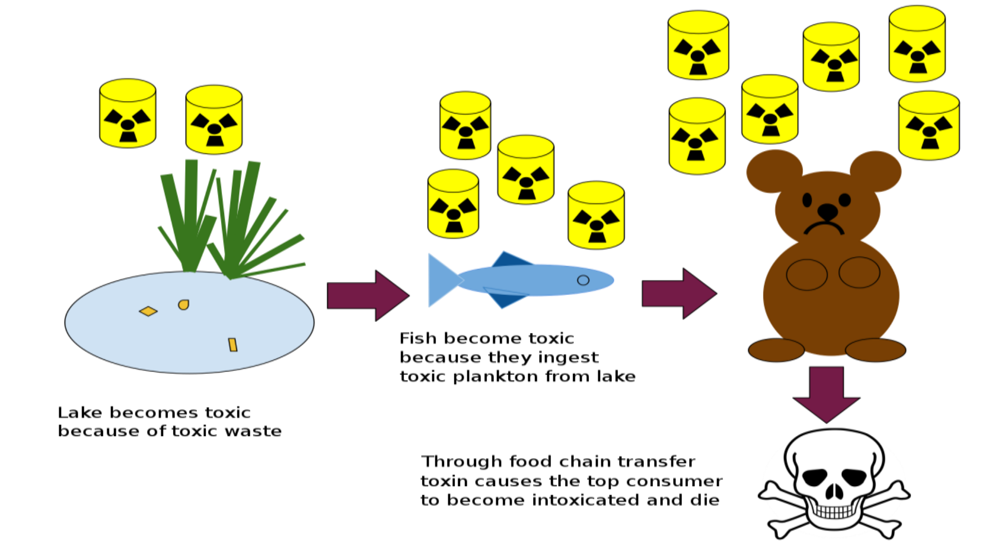

Figure 1: Biomagnification of toxic chemicals in a food chain.

- **Symbiotic Relationships-** It is a relationship in which two species interact in such a way that both are mutually benefitted. Some contaminants may alter the dynamics of this relationship like by affecting one species in the symbiosis such as some contaminants are toxic to mycorrhizae living symbiotically with higher plants. This affects the absorption of nutrients by the plant.
- **Ecological Succession-** The gradual establishment or replacement of a community on a land is called as ecological succession. If it occurs on a barren ground with little or no community prior to the event, it is called as primary succession. Secondary succession occurs after a destructive event, which is much faster than the primary succession as formation of soil is a time consuming process. In the presence of disturbances like contaminants the process of ecological succession is setback many steps.

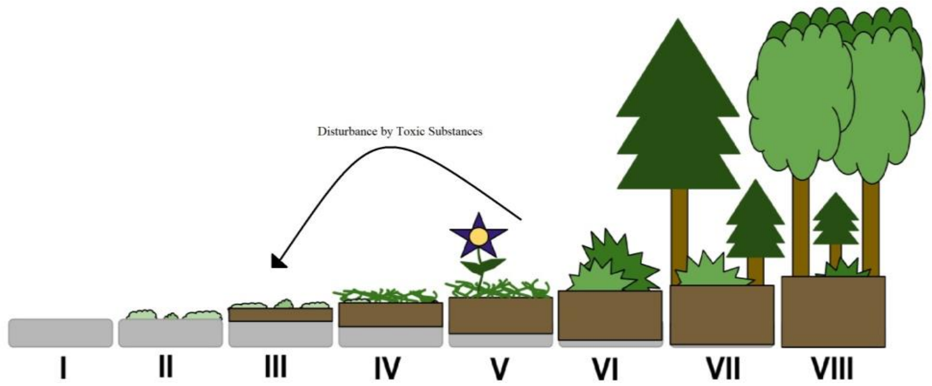

Figure **2: Effect of toxic substances on ecological succession**

Effects on Ecosystem- The interaction of biotic community with the abiotic factors form an ecosystem. The aspects of ecosystem which are affected due to contaminant exposure are:

- **Productivity-** Productivity is the rate of biomass generation in an ecosystem. As the contaminants can directly have effect on growth of individual as well as population the total biomass generation is also affected.
- **Nutrient cycling-** If the contaminants have lethal effects on nitrogen fixing bacteria the whole nitrogen cycle will be affected or atleast its efficiency will be affected. Similarly, it affects on photosynthesis and respiration will affect the carbon cycle as well.

- **Climate-** Climate change is brought on by the greenhouse gases, which is a set of contaminants that include carbon dioxide, methane and nitrous oxide, emitted by anthropogenic sources. The structure and functioning of an ecosystem is directly affected by the climate.

SAQ 1

Identify the correct statements

(i) Ecotoxicology is the study of effects environmental contaminants on humans.

(ii) Ecotoxicology is a relatively younger discipline introduced in 1970s.

(iii) We only study the lethal effects of pollutants in ecotoxicology.

(iv) Environmental contamination can affect the sex ratio and age structure of a population.

(v) Elimination of keystone species from a community will disrupt the whole food chain.

9.5 Branches of Ecotoxicology

9.5.1 Aquatic Toxicology

The science of studying the adverse effects of toxicants on aquatic organisms is called aquatic toxicology. This also includes the studying the toxic effects at the level of cells, individuals, populations, and communities. Aquatic toxicology is an interdisciplinary field which integrates toxicology, aquatic ecology and water chemistry. This field includes freshwater, marine water and sediments. Some common test included in the study of aquatic toxicology are-

Acute Toxicity Tests- are used to evaluate the effects of toxic agents on aquatic species either from a single dose or multiple doses in a short period of time. These tests make evaluation of effects on survival of test organisms usually over a period of 24-96 hour.

Chronic Toxicity Tests- are used to quantify the effects of toxicants on aquatic species over a significant part of the organism's life span, usually one tenth of the organism's life span. These studies make evaluation of sub-lethal effects of toxic contaminants on reproductive efficiency, growth and development, and behavior of the organism caused due to disruption of physiological and biochemical processes.

Sediment Tests- Sediments act as the sink for the contaminants in aquatic ecosystems and frequently reduce the bioavailability of contaminants. Bioavailability is the fraction of a contaminant in the system that is available for ingestion by aquatic organisms, hence capable of imposing a toxic effect. The degree of reduction in bioavailability depends on the physical and chemical properties of toxic agent and those of sediments. Being able to estimate bioavailability is very useful in evaluating the hazard of chemicals present within the sediments.

Bioconcentration tests- Bioconcentration is defined as the process which leads to increase in the concentration of substance in the body of an aquatic organism. Bioconcentration studies are conducted to test the potential of a toxicant to be bioaccumulated in the body of organsms, which might be consumed by a higher trophic level organism including humans. These tests use bioconcentration factors (BCF) to estimate concentrations of contaminants in organisms. The BCF is the ratio of the concentration of test chemical in the tissue of the test organism to the concentration in the water at equilibrium condition.

9.5.2 Wildlife Toxicology

Wildlife toxicology involves study of adverse effects of toxic agents on wild species excluding fish and aquatic invertebrates as they are studied under the field of aquatic toxicology, but together both are integral parts of ecotoxicology (Hoffman, et al., 2003). Wildlife toxicology seeks to identify and evaluate the effects of toxic agents on non-target wildlife species and consequently on the populations in natural environments. Previously more attention was given to certain birds and a few mammal species, which have aesthetic value or economic importance for humans. Only recently wildlife toxicology has been broadened to include the effects of environmental contaminants on reptilian and amphibian species as well as terrestrial invertebrates. Some of the common chemicals affecting wildlife are pesticides, industrial chemicals, fossil and mineral fuels, pharmaceuticals (including human and veterinary drugs), personal care products, metals and fertilizers.

The toxicological studies for the wildlife are done using toxicity tests on non-endangered species. These tests include-

- **Single Dose Acute Toxicity Test-** in which test subject is given at a LC50 and is studied for 24, 48 or 96 hour.

- **Sub-acute Dietary Toxicity Test-** which involve LC_{50} for a 5 day feeding trial.
- **Chronic and Sub-chronic Toxicity Tests-** are conducted for a period of atleast 28 days.
- **Reproductive Studies-** including embryotoxicity, teratogenicity, and neonatal toxicity tests.
- **Behavioral Studies-** like food discrimination and response to fright stimulus.

9.5.3 Soil Ecotoxicology

Soil in itself constitutes a complex and diverse ecosystems on Earth. Other than providing the main environmental support for plants, soil provides habitat for a vast diversity of vertebrates, invertebrates and microbial taxa. Soil provides habitat to a quarter of all of the reported biodiversity. Although organisms may occur in almost all profiles of soil, most of the biological activities occur within the top 30 cm where highest concentration of organic matter is present. Thus ecotoxicological studies can apply to soil ecosystem as well and actually have a very high significance. The ecotoxicological studies in this branch involve standardized laboratory tests using soil invertebrates. They can be considered as main tools for making assessments because they are sufficient for determining the ecological risk level of substances and the safe exposure limits. This type of test provides detailed information on the harmful effects of contamination on soil organisms.

9.5.4 Landscape Ecotoxicology

Mostly ecotoxicologists used to study the effects of contamination on individual species, and only recently a significant amount of research has been carried out on the impacts of toxicants on ecosystem levels. However, for ecotoxicology to be an applied science used for accurate assessment of certain human impacts on natural systems, the effects of pollutants over the entire landscape must be considered, as ecosystem are many times not clearly differentiated, and movement of pollutants between different ecosystems is common phenomenon. Since the pollutants are extensively dispersed their effects have to be investigated at a larger scale. Cairns and Niederlehner defined landscape ecotoxicology as a science of examining “chemicals dispersed over a large spatial scale and their potential for adverse effects on biological systems, including humans.” The word landscape can be variably used to mention areas ranging from a few square kilometers to entire continents. Usually different ecosystems constitute a landscape, however, in some regions a single type of ecosystem might dominate a sufficiently large area in a way that causes the ecosystem boundaries to be blurred. The simple reason for studying ecotoxicology is that pollutants affect the natural environment over a large area. Chemicals can cause ecological stress throughout landscapes in a number of different ways. Firstly, many chemical stressors, such as air pollutants are either emitted or dispersed after emission over huge areas. Secondly, small scale environmental impacts can interact over a large area and add up to become a landscape stress. Thirdly, although a toxicant may affect only one ecosystem directly, it may indirectly affect other ecosystems, which ultimately affects the whole landscape. Many chemical stresses impact natural environment over broad areas, which demands the evaluation of response to such stresses within the boundaries of natural landscape. Besides considering large areas, the effects of contaminant stresses must be observed over long time periods. Finally, a broad scale view in ecotoxicology is gaining importance since human activities are altering the global climate. Changes in climate factors like temperature, humidity and winds will affect the large scale movement of toxicants.

SAQ 2

(i) Choose the most appropriate option

Median lethal concentration represented by LC50 is

(a) 50% of lethal concentration.

(b) An estimated concentration at which 50% of the test population is expected to die.

(c) A concentration at which there is a 50% chance of survival of individual.

(ii) Fill in the blanks

Landscape ecotoxicology is defined as study of chemicals _____________ over a large spatial scale and their effect on ______________, including humans. It is required to study ecotoxicology at a landscape level because chemicals are either emitted or ________________ over an immense area, various small scale impacts can become _______________ events over a large area and toxicant affecting one ecosystem ________________ another resulting in a landscape scale effect.

9.6 Interdisciplinary Significance

Ecotoxicology, as already mentioned is an integration of various disciplines. Different scientific approaches are used to study the concepts of ecotoxicology. The disciplines applied in ecotoxicological studies are-

Chemistry- Various types of contaminants are present ubiquitously in the terrestrial, aquatic and atmospheric environment. Chemistry is applied to analyze and quantify those contaminants in the environment. Also the effect of each such substance can be identified and quantified so as to evaluate the concentration dependence of any such hazardous substance and its associated environmental and human health risk.

Biochemistry- The study of chemical reactions occurring in a living organism is called biochemistry. It involves study of enzymes, DNA and other biomolecules which as previously mentioned are affected by exposure to ecotoxicants.

Microbiology- Microbial activities form the basis of most of the ecological functions. Due to their boundless metabolic capabilities microbes are responsible for the transformation and recycling of organic and inorganic matter. This activity results in the maintenance of soil fertility, nutrient cycling and many other processes. Also symbiotic associations exist between microbes and higher life forms such as nitrogen fixing bacteria and plants, gut bacteria and ruminants, and so on. On the other hand microbes are responsible for the disease and death of higher organisms, which is necessary to control excessive population growth and consequent stress on resources. The interdependence between microbes and higher organisms mean that the toxic effect on one group, imparts consequences on the other.

Biotechnology- It is an applied science which harnesses the living organisms for the benefit of human beings. Agriculture, antibiotics production and food industry are some examples of such applications. The recent advancements in this field allow us to create genetically modified organisms which are more resistant to pests and diseases, toxicant induced stresses and have higher yield of commercially and medically valuable products. Another important application of biotechnology is bioremediation of contaminants in the environment. The tool of genetic modification is helpful in creating organisms with higher resistance to contaminant exposure while at the same time in creating those organisms which are more sensitive to contaminants and thus can be used for biomonitering. Also some microorganism can be genetically modified to enhance their capability of bioremediation to cover a wider range of contaminants.

Ecology- The study of interactions of living organisms among themselves and with their surroundings is called ecology. In order to make gains from the ecosystem we have to be aware of the interactions that determine the distribution and abundance of species on which are crucial for sustenance of human life. It is important for not only in harvesting of species but also in modification of environment to facilitate the yield of economically valuable products. Ecological knowledge is applied to ecotoxicology in order to study the impacts of human activities on ecosystem functioning, especially pollution related effects.

Toxicology- Basic principles of toxicology that apply to ecotoxicology include the concepts of degree of exposure, persistence of pollutants and their distribution in the environment. Toxicology helps to predict the effect of exposure of toxicants on the organisms. Based on the chemical structure of toxin behavior of chemical in the environment can be predicted. Risk analysis which is another aspect of toxicology can be used to quantify the risk of death or other sublethal effects of the contaminant exposure.

Summary

In this we have studied that:

- Ecotoxicology is a multidisciplinary subject which deals with the study of adverse effects of chemicals on living organisms at individual, population, community and ecosystem level. It is called as integration of ecology and toxicology.
- It is a relatively younger discipline which began in 1970s. The term “Ecotoxicology” was used for the first time by Rene Truhaut in an international conference. But the actual pioneer in this field is Truhaut’s assistant Jean-Michel Jouany.
- Chemicals like pesticides, heavy metals, plasticizers, VOCs, dioxins and mycotoxins are some classes of toxic agents present in the environment as contaminants. They interfere with the biological systems in different ways such as, disruption of enzymatic activities, impairment of immune system, disruption of endocrine system and direct effect on genetic material.

- The impacts can be lethal, sublethal, genetic, teratogenic, reproductive or behavioral. At population level they can cause change in sex ratio, age structure, reduce growth rate population density. At community and ecosystem level they can cause reduction in biodiversity, ecological succession, productivity, and efficiency of nutrient cycling.
- Depending upon the environmental realm ecotoxicology can be branched into aquatic and terrestrial with further branching into sediment and sediment and soil ecotoxicology in both fields, respectively. In order to study the toxicological effects at a larger scale another branch called landscape ecotoxicology is introduced.
- Various tests are conducted to study ecotoxicology which include acute toxicity tests, chronic toxicity tests, reproductive studies and behavioral studies. In some studies sensitive organisms called bioindicators are also used to monitor the toxicity of contaminants.
- Ecotoxicology used different scientific approaches like ecology, chemistry, biotechnology, and toxicology in conducting its studies and is itself applied to various other fields for studying distribution and effects of contaminants.

