

## Plant Responses and Adaptation to Metals/Metalloid Toxicity

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### Metals

The term “metals” refers to elements with very good electrical conductance (this property declines with decreasing temperature) and that exhibit an electrical resistance that is proportional to the absolute temperature. From a chemical point of view, the term ‘heavy metal’ is strictly ascribed to transition metals with atomic mass over 20 and has a specific gravity of above  $5 \text{ g cm}^{-3}$  or more. In biology, “heavy” refers to a series of metals and also metalloids that can be toxic to both plants and animals even at very low concentrations. The other metals are referred to as light metals ( $<4.5 \text{ g cm}^{-3}$ ). The specific gravity of water is 1 at  $4^\circ\text{C}$ . Simply stated, specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some well-known HMs with a specific gravity that is 5 or more times that of water are arsenic (As), 5.7; cadmium (Cd), 8.65; iron (Fe), 7.9; lead (Pb), 11.34; and mercury (Hg), 13.54. In fact, some HMs known as “trace metals”, for example, Fe, Cu, Zn, Mo, nickel (Ni) and cobalt (Co) are essential for the growth and metabolism of organisms at low concentrations, and microorganisms possess mechanisms of varying specificity for their intracellular accumulation from the external environment. In contrary, many other HMs like Pb, tin (Sn), Cd, Al, Hg have no essential biological function but can still be accumulated in biomass and are freely transferred from one organism to another through the food chain.

### Sources of metals/metalloids

#### Natural sources

The natural or geogenic sources of HM include: natural weathering of the earth’s crust. Crustal material that is either weathered on (dissolved) and eroded from (particulate) the Earth’s surface or injected into the Earth’s atmosphere by volcanic activity. These two sources comprise 80% of all the natural sources; forest fires and biogenic sources account for 10% each (Nriagu, 1990). Naturally, HM particles come up by soil erosion and released in the atmosphere as windblown dust. In addition, some particles are released by vegetation. The natural emissions of the 5 major HMs are 12,000 (Pb); 45,000 (Zn); 1,400 (Cd); 43,000 (Cr); and 29,000 (Ni) metric tons per year, respectively (Nriagu, 1990) which indicated that an abundant quantity of metals are emitted into the atmosphere from natural sources.

#### Anthropogenic sources

There are a multitude of anthropogenic emissions in the environment. Most of the HMs occurrences in urban soils tend to originate from anthropogenic sources such as industrial, urban development and road traffics. The key sources of HMs are mining and smelting. Mining releases HMs to the environments like soil and water as tailings and to the atmosphere as metal-containing dust. Smelting, on the other hand, discharges metals to the environment in consequence of high-temperature refining processes. Other important sources of HMs to the terrestrial and aquatic environment include fossil fuel combustion, municipal waste disposal, cement production, automobiles, use of commercial fertilizers and pesticides, animal waste etc. Increasing demand of fossil fuels, agrochemicals as well as rapid urbanization causing more anthropogenic HM inputs. However, the two main pathways for HMs to become incorporated into air–soil–sediment–water are transported by air and water.



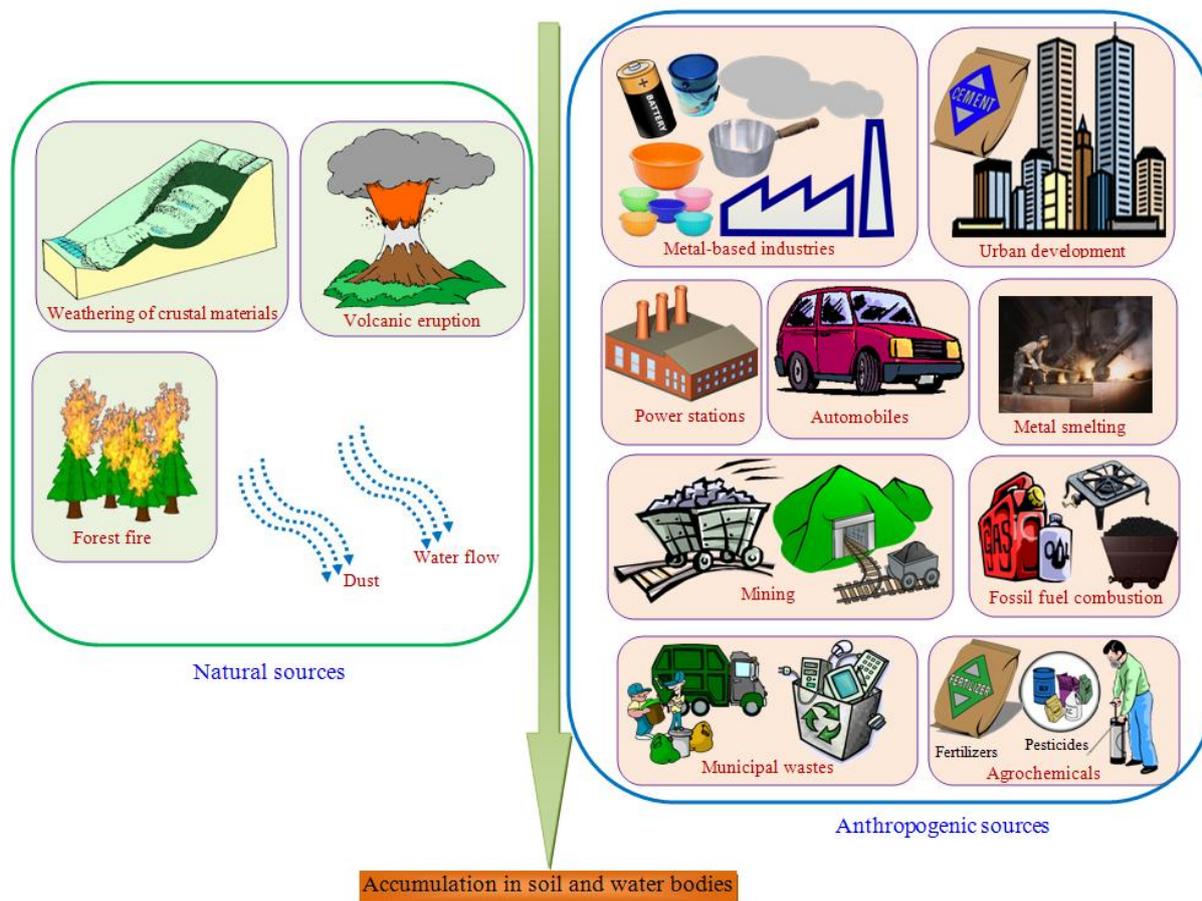


Fig. 1. Sources of heavy metals

Table 1 Anthropogenic sources of major heavy metals

Metal	Anthropogenic sources
<b>Cd</b>	Mining, ore dressing, and smelting of nonferrous metals; battery manufacturing industry, cigarettes, processed and refined foods, large fish, shellfish, tap water, auto exhaust, plated containers, galvanized pipes, air pollution from incineration and occupational exposure.
<b>Pb</b>	Residue resulted from the production of Pb electric accumulator; residue and sludge resulted from Pb caster and product industry, tap water, cigarette smoke, hair dyes, paints, inks, glazes, pesticide residues and occupational exposure in battery manufacture and other industries; waste resulted from the production and apply of Pb compound
<b>As</b>	Mining, ore dressing, and smelting of nonferrous metals; production of As and As compounds; petroleum and chemical industry; pesticides, beer, table salt, tap water, paints, pigments, cosmetics, glass and mirror manufacture, fungicides, insecticides, treated wood and contaminated food, dyestuff and tanning industry
<b>Hg</b>	Production and apply of Hg catalyst in chemical industry; Hg battery manufacturing; smelting and restoring of Hg; Hg compound production; pesticide and medicine making; production and apply of fluorescent light and Hg lamp; Hg slime resulted from caustic soda production; dental amalgams, large fish, shellfish, medications, manufacture of paper, chlorine, adhesives, fabric softeners and waxes.
<b>Cu</b>	Mining, ore dressing and smelting of nonferrous metals, Cu water pipes, Cu added to tap water, pesticides, intra-uterine devices, dental amalgams, nutritional supplements - especially prenatal vitamins, birth control pills, weak adrenal glands and occupational exposure.
<b>Zn</b>	Mining, ore dressing, and smelting of nonferrous metals; metal and plastic electroplate; pigment, beaded paint and rubber working; Zn compound production; Zinoky battery product industry

