LECTURE II

Metal Ions in the Environment

Διατμηματικό Πρόγραμμα
Μεταπτυχιακών Σπουδών
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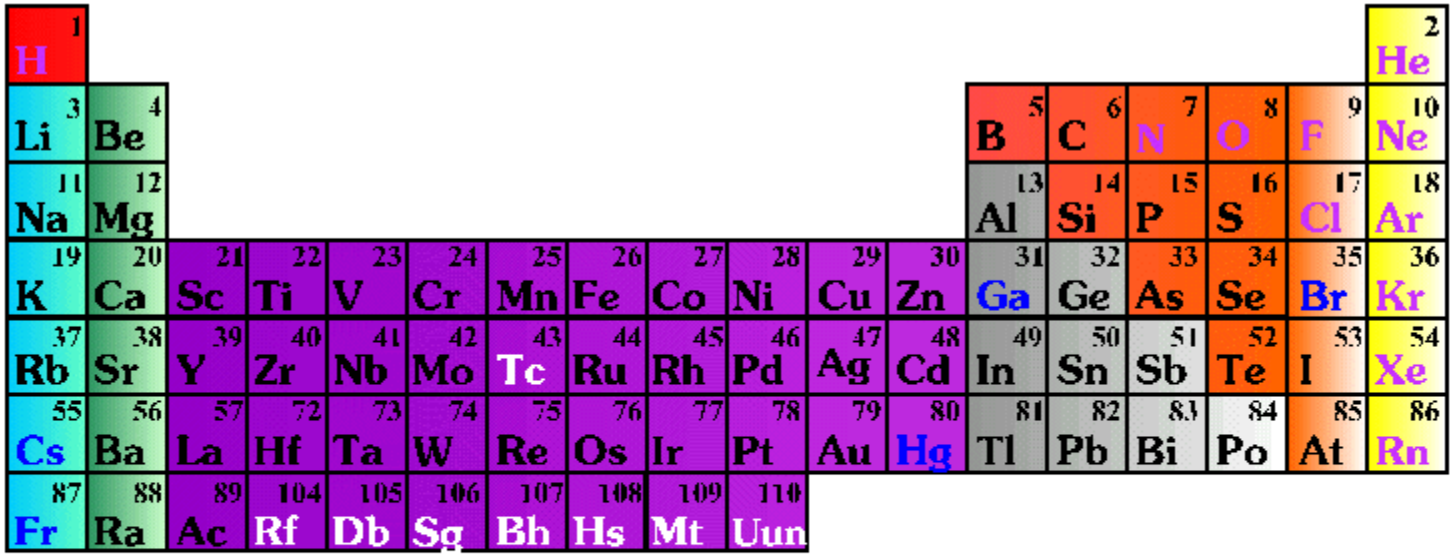
Outline of presentation

- Biology of systems in environmental chemistry of metallotoxins, with consequences on cellular health
- Interactions with thermodynamic and kinetic processes involving metal ions and biomolecular targets

Periodic Table of elements



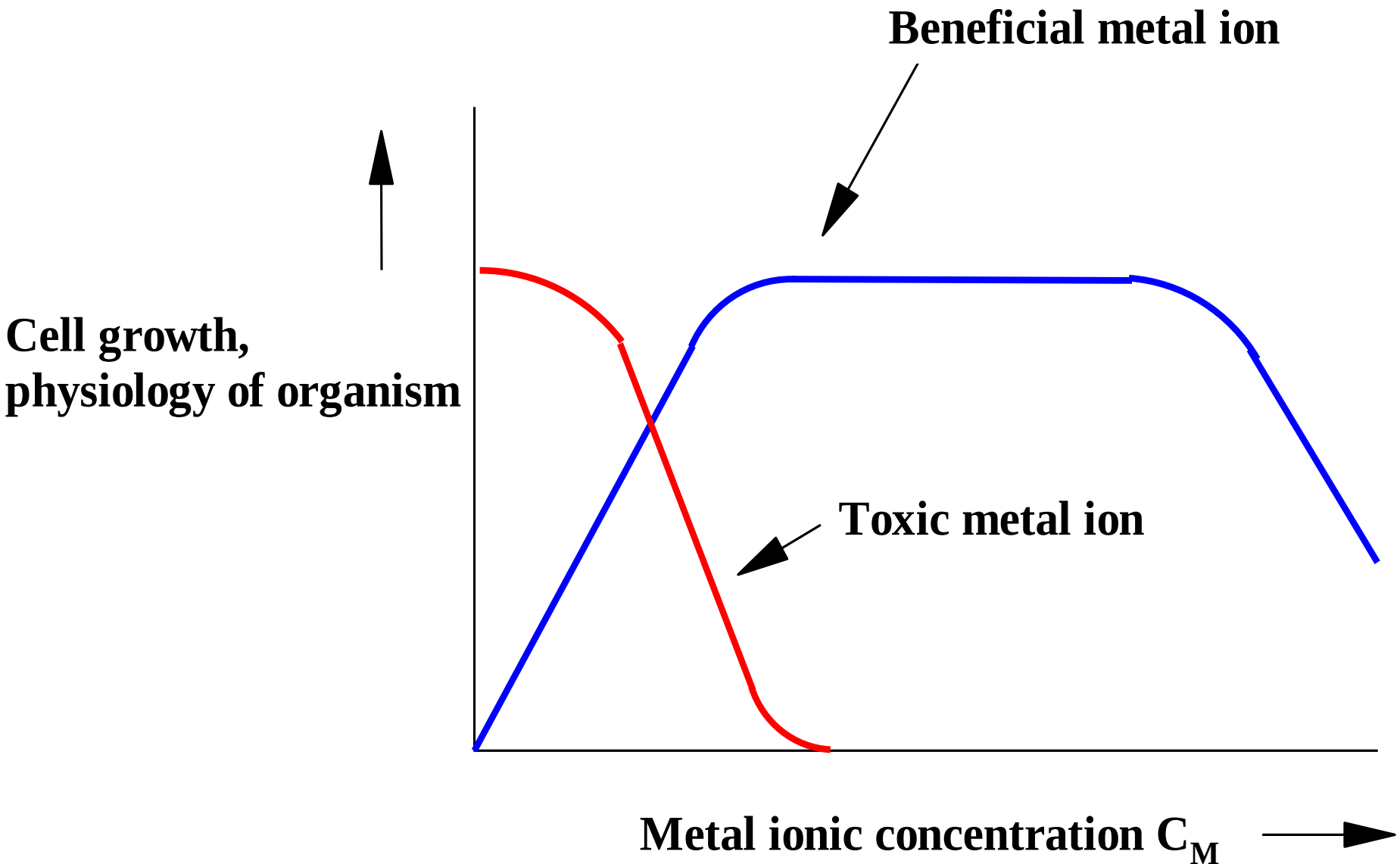
Description

Li	Solids	Cs	Liquids	Ar	Gases	No	Artificial
	Alkali metals		Alkaline earth metals		Transition metals		Rare earth metals
	Other metals		Noble gases		Halogens		Other non-metals

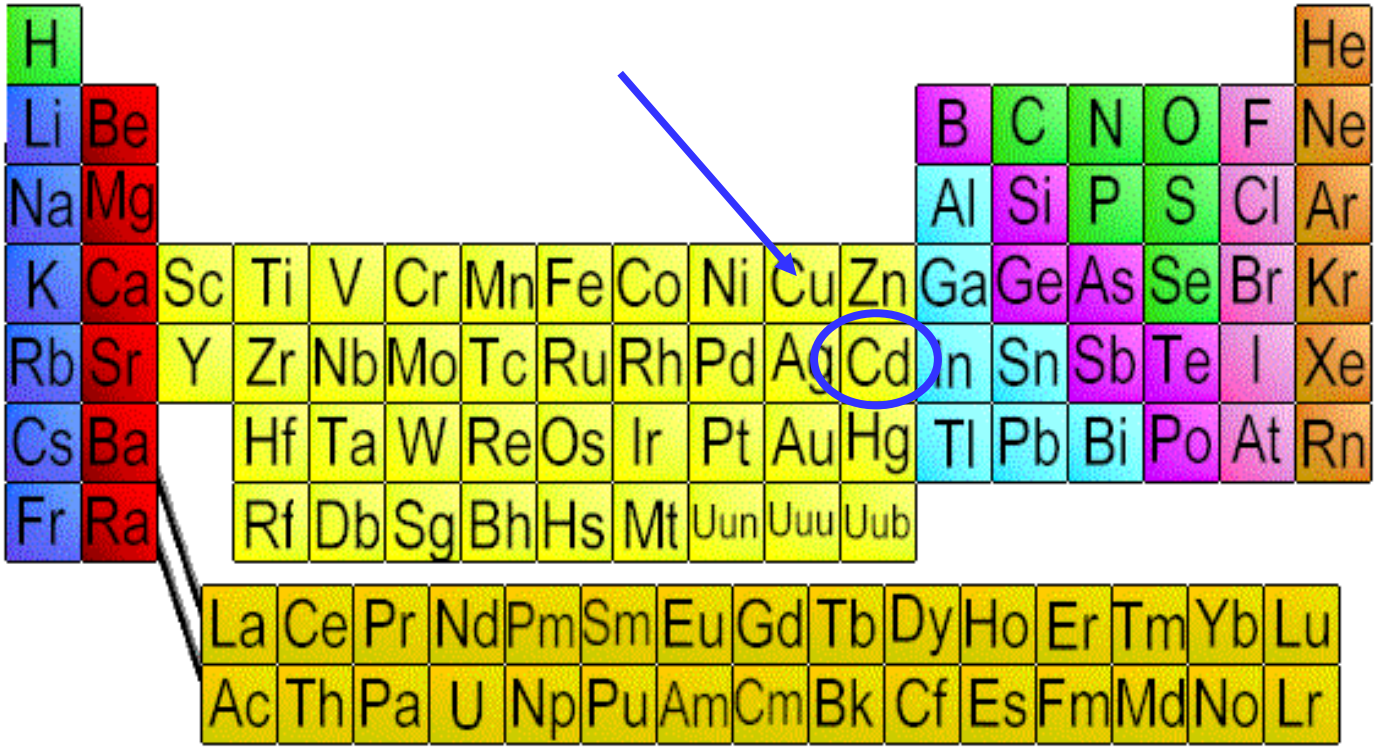
Introduction

- **The introduction of (neuro)toxic metal ions in the environment presents a definite health hazard. Anthropogenic activities linked to modern industrial operations (Cd-Ni batteries, coal burning, etc.) burden the environment with toxic metal ions, thereby affecting the ecosystem and all life forms detrimentally. Clear examples of such metals are cadmium, mercury and aluminum.**

Schematic representation of beneficial and toxic metal ionic concentration to cells



Cadmium in the natural order



Atomic Number	48
Atomic weight	112.40
Specific gravity	8.65 at 20°C
Melting point	321°C
Boiling point	765°C

Electronic configuration

Cd	**[Kr] $5s^2 4d^{10}$**
Cd(II)	**[Kr] $5s^2$**

Cadmium in nature

- **Cadmium (Cd) is an environmental pollutant - anthropogenic activities (Sanita di Toppi and Gabbrielli, 1999). Major sources of Cd pollution are industrial processes and phosphate fertilizers (Pinot et al., 2000).**
- **Long biological half-life: 6-38 year (kidneys), 4-19 years (liver)**
- **Non-essential transition metal - highly toxic.**
- **Taken up in excess by plants, it directly or indirectly inhibits physiological processes, such as respiration, photosynthesis, cell elongation, plant-water-relationship, nitrogen metabolism, and mineral nutrition, resulting in poor growth and low biomass (Hsu, 2008).**

Greenockite

CdS

Named after Lord Greenock, Scotland.

