Hinc
Patriam
Sustinet

INSTITUTO
SUPERIOR DE
AGRONOMIA
Universidade de Lisboa

Contaminação com metais pesados nos ecossistemas

Efeitos nos seres vivos

(Heavy metals in ecosystems)

Química Ambiental

Mestrado em Engenharia do Ambiente

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ISA, 2014/2015

What is a *heavy metal*?

The term heavy metal is difficult to define precisely.

Usual definition: group of metals and metalloids with a density higher than 5 g/cm^3 (does not include all the elements of environmental concern)

Other terms used: toxic metal (*metal tóxico*), trace metal/element (*elemento vestigial/traço*), PTE – Potentially Toxic Elements (*EPT – Elementos Potencialmente Tóxicos*).

However the "heavy metal" designation is still commonly used, usually with a pejorative sense, although several heavy metals are essential for plants and animals.

Heavy metal toxicity: nutrients… or pollutants…

Several metals have biological functions in living beings

At high concentrations even the essential elements can become toxic

-Macronutrients (N, P, K, S, Ca, Mg)

-Micronutrients essential for plants (Cu, Fe, Mn, Mo, Zn, Ni, B, Cl)

- Essential elements for animals: **Cu**, Co, Fe, Mn, Mo, **Zn**, **Cr**, Ni, Se, Sn:

Toxic elements:

-Cd, Hg, Pb, As:
-Non essentail elements for living organisms – no known biological function
-Accumulate in living organisms (bioaccumulation)
-Afect essential physiological processes
(ex: Cd replaces Zn originating inactive enzymes)