Terminal Questions

1. What is the difference between ecotoxicology and classical toxicology?

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Describe the ways in which the toxic agents can affect the endocrine system of an animal.

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2. What are affects of the affects of contaminants at a community level in an ecosystem?

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3. (a) What are the events which led to creation of the discipline of ecotoxicology?

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(b) Who were the pioneers in the field of ecotoxicology?

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4. Describe the multidisciplinary aspect of ecotoxicology.

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Answers

Self-Assessment Questions

1) ii, iv, v
2) i) b

   ii) dispersed, biological system, spread, interactive, can affect

Terminal questions

Hint

1) Toxicology is defined as the study of adverse effects of chemicals on living organisms but ecotoxicology is the science of studying the effects of chemicals on the living organisms at individual, population, community and ecosystem level.
2) Some environmental contaminants have the ability to mimic natural hormones and can thus cause overstimulation. The others bind to the hormone receptors and interrupt the signal needed by the body to initiate a process. Still some others block the pathways of hormonal synthesis and functioning.
3) Ecotoxicants have detrimental effects on sensitive species which can lead to loss of biodiversity. Similarly species loss can lead to disruption of food

chain/web affecting the energy transfer in the community. Harmful effects on one partner in a symbiotic relationship render ill effects on other as well. Disturbance by chemical stresses sets back the natural process of ecological succession.

4) Hint:
   a) The pollution events that occurred after World War 2 were responsible for raised awareness towards the impact of human activities on the environment. This followed by publication of Silent Spring by Rachel Carson after a series of events lead to foundation of ecotoxicology.
   b) Rene Truhaut was the first person who used the term “ecotoxicology”, at an environmental conference in Stockholm in 1969. But this idea actually belongs to his assistant Jean-Michel Jouany who later with his colleague Jean-Marie Pelt established the principles of ecotoxicology.
5) Ecotoxicology is the integration of various disciplines ecology, toxicology, biology, biotechnology and others. It derives its principles and methodology from toxicology, studies nature of contaminants using and uses ecological knowledge to understand their effects on ecosystem.

Unit 10: The Ecotoxicants

Unit Structure

10.0 Objectives

After reading this unit you will able to -

- Define and use in proper text the term ecotoxicology and the concept of ecotoxicants
- Elucidate the natural and synthetic ecotoxicants
- Outline the classes of ecotoxicants based on their characteristic and presence in the environment
- Identify the toxic effects of ecotoxicants on ecosystem
- Describe the importance of ecotoxicology

10.1 Introduction

As we know, chemicals were used frequently in different sectors i.e. agriculture, transport, industries etc in today's life. The frequent use of these chemicals poses risks to many living beings and the environment due to their mishandling and improper disposal. These chemicals have deleterious effects on the ecosystem which becomes a major concern for the ecologists brought up with the discipline that combines the ecology and toxicology called as ecotoxicology. It covers the broader range from biochemical processes to the molecular level to ecosystem as a whole. The important aspect of the ecotoxicology is the pathway by which the toxicant enters in the different levels of the ecosystem. This unit will help to know about the ecotoxicants, their sources, types and effects on ecosystem levels.

10.2 Ecotoxicants

The usage of the chemicals by human society for commercial purpose is huge. Now a day, much development takes place in communication and transport sector that can lead to the usage of variety of chemicals like in electrical technology-lithium, cadmium and mercury has been used in increasing amounts along with other metals. Other examples are usage of PCBs (polychlorinated biphenyls) in 1929 in dielectric fluids, refrigerations and air conditioning systems utilized chlorofluorocarbons extensively. The environmental degradation by these contaminants was first noticed when the book 'silent springs' by Rachel Carson in 1962 was published. The studies of toxic effects of the contaminants dates back in ancient times and also attempts were to find the remedial measures marks the start of toxicology. The term 'ecotoxicology' was first coined in 1969 by Truhaut who explain it as the 'the branch of science which deals with the study of toxic effects occurred by the pollutants (natural or synthetic) to the ecosystem constituents including human'. The explanation given was evolved to cover the broader aspects of ecosystem. It can also be defined as the science concerned with the study and prevention of adverse effects of the anthropogenic chemical emissions on the ecosystem structure, functions and biodiversity. The approach has been used to assess the effects of these toxicants and ensure their safe and effective use. The toxicants, which are commonly used in our society and has adverse effects on the ecosystem is known as the 'ecotoxicants'. It can be released from industry (petroleum hydrocarbons, heavy metals, solvents, acids, and alkalis), agriculture (herbicide and insecticides) and domestic activities (pesticides, petroleum). The Ecotoxicants are variable in their chemical nature, behavior and also variable effects on different biological organization.

10.3 Types and Sources of Ecotoxicants

The toxic chemicals that pollute the ecosystem called as the ecotoxicant. It is of two types- natural and man-made (synthetic). But the common feature is their deleterious effect on living beings even at small doses. The most important aspect of ecotoxicants is whether it is available to organism. Pollutants are released from various sources and enter air, water and soil which in turn used by plants or animals and have affected by their toxicity. Variety of toxins is present naturally and stored in tissues of plants which is used by animals if eaten. Some natural toxins are present very deep inside the earth crust like crude oil which can't contaminate the ecosystem without human interference. So, some biological agents rarely become pollutants which might be due to human activity. Some common types of natural and manmade ecotoxicants are discussed below-

10.3.1 Natural Ecotoxicants

a) Toxins of biological origin: There are many substances present in the environment naturally and acts as an ecotoxicant with elevated concentration. Few ecotoxicant examples are-botulin, mycotoxins, cynanotoxins and microcystins, saxitoxins and brevetoxins produced from clostridium boyulinum, fungal species, blue green algae and dinoflagellates, respectively. They can cause fish deaths and serious health problems in humans.

b) Metals and other elements: All the elements (toxic or non toxic) are present in soil, oceans, sediments at low levels. Most of them called as micronutrients essential for living beings in synthesizing certain biomolecules (iron, cobalt, chromium, copper, iodine and manganese etc.). But their levels are increased due to human activities like mining, manufacturing which can affect the environment such as presence of high concentration of lead due to the usage of leaded fuel motor vehicles, tailing of mine activities contain metal poses risk to the surrounded area, aquatic life. Large amount of metals are processed by the process of smelting which can cause pollution through the smoke stacks and their release in to the water bodies.

c) Inorganic Toxicants: Sulphur dioxide, carbon monoxide and other inorganic substances are released into the air through volcanic eruptions. These substances reacted to the atmospheric vapors or in direct contact with water transformed into acids. The concentration of carbon dioxide above 2% can be toxic. The concentration of these toxicants can be increased due to human activities such as fertilizer or coal and petroleum fuel burning which affects the global weather patterns.

d) Natural organic toxicants: Some organic toxicants are produced naturally such as bushfire majorly a source of air and water contamination with polycyclic hydrocarbons (PAHs).

10.3.2 Synthetic Ecotoxicants

Variety of synthetic compounds is present used for different purposes that affect the ecosystem. For example pesticides and fertilizers used in agricultural sector, PCBs and CFCs used for industrial purpose and other chemicals like dyes, surfactants, detergents, pharmaceutical drugs and explosives used for defense activities etc. The discharge of these chemicals in the environment through manufacturing, usage, accidental spillage or improper disposal can cause damage to the living beings and degrades their environment.

10.4 Classification of Ecotoxicants

The ecotoxicants can find their way in the ecosystem through point and non point sources. Non-point sources includes agricultural runoff from land, contaminated ground water, bottom sediments, urban runoff etc. and point sources include discharge from the industrial plants, disposal sites of waste treatment plants. Ecotoxicants can be divided into two broad categories based on their characteristic feature and their presence in the environment shown in figure 1.

a) The major classes of ecotoxicants categorized into five categories based on their characteristic features-
   (i) Inorganic ions
   (ii) Organic
   (iii) Organometallic compounds
   (iv) Radioactive isotopes
   (v) Gaseous contaminants

   (i) Inorganic contaminants- It includes two types i.e. metals and anions. Metals are present naturally in the environment but turned to be pollutant due to human activities like smelting, mining and damage the environment. In 1998, environment disaster happened in western Europe due to metal rich mining waste spill near Donana National Park, south west Spain. It has been seen that the human activities can contribute to global metal cycle in a larger extent. Heavy metals are also produced toxic effects and contaminate the exposed ecosystem. For example- Aluminium is an important contaminant in acidified lakes where it solubilized and becomes toxic to the fauna. The gills of the fishes are susceptible to the aluminium poisoning. Other category of inorganic pollutants is the anions such as nitrates and phosphates. Nitrate fertilizers are used in the agriculture sector from where it runoff to the nearest water body and leads to eutrophication ultimately affects the fauna of the water ecosystem due to oxygen starvation. Nitrites are also able to bind with haemoglobin and reduces it capacity to bind with oxygen and causes the blue baby syndrome or methemoglobinemia in the infants. Phosphate ion can also affect similarly when used as a fertilizers or in the washing powders.

(ii) Organic pollutant: Compounds that contains carbon are described as organic. Carbon has an ability to form complex organic compounds by forming stable bonds. Organic pollutants include hydrocarbons, polychlorinated biphenyls (PCBs), polychlorinated benzodioxins (PCDDs), insecticides (organochlorine, organophosphorous, carbamates, pyrethroid), detergents etc. PCBs are highly stable compounds and used in hydraulic fluids, coolant–insulation fluids in transformers, and plasticizers in paints. Organochlorine insecticides include DDT and their related compounds having low water solubility and high lipophilicity. They are highly persistent and form stable metabolites. Some of them are nerve poisons. Organophosphorous insecticides are more toxic than organochlorine insecticides and accumulate in fats and oils of organisms. It can inhibit the activity of acetylcholinesterase activity. Like the organochlorine pesticides, organophosphorus pesticides are neurotoxicants. They behave it by blocking acetylcholinesterase, an enzyme essential in breaking down the neurotransmitter, acetylcholine.

(iii) Organometallic Compounds: Some metals combined with organic compounds formed organometallic compounds. Organomercury specie was made in the anoxic sediments naturally by microbial processes. The toxicity of the metal were increased when they deliberately or accidently bind to the organic ligand. Organolead and organomercury compounds were applied to control the caterpillars on fruit crops and as antifungal seed dressings respectively. When these compounds leach to the environment can affect the non target organisms.

(iv) Radioactive Isotopes: Due to development of the nuclear energy and atomic weapons, radioactive waste finds their way in the environment. Everyone is now exposed to the radiations from cosmic rays and the natural decay of the radioactive decays. The factors which determine the harmful effects of radiations on organisms are-a) nature and intensity of the radioactive decay, b) half life of the isotope and c) biochemistry of the radioactive element. Some radioactive elements are the biochemical analogues of some essential elements and find their route to the living tissues. The strontium-90 isotope followed the way similar to calcium in humans.

(v) Gaseous pollutants: It includes ozone and oxides of carbon, nitrogen, sulphur. Ozone is formed in the photochemical smog when oxides of nitrogen from vehicular exhausts react with moisture in presence of sunlight. Ozone in high concentration irritates the respiratory epithelia of animals and affects the growth of some plants. Volcanic eruption and fossil fuel burning produced sulphur dioxide which in turn react with water droplets forming sulphuric acid which fall as acid rain and damages the leaves and roots of the plants.

b) The major classes of ecotoxicants categorized into three categories based on their presence in the environment-

(i) Toxicants in Air
(ii) Toxicants in water
(iii) Toxicants in Food

(i) Toxicants in Air: Large number of chemicals has adverse effects on health when present in air so there is a need to control their manufacturing and handling. The common toxicant present in the air are- Carbon monoxide, nitrogen gases (NO, NO_2, NH_3 and N_2O), sulphur gases (SO_2 and H_2S), hydrocarbons (CH_4), photochemical oxidants (O_3, benzopyrene, peroxybenzoyl nitrate and peroxyacetyl nitrate) and lead from vehicular emission. EPA (Environment Protection Agency), USA and Occupational Safety and Health Administration and consumer Product Safety Commission (1978) enlisted 24 substances as hazardous in the atmosphere. Some of them are- arsenic, asbestos, acrylonitrate, berilium, benzene, cadmium, chlorinated solvents, chromates, chloroflourocarbons, ethylene bromide, lead, mercury, ozone, PCB, sulphur dioxide etc.

(ii) Toxicants in water: Large amount of toxicants can reach the water ecosystem intentionally or otherwise. The toxicants present in aquatic system can be categorized into categories i.e. trace elements and heavy metals in natural and waste water and pesticides in water. Some essential micronutrients are naturally available in water but heavy metals make their way from the industrial effluents and have deleterious effects on aquatic life. For example oil spillage in the water body make a layer over water due to the difference in their densities. Due to which diffusion of atmospheric oxygen into the water body diminished which in turn decreased the dissolved oxygen level of the water body results into the adverse effect on well being of the aquatic organisms. Pesticides (organochlorine, carbamates, organophosphates etc.) enter in the aquatic system through agricultural runoff effects the life of water organisms.

(iii) Toxicants in food: Many food additives are added to the food to increase its flavor and also for its preservation for long time. But it has been found that several food additives are associated with toxic hazards and health risks. Some additives like cyclamates, safrole and diethyl stilbestrol has been banned and some (saccharin, monosodium glutamate etc.) are under investigation and are likely to be banned.

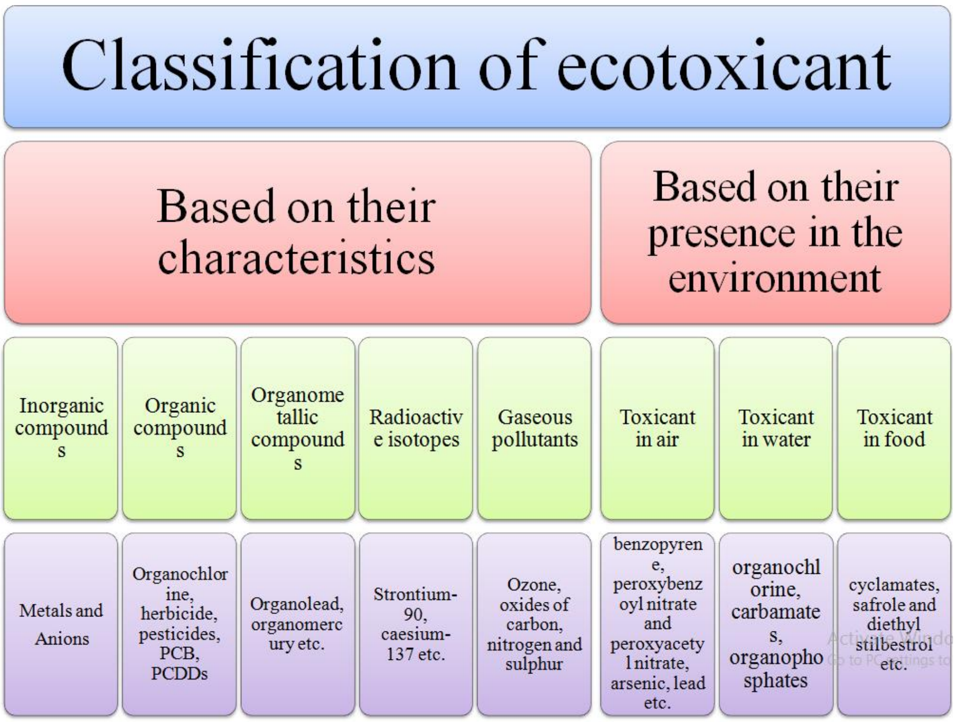

Figure 1: Classification of Ecotoxicants

10.5 Ecotoxicants Effects:

The ecotoxicant effects are the alteration in the state or dynamics of the organism or different levels of biological organization due to the exposure to chemicals (shown in figure 2). These levels include the sub cellular level, cellular level, tissues, individuals, population, community and ecosystem.

a) Toxic effects on individuals: Toxicological effects can seen in an individual when it reaches to the site of its action within the organism. It enters the organism through stomach, gills, lungs etc. and distributed throughout by body fluids and several physiological effects were shown in an organism due to the toxicity of the compound. There are several organic and inorganic compounds which can affect the individual well being described below-

(i) Inorganic compounds: It incapacitates the function of the essential biomolecules like CO which can bind to the haemoglobin and prevents the oxygen binding with it. Some other toxicants like arsenic (As), cadmium (Cd), Chromium (Cr), mercury (Hg), Copper (Cu), lead (Pb) are also make covalent bonds with the biomolecules and disrupts their functioning which are described below-

- In Phosphorylation processes, Arsenic and antimony competes with phosphorous disrupts the ATP production and also arsenic compounds are neurotoxic.
- Hexavalent chromium ($Cr6+$) is mutagenic, and nickel is carcinogenic.
- Enzymes involved in haemoglobin synthesis were inhibited by lead and it also interferes with calcium ions during nerve conduction.
- Cd if inhaled though smoking can cause cancer and it also replace zinc, calcium and magnesium in some metabolic systems.
- Organomercurial forms are toxic and can penetrate in the tissue and reache the nervous system.
- Copper ions disrupt the sodium regulation in fish; accumulates in the algal and fungal spores and prevents their germination and in mammals it can cause cirrhosis.