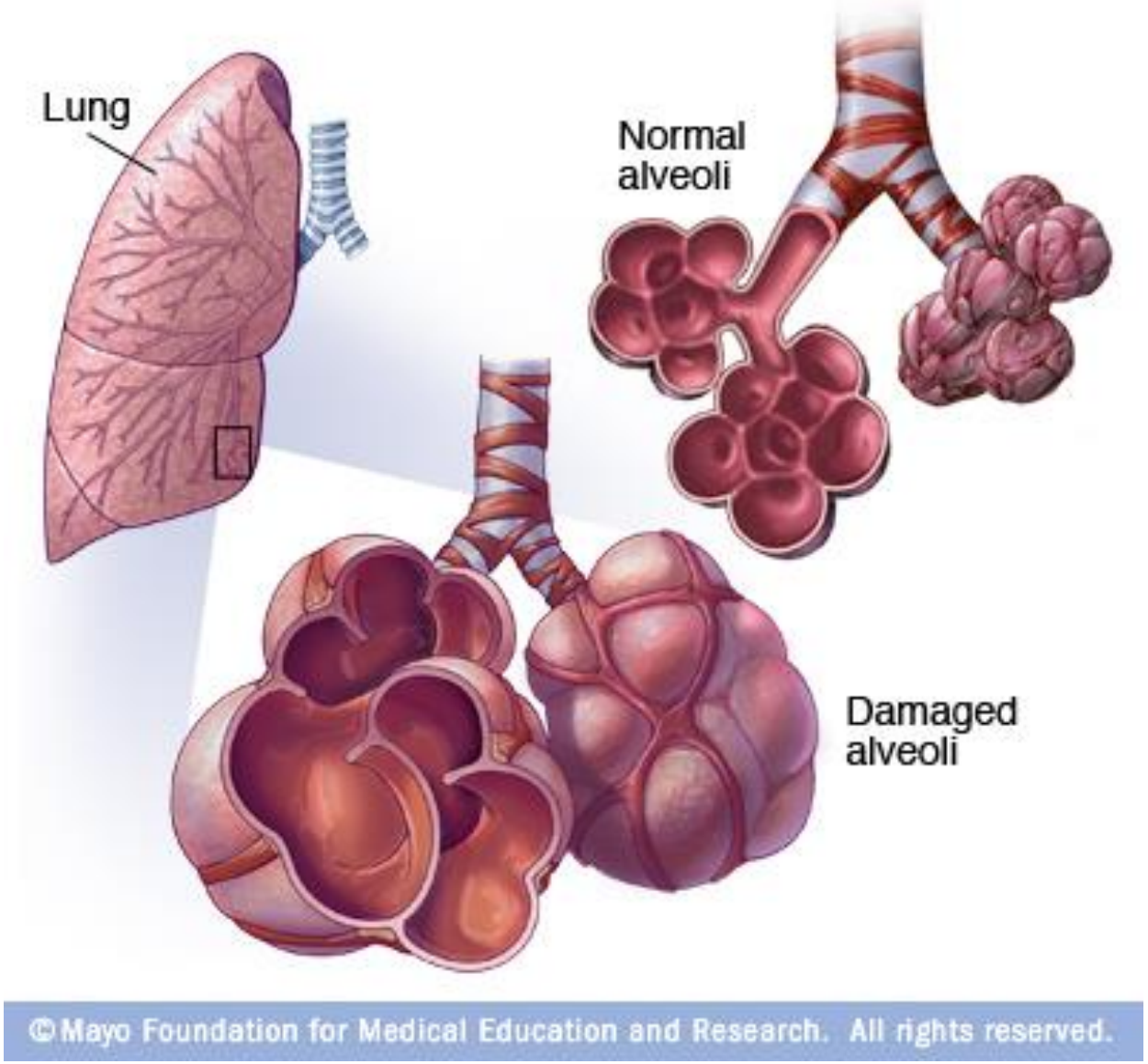
Cadmium as metal ion and a toxin

- Cadmium is one of the most important environmental pollutants.
- It is an element found in ample quantities in the form of minerals in the lithosphere of the planet.
- Cd(II) is not bioessential. It is absorbed by organs such as the liver and ends up being accumulated in the kidneys, with the latter being an important biotarget.
- Among some of the diseases attributed to Cd(II) toxicity are proteinuria, aminoaciduria, cadmium-induced renal tubular dysfunction, and cadmium-induced creatinuria.
- The exact mechanisms by which cadmium toxicity arises, however multifaceted, remain unknown.

Metals in the environment
Cadmium

- Occupational and environmental pollution with cadmium can result from heavy metal mining, metallurgy and industrial use, manufacturing of nickel–cadmium batteries, pigments, plastic stabilizers and anti-corrosive products.
- Cigarette smoke is a serious source of Cd(II) as well as food, water and air contamination.
- Chronic intoxication is associated with obstructive airway disease, emphysema, irreversible renal failure, bone disorders and immuno-suppression. In humans, cadmium exposure has been associated with cancers of the prostate, lungs and testes (it is classified as a carcinogen).

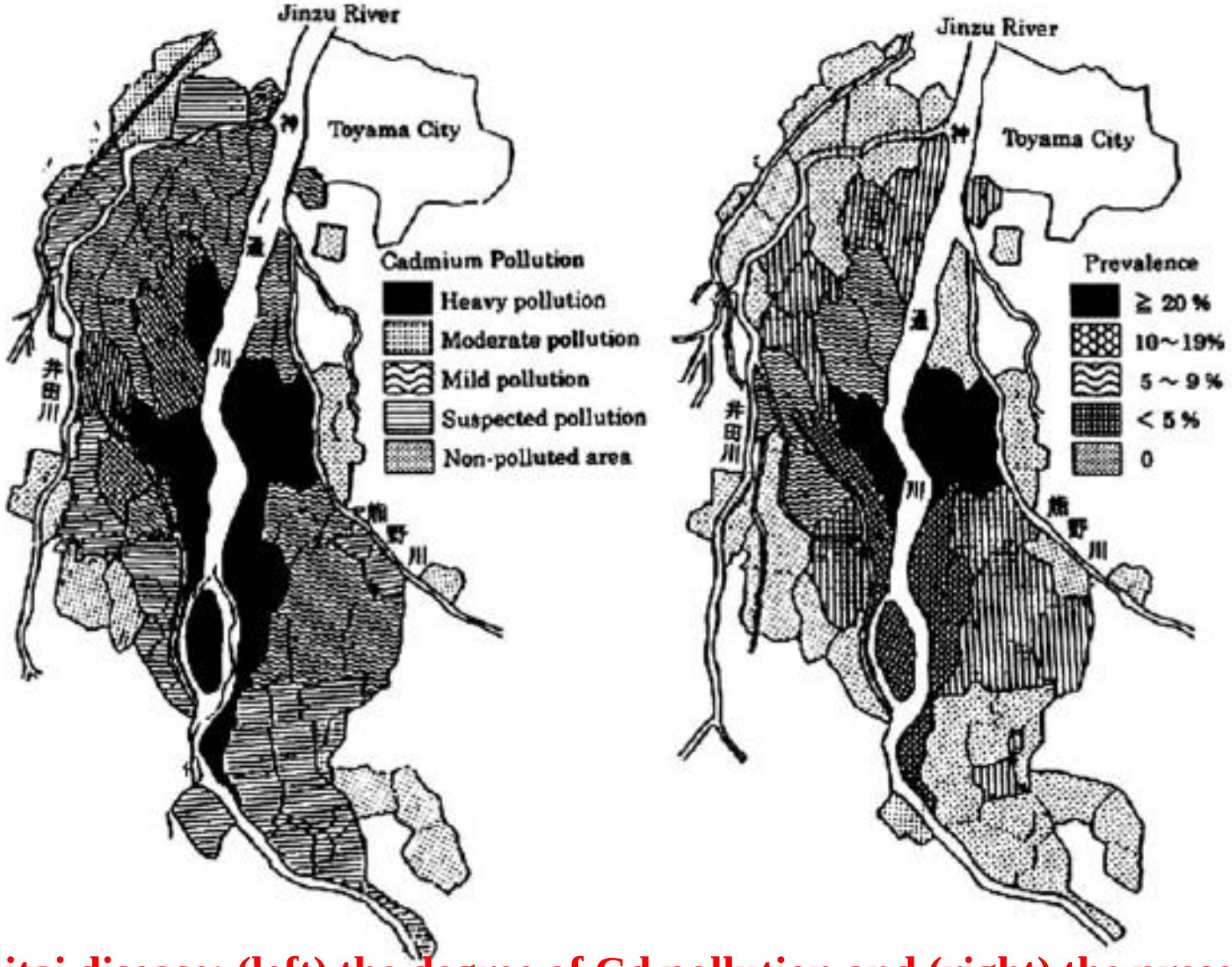
Emphysema – A lung disease



Metals in the environment
Cadmium

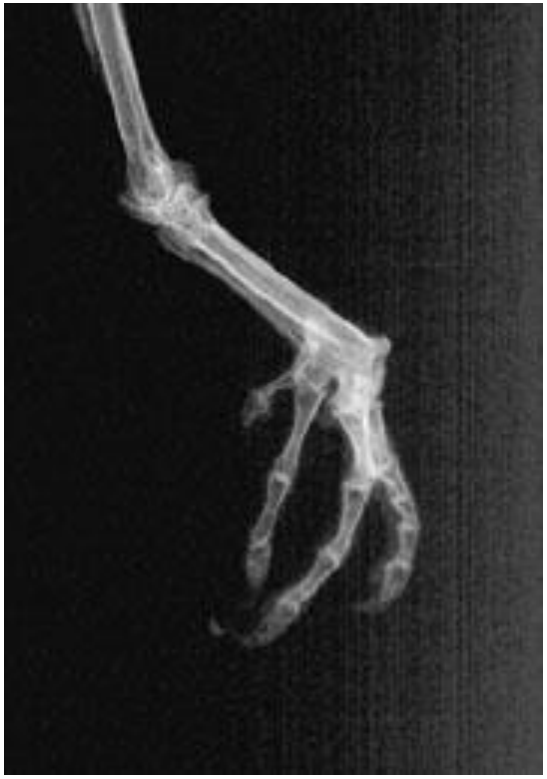
- Severe chronic poisoning from Cd(II) ingestion causes itai-itai disease
- First discovered in Toyama Prefecture in Japan in the 1950s.
- Softening of the bones and kidney failure.
- The source of Cd(II) was a zinc mine polluting the Jinzu river with Cd(II) ending up in the cultivated rice (the food chain).

Metals in the environment
Cadmium

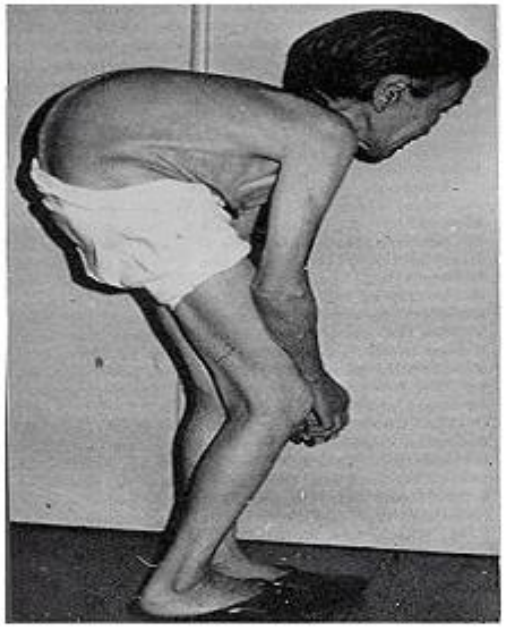


Itai-itai disease: (left) the degree of Cd pollution and (right) the presence of the disease in women over 50 years of age.

The effects of Cd(II) on human health
Itai-Itai Disease

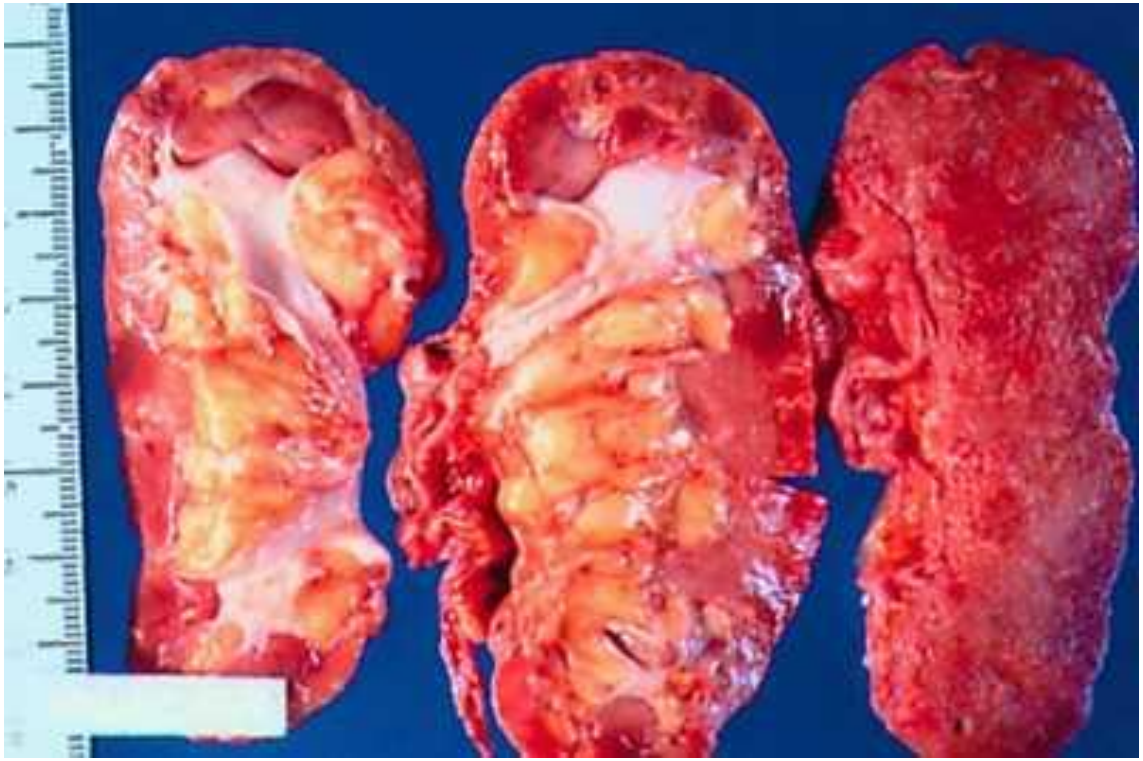


This X-ray image of a ptarmigan shows a fracture caused by calcium deficiency triggered by cadmium-damaged kidneys.