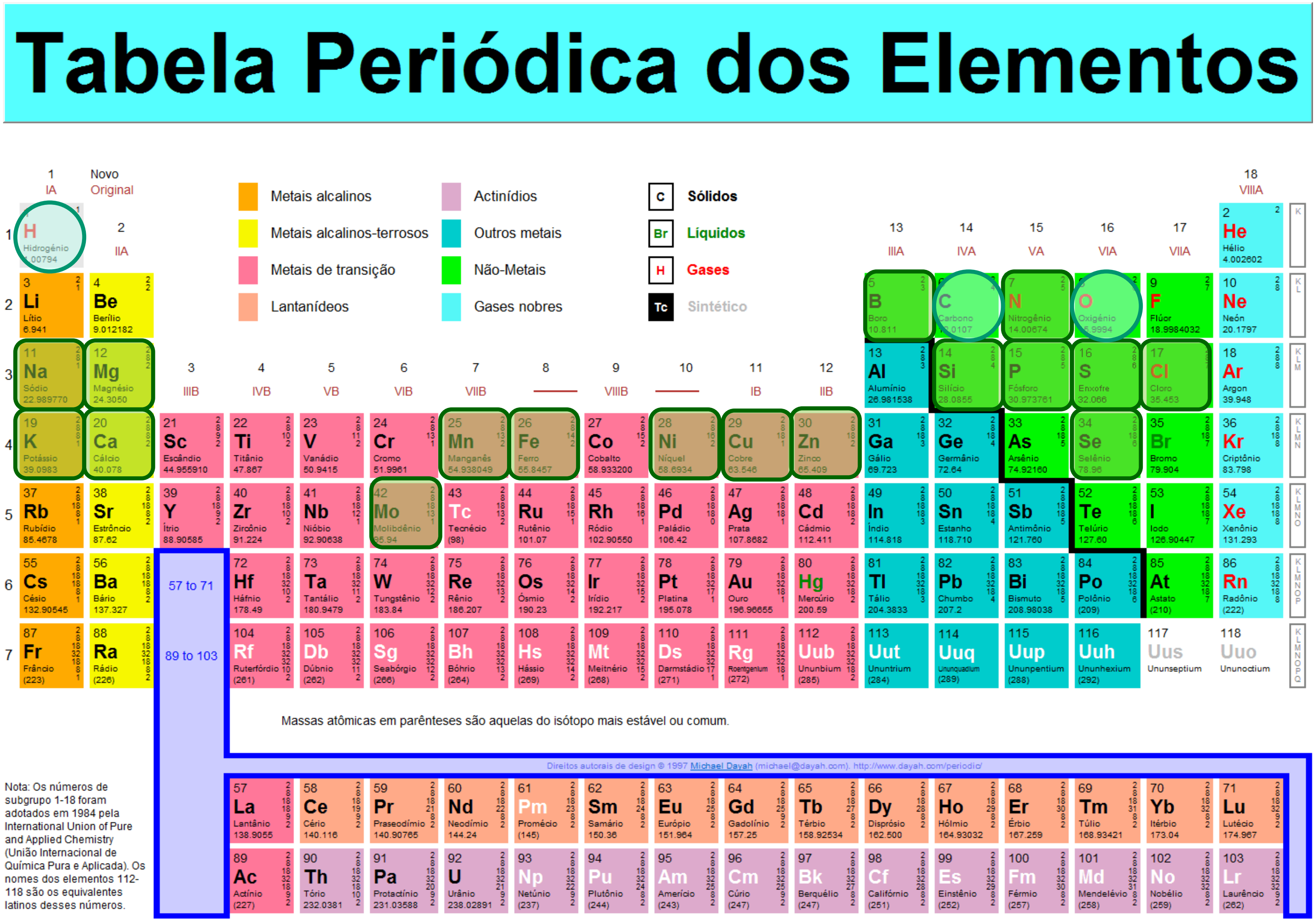

Tabela Periódica dos Elementos

Novo
Original

- Metais alcalinos
- Metais alcalinos-terrosos
- Metais de transição
- Lantanídeos
- Actinídios
- Outros metais
- Não-Metais
- Gases nobres