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Metal	Anthropogenic sources
Ni	Residue resulted from the production of nickeliferous compound; Abandoned nickeliferous catalyst; nickeliferous residue and waste from electroplate technology; nickeliferous waste resulted from analysis, assay, and testing activity; hydrogenated oils (margarine, commercial peanut butter and shortening), shellfish, air pollution, cigarette smoke, plating and occupational exposure.
Cr	Cr compound production; leather working industry; metal and plastic electroplate; dyestuff and dying by acidic medium; production and apply of dyestuff; metal Cr smelting
Al	Cookware, beverages in aluminum cans, tap water, table salt, baking powders, antacids, processed cheese, anti-perspirants, bleached flour, antacids, vaccines and other medications and occupational exposure

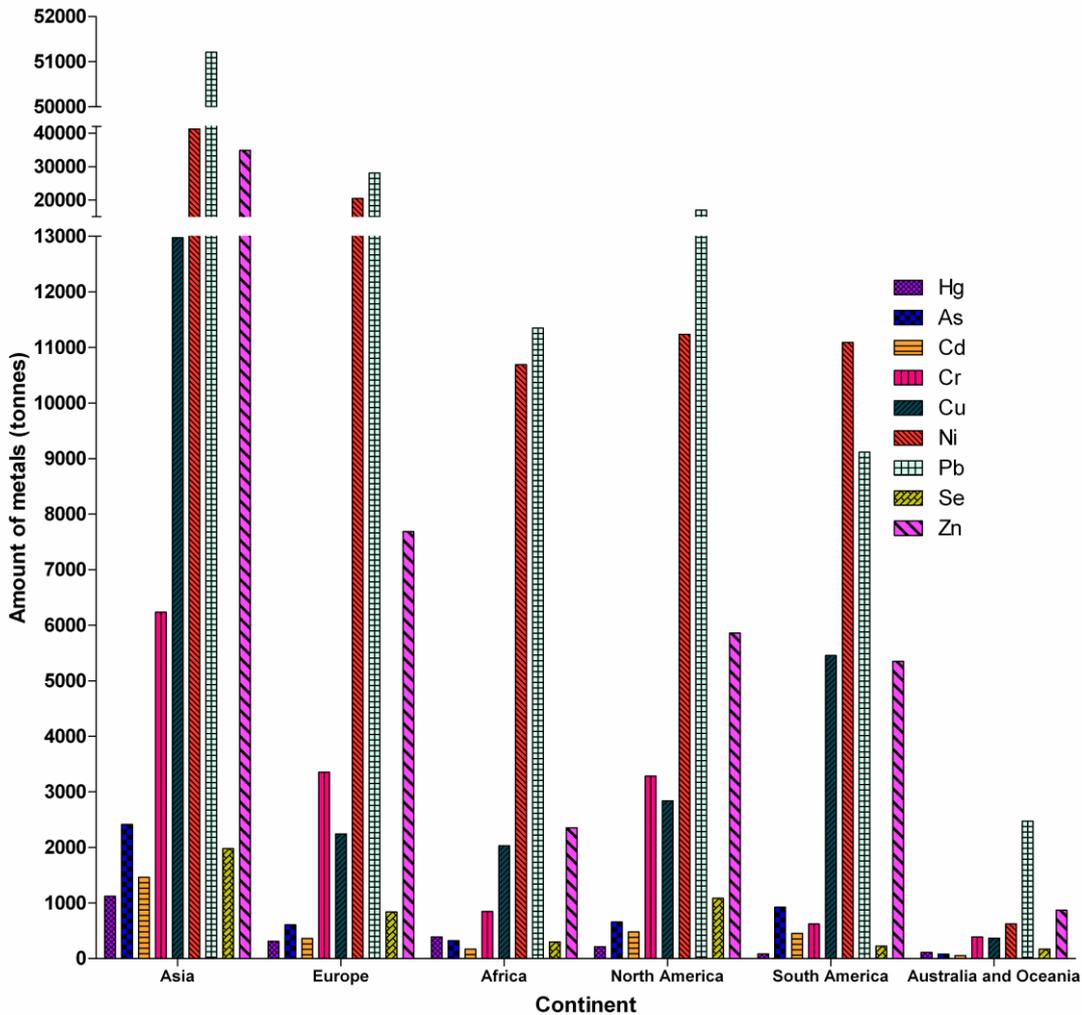


Fig. 2 Worldwide Emission of heavy metals from major anthropogenic categories to the atmosphere

In recent years As contamination in drinking water as well as in soil has been studied extensively as it adversely affect the human life and plants survival. Arsenic is a ubiquitous element and is assumed to be the 20<sup>th</sup> most abundant element in the biosphere. Being a metalloid As can present in soil, water, air and all living matter in any of the form of solid, liquid and gas. Arsenic, primarily in the inorganic form, is present in the earth's crust at an average of 2-5 mg kg<sup>-1</sup>. However, As contamination has become a widespread problem in many parts of the world. Arsenic contamination in natural aquifers has occurred in Argentina, Bangladesh, Cambodia, Chile, China, Ghana, Hungary, India, Mexico, Nepal, New Zealand, the Philippines, Taiwan, the United States and Vietnam.

Arsenic pollution has occurred most severely in Bangladesh and India (West Bengal). It is estimated that more than 35 million people are consuming As-polluted ground water alone in Bangladesh where

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underground water is used mainly for drinking, cooking and other household activities. Until now, lots of effort has been given to find safe drinking water there but no suitable measure has been established yet. In addition to the drinking water problem, continued irrigation with As-contaminated water increases the extent of As contamination in agricultural land soil in Bangladesh.

In a surveys on As contamination in 60 of 64 districts of Bangladesh, it was observed that many tube wells of shallow depth (less than 100 m) exceed the As concentration level of  $0.05 \text{ mg L}^{-1}$  (Bangladesh Standard for arsenic in drinking water) in almost all 60 districts. In a study conducted by BGS/DPHE (2000), it was observed that about 61% of samples exceed  $0.01 \text{ mg L}^{-1}$  (WHO Guideline for As concentration in drinking water), about 45% samples exceed  $0.05 \text{ mg L}^{-1}$ , and 2% exceed  $1 \text{ mg L}^{-1}$  of As concentration in shallow tubewell. Some studies reported As concentration in uncontaminated land of Bangladesh, which varies from  $3\text{-}9 \text{ mg As kg}^{-1}$  (Ullah, 1998; Alam and Satter, 2000). On the other hand, elevated As concentrations were observed in many studies in agricultural land soil irrigated with As-contaminated water, which is in some cases found about 10-20 times higher than As concentration in non-irrigated land.