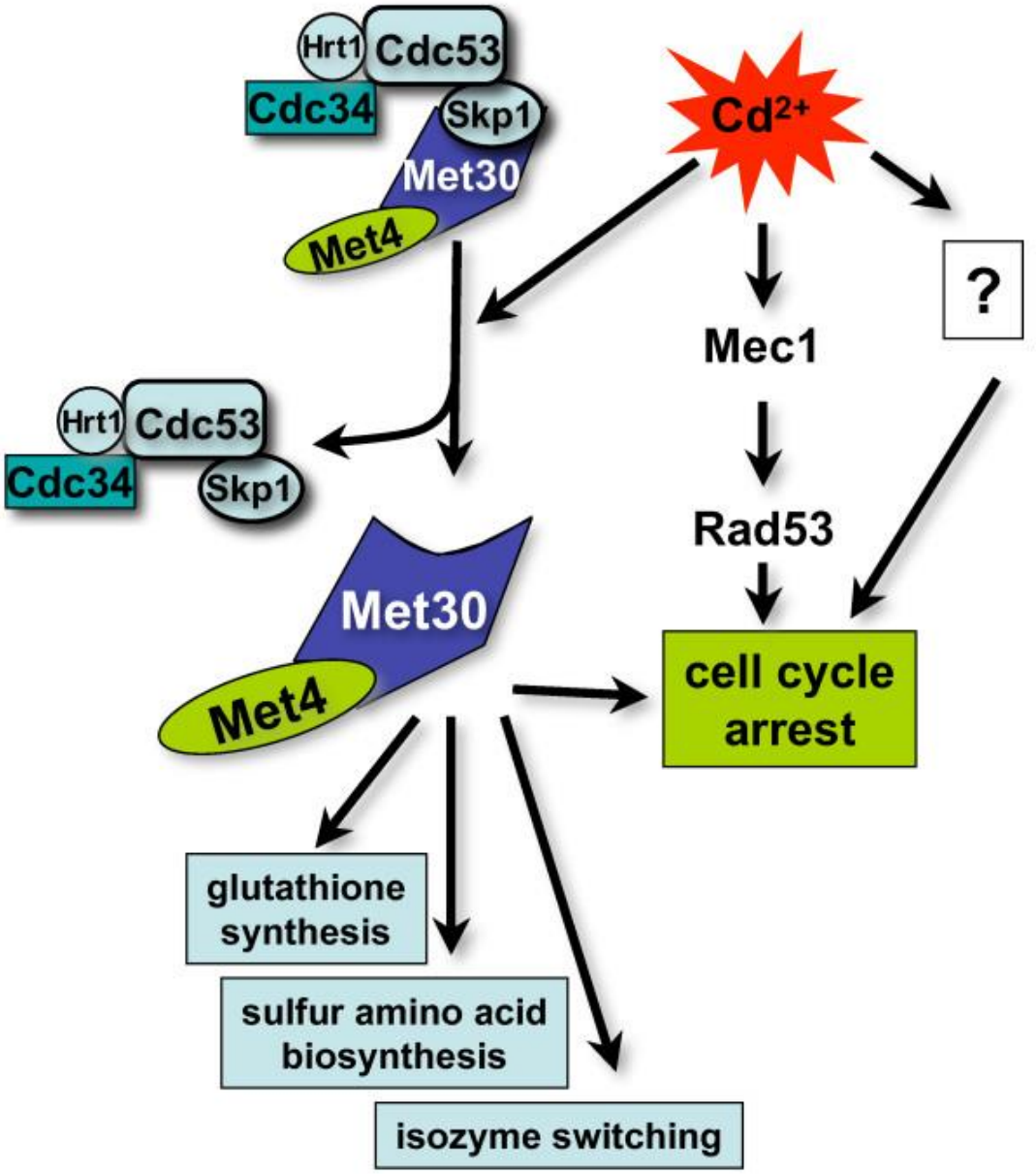
Itai-itai disease which literally means "It hurts, it hurts"

The effects of Cd(II) on soft tissue human organs
Itai-Itai Disease



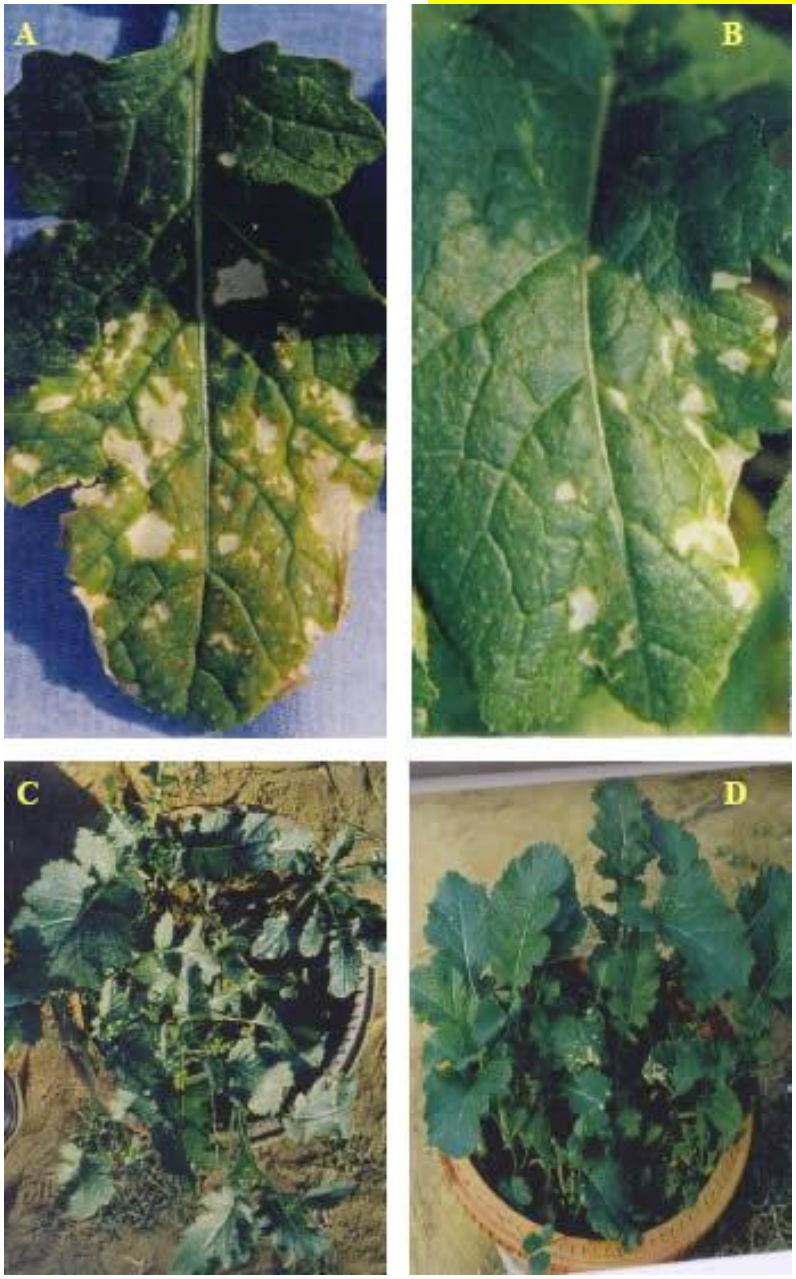
Atrophic kidney of itai-itai disease (macroscopic findings)

Cd(II) stress in cell physiology



Cadmium blocks Met4 ubiquitination to induce a Met4-dependent transcription program, which includes induction of GSH1 expression ("sulfur sparing response“). Hence, upon cadmium stress, yeast cells repress expression of several glycolytic enzymes and instead strongly induce expression of isozymes with a significantly lower content of S-containing amino acids. **Isozyme switching** has been proposed to help cells dedicate more of their sulfur resources to GSH synthesis. **Isozyme switching** could also make glycolysis less vulnerable to the toxic effects of cadmium because the induced isozymes have a markedly reduced number of -SH groups, which are the main targets for Cd(II)-induced protein damage.

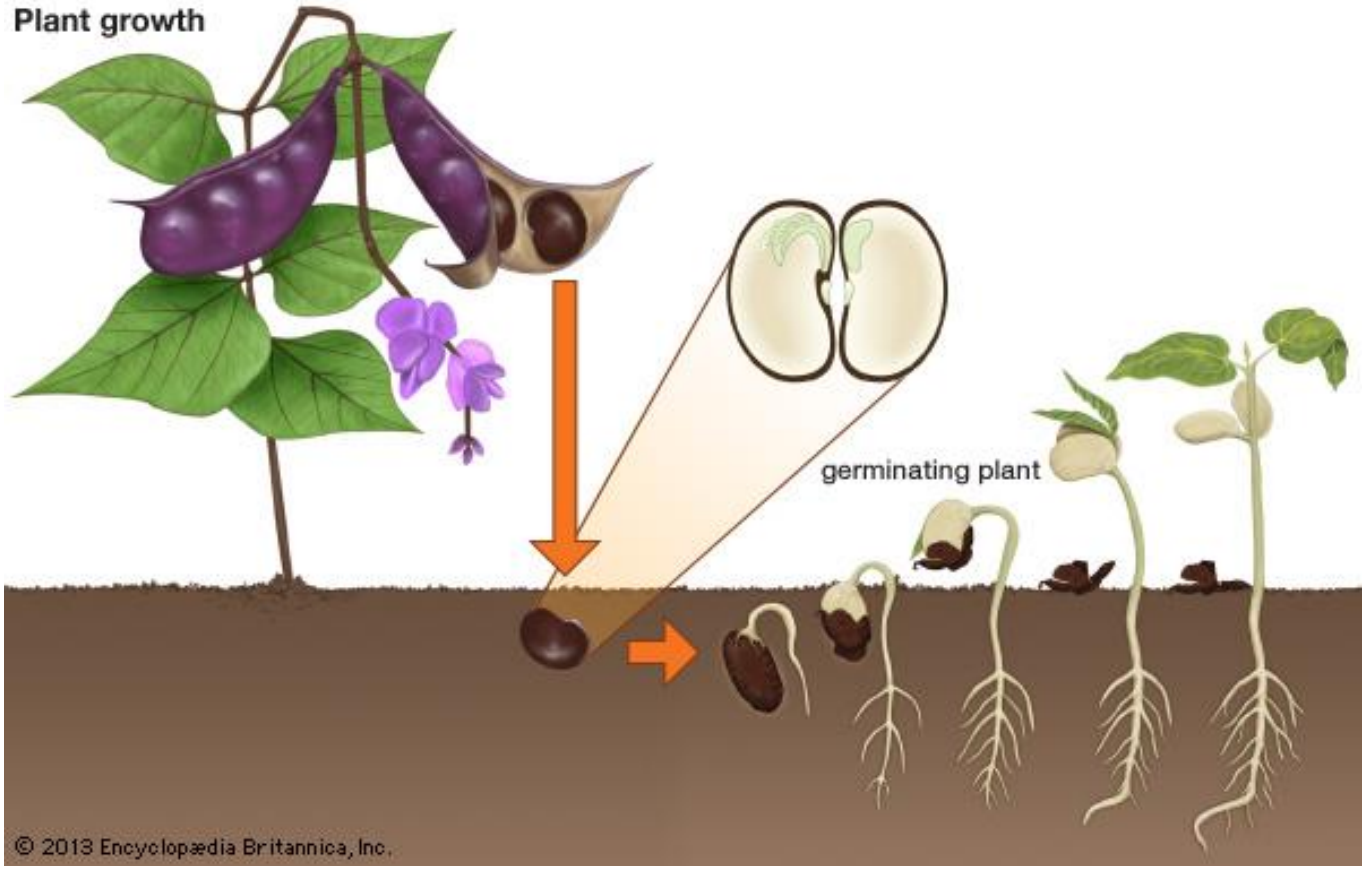
The effects of Cd(II) on plants



- Cadmium (Cd), a toxic element, is dispersed in the natural and agricultural environments mainly through human activities and has a long biological half-life (10-25 years).
- Cd(II) inhibits seed germination, plant root elongation and growth.
- Cd(II) is one of the non-essential heavy metals, toxic to flora and fauna, which is easily taken up by plant roots and translocated to the aerial plant parts.
- Cadmium accumulation reduces photosynthesis, disturbs plant-water relations and the uptake and translocation of nutrients, and results in visible injury symptoms and/or plant death

Some plants are resistant to Cd(II) absorption (tomato, cabbage) compared to soybean, spinach and curly cress.
Plants react to Cd(II) by producing S-rich peptides (phytochelatins) that complex it and neutralize it.