C **Sólidos**
Br **Líquidos**
H **Gases**
Tc Sintético

	1 IA	2 IIA	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8	9 VIIIB	10	11 IB	12 IIB	13 IIIA	14 IVA	15 VA	16 VIA	17 VIIA	18 VIIIA	
1	1 H Hidrogénio 1.00794 1																	2 He Hélio 4.002602 2	K
2	3 Li Lítio 6.941 2 1	4 Be Berílio 9.012182 2 2											5 B Boro 10.811 2 3	6 C Carbono 12.0107 2 4	7 N Nitrogênio 14.00674 2 5	8 O Oxigénio 15.9994 2 6	9 F Flúor 18.9984032 2 7	10 Ne Neón 20.1797 2 8	K L
3	11 Na Sódio 22.989770 2 8 1	12 Mg Magnésio 24.3050 2 8 2											13 Al Alumínio 26.981538 2 8 3	14 Si Silício 28.0855 2 8 4	15 P Fósforo 30.973761 2 8 5	16 S Enxofre 32.066 2 8 6	17 Cl Cloro 35.453 2 8 7	18 Ar Argon 39.948 2 8 8	K L M
4	19 K Potássio 39.0983 2 8 8 1	20 Ca Cálcio 40.078 2 8 8 2	21 Sc Escândio 44.955910 2 8 9 2	22 Ti Titânio 47.867 2 8 10 2	23 V Vanádio 50.9415 2 8 11 2	24 Cr Cromo 51.9961 2 8 13 1	25 Mn Manganês 54.938049 2 8 13 2	26 Fe Ferro 55.8457 2 8 14 2	27 Co Cobalto 58.933200 2 8 15 2	28 Ni Níquel 58.6934 2 8 16 2	29 Cu Cobre 63.546 2 8 18 1	30 Zn Zinco 65.409 2 8 18 2	31 Ga Gálio 69.723 2 8 18 3	32 Ge Germânio 72.64 2 8 18 4	33 As Arsênio 74.92160 2 8 18 5	34 Se Selênio 78.96 2 8 18 6	35 Br Bromo 79.904 2 8 18 7	36 Kr Criptônio 83.798 2 8 18 8	K L M N
5	37 Rb Rubídio 85.4678 2 8 18 8 1	38 Sr Estrôncio 87.62 2 8 18 8 2	39 Y Ítrio 88.90585 2 8 18 9 2	40 Zr Zircônio 91.224 2 8 18 10 2	41 Nb Nióbio 92.90638 2 8 18 12 1	42 Mo Molibdênio 95.94 2 8 18 13 1	43 Tc Tecnécio (98) 2 8 18 13 2	44 Ru Rutênio 101.07 2 8 18 15 1	45 Rh Ródio 102.90550 2 8 18 16 1	46 Pd Paládio 106.42 2 8 18 18 0	47 Ag Prata 107.8682 2 8 18 18 1	48 Cd Cádmio 112.411 2 8 18 18 2	49 In Índio 114.818 2 8 18 18 3	50 Sn Estanho 118.710 2 8 18 18 4	51 Sb Antimônio 121.760 2 8 18 18 5	52 Te Telúrio 127.60 2 8 18 18 6	53 I Iodo 126.90447 2 8 18 18 7	54 Xe Xenônio 131.293 2 8 18 18 8	K L M N O
6	55 Cs Césio 132.90545 2 8 18 18 8 1	56 Ba Bário 137.327 2 8 18 18 8 2	57 to 71	72 Hf Háfnio 178.49 2 8 18 32 10 2	73 Ta Tantálio 180.9479 2 8 18 32 11 2	74 W Tungstênio 183.84 2 8 18 32 12 2	75 Re Rênio 186.207 2 8 18 32 13 2	76 Os Ósmio 190.23 2 8 18 32 14 2	77 Ir Irídio 192.217 2 8 18 32 15 2	78 Pt Platina 195.078 2 8 18 32 17 1	79 Au Ouro 196.96655 2 8 18 32 18 1	80 Hg Mercúrio 200.59 2 8 18 32 18 2	81 Tl Tálio 204.3833 2 8 18 32 18 3	82 Pb Chumbo 207.2 2 8 18 32 18 4	83 Bi Bismuto 208.98038 2 8 18 32 18 5	84 Po Polônio (209) 2 8 18 32 18 6	85 At Astato (210) 2 8 18 32 18 7	86 Rn Radônio (222) 2 8 18 32 18 8	K L M N O P
7	87 Fr Frâncio (223) 2 8 18 32 18 8 1	88 Ra Rádio (226) 2 8 18 32 18 8 2	89 to 103	104 Rf Ruterfórdio (261) 2 8 18 32 32 10 2	105 Db Dúbnio (262) 2 8 18 32 32 11 2	106 Sg Seabórgio (266) 2 8 18 32 32 12 2	107 Bh Bóhrio (264) 2 8 18 32 32 13 2	108 Hs Hássio (269) 2 8 18 32 32 14 2	109 Mt Meitnério (268) 2 8 18 32 32 15 2	110 Ds Darmstádio (271) 2 8 18 32 32 17 1	111 Rg Roentgenium (272) 2 8 18 32 32 18 1	112 Uub Ununbium (285) 2 8 18 32 32 18 2	113 Uut Ununtrium (284)	114 Uuq Ununquadium (289)	115 Uup Ununpentium (288)	116 Uuh Ununhexium (292)	117 Uus Ununseptium	118 Uuo Ununoctium	K L M N O P Q

Massas atômicas em parênteses são aquelas do isótopo mais estável ou comum.