(ii) Organic compounds:

- Inhibitors of biosynthetic processes: Some toxins target the specific metabolic processes like antibiotics, sulfonamides and sulfonylurea herbicide hinders the synthesis of specific proteins in bacteria, fungi and plant cells, respectively. The biosynthesis of essential aromatic amino acids (histidine, phenylalanine, tryptophan, tyrosine), was inhibited by glyphosate. Chitin production in arthropods was inhibited by benzoylurea insecticides (e.g. lufenuron) that hampers the moulting and stopped the development in those organisms.

- Anticoagulants: It can hinder the vitamin K regeneration leads to the dysfunctional hepatocytes that restrain the blood clotting and in some cases causes haemorrhage.
- Endocrine disrupting chemicals: the endocrine system regulates the metabolism and reproduction in an organism. Some chemicals can disrupt the functioning of the endocrine system and manifested the abnormal behavior in the reproductive system that includes dysfunction, change in the secondary sexual characters and abnormal blood steroid levels. For example PCB disrupt the aryl hydrocarbon receptor in the cytosol that triggers the induction of the detoxification complex P450, the metabolite produced competes with the thyroxine, decreases the level of retinol in blood and lead to vitamin A deficiency.
- Auxin-type disrupters: Phenoxy and pyridine herbicides (e.g. 2,4-D and triclopyr respectively) stop plant growth by mimicking plant hormones (auxins).
- Germination inhibitors: various herbicides and fungicides (Thiocarbamates (e.g. thiobencarb), chloroacetamides (e.g. metolachlor) and dinitroanilines (e.g. trifluralin)) hinders the manufacturing of proteins, lipids or fatty acids that are necessary for plant germination.
- Mutagenic, carcinogenic and teratogenic: Aromatic compounds and PAH causes mutations in the genome leads to cancer while dioxins and related compounds like PCB have teratogenic effects.
- Neurotoxic: Insectides like organochlorine, DDT alters the nervous impulse and causes convulsions and paralysis. Acetyl-cholinesterase receptor at the neuronal synapses was inhibited by the organophosphorous (e.g. chlorpyrifos) and carbamate (e.g. carbaryl) insecticides.
- Photosynthesis inhibitors. Herbicides like triazines (e.g. atrazine), urea-derivatives (e.g. diuron), bromoxynil, etc. hinders the photosystem II electron transport process, carried out in the chloroplasts within plant cells and algae.
- Respiratory inhibitors. The cytochrome C - oxidoreductase or cytochrome BC1 complex that are associated in electron-transfer mechanisms to make ATP in

the mitochondria can be inhibited by some organometallic compounds (e.g. TBT), strobilurin fungicides and other toxicant groups.

b) **Toxic effects on population:** Effects of toxins on individual rarely occur due to the spread of toxic chemicals over large area that can affect number of organisms at the same time or at population level. Due to exposure of these chemicals to the population alters the structure of population removing those who are sensitive to the stress conditions. In aquatic population, it can lowers down the fish eggs and fry as they are sensitive groups of the population.

c) **Toxic effects on ecosystems:** To address the effects of toxicants on ecosystem can leads to investigate the large natural ecosystem (mesocosms) and small artificial ecosystem (microcosms). Exposure of toxicants to the individual organism altered their population and their ecosystem. For example birds exposure to DDT resulted in to the weak eggs shells in the population which in turn reduced the population of hawks and eagles. Due to diminished population of predators, prey population (rodents) increased affecting the other terrestrial ecosystem. Alteration in a population of an ecosystem can modify the food web that resulted in the change in the energy and matter transfer patterns. The attributes of an ecosystem such as total respiration, total primary production, and nutrient cycling rates and predator- prey relationship often changed.

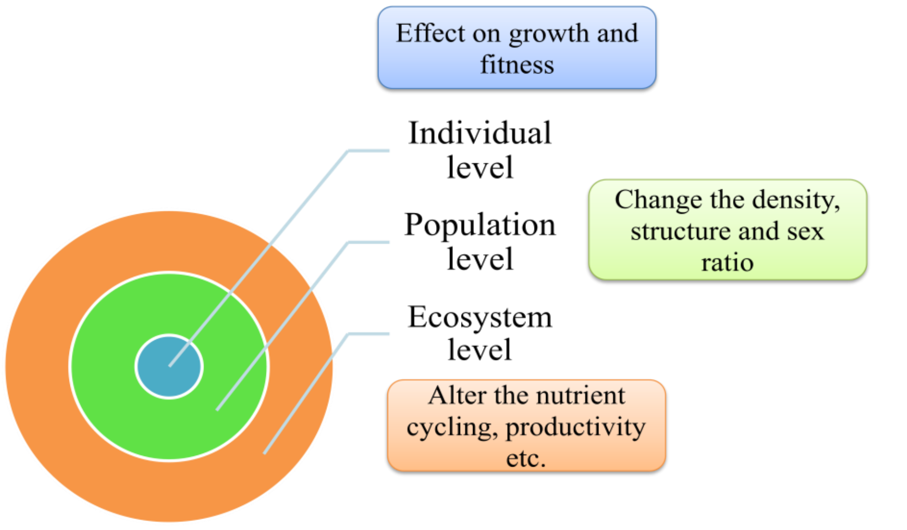

Figure 2: Toxic Effects of the Ecotoxicants on Different Organization

10.6 Nature and Significance of Ecotoxicology:

- ✓ It enables to predict and prevent the undesirable events in the natural environment by executing ecotoxicity tests and risk assessment of the chemicals that enters the environment.
- ✓ It also used in detailed monitoring studies of fish and invertebrates in the polluted water bodies looking at species at many levels in a food chain.
- ✓ It is essential to observe the physiological and biochemical response of an organism when exposed to a toxicant.
- ✓ It can help to assess the population change particularly genetic alteration after exposure to the toxic substances.
- ✓ It can help to predict the fate and effects of chemicals within the ecosystem by use of various models developed by ecotoxicologists.

Summary

In this unit, we have studied that:

- The ecotoxicology is the multidisciplinary field that integrates the study of toxicology, ecology, biochemistry and genetics. It is the study of effects of toxicants on different biological organization i.e. at species, population, community and ecosystem level.
- The different types of ecotoxicants from different sources i.e. natural and man-made. Natural ecotoxicants includes metals, organic and inorganic toxicants while synthetic ecotoxicant includes fertilizers, pesticides, dyes etc.
- The toxicants present in the environment i.e. air, water and food that made their path through different point and non- point sources.
- The effects of ecotoxicants at different levels of organization which affects the characteristics of the ecosystem i.e. modify food chain and total energy transfer etc.
- The ecotoxicology helps to assess, monitor the fate and exposure of the different toxicants present in the ecosystem.

Terminal Questions

1) What do you understand by the term ecotoxicology and ecotoxicant?

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2) Explain the different types of ecotoxicants from different sources?

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3) Classify the toxicants according to their characteristic and presence in the environment?

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4) Describe the toxic effects of the toxicants at different biological organization in detail?

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5) Explain, why the study of ecotoxicology is important?

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Answers

Hints

1) Integral study of toxicology and ecology. The substances have toxic effects on ecosystem constituents.

2) Two broad types of ecotoxicants-Natural (present in the environment naturally) and man-made (manufactured synthetically)
3) Classified into three main classes according to their present in environment- i) toxicants in air, ii) toxicants in water and iii) toxicants in food. The major classes according to their characteristic features are inorganic, organic, Organometallic, radioactive and gaseous contaminants.
4) The toxicants can affect the organism individually which can alter the population of an organisms ultimately leads to the medications in the whole ecosystem.
5) It enables the ecotoxicologists to predict, assess, monitor the fate and exposure of the toxicants to the different levels of biological organization and find the remedial measures for it.

Unit 11: Toxic Effects

Unit Structure

11.0 Objectives

After reading this unit students should be able to:

- List the major types of eco-toxicants and their sources.
- Describe effect of various toxicants on terrestrial and aquatic systems.
- Understand the effect of toxicants on human health.
- Understand various human activities resulting in environmental toxification and deterioration.

11.1 Introduction

The Chemicals present in the environment can be harmful to the structure and function of the ecosystem. Chemical substances in the environment are the major cause of global problems. Natural substances as well as man-made artificial (xenobiotics) compounds are harmful when unusually distributed in the environment and also enter the element cycle of the ecosystem. There are large number of toxic chemicals produced by plants, fungi and animals. Various natural activities like soil leaching, volcanic eruptions and anthropogenic inputs are the different sources through which metals are introduced in the aquatic environments, the sea being the final acceptor of these toxic compounds (Ramade, 1992). The science of toxicology is the study of toxins (poisons) and their effects on living systems. Toxicology is a broad field derived from biochemistry, pathology, histology, pharmacology and several other disciplines.

In 1960's the term ecotoxicology was introduced for the study of environmental toxicity caused by both natural and artificial substances on living organisms present in the nature. Environmental toxicology or ecotoxicology specifically deals with the interactions, transformation, fate and effects of natural and synthetic chemicals in the biosphere (Individual organisms, populations, community and whole ecosystems).

According to Newman (1998), ecotoxicology is the science of contaminants and their effects on the constituents of biosphere. Thus, ecotoxicology can be defined as the study of toxic effects of chemicals like hazardous wastes, industrial chemicals, heavy metals, pesticides and fertilizers on the organism, population, community or ecosystem. It is a multidisciplinary science and represents a multi step process to describe the sources, pathways and effects of chemicals that are harmful in the environments. The main reasons for measuring the ecological effects of chemicals are:

- To predict how toxic substances are likely to impact ecological system. Various tests are done to chemicals prior to their release into the environment and on existing chemicals for which actual effects are still unknown.
- To assess various changes occurring in ecological systems under the influence of released substances, so to assess the environmental risks of the chemicals.

11.2 Basic functions described by environmental toxicology

i) Fate and transport of chemicals in the biosphere and the living organism which arereleased into the environment.

ii) The interaction of the material with the site of action.

iii) Impact of molecular interaction of toxicants upon the function of the ecosystem.

There are many sources of toxic and hazardous chemicals in the environment whose route or method of exposure and their persistence in the environment as well as characteristics of the target organism (Figure 1) that determine the danger of the chemical. The dose, entry route, duration of exposure and sensitivity of the organism all play important role in determining toxicity.

ToxicityFactors related to toxic agents

1. Chemical composition and reactivity
2. Physical characteristics (such as solubility, state)
3. Presence of impurities or contaminants
4. Stability and storage characteristics of toxic agent
5. Availability of vehicle (such as solvent) to carry agent
6. Movement of agent through environment and into cells

Factors related to exposures

1. Dose (concentration and volume of exposure)
2. Route, rate, and site of exposure
3. Duration and frequency of exposure
4. Time of exposure (time of day, season, year)

Factors related to organisms

1. Resistance to uptake, storage, or cell permeability of agent
2. Ability to metabolize, inactivates, sequester, or eliminate agent
3. Tendency to activate or alter nontoxic substances so they become toxic
4. Concurrent infections or physical or chemical stress
5. Species and genetic characteristics of organism
6. Nutritional status of subject
7. Age, sex, body weight, immunological status, and maturity

Figure.1: Factor in Environmental Toxicity
(Source: U.S. Department of Health and Human Services, 1995)

11.3 Types of Eco-toxicants

There are more than 200,000 man-made organic compounds and toxicity of most of these compounds are not yet known. The risk of these compounds can be assessed by following information:

Estimated amount to be produced

i) Intended uses

ii) Tendency to disperse in the environment

iii) Its persistence

iv) Conversion of toxicants into different forms

Effects of these compounds on individual and ecosystem.Broadly, the chemical agents can be categorized in two types:

i) Toxic chemicals

ii) Hazardous chemicals

Hazardous chemicals generally include various flammable substances, explosives, sensitizers, irritants, acids and caustics. Toxic compound affect individual's health by affecting various physiological processes and pose a threat to the ecosystem. The common environmental toxic includes pesticides, heavy metals, oils and petroleum products, domestic wastes, industrial wastes and radioactive substances. Toxic compounds may be of general nature, killing many cells or they may be extremely specific in their target and mode of action.

Xenobiotic substances are those which are foreign to a living system. The various kinds of organic and inorganic substances along with their harmful effects on human health are listed in the Table.1.

Table.1: Major Toxic substances, their source and effect.

Toxic/hazardous substance	**Source**	**Toxicological effect**
Lead	Paint, gasoline	Inhibition of synthesis of haemoglobin, affects central and peripheral nervoussystem.
Elementary Mercury	Coal combustion	Causes insomnia, depression and irritability
Ozone	Reaction of NOx and VOC (volatile organic compound) in presence of sunlight.	Irritates eye, upper respiratory system andeyes
Arsenic	Treated lumber	Coagulates protein and forms complexes with co-enzymes, asrsenicosis manifest in skin discoloration and other symptoms.

Beryllium	Combustion of coal and fuel oil.	Causes lung fibrosis and pneumonitis
Cadmium	Batteries	Causes painful bone disease and kidney damage
Cyanide	Vehicle exhaust	Rapidly acting poison
Hydrogen fluoride	Aluminum production or phosphate fertilizerplants.	Fluorosis (bone abnormality and mottledsoft teeth)
Hydrogen sulphide	Petroleum refineries	Headache, dizziness, affects central nervous system
Asbestos	Asbestos mining	Asbestosis (a pneumonia condition)
Silica	Industrial and chemical process like glassmaking.	Silicosis
Phosphine	Insecticide use	Depression of central nervous system
DDT	Pesticide use	Breast and other cancer, male infertility, miscarriage, nervous and liver damage.
Carbon monoxide	Fuel combustion	Causes disorder of respiratory system and heart.