The effects of Cd(II) on see germination and plant growth

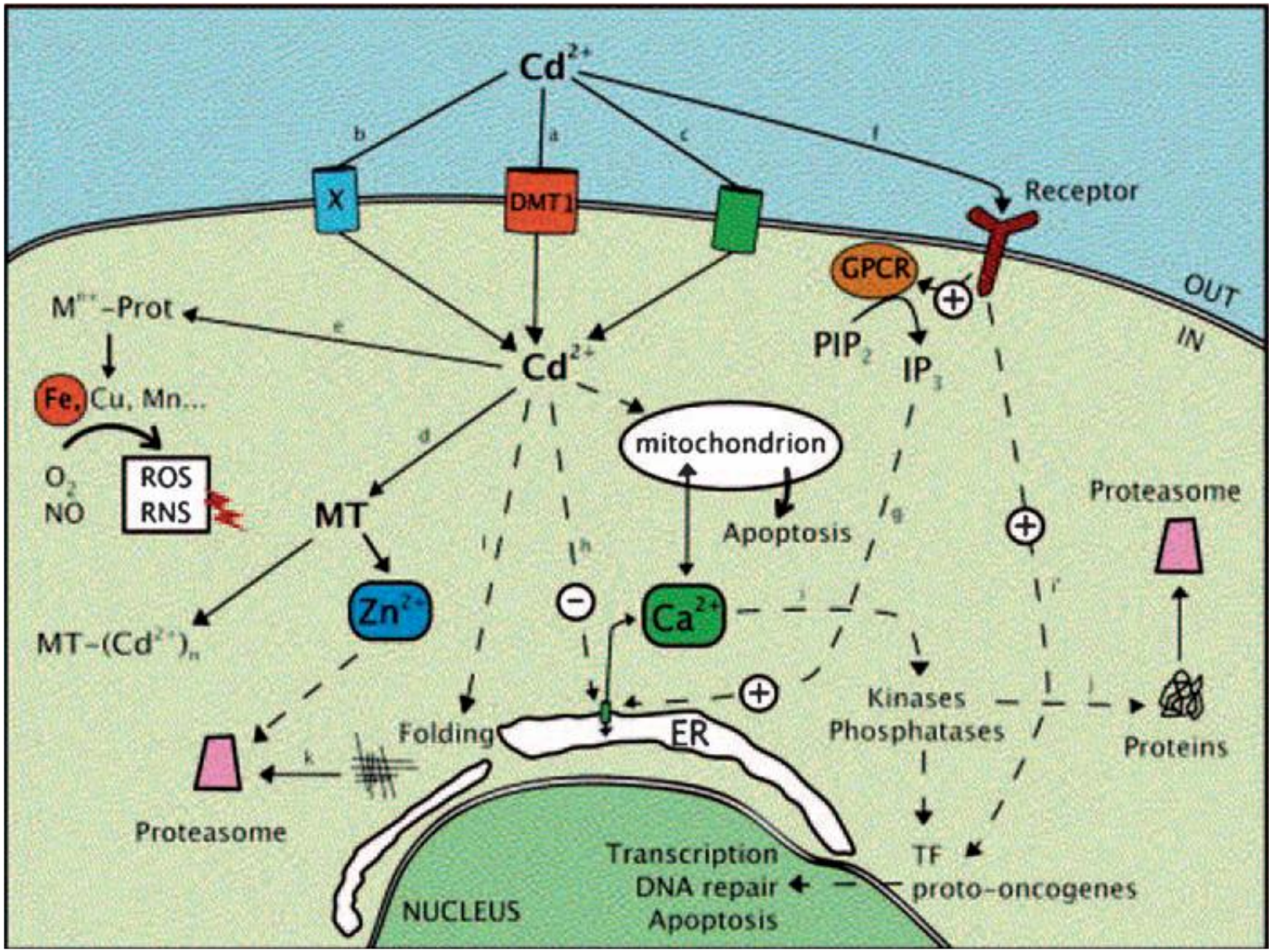


Accumulation of several metals in plants

Concentration (in ppm – Dry weight)			
Metal	**Soil**	**Plant**	**Plant:Soil ratio**
Lead (Pb)	**10**	**4.5**	**0.45**
Zinc (Zn)	**50**	**32**	**0.6**
Cadmium (Cd)	**0.06**	**0.64**	**10**

Cadmium Content in Selected Foods

Type of Food	Cadmium Content (µg/g of wet weight)
Dairy Products	0.001
Milk	0.0015-0.004
Wheat flour	0.07
Leafy vegetables	0.14
Potatoes	0.08
Garden fruit and other fruit	0.07
Sugar and adjuncts	0.04
Meat, fish, poultry	0.03
Tomatoes	0
Grain and cereal products	0.06



Schematic representation of cadmium traffic in animal cells (Martielli et al. 2006)

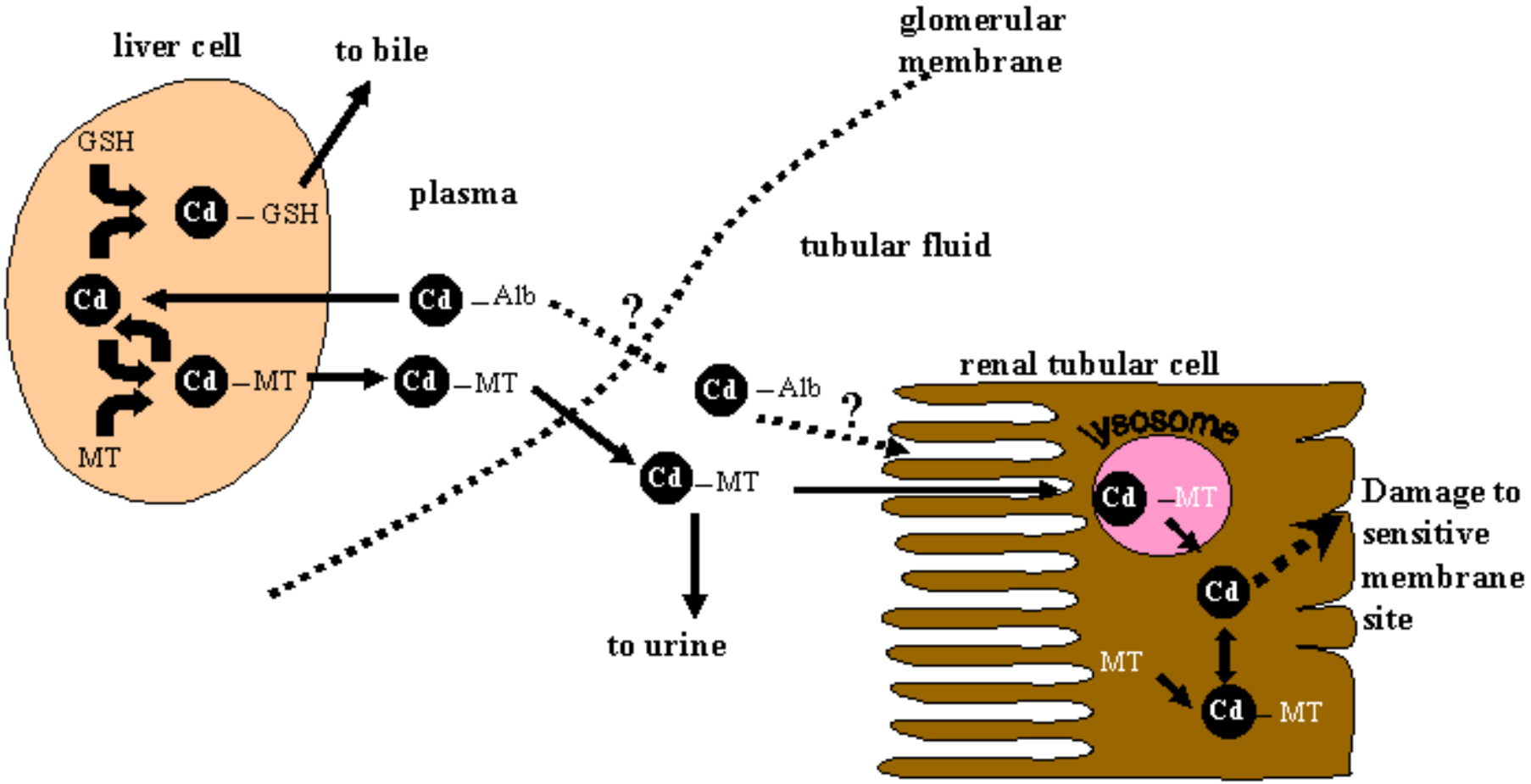
Metals in the environment
Cadmium

- It exacerbates ROS production.
- It interferes with gene expression and DNA repair. Cadmium is a genotoxic pollutant known to target proteins that are involved in DNA repair and in antioxidant defense, altering their functions and ultimately causing mutagenic and carcinogenic effects.
- It is a good soft Lewis acid looking for easily oxidized soft Lewis bases (sulfur containing amino acids). So, it would be expected to displace Zn(II) from proteins, where the zinc coordination environment is dominated by sulfur. The close similarities in the ionic radii of Cd(II) and Ca(II) (0.95 and 1.00 Å, respectively) favors exchange of the two metals in calcium-binding proteins. Cadmium can also interfere with iron.
- It enters neurons through VDCC channels and NMDA receptors.
- It enters metallothioneins (MT), competing for Zn(II). MTs are known for their ability to detoxify heavy metal ions such as Cd(II).
- A considerable number of transcription factors have reactive cysteine residues, which enable them to respond to the redox conditions in the cell. Since cadmium perturbs redox homeostasis, it can affect this class of transcription factors. If cadmium can displace the tetra-coordinate zinc atoms in zinc finger-containing transcription factors, it will affect them as well.

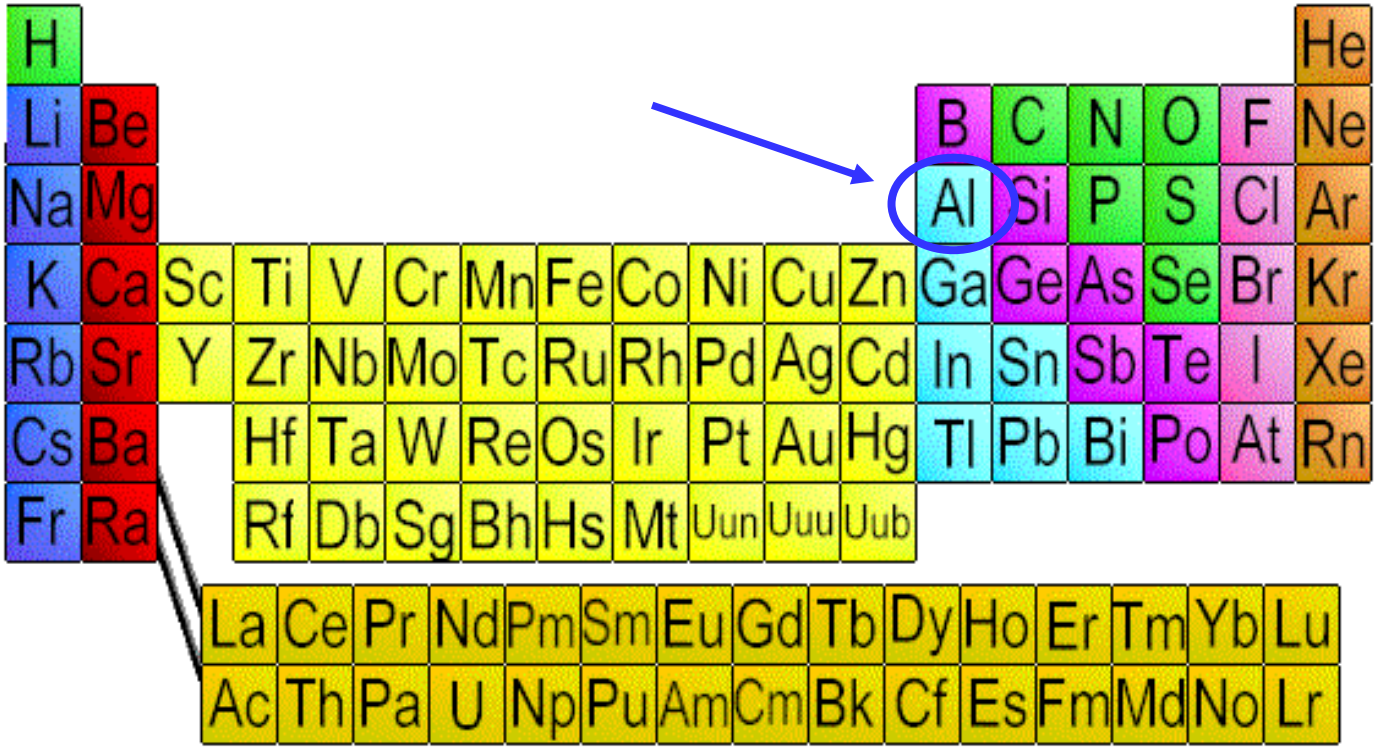
Cadmium

- Cadmium has also been shown to decrease the solubility of specific proteins.
- It can also affect protein folding.
- Cadmium is not strongly mutagenic. It is known that it causes increased oxidative DNA damage and that it inhibits the DNA repair systems. It has also been found to induce cell death both by necrosis and apoptosis. Since the latter is extremely calcium-dependent, it seems likely that the pro-apoptotic effects of cadmium are due to its interference with calcium homeostasis.