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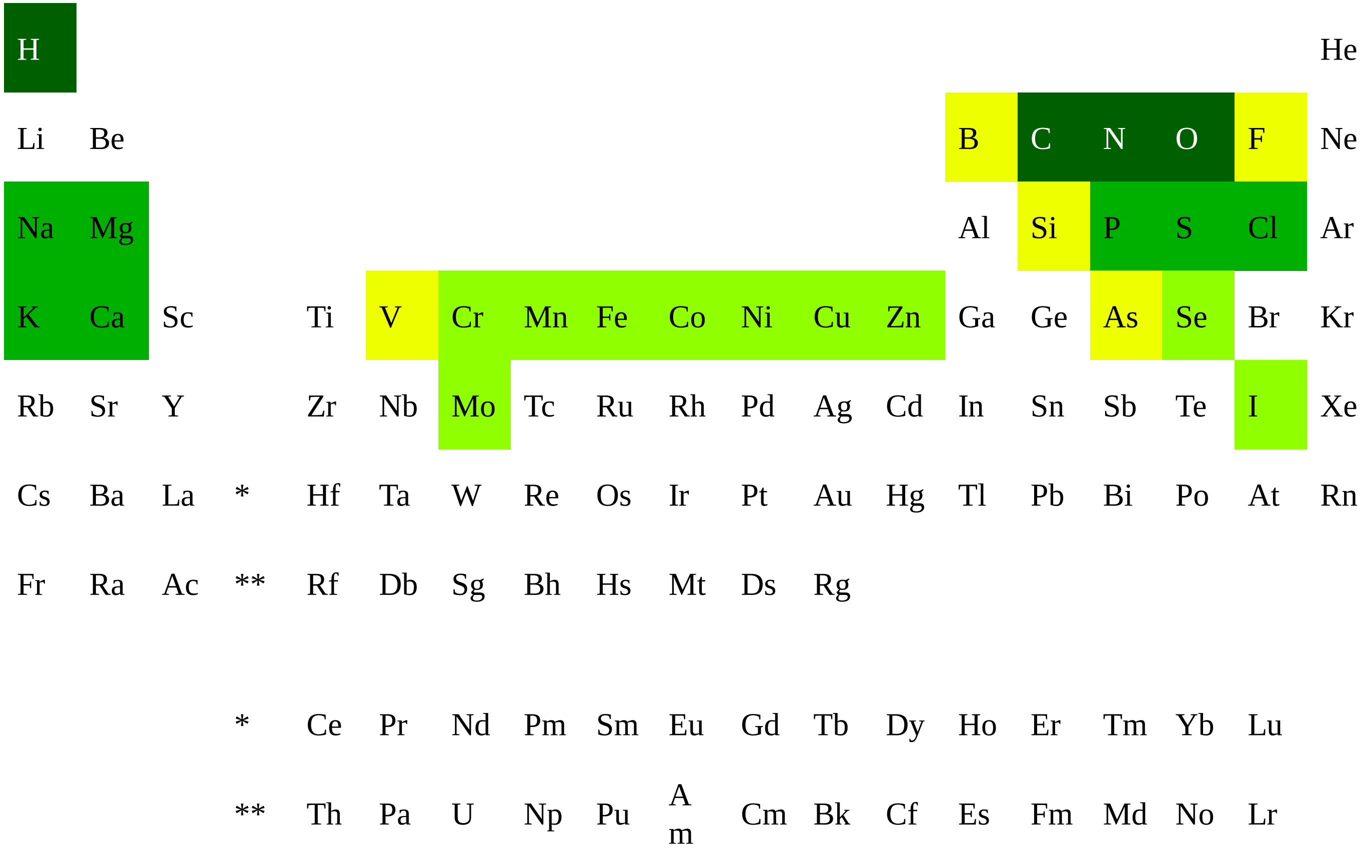
57 La Lantânio 138.9055 2 8 18 18 9 2	58 Ce Cério 140.116 2 8 18 19 9 2	59 Pr Praseodímio 140.90765 2 8 18 21 8 2	60 Nd Neodímio 144.24 2 8 18 22 8 2	61 Pm Promécio (145) 2 8 18 23 8 2	62 Sm Samário 150.36 2 8 18 24 8 2	63 Eu Európio 151.964 2 8 18 25 8 2	64 Gd Gadolínio 157.25 2 8 18 25 9 2	65 Tb Térbio 158.92534 2 8 18 27 8 2	66 Dy Disprósio 162.500 2 8 18 28 8 2	67 Ho Hólmio 164.93032 2 8 18 29 8 2	68 Er Érbio 167.259 2 8 18 30 8 2	69 Tm Túlio 168.93421 2 8 18 31 8 2	70 Yb Itérbio 173.04 2 8 18 32 8 2	71 Lu Lutécio 174.967 2 8 18 32 9 2
89 Ac Actínio (227) 2 8 18 32 18 9 2	90 Th Tório 232.0381 2 8 18 32 18 10 2	91 Pa Protactínio 231.03588 2 8 18 32 20 9 2	92 U Urânio 238.02891 2 8 18 32 21 9 2	93 Np Netúnio (237) 2 8 18 32 22 9 2	94 Pu Plutônio (244) 2 8 18 32 24 8 2	95 Am Amerício (243) 2 8 18 32 25 8 2	96 Cm Cúrio (247) 2 8 18 32 25 9 2	97 Bk Berquélio (247) 2 8 18 32 27 8 2	98 Cf Califórnio (251) 2 8 18 32 28 8 2	99 Es Einstênio (252) 2 8 18 32 29 8 2	100 Fm Férmio (257) 2 8 18 32 30 8 2	101 Md Mendelévio (258) 2 8 18 32 31 8 2	102 No Nobélio (259) 2 8 18 32 32 8 2	103 Lr Laurêncio (262) 2 8 18 32 32 9 2