11.3.1 Toxic elements and Elemental forms

It includes ozone, white phosphorous, elemental halogens (Fluorine and Bromine), and Heavy metals (Cadmium, lead, Arsenic and mercury). Lead is one of the most prevalent poisons found on the earth. It occurs in the environment due to vehicle exhaust, house paint, ceramic glazing, batteries, cosmetics, lead mining, smelting and coal combustion. Inhalation of lead causes lowering of IQ and heart diseases in the human beings.

11.3.2 Toxic inorganic compounds

The toxic inorganic compounds include hydrogen cyanide, carbon monoxide, oxides of nitrogen (NO and NO2), hydrogen halides etc. Silica occurs in a variety of rocks. It causes an occupational disease commonly called as silicosis when people are exposed to silica dust. Asbestos is a group of fibrous silicates whichis commonly used as fire retardant, house flooring and walls, the inhalation of asbestos particles results in a disease called as asbestosis. It is a degenerative lung disease andresponsible for lung cancer.

11.3.3 Toxic organic compounds

Some organic compounds and their toxic effects are shown in the table 2.

Table.2: List of some common Organic compounds and their toxic effects.

Organic compounds	Toxic effects
Hydrocarbons	
Alkane hydrocarbons	Inflamed dry, scaly skin
Alkene and alkyne Hydrocarbons	Phototoxic to plants, irritant to eyes and respiratory system
Benzene and Aromatic Hydrocarbons	Skin irritant, fatigue, headache, appetite loss
Polycyclic Aromatic Hydrocarbons	Benzo pyrene is the cause of cancer
Phenols	
2-Nitrophenol, Pentachlorophenol, O-Cresol, m-Cresol, p-cresol	Protoplasmic Poison, malfunctioning of respiration and circulatory system.
Aldehydes and Ketones	
Formaldehyde	Hypersensitivity, lung cancer
Acetone	Dermatitis
Pesticides	
DDT, Aldrin, Dieldrin, endosulfan etc.	Affect nervous system, loss of memory, causes cancer, nerve damage and brain damage.
Parathion, Malathion	Skin and respiratory disease.
Carbaryl, carbofuran, paraquat	Systemic Insecticide, systemic poison

11.3.4 Radionuclides

These are unstable isotopes of elements which continuously undergo decay and as a result emit one or another type of radiation. These are either present naturally or produced in nuclear power plants, use of nuclear weapons, research and medical purposes. It includes Uranium (U), Krypton (Kr), Plutonium (Pu), Thorium (Th) and many more. Table 3 presents some radioactive isotopes their sources and principal modes of decay.

Table.3: Environmental Half-lives of Some Chemical Contaminants and radionuclides

Contaminant	Half-life	Source
DDT	10 Years	Soil
TCDD	9 Years	Soil
Atrazine	25 Months	Water

Benzoperylene(PAH)	14 Months	Soil
Phenanthrene(PAH)	138 Days	Soil
Carbofuran	45 Days	Water
$Uranium^{235}$	$7.1X\ 10^8$ years	Nuclear reactor
$Uranium^{238}$	$4.47X\ 10^9$ years	Nuclear reactor
$Cesium^{134}$	2 years	Nuclear reactor
$Cesium^{137}$	30 years	Nuclear reactor
$Carbon^{14}$	5760 years	Nuclear reactor
^{40}K	$1.3X\ 10^9$	Nuclear reactor
Radon (Rn^{222})	3.8 days	Nuclear reactor
Strontium (^{89}Sr)	52 days	Nuclear reactor
Strontium (^{90}Sr)	28.6 years	Nuclear reactor

11.4 Distribution and fate of toxic substances

There are number of ways through which toxic materials enter in the body of living organisms like water, air, food, accidental exposure, medicinal drugs and many more. The solubility of the toxic chemicals determines the movement of toxic in the body of living beings and in the various components of the environment. Water soluble compounds spread rapidly in the both aquatic and terrestrial system and within the body of the living organisms. Fat soluble compounds penetrate the tissues which are not used during metabolic processes.

Some toxic chemicals are persistent for longer duration in the environment. Some of these chemicals are chlorofluorocarbons, chlorinated hydrocarbons and pesticides. They show additive effect when they are present together in the environment. It is called as synergistic effects in which one chemical which is less toxic when comes in contact with some other chemical show toxicity several folds higher than the individual effects.

The toxicity can be categorized as acute and chronic. The acute toxicity occurs as a result of single exposure to chemicals which immediately show its harmful effects. However, chronic toxicity may occur due to prolonged exposure of sub-lethal dose. The appearance of symptoms of chronic toxicity takes several years.

11.5 Dose Response Relationship

The dose of the chemicals determines the extent of an adverse effect of toxic substance on an organism. The dose response relationship is used to examine the time dependent effect of exogenous and endogenous chemicals and physical agents. Dose can be defined as the amount of toxicant per unit of body weight to which an organism is exposed. For example, a dose of lead to a bird might be 5 mg Pb/kg body weight. Sometimes dose is expressed as dose per unit time. (e.g., 5mg lead/kg body weight/day). The dose response is a quantitative relationship which recognizes that the effect of a certain chemicals depends upon the concentration of the toxic factors (figure 2).

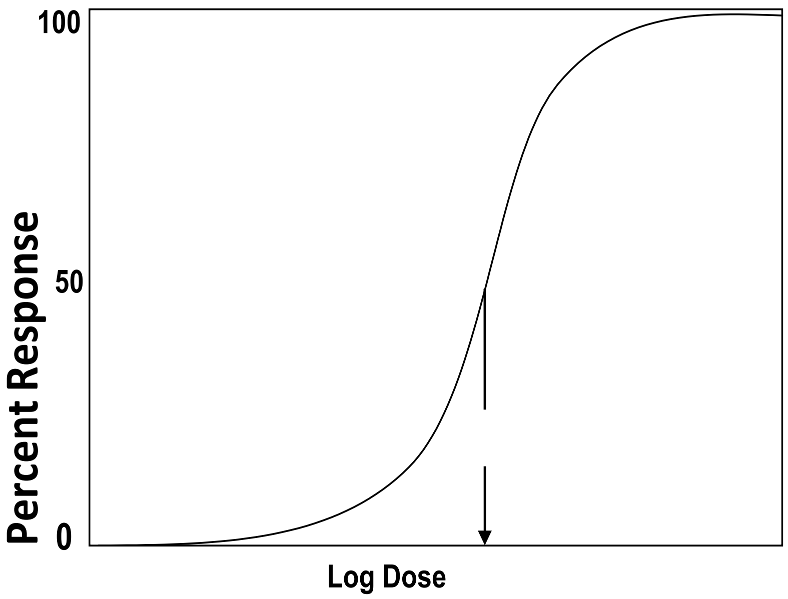

Figure.2: Dose response Curve

The dose dependency can be shown with the help of dose response curve (e.g. by giving different doses of a toxicant to a uniform population of test animals and the cumulative percentage of death is plotted as a function of log of the dose). Generally, the dose response curve is S- shaped. The dose corresponding to the midpoint is the statistical estimate of the dose that kills 50% of population and is written as LD_{50}. The dose response curve may differ among different kind and strain of organisms, type of tissues and population of cells (table 4).

Table.4: LD_{50} of some chemical substances.

Substance	LD_{50} (mg/kg)	Effect
Benzene	930	Primary irritant, Tumorigenic, Mutagen, Reproductive effector
DDT	80	
Phenol	310	
Parathion	2	

The ED_{50} (effective dose 50%) is the dose which affect 50% of the treated population. The ED_{50} is more refined as compare to LD_{50} and can be used to indicate responses such as reduced enzyme activity, decreased reproductive success and onset of disease symptoms.

The goal of dose response assessment is to obtain a mathematical relationship between the amount of toxicant to which an organism is exposed and the risk of an adverse outcome from that dose. The toxic effect due to a combination of chemicals could be of following types:

- **Independent:** Each chemical produces a different effect or has a different mode of action.
- **Additive:** The combined effect of two or more chemicals is equal to the sum of the individual effects of the chemicals.
- **Synergistic:** The effect is more than additive as two or more chemicals can react to create a greater effect than any one of them alone.
- **Antagonism:** The effect is less than additive.

11.6 Effect of toxins

11.6.1 Teratogens

Teratogens are chemicals or other factors that specifically cause abnormalities during embryonic growth and development. Thalidomide is the most commonly known teratogen. It is a sedative drug used by pregnant women which causes abnormality in developing embryo. It leads to phocomelia in which a baby develops seal like limbs i.e., without forelimbs or hindlimbs. Perhaps the most prevalent teratogen in the world is alcohol. Drinking of alcohol during pregnancy can lead to fetal alcohol syndrome. It is a cluster of symptoms including craniofacial abnormalities, delay in fetal developmental, behavioral changes and mental retardation. Moreover, only one alcoholic drink per day during pregnancy results in decreased child birth weight. According to certain estimates, between 300,000 and 600,000 children born every year in the United States are exposed in the womb to unsafe levels of mercury.The effects are subtle, but include reduced intelligence, attention deficit, and behavioral problems. The total cost of these effects is estimated to be $8.7 billion per year.

11.6.2 Carcinogens

A carcinogen is a chemical or other factor which causes cancer. There are number of elements or chemicals in the environment which are carcinogenic. It includes synthetic chemicals, radiation, air pollutants, heavy metals, smoking and chewing tobacco. The carcinogens can be biological, chemical or physical agents. The biological agent includes retrovirus and HIV, the chemical agents includes nitrosamines and polycyclic hydrocarbons. Xenobiotic chemicals also act as carcinogens. The physical agent includes ultraviolet radiations and ionizing radiations. Cancer rates increased in most industrialized countries during the twentieth century and is now the second leading cause of death in the United States, killing more than half a million people in 2002. According to the American Cancer Society, 1 in 2 males and 1 in 3 females in the United States will have some form of cancer intheir lifetime.

11.6.3 Mutagens

Mutagens are agents, such as chemicals and radiation which causes sudden and permanent changes in the genetic material (DNA) of living organisms. This can lead to birth defects if the damage occurs during embryonic development. Later in life, genetic damage may trigger neoplastic (tumor) growth. When damage occurs in reproductive cells, the results are passed on to future generations. Number of chemicals, pesticides, radiations is mutagens. Chemicals frequently reacting with DNA are generally mutagens and also act as carcinogens.

11.6.4 Allergens

Allergens are those substances that activate the immune system. Formaldehyde is one of the most common examples of a widely used chemical that acts as a powerful sensitizer of the immune system. It is directly allergenic and also activates the reactions to other substances. Itis widely used in plastics, wood products, insulation, glue, and fabrics. Formaldehyde concentrations in indoor air can be thousand times higher than in normal outdoor air. Some people suffer from sick building syndrome characterized by headaches, allergies, chronic fatigue and other symptoms caused by poorly vented indoor air contaminated by mold spores, carbon monoxide, nitrogen oxides, formaldehyde and other toxins released from carpets, insulation, plastics, building materials and other sources.

11.6.5 Endocrine disrupters

These are chemicals that disrupt normal hormone functions. Hormones are chemicals released into the blood stream by cells in one part of the body which regulate development and function of tissues and organs elsewhere in the body.

In some cases, very small concentrations (pictogram) may be enough to cause developmental abnormalities in sensitive organisms. Because these chemicals often cause sexual dysfunction (reproductive health problems in females or feminization of males), these chemicals are sometimes called environmental estrogens or androgens. There are number of compounds like phthalates, alkyl phenols, organo-chlorine compounds and polycyclic hydrocarbons are xenoestrogens. Fall in sperm count of male and rise in hormone related cancer such as breast cancer are probably due to environmental estrogen.

11.6.6 Neurotoxins

Neurotoxins are a special class of metabolic poisons that specifically attack neurons. The nervous system is important in regulating body activities and disruption of its activities is fast-acting and devastating. Different types of neurotoxins act in different ways. Heavy metals such as lead and mercury kill nerve cells and cause permanent neurological damage. Anesthetics (ether, chloroform, halothane, etc.) and chlorinated hydrocarbons (DDT, Dieldrin and Aldrin) disrupt nerve cell membranes necessary for nerve action. Organophosphates (Malathion, Parathion etc) and carbamates inhibit activity of acetylcholinesterase, which is an enzyme that regulates transmission of nerve impulse between nerve cells and the tissues or organs they innervate. Most neurotoxins are extremely toxic and fast-acting.

11.7 Uptake of toxicants

Uptake is the absorption of substance into extracellular fluid for systemic circulation; the fate of the material taken up will be governed by metabolic process. In human, radionuclides have been shown to be absorbed through skin. Radioactive iodide applied to human skin appeared in thyroid gland. The substance inhaled by man may be deposited in nasopharyngeal, tracheobronchial or the alveolar regions of lung. In each of these, the particles are absorbed into the extracellular fluid or transported up to the pharynx in mucous, propelled by ciliary action. After reaching the pharynx the material is swallowed into the digestive tract. Materials taken up from alveoli may go to the blood stream or may be retained in the bronchopulmonary lymph nodes.

Dieldrin is absorbed rapidly from the intestinal tract and transported to liver mainly through the portal venous system. However, metabolic conversion is much slower, only small part is metabolized and excreted, the major part being redistributed into the storage depot of adipose tissue. Methylmercury is absorbed through the gills by fresh water fish and this respiratory uptake is shown to be dependent on metabolic rate. For terrestrial plants, Strontium-90 uptake has been investigated experimentally and it is shown that foliar deposition is much more important than uptake by the root. For heavy metals also, foliar deposition is more important route for contamination. The understanding of retention of toxicants by organism is useful in determining the toxic effect. It can be obtained by estimation of substance remaining in the body or by measuring the total amount excreted per unit time. Monitoring of excretion value in contaminated organism is also useful. As the most common route of excretion is urine, so the analysis of urine is useful to estimate the toxicant. In case of monitoring of workers contaminated by toxicants, sample of urine and faces are analyzed. For human, ICRP (International Commission on Radiological Protection, 1975) has given information on 30 elements excreted in the urine and faces. Sometimes the concentration of heavy metals in the hair can be measured to calculate the concentration in the body. For e.g. the concentration of methyl mercury in hair is more than 300 times that in blood. Intake of toxicant from the physical environment, either through epidermis, respiratory organ or gut of terrestrial animals depends upon the precise form of toxicant, both chemical species and the degree and type of physical aggregation.

11.8 Effects of toxicants on terrestrial environment

The various toxic compounds affect growth in plants, invertebrates and microorganisms. Effects on plant biomass production are related to contamination of soil or air including deposition on plant surface. The toxic compounds also affect the above ground and foliar invertebrates which represent food for other organisms and perform essential role as pollinators, detritivores, saprophages and pest controller.

The effect on terrestrial vertebrates exposed to contaminated food, air, water and soil has social or economic consequences. Poisoned birds and mammals probably constitute the highest social concern, while reproductive effects although less evident represent a higher ecological hazard. Accumulation of toxic compounds in food and through food chain represents an additional concern related to the consumption of this food by human and domestic animals.

11.9 Effects on aquatic system

As a result of human activity large amount of pollutants and toxicants are released into river, lakes, estuaries and oceans. It is well established that heavy metals like lead, cadmium, mercury accumulate in the aquatic organisms. Mercury tends to accumulate in clams. Pesticides affect crustaceans, fishes and mollusks. Oil pollution (Oil spill) adversely affects the echinoderms. It is also well established that higher concentration of pesticides and heavy metals accumulates in the aquatic system through food chain.