Cadmium metabolism in humans



Aluminum in the natural order



Atomic Number	13
Atomic weight	26.9815386 (8)
Specific gravity	2700 kg m^{-3}
Melting point	2519 °C
Boiling point	765°C

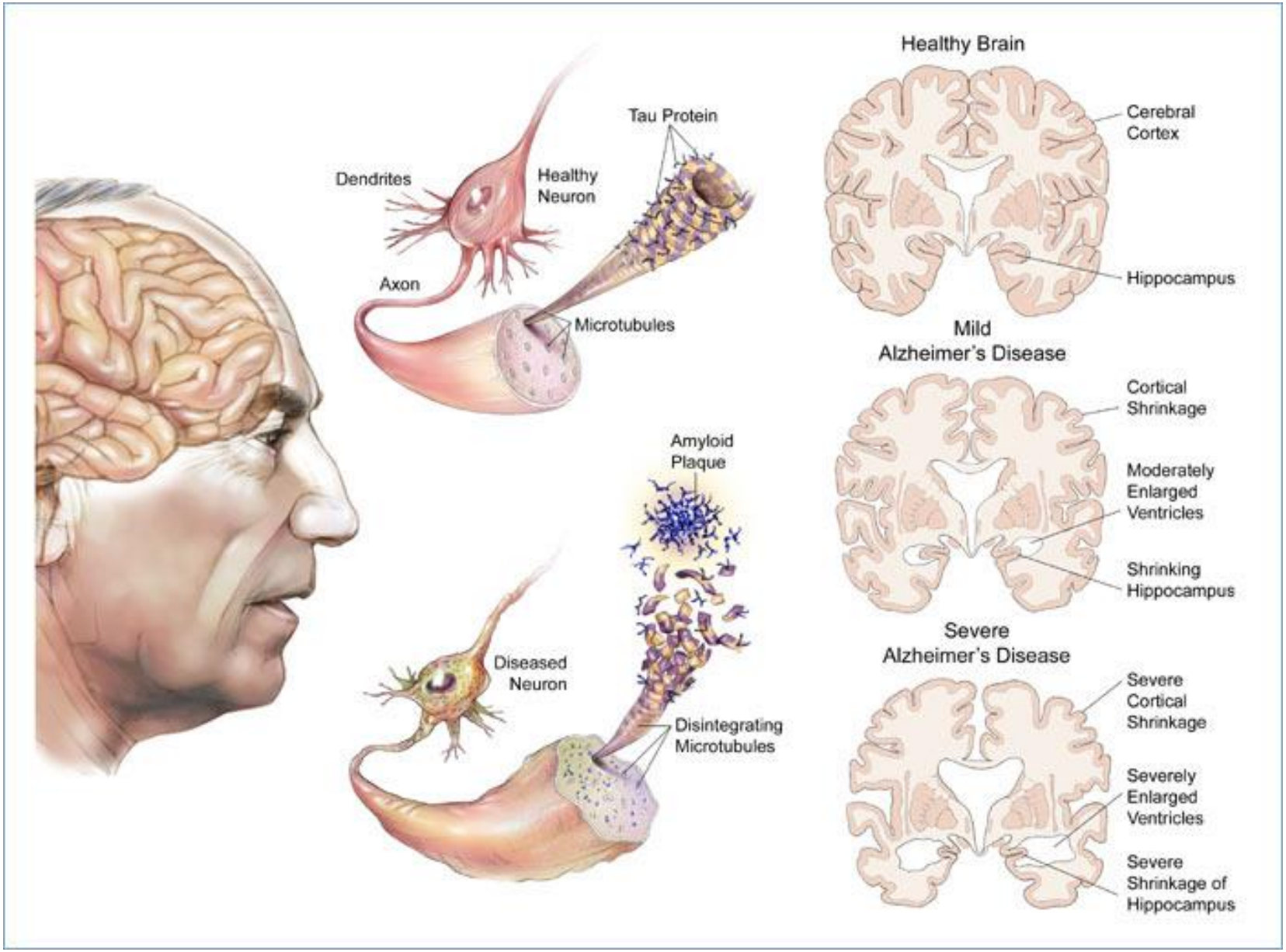
Electronic configuration

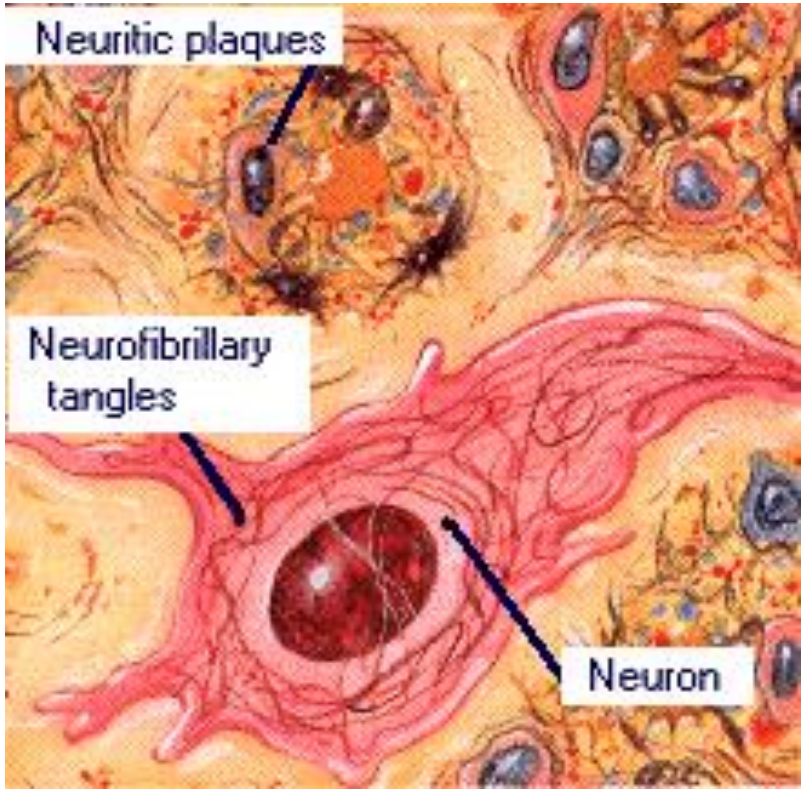
Al	**[Ne] $3s^2 3p^1$**
Al(III)	**[Ne] $3s^0$**

Aluminum

- Soil acidification leads to toxic levels of aluminum and manganese, and sub-optimal levels of phosphorous. Acidification of soils leads to acidification of rivers and streams, increasing the solubility of aluminum, with direct consequences on fish populations, and eventually on water supplies to the general population.
- The chemistry of aluminum combines features in common with two other groups of elements, namely (i) divalent magnesium and calcium, and (ii) trivalent chromium and iron. The toxic effects of aluminum are more related to its interference with calcium-dependent processes, whereas its access to tissues is probably a function of its similarity to ferric iron. Al(III) is a much stronger acid than Mg(II) and Ca(II), which makes it a powerful competitor for oxygen donor ligands for these two important biological cations, and leads to profound interference with their metabolism.

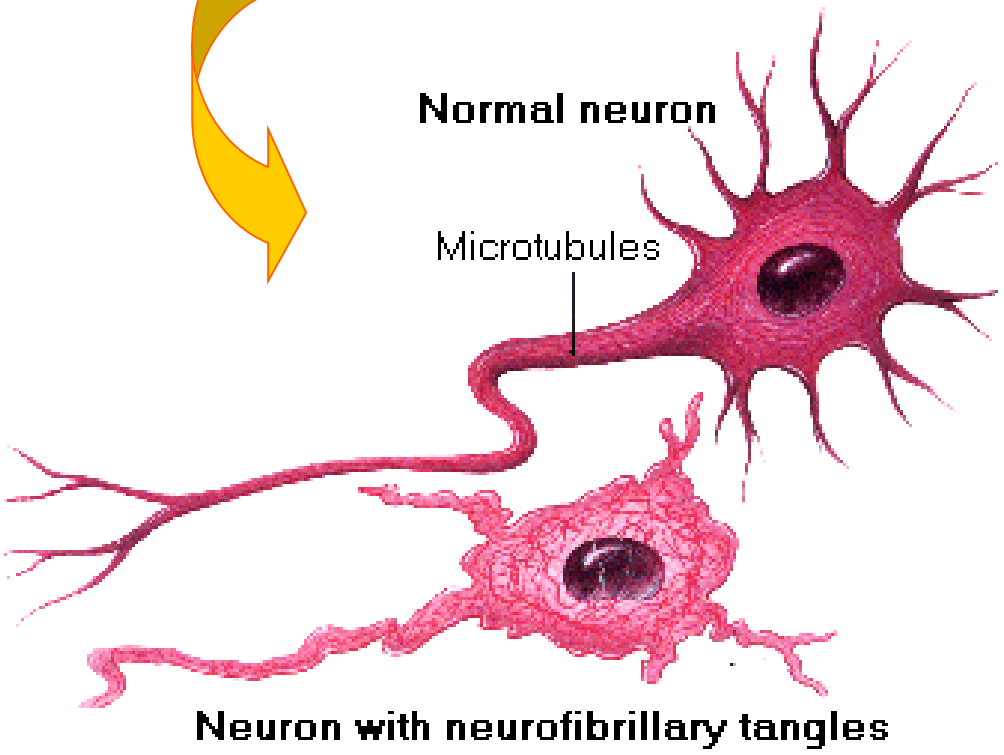
The clinical picture in the disease





Senile plaques in Alzheimer's brains

Neurofibrillary tangles in Alzheimer's brains

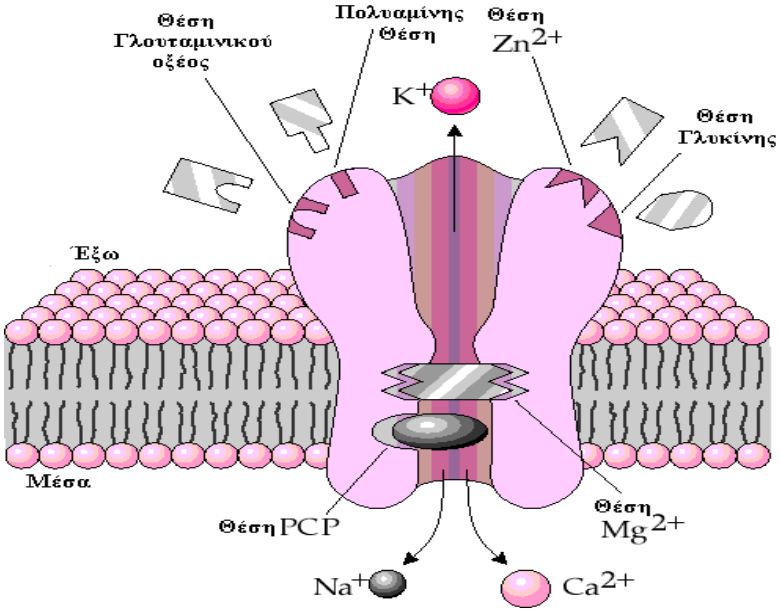


The neurotoxic case of Al(III)

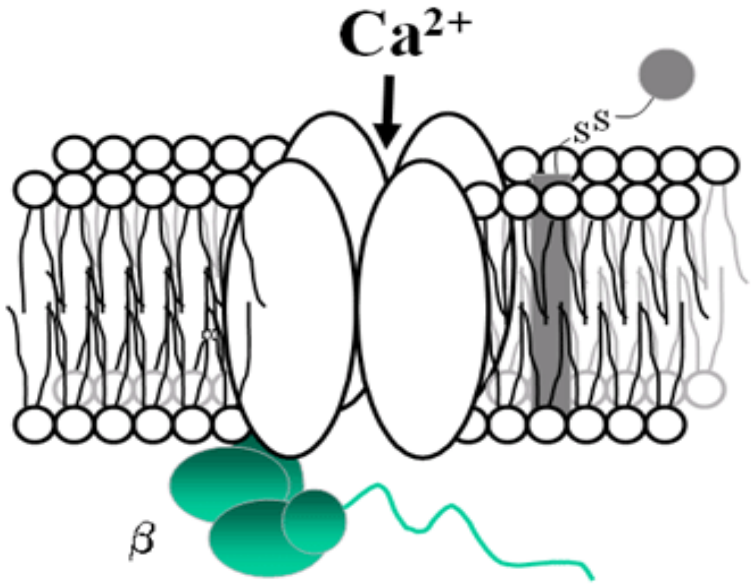
- **Aluminum has increasingly invaded human physiology (environmental deterioration, increasing human exposure, dietary habits, etc.)**
- **Causative agent in dialysis encephalopathy, osteodystrophy, and microcytic anemia.**
- **Present in the brain of Alzheimer's patients (in senile plaques and NFTs) (Al up to 6 μM)**
- **Heavy use in activities facilitating contact with human physiological structures (water treatment plants)**
- **Causes precipitation of amyloid peptide and NFTs**
- **It is a neurotoxin – Camelford incident (Al up to 23 μg/g dry weight)**

Al(III) perturbs Ca(II) homeostasis in the neurons, as a result of which the latter are led to apoptosis

Ca(II) transmembrane channels (N-Methyl D-Aspartate, NMDA & Voltage Dependent Calcium Channels, VDCC) constitute the main targets of toxic metal ion chemical reactivity