Nota: Os números de subgrupo 1-18 foram adotados em 1984 pela International Union of Pure and Applied Chemistry (União Internacional de Química Pura e Aplicada). Os nomes dos elementos 112-118 são os equivalentes latinos desses números.

Important elements in human diet



H																		He
Li	Be												B	C	N	O	F	Ne
Na	Mg												Al	Si	P	S	Cl	Ar
K	Ca	Sc		Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y		Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	**	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg							
			*	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
			**	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

The four organic basic elements

Quantity elements

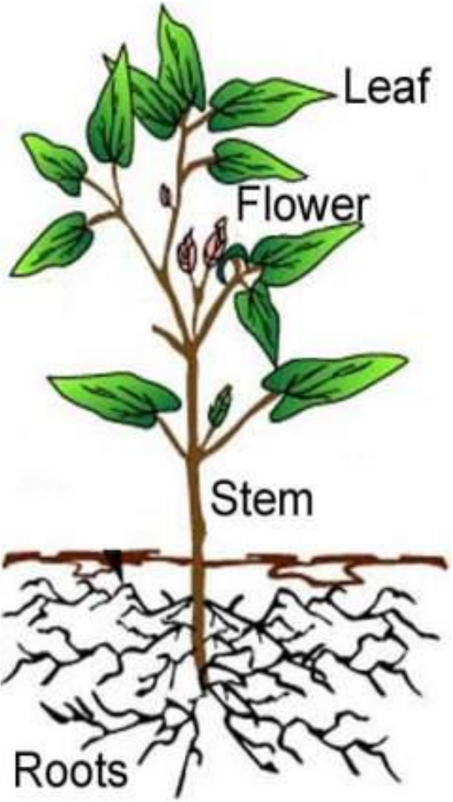
Essential trace elements

Suggested function from biochemistry and handling but no identified biological function in humans