### Heavy metal transport in soil-plant-water system and their uptake

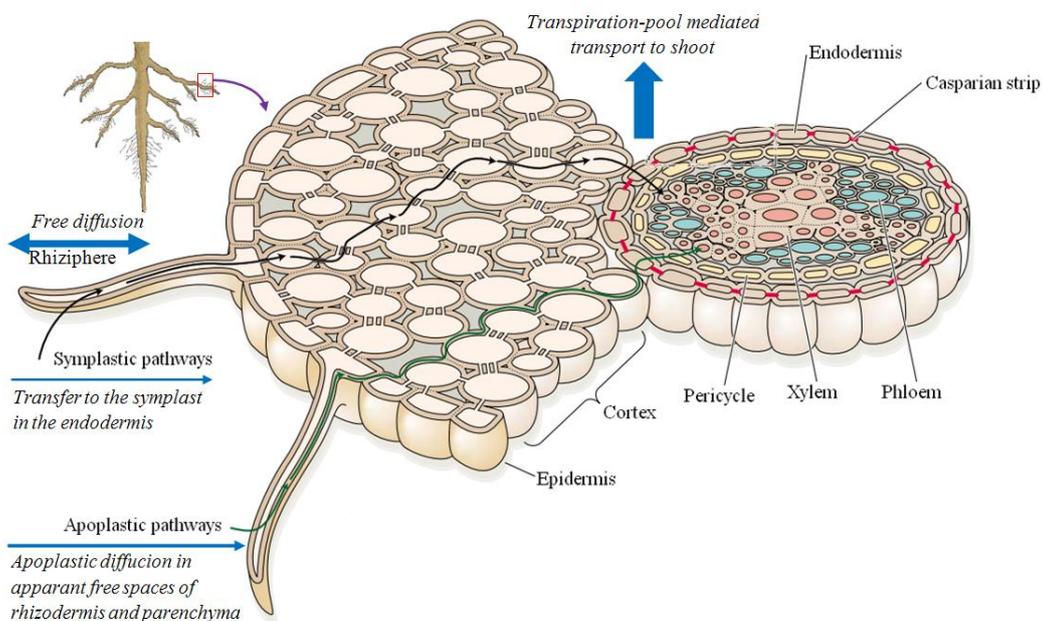


Fig. 3 Heavy metal uptake and transport by plants

In soils, HMs are retained in three ways:

- i. by adsorption onto the surface with mineral particles,
- ii. by complexation with humic substances in organic particles and
- iii. by precipitation reactions.

In general, only a fraction of soil metal is readily available (bioavailable) for plant uptake. Maximum amount of HMs in soils are presents as insoluble compounds; however, different rhizopheric activities of plants enhances the availability of the metals and facilitates the uptake by roots. After a series of complicated physical and chemical reactions, HMs in soil are absorbed by plant. In fact, the metal uptake by roots may take place at the apical region or from the entire root surface depending upon the type of metals, the uptake capacity and growth characteristics of the root system.

Uptake of most of the metals is performed by the younger parts of the roots where, the casparian strips are not fully developed. Two different processes have been suggested for metal uptake: (a) passive uptake, which is driven by the concentration gradient across the membrane and (b) active uptake which is substrate-specific, energy-dependent and carrier mediated. To initiate the uptake

process by roots, metal species must occur in soluble form adjacent to the root membrane. The availability of metal species in soluble form has a strong influence on its uptake, mobility and toxicity within the plant. Uptake studies of metals reveal that many metal pollutants such as As, Cd and Pb are taken up in the rice roots against the concentration gradient.

Toxicity of metals within the plant tissues could be due to direct interaction of metals with biomolecules such as enzymes, or displacement of cations from specific sites of enzymes and other biomolecules. Heavy metals uptaken by plant then released into the atmosphere through volatilization. A part of metals is leached down to the underground water or run-off to the surface water. These contaminated metals then enter into the food chain directly or indirectly.