11.10 Effect of toxicants on human health

Large number of toxicants or pesticides are taken by humans through ingestion, inhalation or absorption through the skin. These toxicants usually affect one or more organs like lung, skin or alimentary canal. Inhalation of asbestos affects lungs causing irritation in lung lining and finally lung impairment. Sometimes asbestos cause's dermatitis when it irritates skin cells.

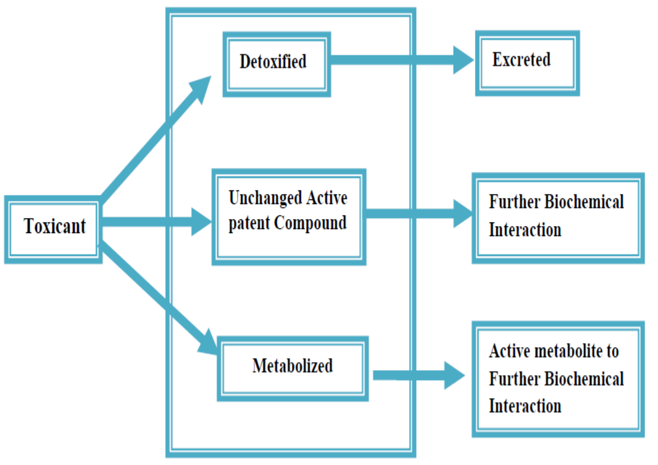

Figure.3: Processing of toxicants involving kinetic phase.

Toxins when absorbed in the body are metabolized, transported or excreted. During the metabolic process, they have adverse biochemical effects and causes manifestation of poisoning. These processes are characterized by kinetic phase and a dynamic phase. In kinetic phase, a toxic substance may undergo absorption, metabolism, temporary storage, distribution and excretion. When a toxicant remains unchanged during the metabolic process, it is available for further biochemical interactions as an active parent compound. Sometoxicants are detoxified and excreted or converted to non-toxic active metabolites (figure 3).

In the dynamic phase, a toxicant interacts in cells, tissue, or organ in the body and leads to various types of toxic effects. The dynamic phase comprises primary reaction with the receptor or target organ, biochemical effects (e.g., impairment of enzymatic reaction etc) and change in behavioral and physiological responses (figure 4). The immediate and readily observable manifestation of poisoning is changes in temperature, pulse rate, respiration and blood pressure. Central nervous system poisoning may be manifested by convulsion, paralysis, hallucination and ataxia (lack of coordination of voluntary movements of thebody). The chronic responses to toxicant exposure are mutations, cancer, birth defects and effects on immune system.

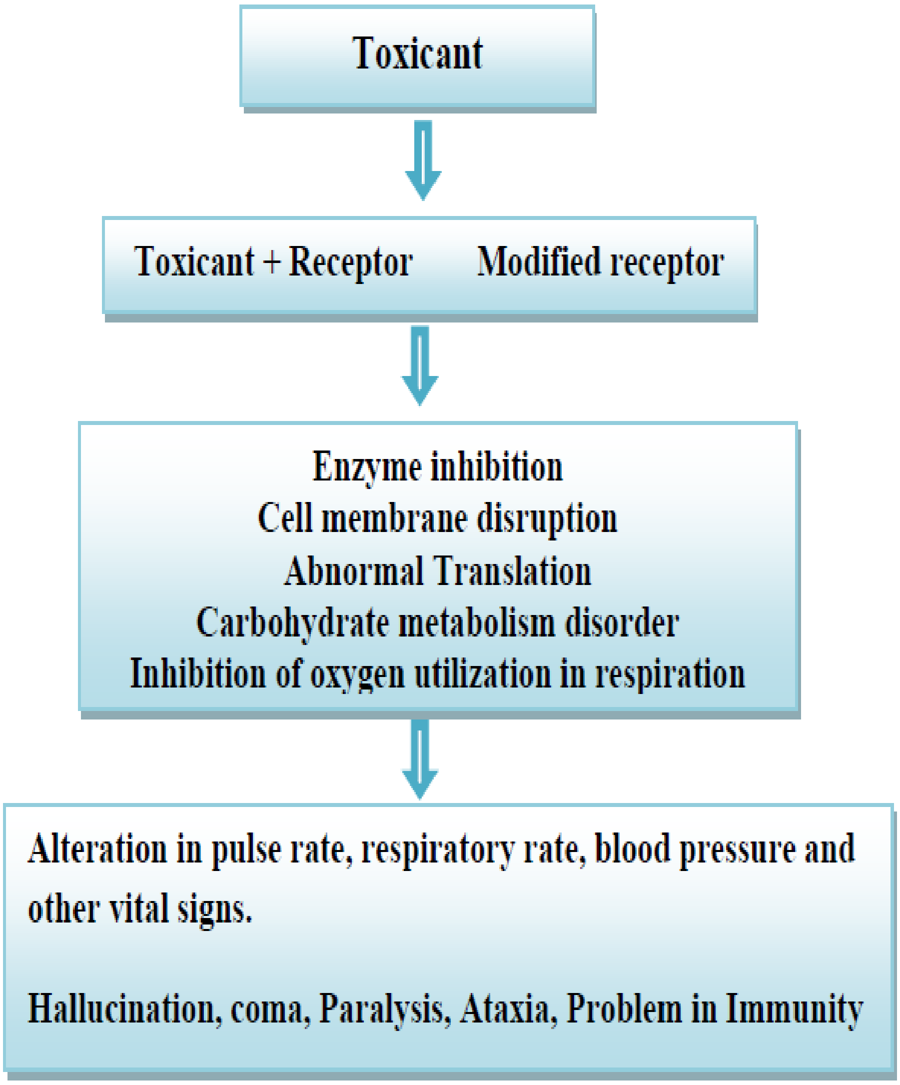

Figure.4: Dynamic phase of Toxicant action (Manahan, 2000).

11.10.1 Neurotoxins

These toxins specifically affect nerve cells. Lead and mercury kill nerve cell and sometimesit results in permanent neurological damage (figure 5).

11.10.2 Effects of lead

Lead is extremely harmful to humans as it damages brain, kidney, reproductive organs and cardiovascular system. Lead poisoning causes decrease in IQ level, delinquency, attention order in children, aggressive behaviour, higher blood pressure and hypertension in adults and risk of heart disease.

11.10.3 Effect of radionuclide

The radioactive elements like uranium possessed a great threat to environment and occupational threat to workers. Radioactive waste from nuclear power plant affects the ecosystem, contaminate soil, sediments and ground water. There are two types of radiations viz., ionizing radiation and non-ionizing radiation. Non- ionizing radiations from sun is vital to life whereas ionizing radiation produces more complex biological effects. Alpha radiation has a low penetration power, beta particles can penetrate up to a centimeter, whereas, gamma radiation can pass through a living organism and can only be blocked by a thick concrete wall or lead.

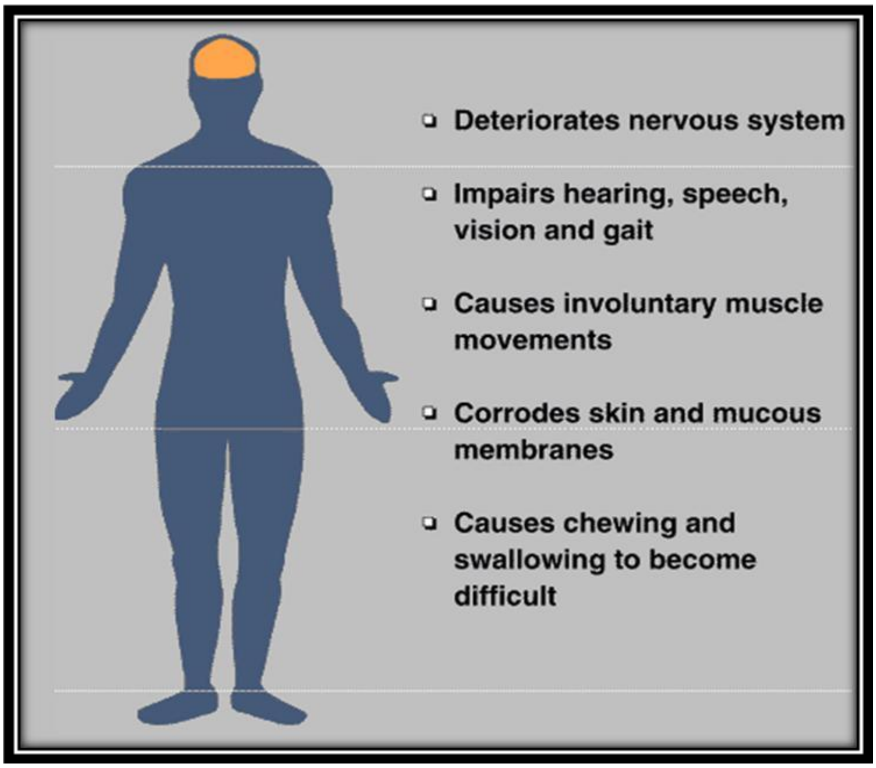

Figure.5: Effect of Mercury on Human body.

Exposure to radioactive radiation causes leukemia, eye cataract and various forms of cancer. These radionuclides when enter the food chain, become more harmful. In food chain, these hazardous substances are taken by grazing animals and humans. Some radionuclides are selectively concentrated within the body of organisms. e.g., iodine is selectively absorbed in thyroid gland and phosphorous is absorbed in bones.

Recently, radioactive Radon (Rn) gas in home has received a great attention. Most of it come from natural sources and is also the product of decay of ^{238}U. It is colorless, odorless and tasteless gas and has been reported as a large indoor air health threat which can greatly increase the risk of lung cancer. When radon is inhaled, it is trapped in lung and releases radiations during its breakdown that damage the lung tissue.

Summary

The chemicals present in the environment can be harmful to the structure and function of the ecosystem. These chemicals are either produced naturally or artificially by humans. In 1960's the term ecotoxicology was introduced which deals with the interactions, transformation, fate and effects of natural and synthetic chemicals in the biosphere including individual organisms, populations, community and whole ecosystems. There are number of ways through which toxic materials enter into the body of livingorganisms like water, air, food, accidental exposure, medicinal drugs and several other means. The toxicity can be categorized as acute toxicity which occurs as a result of single exposure to chemicals which immediately show its harmful effects and chronic toxicity which occur due to prolonged exposure of sub-lethal dose. The dose response relationship is used to examine the time dependent effect of exogenous and endogenous chemicals and physical agents.

Toxicants adversely affect living organisms in different ways. Toxins that specifically cause abnormalities during embryonic growth and development are called teratogens. Thalidomide is the most commonly known teratogen which causes abnormality in developing embryo. Many chemicals in the environment are carcinogenic. It includes synthetic chemicals, radiation, air pollutants, heavy metals and smoking. Mutagens are agents, such as chemicals and radiation which causes sudden and permanent changes in the genetic material of living organisms. This can lead to birth defects or may trigger neoplastic (tumor) growth. Certain chemicals act as allergens that activate the immune system. Formaldehyde is one of the most common examples that act as a powerful sensitizer of the immune system.

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Unit 12: Toxicants and Earth Environment

Unit Structure

12.0 Objectives

After reading this unit students should be able to:

- Explain the pathways, dispersion and transportation of the toxic substances
- Describe the biosorption process of heavy metals.
- Explain the concept of bio-accumulation and bio-magnification

12.1 Introduction

The study of the detrimental effects of diverse biological, physical, and chemical (substance) agents on living organisms falls under the umbrella of the interdisciplinary branch of research known as environmental toxicology, or ecotox. A branch of environmental toxicology called ecotoxicology focuses on understanding how toxicants impact populations and ecosystems. Through the publication of her book Silent Spring in 1962, environmental toxicology's founder Rachel Carson established the subject as its own. The principles of hazardous material dispersion, heavy metal biosorption, bio-accumulation, bio-magnification, etc. are provided in this section. Additionally, this unit will provide a fundamental knowledge of the biosorption process of heavy metals. At different times throughout its life cycle, an organism might be exposed to toxins. According on an organism's position in its food web, the amount of toxicity might change. Bioaccumulation, bio-magnification, biodegradation, and other words are only a few that are related to toxicity. With regard to chemical dispersion, destiny, concentration, and related exposures to animals, ecosystems, and people, there are significant gaps in the knowledge that this course aims to fill.

12.2 Worldwide Distribution of Toxic Substances

Europe is one of the regions in the world that produces the most chemicals, accounting for roughly 38% of worldwide supply. Since 1993, both for production of all chemicals as well as of dangerous compounds, the chemical strength of the EU GDP has been rising. About 20–70,000 categories of compounds or substances, many of which are produced from organic chemistry with a chlorine basis, are available on the European market. Few people are aware of the hazards or toxicities that most of these compounds pose to humans or the environment. Despite advancements in multi-media modelling, data on the amounts of harmful compounds produced or sold often have little utility for projecting dispersion and prospective exposures, which are still difficult to quantify due to growing non-point sources of emissions and recycling processes.

Information on transformations, byproducts, degradations or bio-degradation, and exposures to mixes is also lacking. The majority of existing monitoring programmes concentrate on mobile media (water, air), frequently excluding sediments, soil, and consumer goods. Burning fossil and other organic fuels is also believed to be responsible for a significant, but declining, portion of the environmental load of 280 different carcinogenic dibenzofurans (PCDF), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dibenzo-p-dioxins (PCDD), which are primarily from waste and air emissions. Depending on the weather, less than 5% of the pesticide used can reach its intended target during application due to losses from washings and volatilization.

If existing trends and policies continue, the majority of the EU nations' output of chemicals might increase by 30 to 50% as a result of rising economic activity, including road transport and agricultural production. Emissions of mercury, cadmium, chromium, copper, lead, and other pollutants are predicted to grow significantly over the next several decades; some nations even have plans to phase out these pollutants. The concentration of PAH (polyromantic hydrocarbon), hexa-chloro-benzene (HCB), and xylene emissions is projected to rise, but emissions from pesticides, fertilizers, and POPs (such dioxins/furans and PCBs) will continue to decline.

However, the effects of some new trends in chemical management, such as: increased eco-efficiency; a shift from goods to services; increased use of caution; the inclusion of external environmental costs in prices, such as through taxes; increased evidence of low-dose effects; and increased public education.

12.3 Mechanisms of Toxic Substances' Circulation and Exposure

The path via which a person is exposed to a harmful substance is referred to as the circulation mechanism of a pollutant. Any item that, if ingested, might be harmful or have a negative effect on health is considered toxic. Chemicals like petroleum, hydrogen sulphide, dioxin, mercury, snake venom, arsenic, caffeine, kola, and cocoa are a few examples. A journey that an agent takes from its source to a person is referred to as an environmental pathway. Any organism or population can only come into touch with agents that arrive from a certain location through this unique mechanism. Strategies to preserve the health, such reducing low-dose interaction, need ecological channels and circumstances. Intake of harmful substances into the body can occur in a variety of recognised ways.

12.3.1 Pathways of Direct Exposure

A straightforward and singular route is frequently linked to exposures to substances at high dosage rates. A direct inhaling or eye contact, for instance, will result in the maximum intake of chemicals released into the air. Direct consumption of polluted water, transfer from ambient air to the interiors of buildings and vehicles, and transfer from ambient air are three additional significant exposure pathways. By wearing protective clothes, this exposure can be avoided.