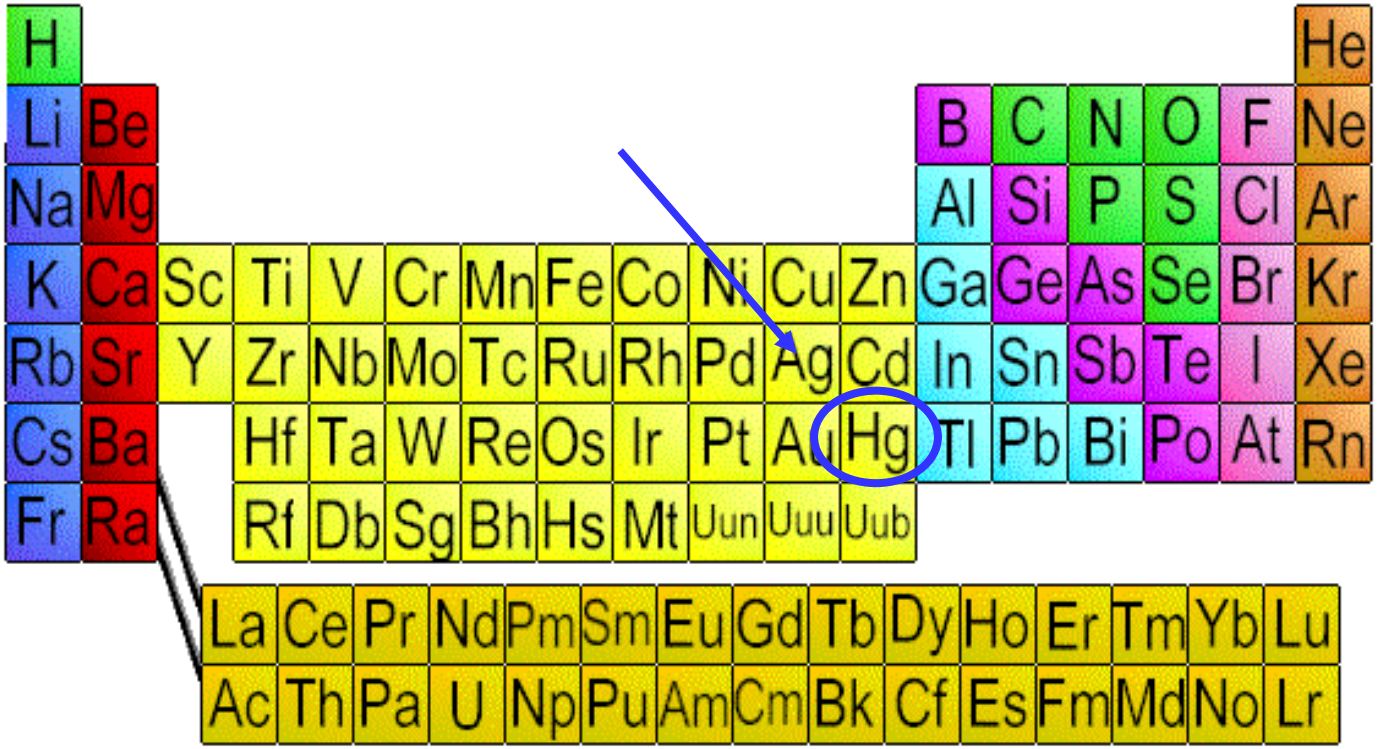


NMDA



VDCC

Mercury in the natural order



Atomic Number	80
Atomic weight	200.59 (2)
Specific gravity	14190 kg m^{-3}
Melting point	-38.83 °C
Boiling point	356.73 °C

Electronic configuration

Hg	**[Xe]$4f^{14}5d^{10}6s^{2}$**
Hg(II)	**[Xe] $4f^{14}5d^{10}6s^{0}$**

Mercury – a potent metallotoxin

Mercury is one of the most toxic heavy metals and has significant industrial and agricultural uses

(Shaolin and David, 1997; Hobman et al., 2000).

These uses have led to severe localized mercury pollution in aquatic systems and in soils

(Stanisich et al., 1977; Hobman and Brown 1997).

The Detoxification Mechanism

- To prevent the detrimental effects of mercurial compounds, many bacterial species have evolved a sophisticated detoxification system, in which mercurials and Hg(II) are actively transported into the intracellular space, where ultimate reduction of Hg(II) to much less toxic Hg(0) leads to its elimination from the cell.
- The crucial two-electron reduction step in this pathway is catalyzed by the cytoplasmic flavoenzyme mercuric ion reductase (Ghosh et al., 1996; Essa et al., 2002). Hg(0) appears to be eliminated by passive diffusion from the cell under normal physiological condition (Hobman and Brown, 1997a). The high vapor pressure of elemental mercury results in the volatilization of mercury from aqueous media (Chang et al., 1993; Ogunseitan,1997; Ogunseitan, 1998).

Critical aspects of the Detoxification Mechanism

- It is a plasmid encoded system
- It manages both the uptake and transport of Hg(II) as well as detoxification
- It targets the clean up of the environment of bacterium
- The expression of genes encoding for proteins in detoxification is regulated by metal ion concentration

Routes of mercury invasion of abiotic and biological systems

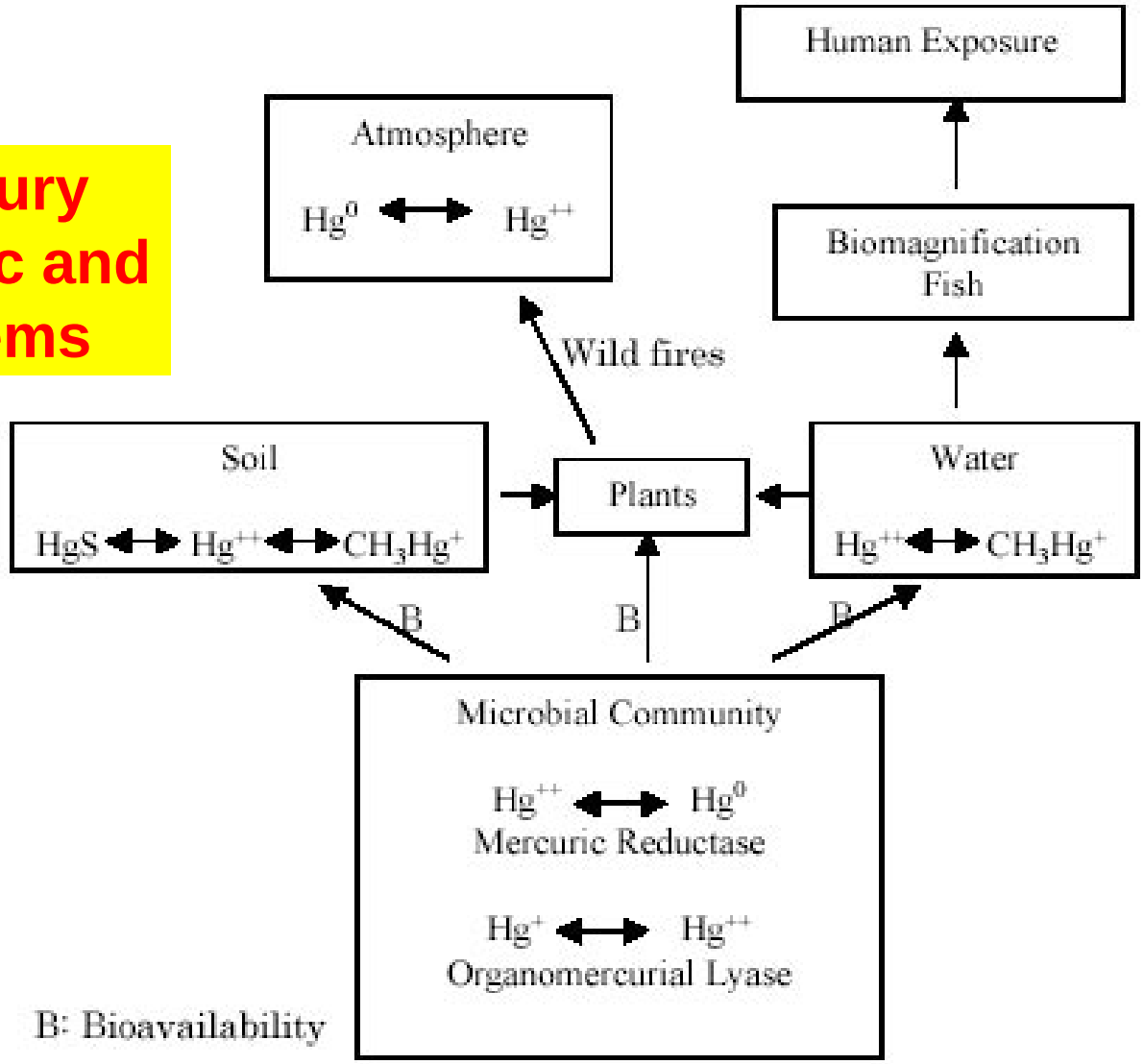
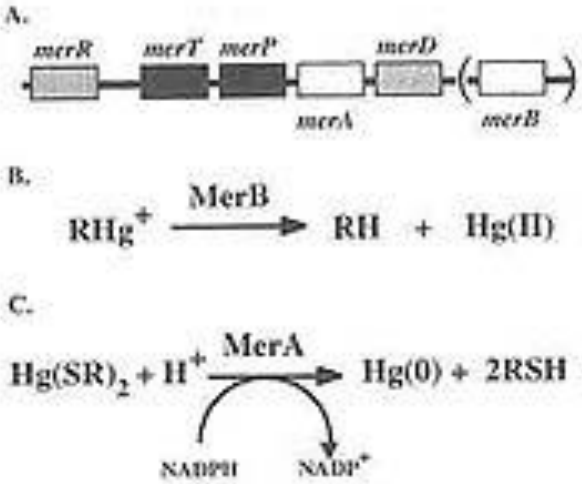
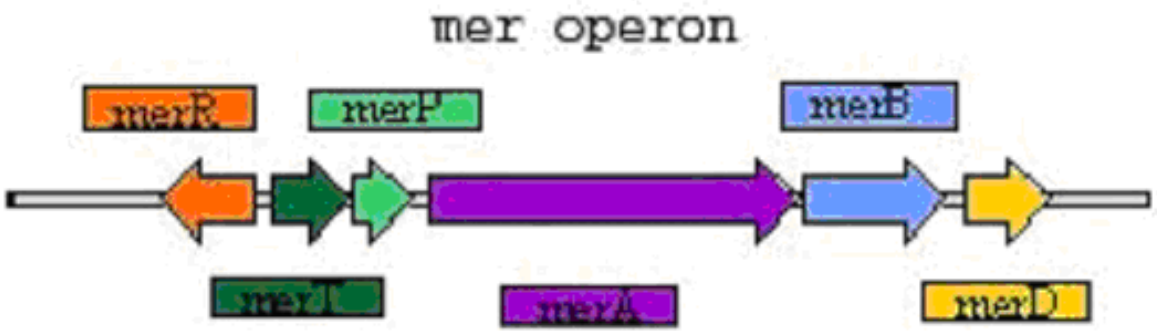
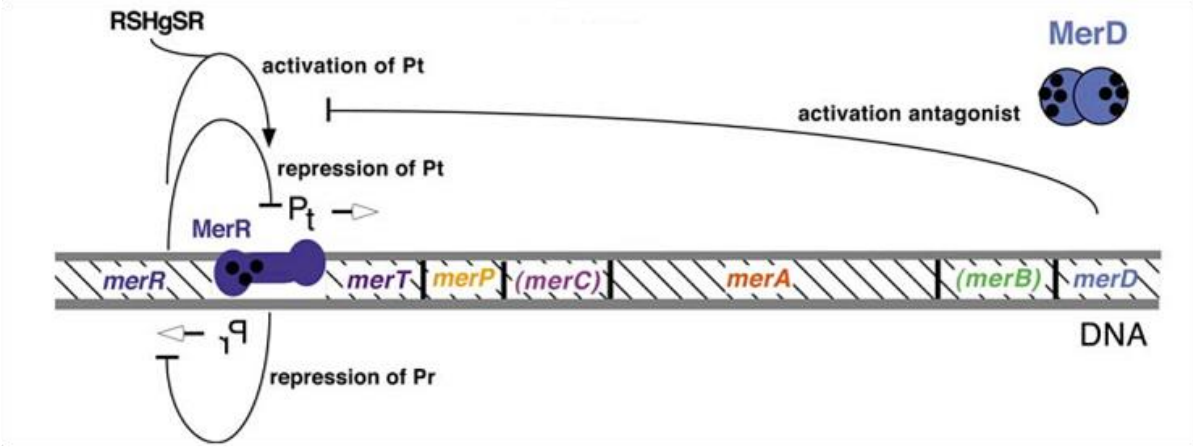


Figure 2. Schematic diagram showing the fate of mercuric compounds released from wildfires as mediated by microbial community enzymatic activities.