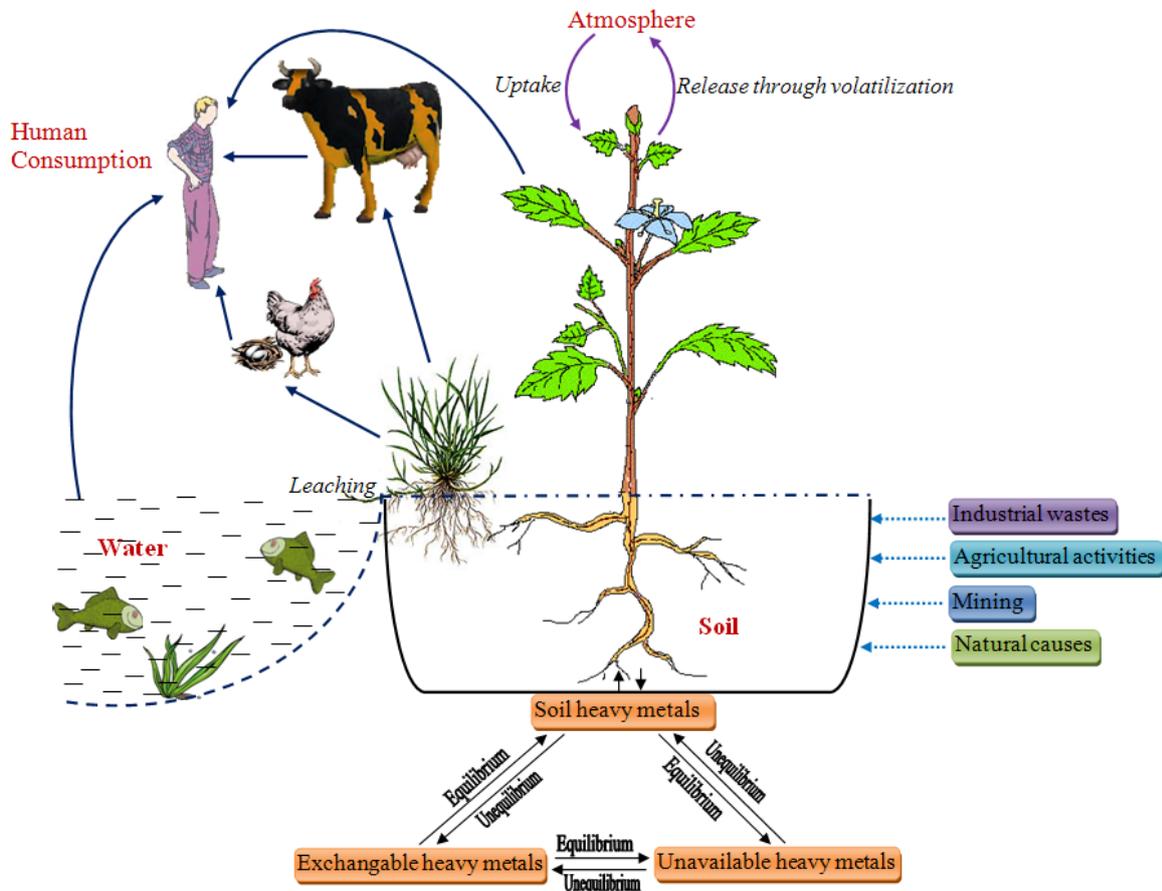


Fig. 4 Interaction among plants, heavy metals, soils and human activities

### Metal toxicity in plants

Making a generalization about the effect of HMs on plants is difficult due to the multidimensional variations in parameters under different concentrations, types of HMs, duration of exposure, target organs of plants, plant age, etc. Several physio-biochemical processes in plants cells are affected by HMs. The most obvious plant reaction under HM toxicity is the inhibition of growth rate. Heavy metals also cause chlorosis, necrosis, leaf rolling, inhibition of root growth, stunted plant growth, altered stomatal action, decreased water potential, efflux of cations, alterations in membrane functions, inhibition of photosynthesis, altered metabolism, altered activities of several key enzymes, etc. (Fig. 5). Direct phytotoxic effects of HMs include their direct interactions with proteins, enzymes, displacement of essential cations from specific binding sites, causing altered metabolism, inhibiting the activities of enzymes, etc.. Initially, a HM interacts with other ionic components present at the entry point of a plant root system. Later, the HM ion reacts with all possible interaction partners within the cytoplasm, including proteins, other macromolecules and metabolites. After that, HMs influence homeostatic events, including water uptake, transport and transpiration and thus symptoms start to develop and become visible, eventually leading to the death of plant.

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Fig. 5 Common effects of toxic metals in plants

### Heavy metals and seed germination

The germination rate and seedling growth are largely affected by HM toxicity and it is often used to assess the abilities of plant tolerance to metal elements. Since seed germination is the foremost physiological process affected by toxic elements, the ability of a seed to germinate in a medium containing any metal element would be a direct indicative of its level of tolerance to this metal.

### Heavy metals and plant growth

Inhibition of plant growth is often used as an indicator for toxic HMs (Lal, 2010). Heavy metals either retard the growth of the whole plant or plant parts (Shanker et al., 2005). In general, plant roots show rapid changes in growth pattern than shoots as they have the direct contact with the contaminated soils (Fig. 7).

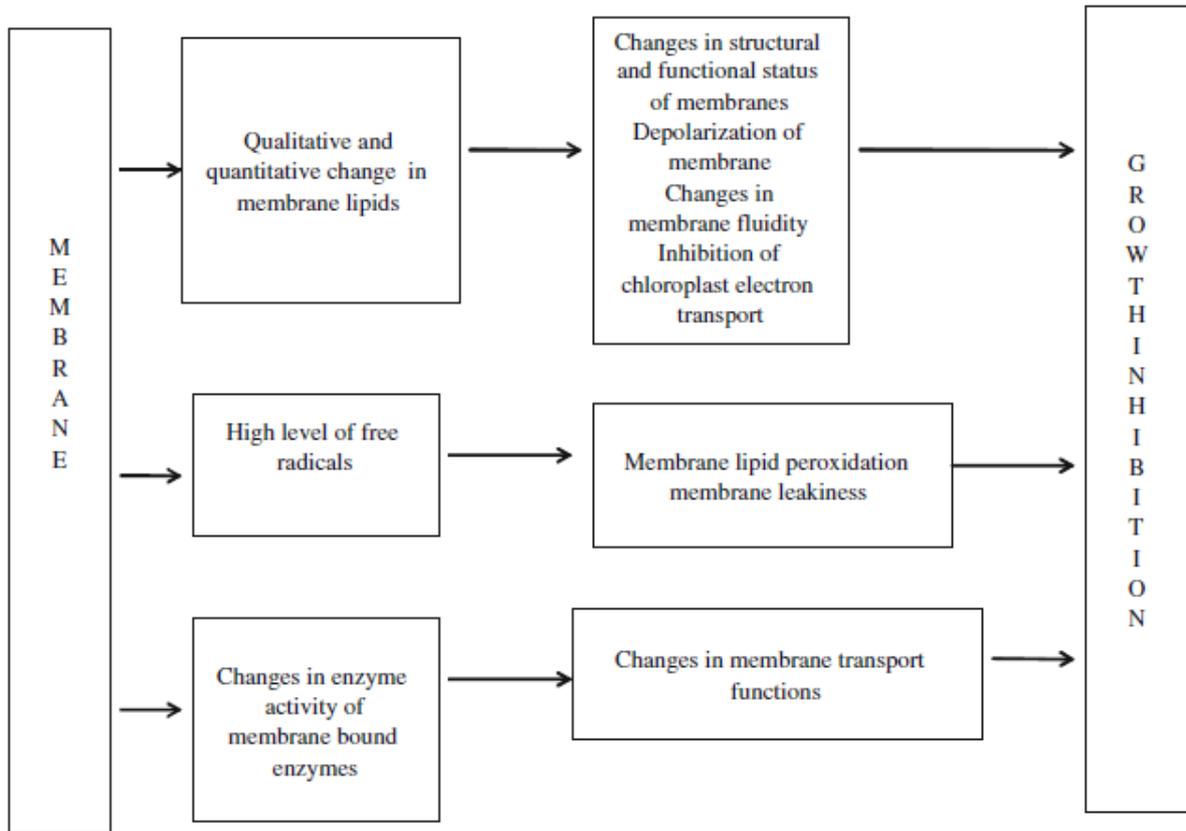


Fig. 7 Heavy metal-induced growth inhibition in plants

**Water relations**

The toxic effects of HMs in plants connected with water relations, especially in the early stage of plant growth. Heavy metals influence on membrane transport and an inhibition of root growth and enzyme activities. Heavy metals can also decrease in number and size of leaves, stomatal size, number and diameter of xylem vessels, increased stomatal resistance, increase of leaf rolling and leaf abscission, higher degree of root suberization. Many plant studies revealed that the application of HMs increased the stomatal resistance and in this way decreased the rate of transpiration. It is assumed that stomatal closure after the application of HMs is a consequence of stress due to water deficiency. This leads to increased levels of proline and ABA which are known indicators of drought stress.

**Photosynthesis**

One of the most obvious effects of HMs is the reduction of photosynthesis in plants. Heavy metals directly affect light and dark phases of photosynthesis. They also decrease the photosynthetic pigment content and change stomatal function which ultimately affects the photosynthesis (Fig. 8). It seems that nearly all of the components of the photosynthetic apparatus are influenced by almost all HMs, including chlorophyll and carotenoid content, chloroplast membrane structure, light-harvesting and O<sub>2</sub>-evolving complexes, photosystems and constituents of the photosynthetic electron transport chain. Several enzymes involved in the Calvin cycle are also inhibited; especially Rubisco and PEP carboxylase which inhibit the photosystem II (PSII) activity and electron transport. Heavy metals also change the chloroplast ultrastructure and pigment composition, inhibition in net photosynthetic rate, decreased carboxylation efficiency of RuBisCO, inhibition in photosystem II (PSII) activity, and electron transport.