12.3.2 Pathways to Indirect Exposure

Agents are frequently exposed at low dosage rates through a variety of intricate and indirect routes. Chemicals, for instance, may be deposited on vegetation, travel to automobiles and buildings, and then be transported from soil to food. The agents might potentially be dispersed directly or via airborne runoff from water sources onto surface waters.

When soil particles are breathed, soil pollutants that are connected to them can be re-suspended and inhaled with the dust. Outside or inside, people might breathe in suspended particles. Recent research has demonstrated that a sizeable portion of the fine and coarse particles in the interior environment come from outdoor sources. By means of procedures like resuspension, deposition, and soil tracking—i.e., the process by which soil particles are transported into the indoor environment by the shoes and clothes of human occupants—soil enters the indoor environment.

A disposition might involve a multitude of behaviours that expose skin to soil pollutants. An adult working outside may have a significant amount of dirt on their skin. Chemicals that are lipid-soluble have a significant propensity to migrate from the skin's soil layer to the lipid-rich layer. However, the speed at which this transfer occurs is frequently quite sluggish, and equilibrium may not be reached for several hours or even days. Root absorption from the root zone and resuspension/deposition, rain splash, or volatilization followed by partitioning from the surface-soil layer are two ways that soil pollutants can be transmitted to edible sections of plants. Food items can become contaminated by plants.

Vaporised volatile pollutants may diffuse via soil pore spaces and into structures. Three key factors are required to calculate the ratio of contaminant concentration in indoor air to reported contaminant concentration in soil gas phase:

(a) Distance between contaminated source and building foundation

(b) The permeability of the soil

(c) The foundation cracks' area in relation to the foundation's overall area.

Exposure is the process through which humans or other living things come into touch with a harmful or dangerous material. There are basically three types of exposure pathways:

(i) Breathing (inhaling)

(ii) Consumption (consuming food or liquids)

(iii) Direct or dermal contact (touching or coming into contact with the skin)

Inhalation is the most frequent method of exposure since most chemicals exist as fumes, mists, vapors, and particles. Certain substances can enter the bloodstream through the skin. An entrance point might be the eyes.

In nature, they are often chemicals, biological agents, physical traits, and radiation. The level of toxicity is how much a chemical substance or hazardous component may expose an organism.

Chemicals can be discharged into the air, water, or land by spraying, leaky or spilled containers, spills, or chimney releases. From their output to where a human comes into contact with them, these chemicals travel along a route. The substances that enter the environment that are not typically there are known as contaminants. Three main routes—breathing, consuming, or touching a product that contains chemicals—allow humans to come into contact with these pollutants. Two things are required to determine the danger to human health from exposure with pollutants.

A full exposure pathway must be followed for a disease to manifest. The following are the components of the exposure pathways:

a) Origin or Source: the point at which a contaminant enters the environment.
b) Media for transportation: the means by which a contaminant is transferred through the environment.
c) Point of exposure: the point at which a person first came into contact with a contaminant.
d) Route of exposure: the manner in which a contaminant enters or travels through the body.
e) Population Receptor: the mechanism by which a population is susceptible to a contaminant.

Additionally, whether exposure to a substance causes disease or toxicity depends on the contaminant's dose, exposure time, pathway of exposure—such as inhalation, ingestion, or skin contact—as well as personal characteristics like sex, age, genetic makeup, way of life, nutritional status, and overall health status (Fig. 4.1).

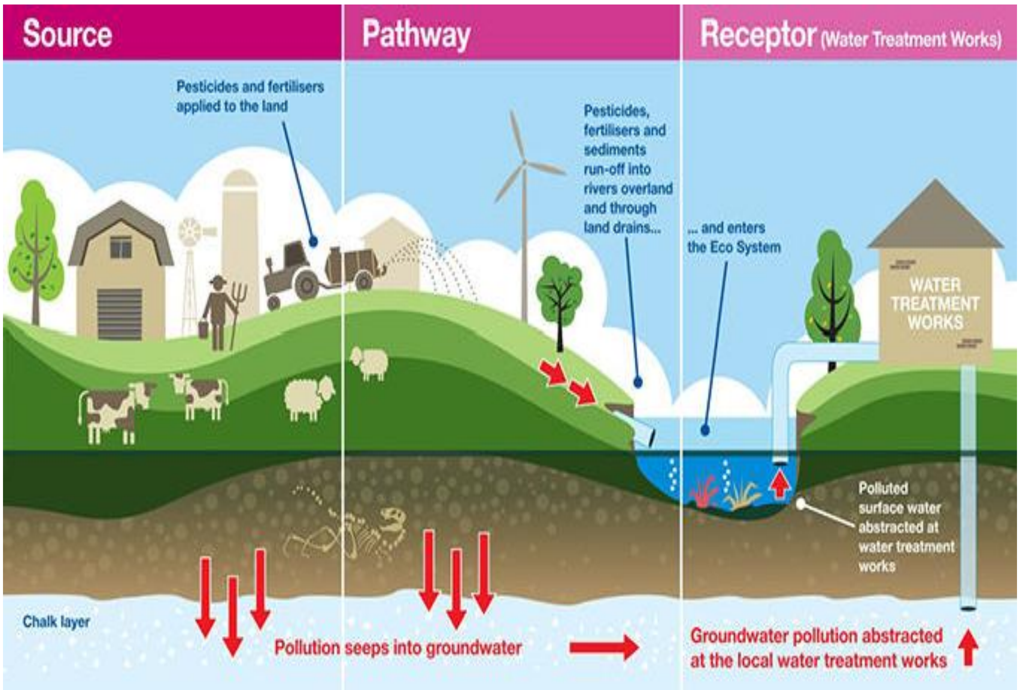

Fig. 4.1: The environmental toxin's exposure and circulation
(Source: https://stakeholder.affinitywater.co.uk/catchment-management.aspx)

12.4 Toxic Substances in Food Chains: Degradable and Non-Degradable

Toxins are harmful chemicals that are created by living things (animals or plants) and act as antigens in the body. The term "biotoxin" is occasionally used to emphasise the compound's biological origin. Better referred to as toxicants, chemicals having the potential to cause harm are those that are created by humans. There are many negative and destructive impacts that toxins and toxicants may have on human health. There are a variety of toxins and toxicants that, in general, act as mutagens (damage DNA or cause mutations), teratogens (cause birth defects or embryonic malformations), or carcinogens (agents that cause cancer). Other toxins and toxicants can also disrupt specific metabolic pathways and cause the dysfunction of certain biological systems, such as the hepatic, nervous system, and immune system, kidneys etc.

Food is the main source of toxin to the human body. The human body is exposed to toxic substances and anthropogenic toxicants such as pesticides, food processing wastes, drugs, industrial wastes, and occasionally non-food plant sources such as heavy metals (mercury, lead, cadmium, cobalt, chromium, etc.) that are also released into the environment by man at potentially dangerous levels and also end up in the human diet. A significant factor in this is the nutrition.

The toxic substances that can be broken down by bacteria, fungi, and other living creatures are known as biodegradable substances. Materials that degrade naturally are affected by temperature and light. If non-biodegradable materials are noxious in nature, they can contaminate the ecosystem and harm local animals for a very long period (Fig. 4.2).

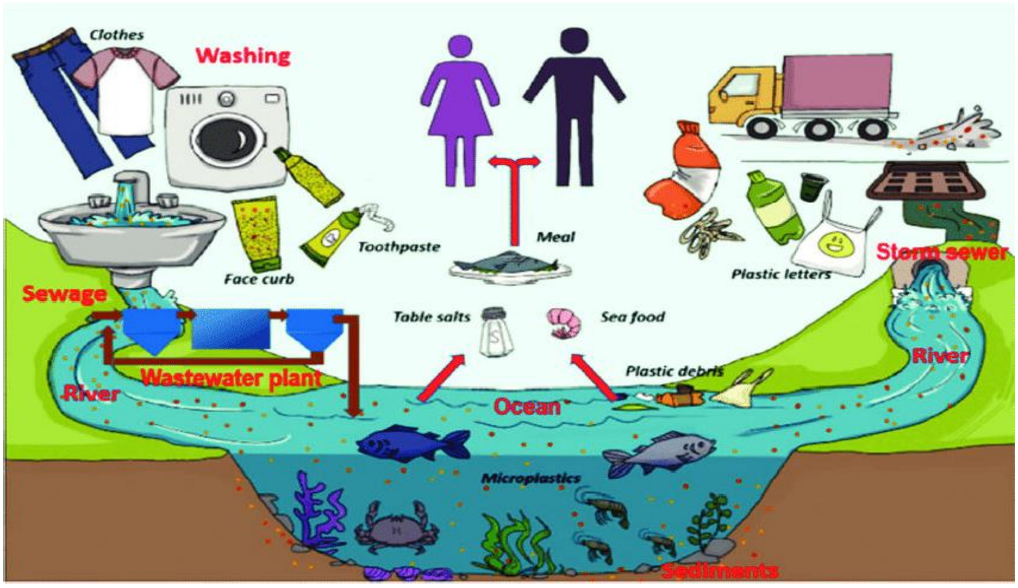

Fig. 4.2: Effects of micro plastic contamination on food chains in aquatic environment.
(Source: Wo et al., 2017

Different cultures promote the use of these materials to create compost, which may subsequently be used to create humus, an organic-rich substance that can be used to fertilise plant soil. By composting, less solid waste is created in cities and towns, reducing the need for landfill disposal. Drains, streams, and sewage treatment facilities, for instance, become clogged with foam made of phosphate complex from the usage of domestic detergents.

A component of detergents called sodium-tri-polyphosphate interacts with grime on surfaces and clothing to remove it. Because of how they interact with material surfaces, these substances are collectively referred to as surfactants. These are non-biodegradable and seem to be bad for both plants and animals. These are swapped out by biodegradable enzymes like amylase and proteases. Tree leaves, food scraps, grass clippings, and other organic materials are examples of biodegradable materials. Non-biodegradable pollutants/contaminants have a negative impact on the environment and organisms, and they enter the food chain (Fig. 4.3).

Chemicals like DDT and other organochlorine insecticides dissolve in fat rather than water. Therefore, these compounds tend to diffuse throughout the tissues that store fat in humans and other animals when they are exposed to them. If exposure is prolonged, bio-accumulation—also known as the buildup of toxic compounds inside the body—occurs. Chemicals that are soluble in fat, like organochlorine insecticides, can accumulate and concentrate in the environment as they migrate up the food chain, a process known as bioaccumulation and bio-magnification. The largest concentration of chemicals are absorbed by animals at the top of the food chain, such as fish-eating birds. In nature, certain chemical compounds are tenacious and do not break down, while others are swiftly converted into innocuous compounds. Rather, they lead to bioaccumulation, particularly in top predators. Mercury and DDT are examples of two persistent hazardous substances.

12.5 Bioaccumulation and Biomagnification

Bioaccumulation is the selective absorption and storage of a great variety of molecules inside the cell. Biomagnification is the tendency of some chemicals to become increasingly concentrated at successive higher trophic level of a food chain or food web. Due to this process, toxic compounds which are low in concentrations in the environment can reach to dangerous level inside cell and tissue of living organisms. The figure 6 explains the biomagnification of toxins in the food chain of a terrestrial environment. The dots represent the organic molecules present in each trophic level. The crosses represent the toxin present in each trophic level. The black dots remain relatively constant in each individual, while the concentration of crosses becomes greater in each succeeding trophic level. The concentration system is due to persistence of the toxins, food chain energetics, and low rate of internal degradation or excretion of the substance. Trophic level I represents the primary producers. Trophic level II represents the primary consumers. Trophic level III represents the secondary consumers. Trophic level IV represents the tertiary consumers.

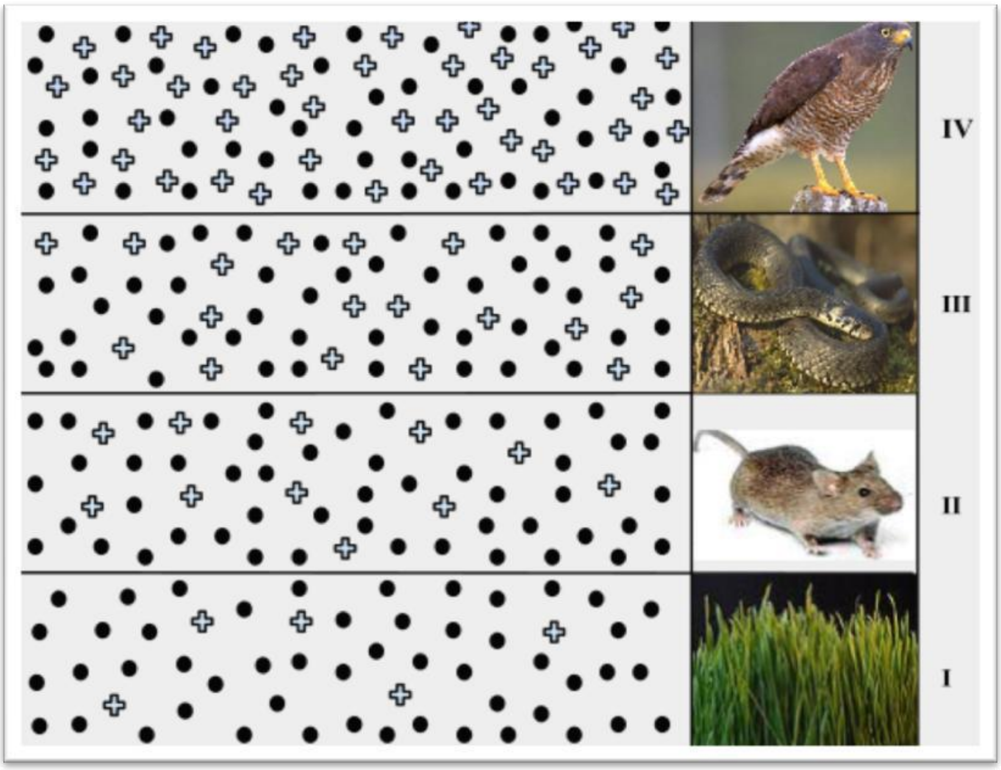

Figure.6: Biomagnification of toxins in the food chain of a terrestrial environment.

The effect of DDT, PCB, heavy metals and radioactive materials get magnified through the food webs. DDT and PCB can accumulate through food chain as these are fat soluble. Due to biological magnification, DDT also interferes with the reproduction of peregrine falcons, bald eagles, brown pelicans, and other predatory birds by causing thinning of egg shells. Metals like mercury and lead can pollute water, air and soil and get into human tissue through food chain. Concentration of some elements is known to increase from lower to higher level. The incidence of neurological disorder affecting many fish eating people in the industrial city of Minamata bay in Japan is a good example of Biological magnification of mercury. This disease is called Minamata disease. The methylation of mercury in the lake sediment or sediments of other aquatic body is the key to the transport of mercury into food chain (Figure 7).

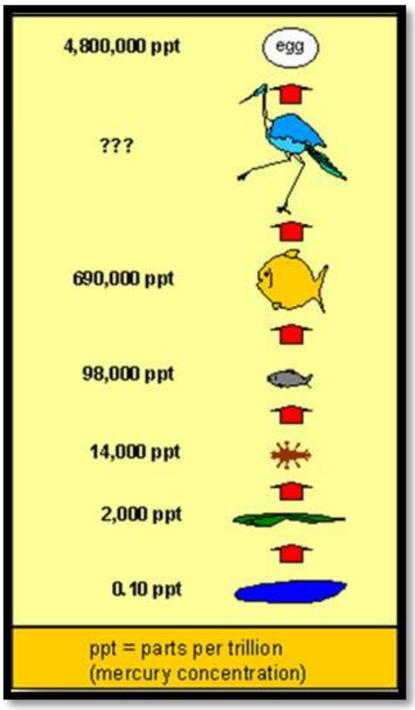

Figure.7: Biomagnification of methyl mercury in aquatic organisms.