The mer operon: A genetic blueprint set for action



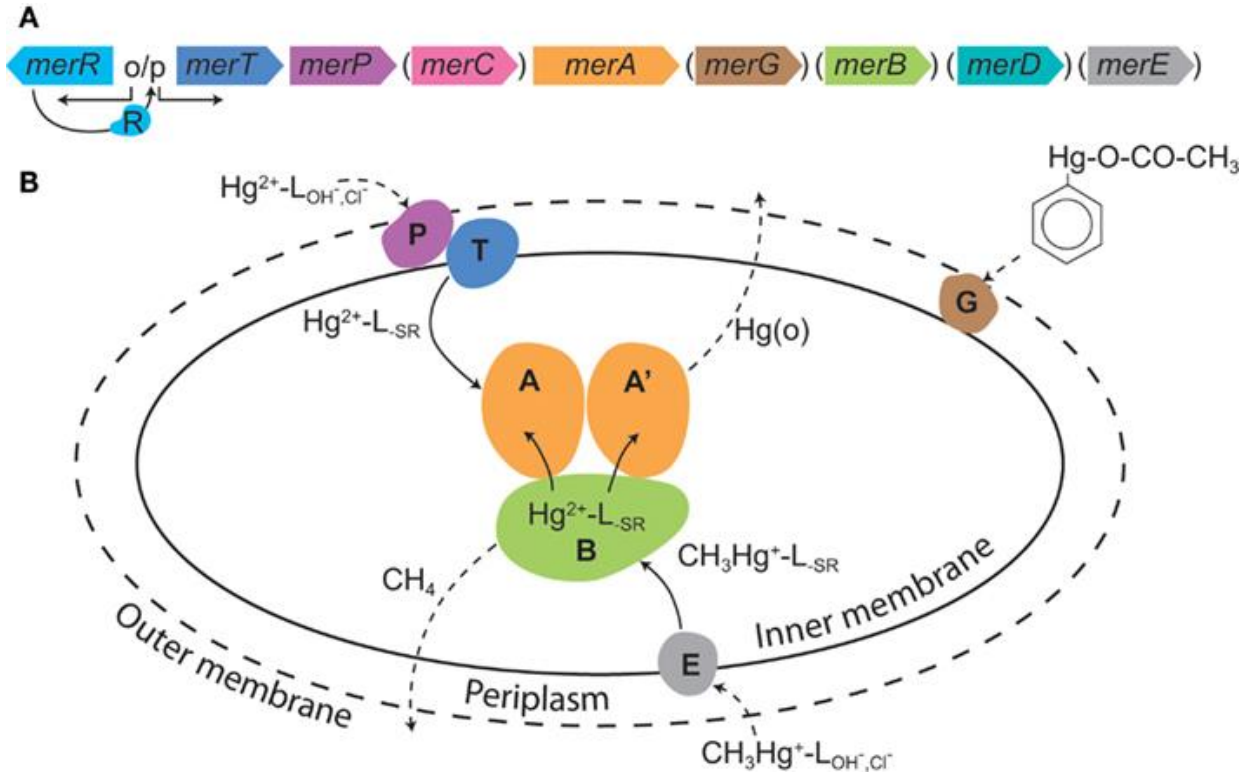




The mercury detoxification reaction sequence

1. $R\text{-}Hg\text{-}X + H^+ + X^- \longrightarrow R\text{-}H + HgX_2$

2. $Hg(SR)_2 + NADPH + H^+ \longrightarrow Hg(0) + 2RSH$



Alkylmercury lyase or Mercuric Lyase (MerB)

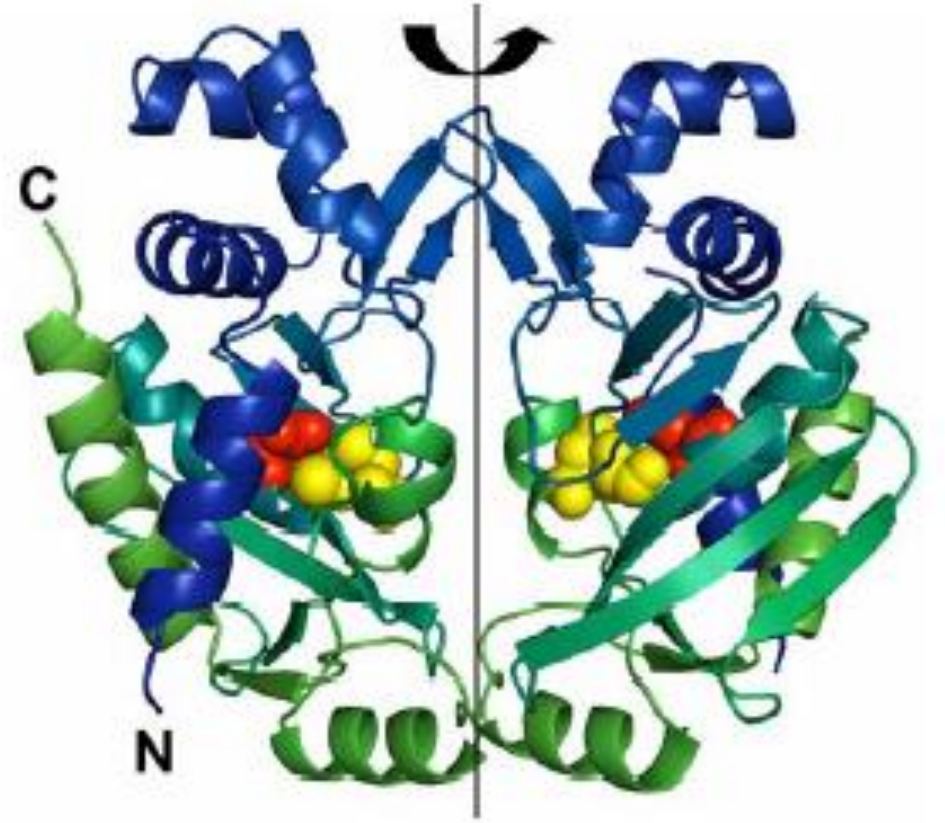
Mercuric Lyase (MerB) catalyzes the first step in the microbial detoxification of organomercurial salts (the other one is catalyzed by mercuric reductase).

Organomercurial lyase catalyzes the protonolysis of the C-Hg bond in a wide range of organomercurial salts (primary, secondary, tertiary, alkyl, vinyl, allyl and aryl) to Hg(II) and the respective organic compound

Hg(II) is subsequently detoxified by mercuric reductase (MerA).

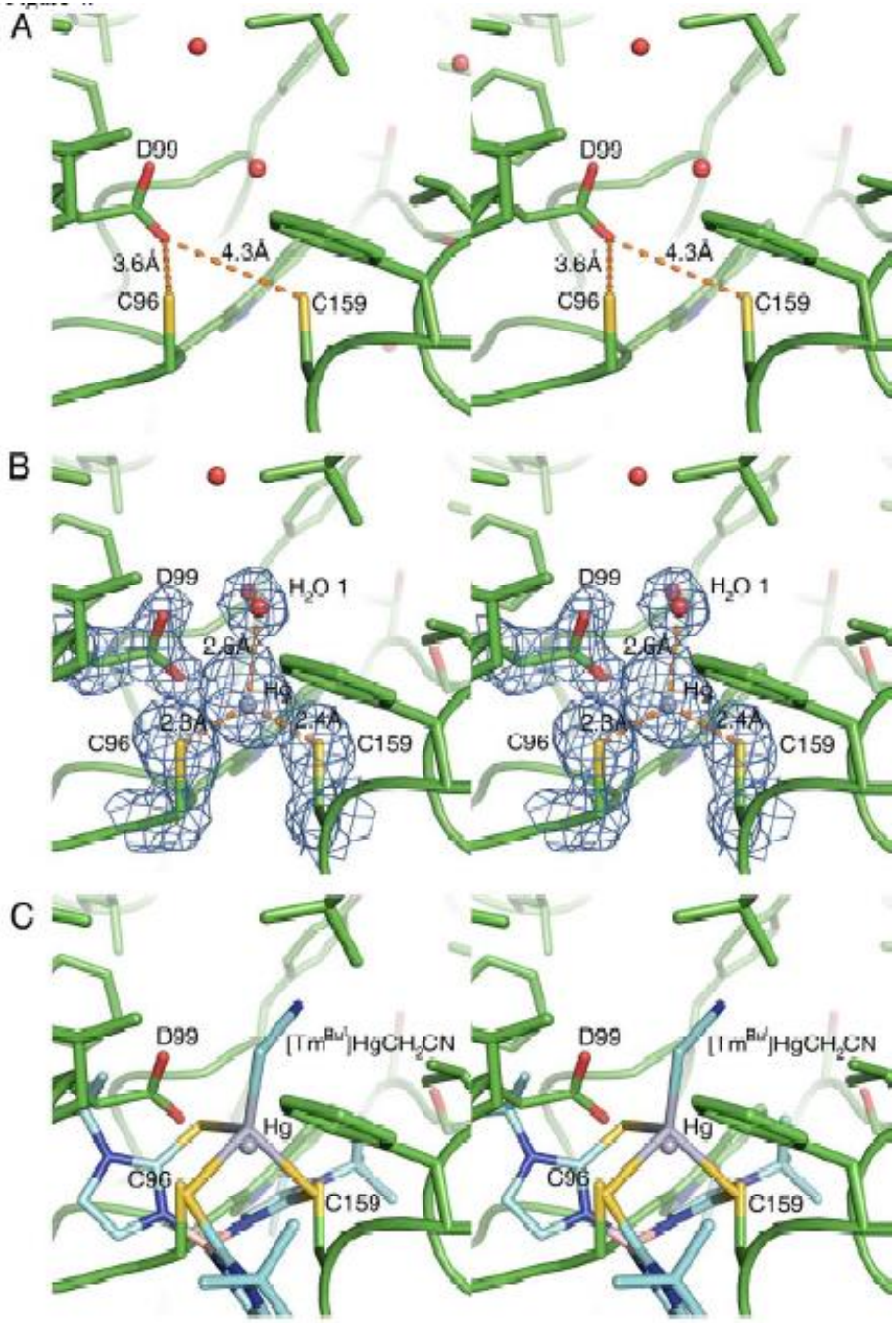
The enzyme has been purified to homogeneity in *Escherichia coli* and has been found to be a 22.4 kDa monomer with no detectable cofactors or metal ions.

MerB structure in the free form

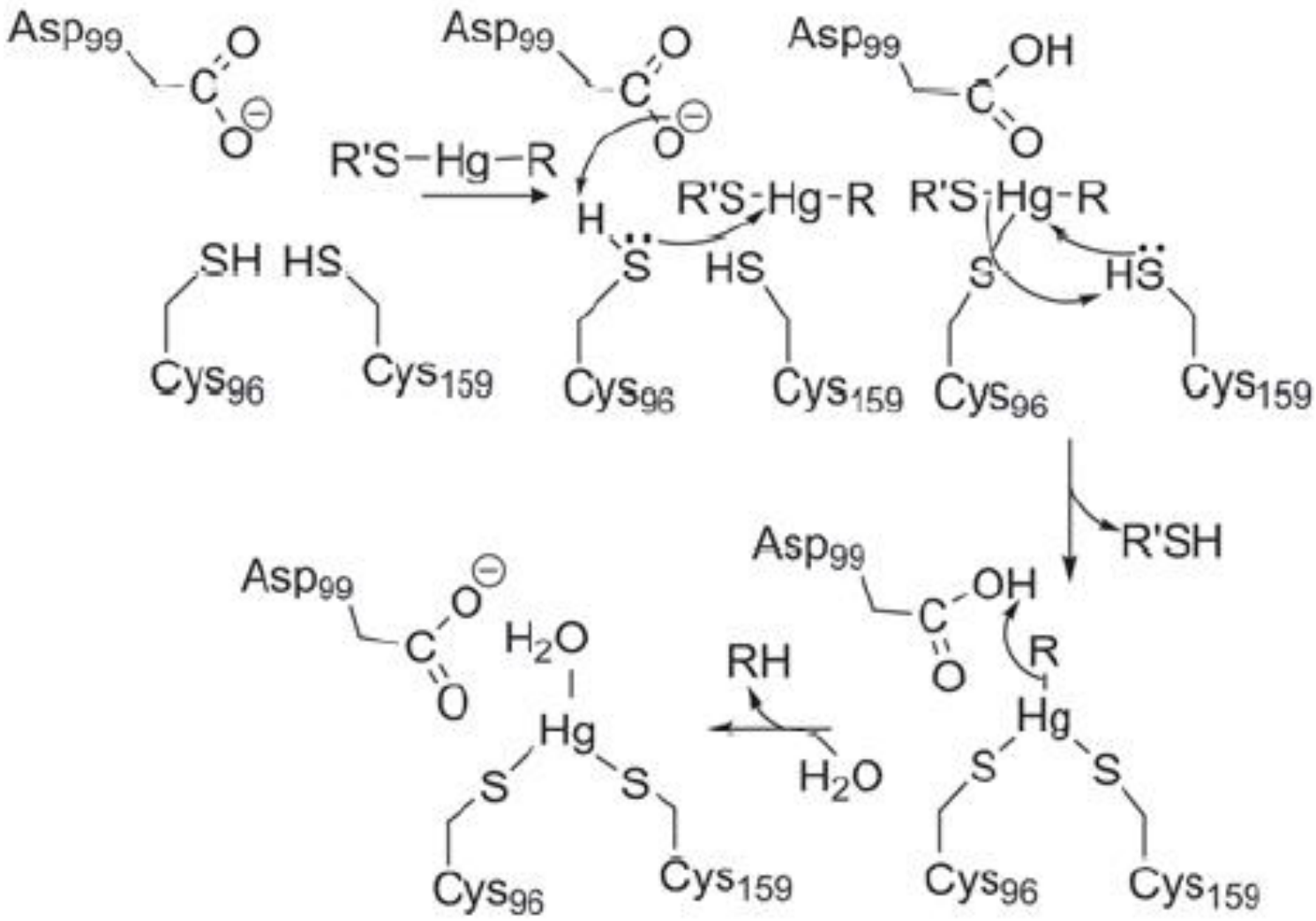


Crystal structure of free MerB at 1.76 Å resolution. MerB forms a dimer in the asymmetric unit, related by a 2-fold pseudosymmetry as depicted by the rotation axis. The two subunit color varies from blue to green, starting from the amino-terminal end of the protein to help distinguish the amino-terminal end of the protein from the C carboxy-terminal end of the protein. Active site residues are highlighted with a colored van der Waals sphere representation

Stereoview of the active site of the free MerB (A) the MerB-Hg complex (B) and an alignment of the MerB-Hg complex with [TmBut]$HgCH_2CN$ (C).