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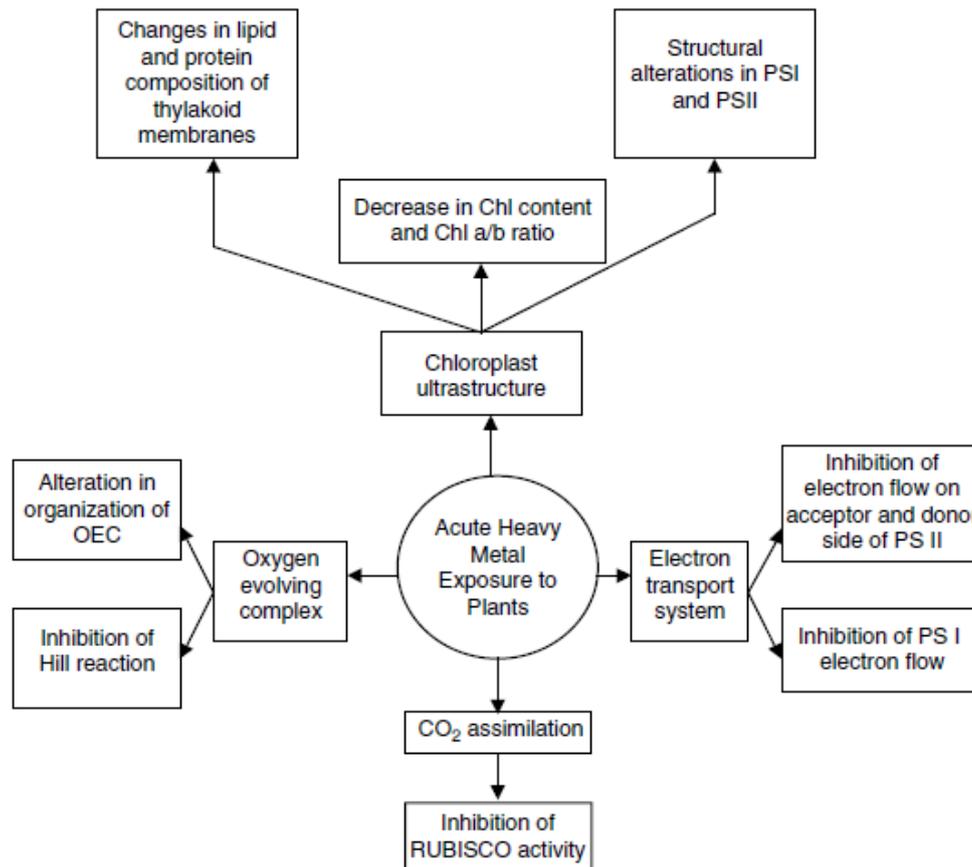


Fig. 8 heavy metal-induced inhibition of photosynthesis in higher plants

## Respiration

Although, some researchers consider that respiration increases under stress, for instance, *Vicia faba* exposed to 1  $\mu\text{M}$   $\text{Cd}^{2+}$  showed increased transpiration. In fact, at toxic concentrations of HMs, respiration is usually inhibited. However, this is true that in some plant species the presence of some HMs at lower concentrations increases respiration.

## Metal toxicity and oxidative stress

There is enough evidence that exposure of plants to excess concentrations of redox active HM results in oxidative injury. Metals of biological significance are of two groups' viz. redox-active (Fe, Cu, Cr, Co) and non-redox-active (Cd, Pb, Zn, Ni, Al). Metals with lower redox potentials than those of biological molecules cannot participate in biological redox reactions. The redox-active metals, unlike non-redox-active metals are directly involved in redox reactions in the cell. Heavy metal uptake by transporters and distribution to organelles is followed by ROS generation, stimulated either by HM redox activity or by the effects of an HM on metabolism in a subcellular site-specific manner.

## Phytoremediation

Phytoremediation is defined as "the engineered use of plants *in situ* and *ex situ* for environmental remediation". This technology has gained recent attention as strategies to clean-up contaminated soils and water. These strategies refer to the use of higher plants and their associated microbiota for the *in situ* treatment of soil, sediment, and ground water. The phytoremediation is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove HMs, including radionuclides, from soil and water. Phytoremediation has recently become a subject of intense public and scientific interest and a topic of many recent reviews. Phytoremediation has been reported to be an effective, non-intrusive, aesthetically pleasing, socially accepted technology to remediate polluted soils. Biologically based remediation strategies, including phytoremediation, have been estimated to be 4 to 1000 times cheaper, on a per volume basis, than current non-biological technologies.

### Different kinds of phytoremediation/Mechanisms

Fundamental and applied researches have unequivocally demonstrated that selected plant species possess the genetic potential to remove, degrade, metabolize, or immobilize a wide range of contaminants by different process (Fig. 9). The phytoremediation process may be divided into following heads based on their nature of remediation process:

1. Phytoextraction/Phytoaccumulation
2. Phytostabilization
3. Phytodegradation/Phytotransformation
4. Rhizofiltration
5. Rhizodegradation
6. Phytovolatilization
7. Phytorestauration

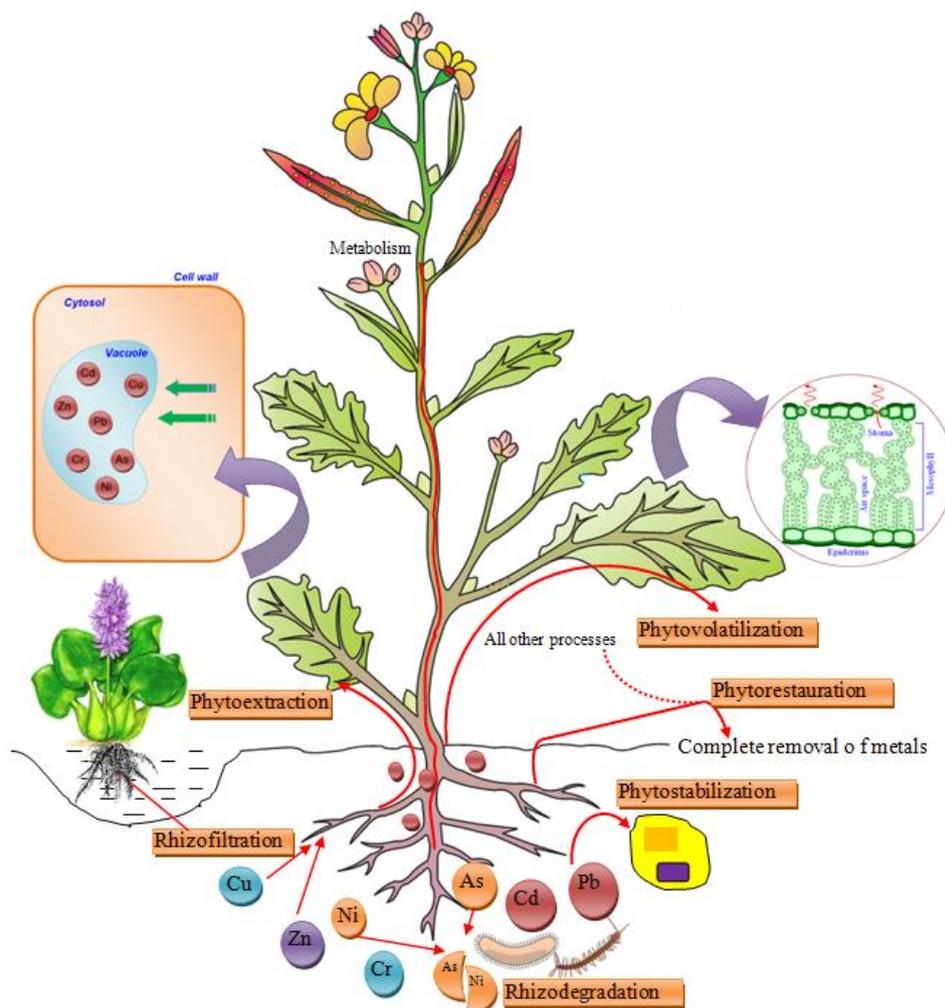


Fig. 9 Different process of phytoremediation of toxic metals

### Phytoextraction/ Phytoaccumulation

The use of plants to remove contaminants from the environment and concentrate them in above-ground plant tissue is known as phytoextraction. Phytoextraction is also called phytoaccumulation. Among the phytoremediation mechanisms, phytoextraction is the most important and have been extensively studied. This remediation technique is employed to recover metals from contaminated soils using non-food crops. In this case metals are absorbed by roots and subsequently translocated within the above ground parts of plants. Some plants possess the genetic and physiological potential

to accumulate, translocate and tolerate high concentration of metals which is concerned to the phytoextraction technology. The discovery of some wild plants having accumulating capacity of high concentration of metals provided the idea of using such plants to remove HMs from soils.