Apart from the toxicity and increase in concentration from lower to higher trophic level, the persistence of the metal in environment makes metal pollution difficult to control. The radioactive dust that falls on the earth after atomic explosion is called radioactive fallout.Fall out radionuclides are being passed on to man via food chain. For example, in Arctic and Sub-Arctic regions of Alaska and Northern Finland reindeer and caribou meat is consumed. ^{137}Cs in man was higher during 1965. From the air, ^{137}Cs gets deposited in the reindeer moss (lichen) which is a primary producer. This lichen is a primary winter food for the caribou; it also showed high concentration of ^{137}Cs in winter. ^{137}Cs also showed food chain concentration which doubled with each trophic level in the food chain.

The most severe nuclear reactor accident occurred on April 16, 1986 at the Chernobyl Nuclear power plant in the Ukraine. The accident of Chernobyl produced a nuclear cloud and deposited much of contamination over Arctic Eurasia. The isotopes of ^{137}Cs, ^{134}Cs, ^{131}I and ^{137}Sr were emitted and people living by the nuclear plant were most adversely affected. Much of the nuclear fallout from the Chernobyl site occurred in Scandinavian countries, Poland and some Northern Europe countries. ^{137}Cs caused much exposure to general public. Radioactive contaminated lichens passed Strontium and Cesium to reindeer. In vertebrate food chain, strontium and cesium usually accumulate, particularly at higher level. In arthropod food chain, radioactive elements like potassium, sodium and phosphorous accumulate while strontium and cesium does not accumulate. Because half-life of ^{137}Cs is about 30 years, its effect will persists for many years on the sites which have been exposed to nuclear fallout.

Toxic pesticides as BHC, DDT are not easily degraded and are long lasting in the environment. Their concentration therefore goes on increasing in water and soil in successive application. For instance, DDT in water may be very low, but the aquatic plants like phytoplanktons may show an average value of about 5.5 ppm, about 265 times increase over the concentration used in initial application of DDT. Plants and animals accumulate DDT in much higher concentration in these bodies, the herbivore, insects and fish feeding on these plants may accumulate DDT in still higher concentration and at the top carnivore level, fish eating birds etc. concentration may reach at very high level. Some shellfish as oyster accumulate DDT 70, 000 times the original concentration, frog 2000 times and sunfish12,000 times.

Maxwell (1973) presented on example of DDT magnification in food chain. The spraying on elm tree resulted in 99 ppm of DDT in soil, 140 ppm in earthworm and more than 400 ppm in Robins that fed on these worms. The following is another example of biological magnification of DDT in aquatic system (Table 5).

Table.5: Biological magnification of DDT in aquatic system.

Component	Concentration of DDT (ppm)
Water	10^{-6}
Zooplanktons	10^{-2}
Minnows	10^{-1}
Needlefish	1.0
Birds	10.00

The polychlorinated biphenyl (PCBs) compounds are group of manmade chemicals which are similar to DDT in their extreme stability. These are introduced in 1929 and are extremely toxic compounds which originate in a great variety of manufacturing process like manufacturing of glass, electric transformers, inks, carbon less copy papers, grinding wheels, ceramics, varnishes, fire resistant coating to wood and plastics, sealants, tyres, plastic coating and many more. PCB can affect the immune system, fertility, child development and increase the risk of certain cancer. Because of the extreme toxicity, the accepted level of PCB in fishto be eaten is only 2 ppm. In higher concentration it causes death. The biological magnification of PCB in great Lakes region of United States is shown in the Figure 8.

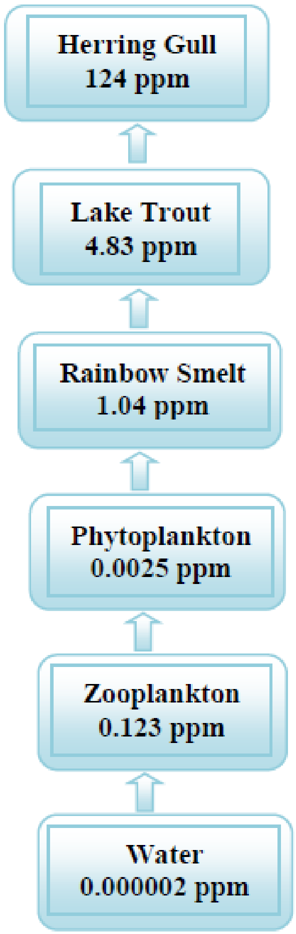

Figure.8: The biological magnification of PCB in great Lakes region of United States.

The DDT molecule is stable and does not decompose under continued exposure to oxygen, sunlight or other reagents. Similarly, from biological point, DDT is also very stable. Only few microbes have the ability to degrade DDT efficiently and the process appear to require anaerobic conditions. In animals, DDT is metabolized very slowly by the liver into breakdown products DDE and DDD which are highly toxic than their parent compound. These factors combine to yield a half-life for DDT in the biosphere estimated at about 15 years.

It is estimated that over 2 billion metric tons of DDT is circulating in the biosphere, of which ¾ is distributed in terrestrial environment and the remaining ¼ in marine environment. DDT enters the air largely through aerial spraying operation, but significant amount enters the air by simply evaporating from water surfaces, plant surfaces and soil. In this fashion, about ¼ of the total annual production of 100,000 metric tons/year reaches the ocean. Even if it is never used again in future it would be still there for many years. DDT is a potent toxicant of water and soil. Many developed countries like U.K., Sweden and Denmark have already banned the use of DDT. In India, the government has banned the use of more than 30 pesticides, and restricted the use of seven pesticides including DDT. India also has BIS standard (Bureau of Indian standard) for pesticide application equipment. However, implementation of legislation and standards at field level needs to be strengthened to prevent misuse and inappropriate use of pesticides.

Summary

The chemicals present in the environment can be harmful to the structure and function of the ecosystem. These chemicals are either produced naturally or artificially by humans. In 1960's the term ecotoxicology was introduced which deals with the interactions, transformation, fate and effects of natural and synthetic chemicals in the biosphere including individual organisms, populations, community and whole ecosystems.

There are number of ways through which toxic materials enter into the body of living organisms like water, air, food, accidental exposure, medicinal drugs and several other means. Toxicants adversely affect living organisms in different ways. Toxins that specifically cause abnormalities during embryonic growth and development are called teratogens. Thalidomide is the most commonly known teratogen which causes abnormality in developing embryo. Many chemicals in the environment are carcinogenic. It includes synthetic chemicals, radiation, air pollutants, heavy metals and smoking. Mutagens are agents, such as chemicals and radiation which causes sudden and permanent changes in the genetic material of living organisms. This can lead to birth defects or may trigger neoplastic (tumor) growth.

Accumulation of toxic compounds in food and through food chain represents concern related to the consumption of this food by human and other animals. Toxins when absorbed in the body are metabolized, transported or excreted. During the metabolic process, they have adverse biochemical effects. These processes are characterized by kinetic phase and a dynamic phase. In kinetic phase, a toxic substance may undergo absorption, metabolism, temporary storage, distribution and excretion. In the dynamic phase, a toxicant interacts in cells, tissue, or organ in the body and leads to various types of toxic effects.

There are certain chemicals in the environment which have the tendency to become increasingly concentrated at successive higher trophic level of a food chain or food web. Thisis known as Biomagnification or Biological magnification. Due to this process, toxic compound reaches to dangerous level inside cell and tissue of living organisms. The effect of DDT, PCB, heavy metals and radioactive materials get magnified through the food webs. Due to biomagnification, DDT interferes with the reproduction of peregrine falcons, bald eagles, brown pelicans and other predatory birds by causing thinning of egg shells. The incidence of neurological disorder affecting many fish eating people in the industrial city of Minamata bay in Japan is a good example of Biological magnification of mercury.

Due to higher toxicity of DDT, many developed countries like U.K., Sweden and Denmark have already banned the use of DDT. In India, the government has banned the use of more than 30 pesticides, restricted the use of seven pesticides including DDT. However, implementation of legislation and standards at field level needs to be strengthened to prevent misuse and inappropriate use of pesticides.

Reference

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Unit 13: Distribution and Fate of Ecotoxicants

Unit Structure

13.0 Objectives

After reading this unit students should be able to:

- To familiarize the concept of biotransformation
- To understand the types and consequences of biotransformation
- To study the factors influencing biotransformation

13.1 Introduction

Ecosystems have a significant role in the movement of poisonous or dangerous compounds through various abiotic elements including air, water, land, etc. As a result, the term "environmental media" refers to the abiotic elements of the natural environment, such as the air, water, and land. In terms of interaction between the agent and the body's external surfaces, exposure to toxic agents is defined. The body's points of contact (such as the skin) and entryways (such as the mouth and nose). The concept that exposure may be connected to ambient concentrations in air, water, sediment, and soil by means of simple parameters is one that underlies many exposure evaluations. To give a complete picture of exposure pathways and pinpoint the main sources of uncertainty, total exposure evaluations also incorporate time and activity patterns, micro environmental data, and other relevant information.

The early phases of an exposure evaluation should consider a soldier's immediate environment, food and drink intake, as well as the layer of dirt, water, or other chemicals that come into touch with their skin. The quantity and relative contribution of each exposure route and pathway must be taken into account in order to determine the overall human exposure to a hazardous chemical and select the best approach for characterizing the exposure. A semi-volatile hazardous air pollutant that has been released into the surrounding air, such an aromatic hydrocarbon, is an excellent example to employ. When this chemical is released, the vapor phase and the condensed phase, or airborne particles, will separate. Pollutants that are both indoor and outside and both particles and vapors are present in the inside air.

Deposition and runoff to aquatic bodies have contaminated drinking water. Vegetation that nourishes animals that produce milk and meat may deposit pollutants on its surface. Once the contours of intake and uptake for the intended group have been identified, the precise potential for contact cannot be calculated. The chemical properties, the manner of material release, and other environmental factors all affect the route.

Transport and transformation processes, source and emission variables, exposure route, and intake or absorption routes are crucial elements for the exposure evaluation. Both long-term (months or years) and short-term (hours or days) exposure characterization processes are possible. Information gathering and compilation (including information on origin, discharges, transit, contact media concentration, and tracking activity) constitute the primary stage.

An agent can enter a body by exposure channels, including touching, breathing, and ingesting. The quickest mode of absorption is inhalation, followed by skin contact and ingestion. The health impacts are determined by the exposure pathways. The types of pollutants, such as vapor or condensed forms, are an additional factor that affects how well an agent will make you feel when you inhale it. Another crucial element in determining the health effect is the length of exposure. The amount and period of peak concentration must be estimated for many toxic chemicals used in non-warfare as well as some warfare agents. It is necessary to evaluate the cumulative effects of exposure over an hour or less in order to characterize the hazardous agent effects with significant acute health consequences. Health impacts could not become apparent until long-term cumulative ingestion for industrial chemicals that are less hazardous.

The paths from source to contact, however, may be more complicated and less clear for low-dose exposures to substances. Agents that have been released into the air, for instance, may land on the ground and cause low-dose exposures through volatilization and resuspension, exposures through dermal contact when dust comes into contact with troops moving through the area, and exposures through ingestion if the chemical agents are washed into a nearby water source by rainfall. The destiny and redistribution of chemicals deposited on the soil can be greatly influenced by a variety of factors, including soil parameters like pH, oxidation potential, moisture content, etc. Many people's exposures may also be caused by a variety of environmental processes, such as the movement and dispersion of harmful substances via the air, water, and land (including sediments and soil).

13.2 Hazardous/Toxic material Transport and dispersion in the atmosphere

The mixing of gases and aerosols in ambient air is primarily facilitated by atmospheric advection and diffusion. On the behaviour of pollutants in the lower atmosphere, meteorological parameters have a significant impact. Thermal qualities (such as stability) and wind-related parameters including velocity, direction, and turbulence are crucial. The atmospheric diffusion equation may be statistically solved using Gaussian distributions to obtain standard models for predicting the time and spatial dispersion of agents to the atmosphere. Using Gaussian statistical solutions, the standards for estimating space and time may be performed.

There are several publications, studies, and computer programmes that provide techniques for determining how widely spread line, point, and volume air pollution sources are. These models are frequently employed and have undergone several calibrations. However, in many situations, such as those involving complex terrain, urban settings, different meteorological conditions (such as when a plume mixes down to the surface as the height of convective cells rises as a result of surface heating), or when there is a strong interaction between the dispersed agent and the surfaces of the ground and vegetation, these models are frequently not sufficient to make predictions.

13.2.1 Hazardous/Toxic material Transport and dispersion in water

Groundwater and surface waterways have been poisoned by various point and non-point sources. In many nations, household garbage is one of the main sources of pollutants in waste. (Layton and others, 1993). Point sources, including residential or commercial wastewater treatment facilities, are located at a particular location (outlet) along surface water. Due to its dispersed character, it is exceedingly challenging to clean up nonpoint sources of water polluted by runoff from sizable urban and agricultural regions. In surface waters, the behaviour of chemicals and biological agents is determined by-

a) Chemical reactivity and the rate of physical transit in the water system.

b) Depending on the type of body of water (such as an ocean, sea, estuary, lake, river, or wetland), physical transport mechanisms might vary significantly.

13.2.2 Soil and vegetation dispersion on land

How a chemical or organism introduced to soil will be transported and/or altered depends, in great part, on how air, water, mineral, and organic components mix together in the soil. In nature, soils are described as diverse. via intricate web interactions Environmental and human health can be impacted by contaminants in soil. The fate of soil pollutants is influenced by a variety of conflicting mechanisms. "Plant interactions with two environmental media (soil and air) are typically in contact with vegetation, but this is not well understood enough to describe a process of predicting CB agent uptake by flora."

13.2.3 Toxic Substance Transformation Processes

Due to the change of chemical and biological substances, the exterior and inward surroundings can have a significant influence on an organism's ability to remain in the environment and on how they are exposed, gathered, and dispersed. Abiotic or biotic processes cause chemical changes to take place. It can alter the quantity of a substance or alter its structure in such a way to modify it up to a significant extent or raise or decrease how toxic it is. For instance, many organic molecules present in the air may be changed into other compounds through transformation processes including photolytic breakdown and oxidation/reduction reactions. The half-life of a molecule for a certain conversion process in environmental medium plays a significant role in determining persistence time for organic compounds. Indirectly from knowledge on compounds that are structurally similar, experimental determination provides precise information on rates and methods of transition for molecules of interest. As a result, complexes with documented statistics deficiencies have far greater difficulty generating quantitative estimations.

13.3 Heavy Metal Biosorption

The term "heavy metal" describes a slightly metallic chemical component that is toxic or fatal, has a relatively high density, and is present in low concentrations. Even highly diluted water solutions can contain heavy metals that bio accumulate. The biomass that has this capacity functions as a chemical compound and a biologically derived ion exchanger. Heavy metals are reported to be biosorbed by the cell walls of several bacteria, fungus, and algae. However, the bioaccumulation process, which is fueled by live cells with active metabolisms, is the opposite of biosorption. Metal removal with microbial biomass is quite efficient. For the metal removal procedure and detoxification of industrial effluents heavy with metals, a whole new class of properly "formulated" bio sorbents can be used.