Mechanism for the cleavage of the carbon-mercury bond and the formation of the MerB-Hg complex.



Mercuric Reductase (MerA)

Mercuric reductase (MerA) has previously been isolated from several bacteria (Furkawa and Tonomura, 1972; Schottel, 1978; Fox and Walsh, 1982; Rinderle et al., 1982; Blaghen et al., 1993). From these studies, the mercury resistance operon is in all cases plasmid-encoded and inducible (Stanisich et al., 1977; Summers and Silver, 1978; Summer et al., 1980). The enzyme has been found to contain FAD, utilizes NADPH as an electron donor and requires an excess of exogenous thiols for activity and was mechanistically similar to other classes of flavoproteins such as lipoamide dehydrogenase and glutathione reductase (Brown et al., 1983; Fox and Walsh, 1983). The presence of thiols ensures that the Hg(II) will be present as dimercaptide, RS-Hg-SH. The overall reaction catalyzed is as follows

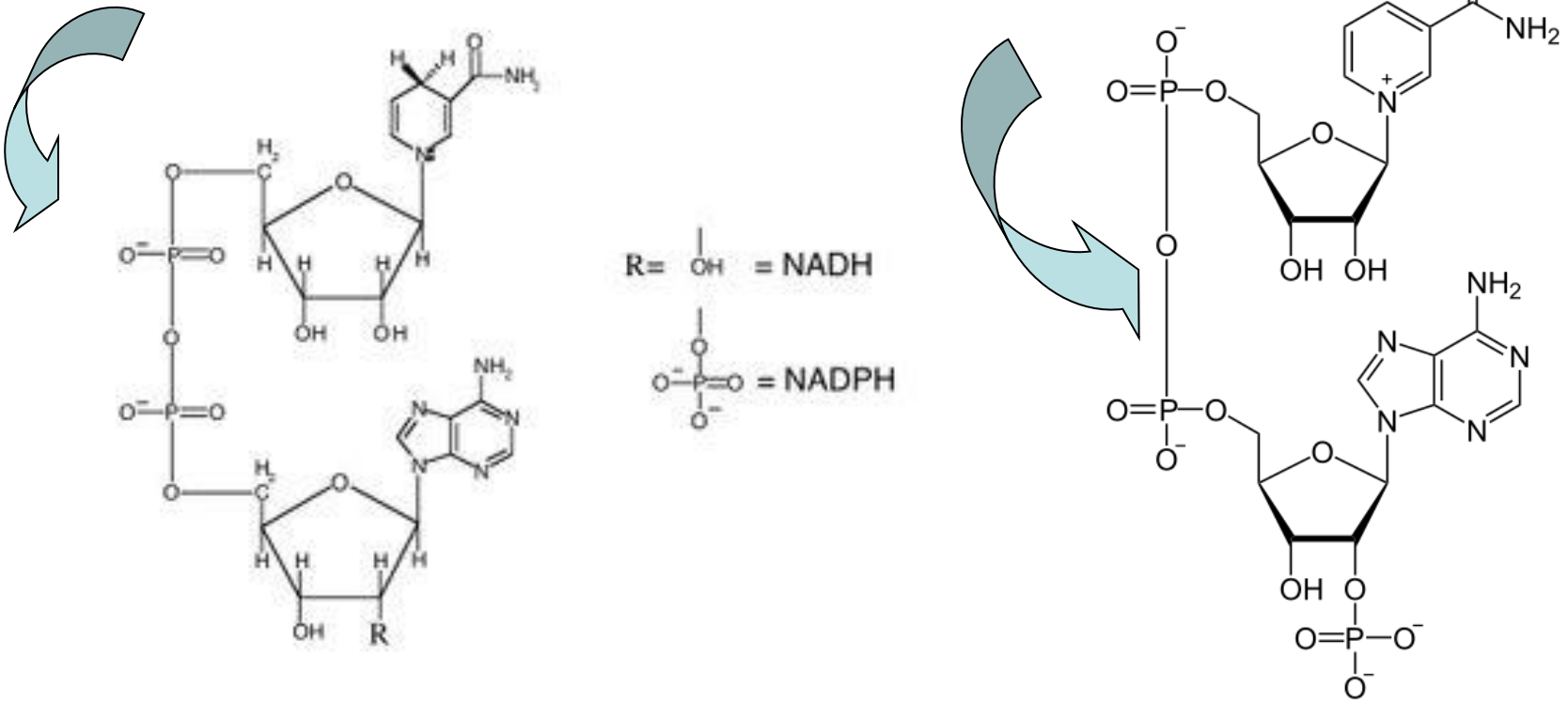
$$NADPH + RS\text{-}Hg\text{-}SR + H^{+} \rightleftharpoons NADP^{+} + Hg^{0} + 2RSH$$

Mercuric Reductase reaction

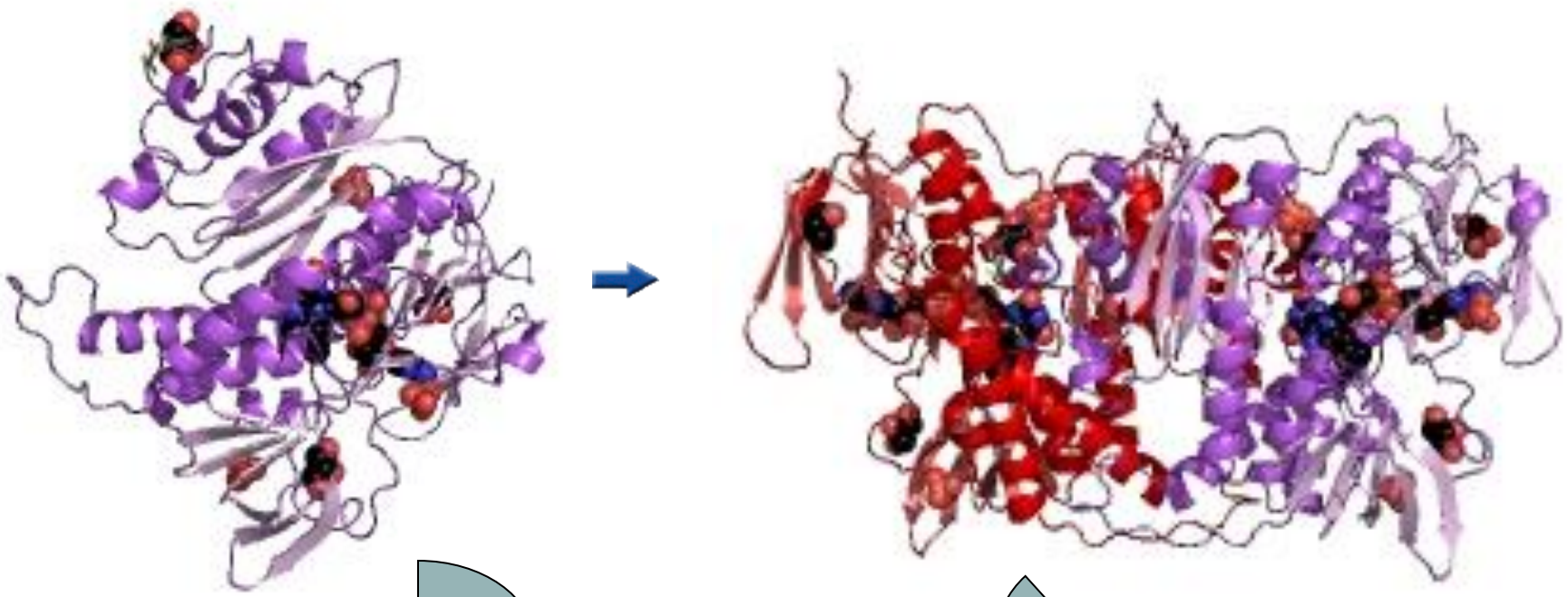
The enzyme belongs to the family of oxidoreductases specifically oxidizing metal ions with NAD^+ or $NADP^+$ as acceptor.

Reaction catalyzed by Hg(II) reductase

$$Hg(II) + NADPH \rightleftharpoons Hg + NADP^+ + H^+$$



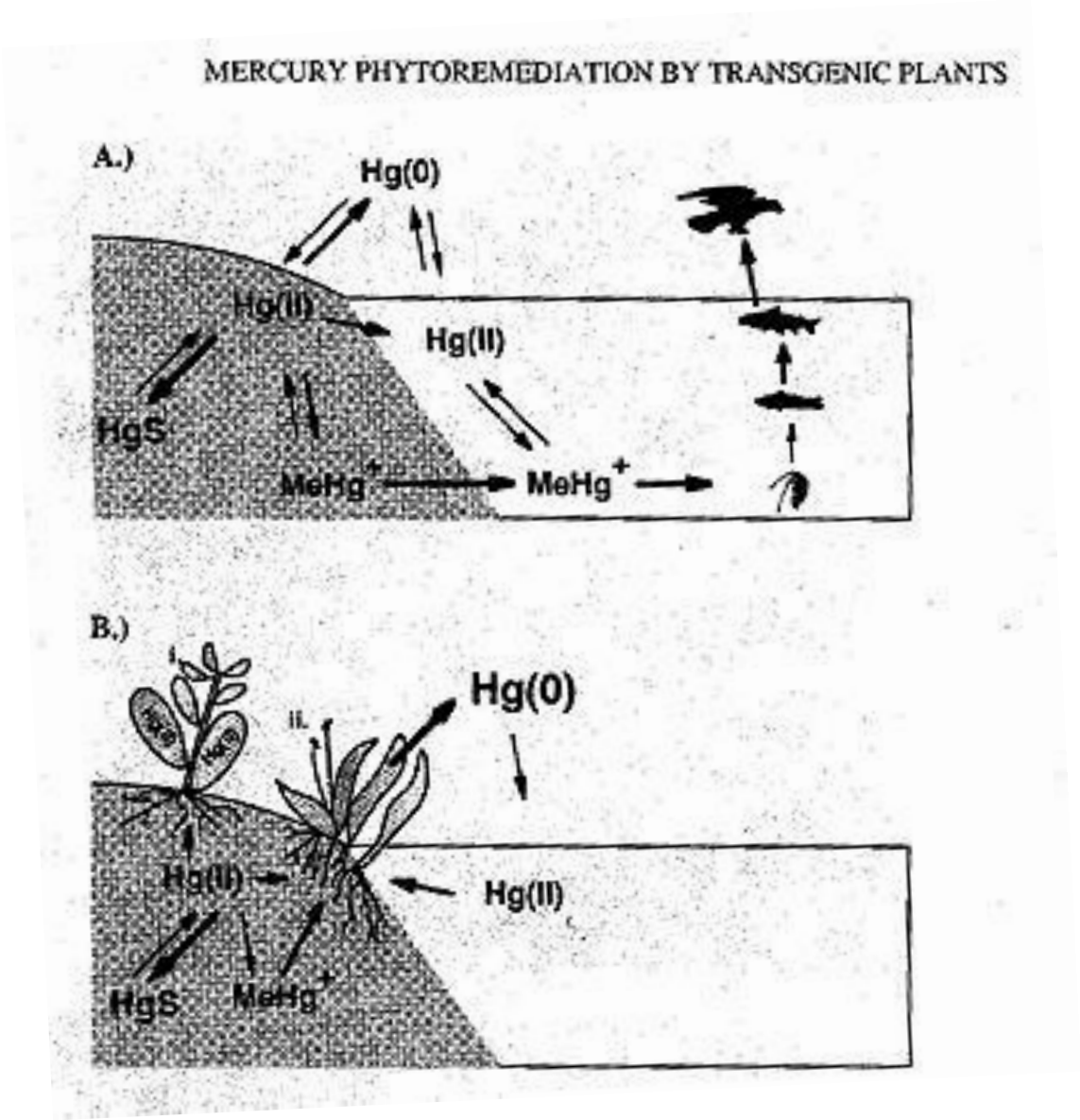
Mercuric Reductase structure



Asymmetric unit

Biological unit, dimer

Mercury phytoremediation by transgenic plants



Conclusions

- Heavy metal ions Cd(II), Hg(II) are metallotoxins with dire consequences for the physiology of the cell and its pathogenic fate. The same goes for the lighter metallotoxin Al(III) involved in neurodegeneration.
- The detoxification system by bacteria in nature employs a genetic locus to control both the detoxification and the clean up of the environment of the bacterium.
- In humans irreversible changes occur that jeopardize cellular integrity and lead to pathological conditions and diseases often ending to death.
- Synthetic and biological studies (structural speciation) can help comprehend the process of toxicity and detoxification at the molecular level.