Phytoextraction process is of two types: i) Natural or continuous phytoextraction; and ii) Induced or chelate-assisted phytoextraction. Natural phytoextraction employs the natural ability of the plant to remediate the soil. Some plants have been identified that have the potential to take up HMs.

At least about 45 families have been identified as hyperaccumulator plants; these are Brassicaceae, Fabaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae.

Considering the pattern of metal accumulation, plants are divided into three main types;

- i. Accumulator plants which accumulate high quantity of metals primarily in shoots;
- ii. Indicator plants which accumulate metal concentrations in different plant tissues corresponding to the increasing available content of metals in soils and
- iii. Excluder plants which maintain low metal concentrations in their shoots even if the external metal concentration in the environment is high i.e. insensitive for uptake and accumulation of metals.

The efficiency of phytoextraction is determined by the accumulation factor, indicating the ratio of HM concentrations in the plant organs (shoots, roots) and in the soil and the seasonally harvestable plant biomass. There are three processes that regulate the movement of HMs from root tips to xylem (root symplast). The sequestration of HMs occurs inside cells; they then subjected to symplastic transport to the stele, and subsequent release into the xylem (Fig. 10). Generally the HM content in various plant organs decreases in the following sequence of root > leaves > stems > inflorescence > seed. However the metal accumulating capacity greatly varies with plant species.

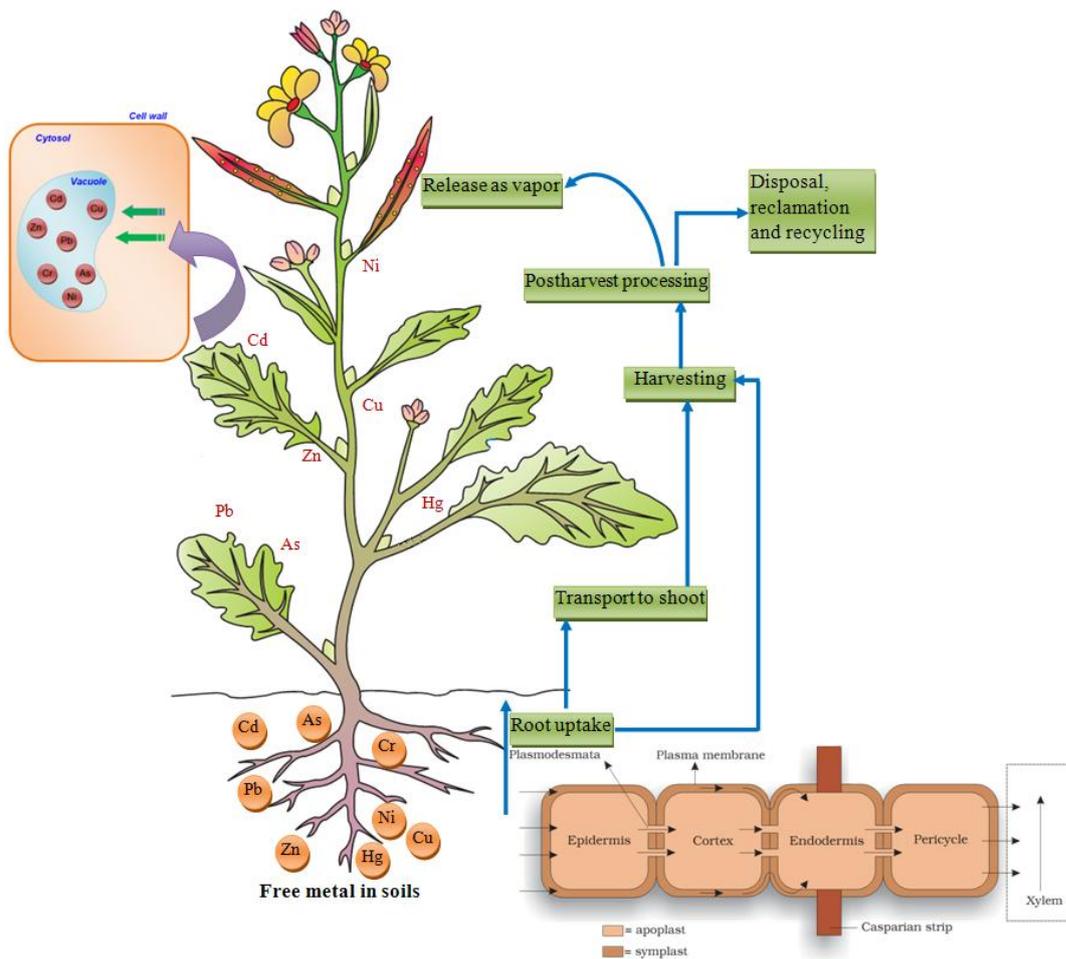


Fig. 10 Phytoextraction of toxic metals

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### Characteristics of a good phytoaccumulator plant

- Plants to be used for phytoextraction should have:
- High metal uptake and translocation accumulation capacity,
- Tolerance to high concentrations metals without showing toxic symptom,
- Fast growth pattern
- High biomass producing capacity
- Extensive root system. The success of phytoextraction depends especially on these characteristics. Ability of plants to withstand difficult soil conditions (i.e. soil pH, salinity, soil structure, water content) is also important.

### Phytostabilization

Phytostabilization is the removal of soil metals through absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone. In this case, contaminated metals around the roots become insoluble and/or immobilized. It depends on roots' ability to limit metal's mobility and bioavailability in the soil (Fig. 11). This process can occur through the sorption, precipitation, complexation, or metal valence reduction. Plants used for phytostabilization are of poor translocators of metal from root to shoots, such as grasses, thus minimizing exposure to toxic elements.