The most effective method of apposition for the removal of heavy metals is sorption packed-column outlines. Because the deposited metals may frequently be liberated from the biosorbent with ease in a powerful wash solution, which also regenerates the biosorbent for ensuing several reuses, it is possible to recover the deposited metals from soaked bio sorbent. The main benefits of bio sorption are its effectiveness, cost-effectiveness, lack of nutritional requirements, and ability to aid in metal recovery. The solid phase sorbent or biosorbent used in the biosorption process contains biological material, while the liquid phase contains contaminants that have been dissolved in the solvent and are known as sorbate or heavy metal ions. Because the absorbent is so effective at binding sorbate, it can do so through a variety of methods. This biosorption process continues until equilibrium is reached between the forms of sorbate that are solid-bound and the remaining amount in the aqueous solution. The effectiveness of the sorbent's likeness to the sorbate controls how evenly it is distributed across the liquid and solid phases.

13.4 Bio-sorbent Substance

Biological substances, including certain microorganisms with strong biosorbent behaviour for metallic ions, which is a feature of the makeup of microbial cells. These sorbents are composed of dormant, inert cells. A small number of sorbents are specific for only a few heavy metals whereas others gather heavy metals in large quantities with little to no binding activity. These are usually classified as (i) and (iii) based on how hazardous they are to the environment, and eradication from the source of effluent discharge is where the focus should be. In addition to safety standards, a person's interest about a particular metal may be a driving force behind how they will ultimately assess the effects of ingesting that metal's biosorbent.

13.5 Biotransformation

Biotransformation are structural modifications of a chemical compound by organisms /enzyme systemsthat lead to the formation of molecules with relatively greater polarity. This chemical process of modification of endogenous or exogenous chemicals into water soluble chemicals aid in the easy elimination of the toxic/foreign chemicals from the body. Natural Biotransformation mechanisms are catalyzed by enzymes in the liver and other tissues. However, the natural transformation process are slow, nonspecific and less productive. There are also other types of biotransformation reactions catalysed by microbes called microbial biotransformation which was developed to acclimatize to environmental changes making them useful in several biotechnological processes. The most significant aspect of biotransformation is that it maintains the original carbon skeleton after obtaining the products. Biotransformation can also be used to synthesize compounds or materials where synthetic approaches are challenging. Biotransformation is of two types:

A. Enzymatic
B. Non-enzymatic

A. Enzymatic biotransformation is the process of elimination of chemicals from the human body catalysed by various enzymes present in the body. In the body, enzymes metabolizing the drugs and xenobiotics are different from food metabolizing enzymes. They are broadly classified into

(i) microsomal
(ii) non microsomal

(i) Microsomal biotransformation is caused by enzymes present within the lipophilic membranes of smooth endoplasmic reticulum. Microsomal enzymes are located on smooth endoplasmic reticulum of liver, kidney, lungs, intestinal mucosa and catalyse majority of drug biotransformation reaction that includes oxidative, reductive, hydrolytic and glucouronidation reactions. The enzymes are inducible bydrugs and diet.

Examples of microsomal enzymes include

- Cytochrome P450 (ferric, ferrous forms) (CYPs)
- NADPH (flavoprotein)
- Flavin monooxygenase systems (FMO)
- Epoxide hydrolase (EH)
- Uridine 5'-diphosphate-glucuronyltransferase (UDPGT)
- Molecular oxygen
- Membrane lipids

(ii) Non-Microsomal Biotransformation are catalyzed by non-microsomal enzymes that are non-specific enzymes. These enzymes are located in the cytoplasm and mitochondria of liver cells, plasma and also in other tissues. They are not inducible but show polymorphism. They catalyze very few oxidative; a number of reductive; and hydrolytic reactions. They also show conjugation reaction except for glucouronidation.

Examples:

- Xanthine oxidase converting hypoxanthine into xanthine.
- Cytotoxic agent 6-mercaptocurine
- Monoamine oxidase involved in non-microsomal metabolism of catecholamine's and noradrenaline.
- Alcohol dehydrogenase responsible for metabolism of ethanol into acetaldehyde
- Tyrosine hydrolases enzymes

The metabolizing enzymes that catalyze biotransformation are either less or completely absent in the neonates leading to decreased metabolism of drugs. This makes the drugs toxic. For example certain syrups are toxic for children despite of minimum alcohol percentage. Drug metabolizing enzymes are controlled by specific genes coding for specific protein (enzymes). However, the specific gene coded proteins vary from species to species. For example, dogs are deficient in acetyl transferase whereas cats are deficient in glucouronyl transferase. Also, some drugs are inactivated in the body fluids by spontaneous molecular rearrangement without the presence of any enzyme. They are eliminated from the body due to hepatic dysfunction. Example: atracurium

B. Non enzymatic Biotransformation is Spontaneous, non-catalyzed and non-enzymatic types of biotransformation are for highly active. Itproduces unstable compounds at varying physiological pH. Some of the example include

- Mustin HCl converted into Ethyleneimonium
- Atracurium converted into Laudanosine and Quartenary acid
- Hexamine converted into Formaldehyde
- Chlorazepate converted into Desmethyl diazepam

13.5.1 Role of biotransformation

Biotransformation causes

- The polarity of the drugs increases after metabolism in the body. This favours easy excretion of the drugs
- Alteration or termination of drug activity
- Converts pro-drug to active compound

- Coverts less active drug to more active drug
- Produces toxic compound

13.5.2 Consequences of biotransformation

1. The physical properties of xenobiotics are generally changed from those favouring absorption (lipophilicity) to those favouring excretion in urine or fecaes (hydrophilicity). However, an exception is the elimination of volatile compounds by exhalation, wherein biotransformation to non-volatile, water-soluble chemicals can retard their rate of elimination. Similarly, biotransformation of xenobiotics in the brain and testis (two organs with a barrier to chemical transport) might also be an obstacle to xenobiotic elimination if the metabolites cannot cross the blood-brain or blood-testis barrier.
2. Xenobiotics exert a variety of effects on biological systems that may be beneficial or deleteriousdepending on the physicochemical properties of the xenobiotic. Example, in pharmacology, chemical modification of drugs by biotransformation helps the drug to exert its pharmacodynamic effect (i.e., it is the metabolite of the drug, and not the drug itself, that exertsthe pharmacologic effect).

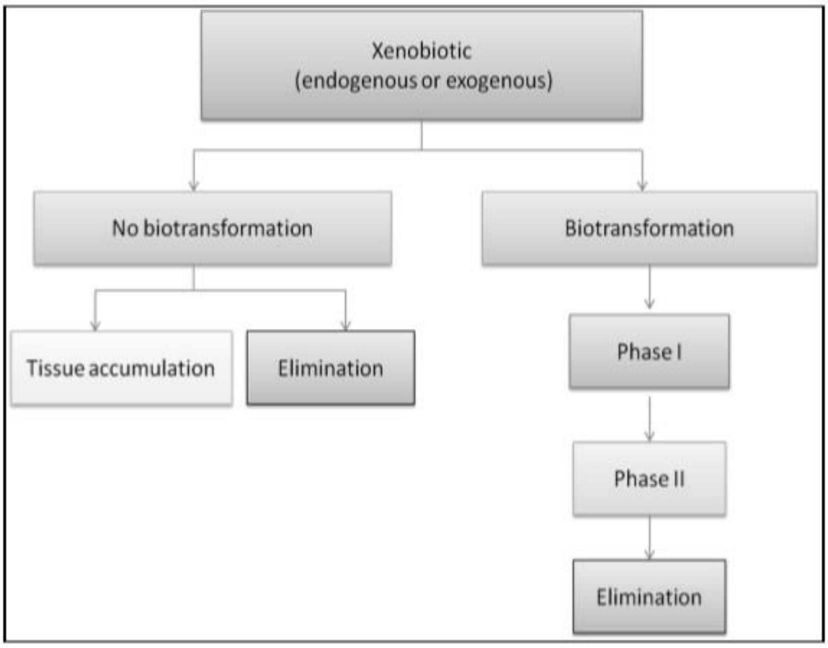

Figure 1 Schematic representation describing the role of

xenobiotic in the presence or absence ofbiotransformation

3. In toxicology, many xenobiotics must undergo biotransformation to exert their characteristic toxic or tumorigenic effect (i.e., many chemicals would be considerably less toxic or tumorigenic if they were not converted to reactive metabolites by xenobiotic-bio transforming enzymes). However, biotransformation terminates the pharmacologic effects of a drug and lessens the toxicity of xenobiotics. Enzymes catalyzing biotransformation reactions often determine the intensity and duration of action of drugs and play a key role in chemical toxicity and chemical tumorigenesis.

13.6 Biotransformation capacity of the organism

Biotransformation capacity of the organism depends on the degree to which the organisms are exposed to xenobiotics. For example, insects that feed on a variety of plants have a greater capacity to biotransform xenobiotics than insects that feed on a limited number of plants. Compared with mammals, fish have a low capacity to metabolize xenobiotics because they can eliminate xenobiotics unchanged across their gills.

13.7 Other factors affecting biotransformation

13.7.1 Presystemic Metabolism/First pass effect/Route of Administration

First pass effect or presystemic metabolism is a phenomenon of drug metabolism wherein the concentration of drug is greatly reduced before it reaches the systemic circulation. Drugs following first pass metabolism have decreased bioavailability. Most of the drugs are metabolized within the liver. Changing the route of administration might change the first pass metabolism. For example Propanolol is 80% metabolized before reaching systemic circulation.

13.7.2 Genetic Variations

Drugs behave differently in different individuals due to genetic variations. These inter individual genetic variations results either in complete absence of the enzyme or its malformation leading to malformed genes. Mostly non microsomal enzyme show genetic variations. Examples: Succinyl choline, a skeletal muscle relaxant is metabolized by pseudo choline esterase. Some people lack this enzyme, due to which metabolism of succinyl choline might not occur. When administered in those individuals, prolonged apnea might result. Different groups of populations might be classified as fast metabolizers and poor metabolizers of drugs. For certain drugs, like isoniazid, fast acetylates as well as slow acetylates are present. Fast acetylates cause rapid acetylation, while poor metabolizers metabolize less. Hepatic acetyl transferase catalyzes acetylation. Slow acetylation might occur due to genetic malformation leading todecreased production. Fast metabolism may lead to hepatotoxicity while poor metabolism might result in peripheral neuropathy. Absence of catalase may lead to achalasia, while G6PD deficiency predisposeserythrocytes to hemolysis as a result of oxidative stress imposed by some commonly used drugs.

13.7.3 Species Differences

Drug metabolizing capacity varies between species. The difference is most established in animals. Somemetabolize drugs rapidly and some do not. Example: Rats and rabbits metabolize drugs more efficientlythan humans. Likewise, in human drug metabolizing capacity varies between races. Example: Drug metabolizing capacity of certain anti-malarial drug is different amongst the Eskimos and the Asians. Eskimos may metabolize drugs more efficiently than the Asians.

13.7.4 Exposure to Pollutants from Environment or Industry

Exposure to external factors such as pollutants and chemicals affects the enzymes of the biotransformation reaction resulting in enhanced or inhibited biotransformation. Smoking and chronic alcoholism might act as enzyme inducers. Similarly, pesticides or insecticides may act as enzyme inducers. Likewise, environmental factors too influence and show effect on biotransformation. In hot and humid climate biotransformation is decreased and vice versa. At high altitude, the occurrence of biotransformation decreases due to decreased oxygen leading to decreased oxidation of drugs.

13.7.5 Age

Age plays a very important role in drug metabolism by biotransformation. Extreme age groups (very young and very old) behave almost the same. Drug metabolizing enzyme develop early but their capacityis low. Thus the rate of metabolism in infants is very low.

Care should be taken in administering drugs in younger patients. True development of enzyme occurs in one to two months. Chloramphenicol (antimicrobial drug) when administered in infant, does not have great efficacy. Toxic effects in the form of grey baby syndrome might occur. The baby may be cyanosed, hypothermic, flaccid and grey in color. Shock and even death might occur if toxic levels get accumulated. Diazepam (sedative hypnotic) may result in floppy baby syndrome in which flaccidity of the baby is seen.

In elderly people, most processes slow down and leads to decreased metabolism. Shrinkage of organs occurs along with decreased liver functions and decreased blood flow through the liver. All these factors decrease the metabolism. Old people that are usually taking multiple drugs are more prone. Henceforth, the drug doses should be decreased in the elderly persons.

13.7.6 Sex

Male have a higher BMR as compared to the females, thus can metabolize drugs more efficiently, e.g. salicylates (others might include ethanol, propanolol, benzodiazepines). Females, during pregnancy, have an increased rate of metabolism. Thus, the drug dose has to be increased. After the pregnancy previous doses can be high and hence the dosage should be decreased and reverted back to normal levels. Example includes phenytoin, whose dose has to be increased during pregnancy (specially second and third trimester).

13.7.7 Drug-Drug Interaction

Toxic effects might result when drug combinations act as enzyme inhibitors and inducers. The dose hasto be adjusted accordingly.

13.7.8 Nutrition

Malnutrition may also affect biotransformation. Depletion of amino acids and glycine may affect drug metabolizing capacity, especially during the phase II, which depends on the food stores. Synthesis of microsomal enzymes depend on nutritional status.

13.7.9 Pathological Conditions

Most of the drugs are metabolized in the liver. Any disease which might cause cirrhosis, viral hepatitis, drugs induced hepatitis, hepatocarcinoma may slow down the metabolizing capacity. Jaundice depresses glucuronic acid conjugation and oxidative function of liver microsomes. Likewise, cardiovascular diseases, although have no direct effect, decrease the blood flow, which may slow down biotransformation of drugs like isoniazid, morphine and propanolol. Similarly pulmonary conditions may decrease biotransformation. Procaine and procainamide hydrolysis is also impaired. Hypothyroidism increases drug metabolizing capacity (increased half-life of antipyrine, digoxin, methimazole, practolol) while hyperthyroidism decreases it.

13.7.10 Circadian rhythm

The rate of hepatic metabolism of certain drugs follow diurnal rhythm in rats and mice. This may be true in humans as well.

13.8 Advantages of Biotransformation

(i) Significance of bioconversion reactions becomes obvious when the production of a particular compound is difficult by chemical methods

(ii) Biotransformation reactions are highly preferred as they are cheaper, more direct than their chemical analogues and the conversion normally proceeds under conditions that are regarded as environmentally acceptable.

(iii) In biotransformation, the enzymes or whole cells provide a remarkable enhancement in reaction rates as well as specificity to exhibit high stereos electivity over the corresponding reactions.

(iv) Microbial transformation offers the advantages of highly selective operation at non extreme pH, near room temperature and reduced levels of toxic waste products.

(v) Biotransformation with recombinant microbial enzymes have been widely used, including applications for the production of hormones, antibiotics, and specialty chemicals.

(vi) The environmental pollution due to biotransformation is almost insignificant or negligible.

(vii) In addition, it is easy to apply recombinant DNA technology to make desired improvements in bio-transformations.

(viii) Another practical advantage of bio-transformations is that it is easy to scale-up the processes due to limited number of reactions.

Summary

In this unit, we have learnt about the ecotoxicology and impact of toxicants on environment. The terms such as bio adsorption, bioaccumulation and bio magnification have been described. Due to environmental pollution, the severity of the effects of toxicants on human beings increases manifold. The unit explains

- Biochemical aspect of various carcinogens and throws light on their toxicity.
- Concept of biotransformation
- Need of biotransformation
- advantages and disadvantages of biotransformation
- types and consequences of biotransformation
- Factors influencing biotransformation

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