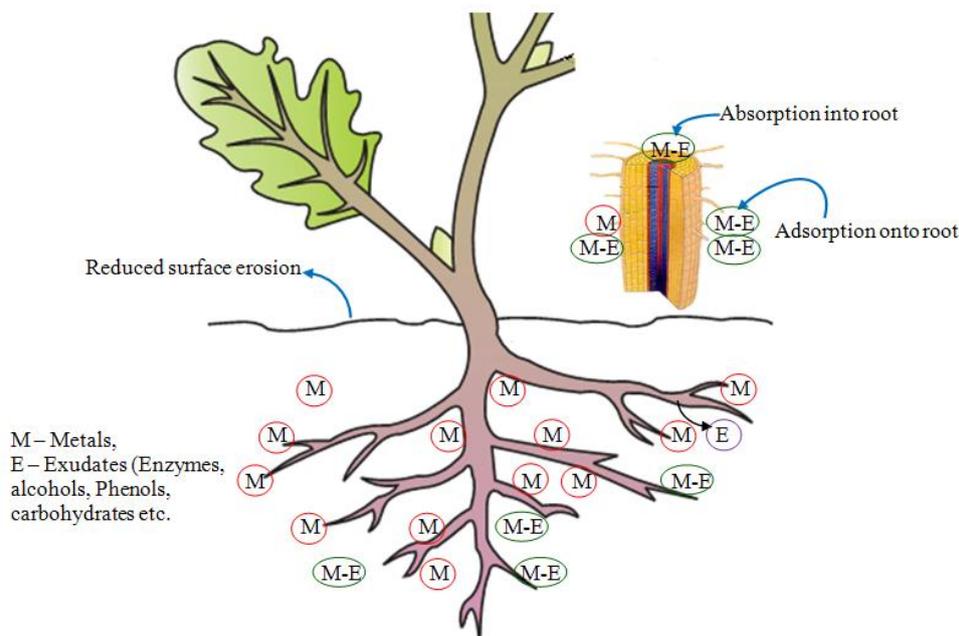


Fig. 11 Phytostabilization of toxic metals

### Phytodegradation/ Phytotransformation

Phytodegradation is often called phytotransformation. In this process, the contaminants taken up by plants is broken down into simpler or less toxic molecules with the help of some compounds (such as enzymes) synthesized by plants and then incorporated/and or metabolized into their vascular systems. Plants synthesize a large number of enzymes as a result of primary and secondary metabolism and can quickly uptake and metabolize organic contaminants to less toxic compounds (Fig. 12). Plants having phytodegradation capacity contain enzymes that can catalyze the degradation of contaminants. These enzymes are generally dehalogenases, oxygenases, and reductases.

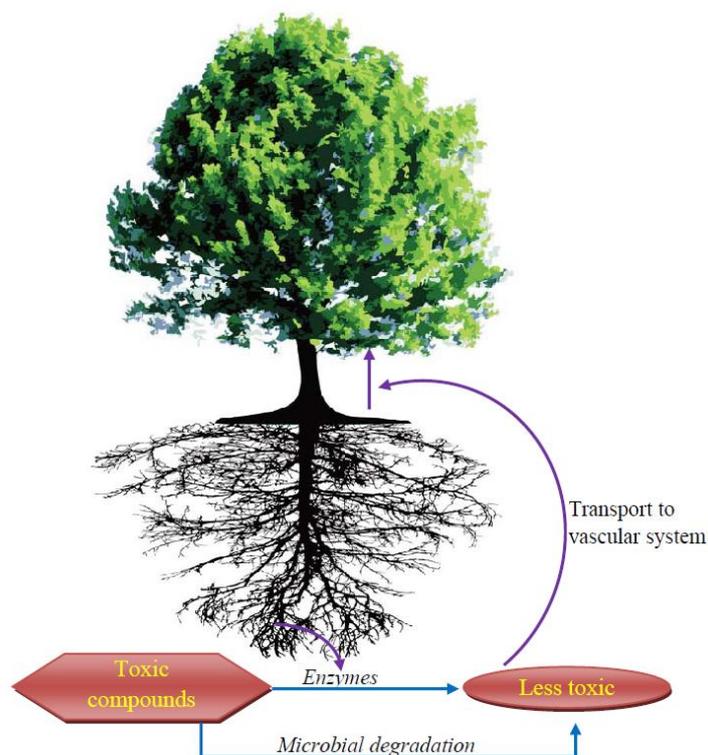


Fig. 12 Phytodegradation of toxic pollutants from soil

### Rhizofiltration

Rhizofiltration is a process of phytoremediation by using terrestrial and/or aquatic plants to uptake, concentrate and precipitate metals from contaminated sources through its root system (Fig. 13). This mechanism highlights the removal of toxic metals derived from aquatic environments such as damp soil and groundwater by the rhizosphere. The ideal plants for rhizofiltration should possess extensive root system and root biomass and the ability to accumulate and tolerate higher amounts of toxic metals and involves easy handling and a low maintenance cost. This technology can be used for HMs like Pb, Cd, Cu, Ni, Zn, and Cr, which are primarily held within the roots. A variety of plants like *B. juncea*, *N. tabacum*, *H. Annuus*, *Spinacia oleracea* and *Z. mays* have been studied for their ability to remove Pb from effluent, with sunflower showing the greatest ability. Mustard has proven to be very effective at removing lead at a wide range of concentrations (4–500 mg L<sup>-1</sup>).

However, some researchers reported the limited potentiality of aquatic plants in rhizofiltration process due to their small, slow growing roots as well as the high water content which complicates their drying, composting, or incineration.

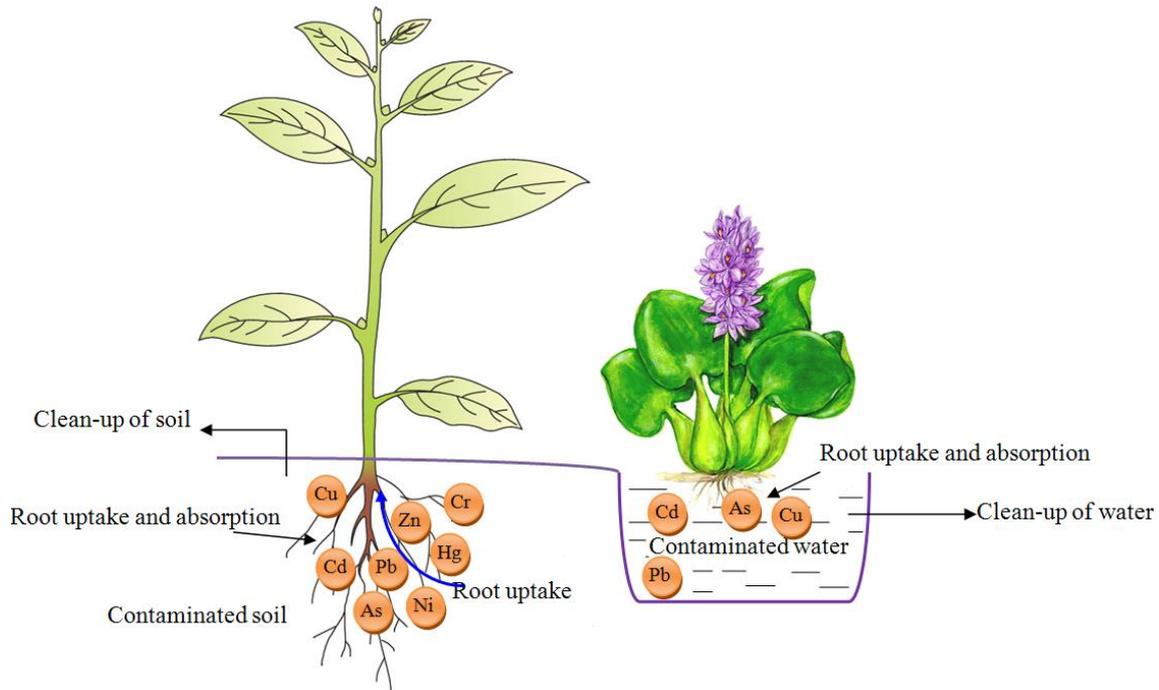


Fig. 13 Rhizofiltration of toxic heavy metals from soil and aquatic environment

**Rhizodegradation**

Rhizodegradation is the breakdown of contaminants in the soil through the microbial activity of the rhizosphere (Fig. 14). It is also called phytostimulation, rhizosphere biodegradation, enhanced rhizosphere biodegradation, or plant-assisted bioremediation/degradation. This process is slower process than phytodegradation. Rhizodegradation takes place in the rhizosphere, by creating beneficial conditions for microbial growth and development in the rhizosphere. Microorganisms such as fungi, bacteria, yeast consume and assimilate organic matter (fuels and solvents). Afterward, these breakdown products are either volatilized or incorporated into the microorganisms and soil matrix of the rhizosphere. However, the microbial population, their activities and the degradation process depends on the types of plants and the soil condition. Grasses with high root density, legumes that fix nitrogen are suitable for microbes and they create a more aerobic environment in the soil that stimulates microbial activity and thus enhances oxidation of organic chemical residues.

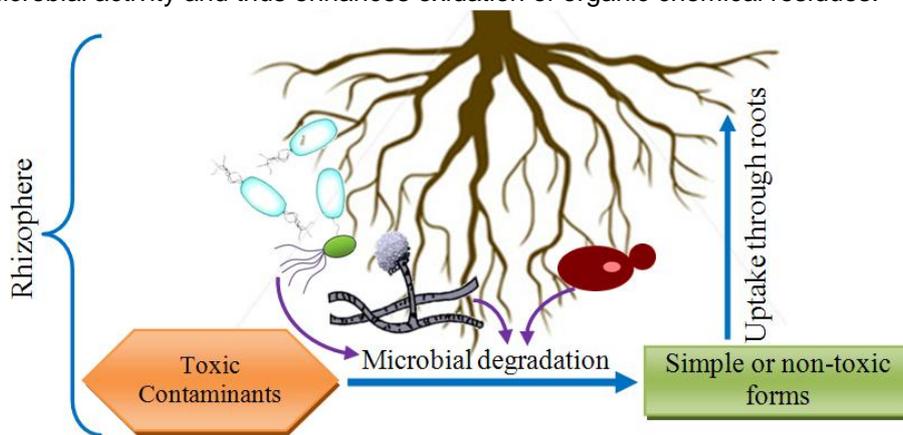


Fig. 14 Rhizofiltration of pollutants

**Phytovolatilization**

Phytovolatilization is a special process of phytoremediation using plants to accumulate toxic compounds from soil followed by transformation into volatile form and release into the atmosphere

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through transpiration (Fig. 15). Phytovolatilization occurs as growing trees and other shrubs and herbs take up water, organic and inorganic contaminants. This process is based on the facts that some of the toxic contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Phytovolatilization is successfully used for the remediation of As, Hg and Se contaminated soils. Some plant species, such as *Brassica juncea*, *Arabidopsis thaliana* and *Chara canescens* are reported to possess capability to absorb HMs and convert them to volatile forms within the plant which ultimately release them into the atmosphere.

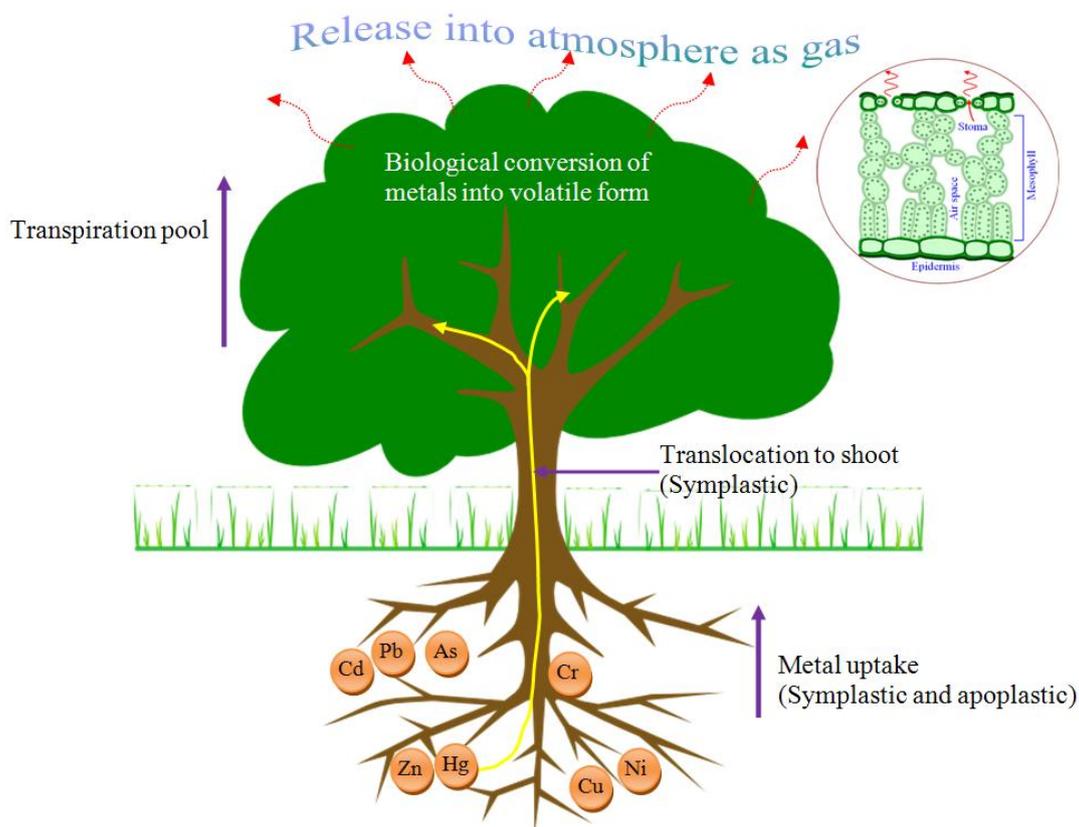


Fig. 15 Phytovolatilization of toxic metals in the environments

### Phytorestauration

Phytorestauration is the complete remediation of metal contaminated soils to make it suitable for normal activities as non-contaminated soils. This process of phytoremediation uses plants that are native to the specific area, with a view to restore the land to its natural state.

### Suitable plants for phytoremediation

The phytoremediation potential of a plant mostly depends on how much metals it can uptake without showing any toxic symptoms and its capacity to transform these toxic metals to regular cellular metabolism. Before considering a plant for efficient phytoremediation, following characteristics should be considered:

- Capacity to take up and degrade higher concentration of toxic metals from the soil and water.
- Translocation and sequestration capacity of the uptaken metal to the cellular systems
- Higher capacity to release the exudates to stimulate the growth of microorganisms and secretion of enzymes required for transformation or degradation of toxic contaminants.
- Extensive root system and higher growth rate
- Tolerant to adverse environmental conditions
- Capacity to tolerate the higher concentration of toxic metals

However, the best plants for a particular phytoremediation task should be selected based on multiple plant characteristics.

### **Limitations of phytoremediation**

Although phytoremediation has several advantages, it also has some limitations.

- This process is restricted to sites with shallow contamination within rooting zone of remediative plants.
- It may take up to several years to remediate a contaminated site.
- It is restricted to sites with low contaminant concentrations.
- Harvested plant biomass from phytoextraction may be classified as a hazardous waste hence disposal should be done carefully.
- Climatic conditions are a limiting factor. Introduction of exotic species may affect biodiversity.
- Consumption of contaminated plant biomass is also a matter of concern.

### **Use of soil amendments**

Biochar, Chetosan

### **Use of growth promoting microorganisms**