Sources of “heavy metal” contamination

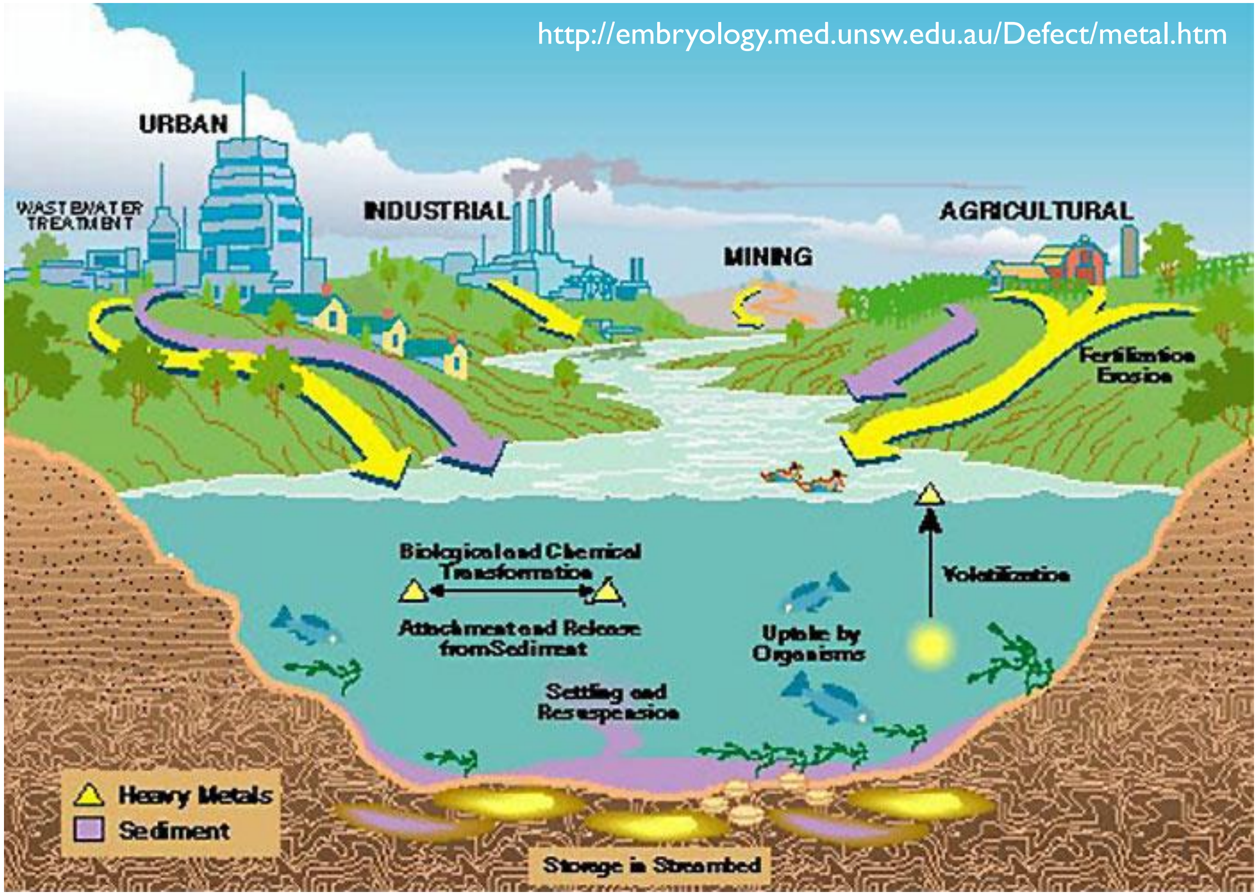
Natural Sources

Anthropogenic Sources

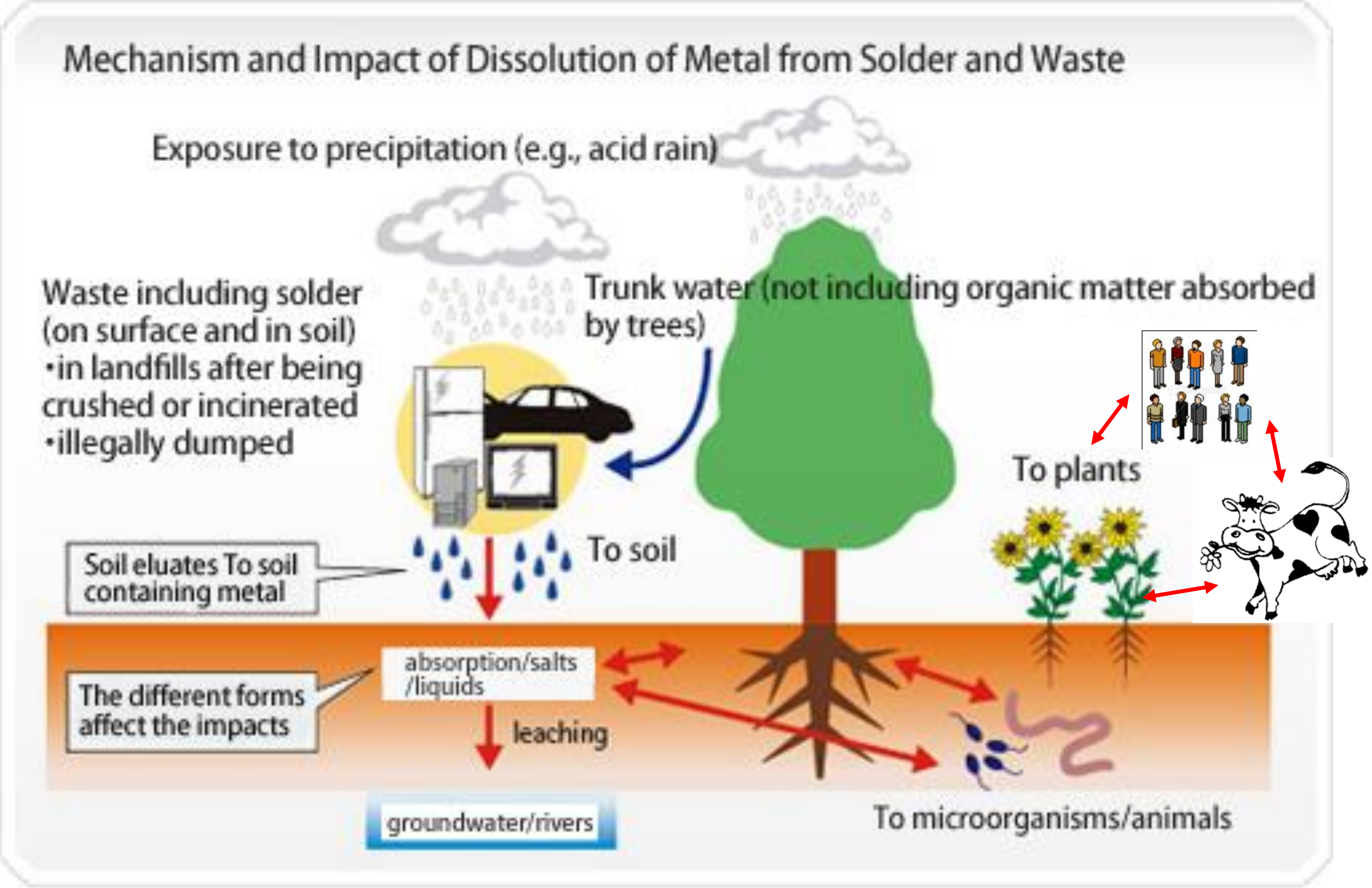


Toxic metals are metals that are not essential for living beings or that are essential but can be toxic at high concentrations (Fe, Cu, Mn, etc.). Some metals can be toxic in certain forms and not in others like Cr(III), essential for humans, and Cr(VI), toxic.

The term <u>trace element</u> is also used as some elements are of environmental concern but are not *heavy* metals, like As and Se.



http://embryology.med.unsw.edu.au/Defect/metal.htm



http://www.nies.go.jp/gaiyo/bunya/aquaterra-e.html

Inorganic pollutants: toxic elements

Metal classification:

- **Heavy metals:** elements with density higher than 5 g/cm^3

  Zn (7.1) Cr (7.2) Cd (8.6)
  Ni (8.7) Co (8.9) Cu (8.9)
  Mo (10.2) Pb (11.4) Hg (13.5)

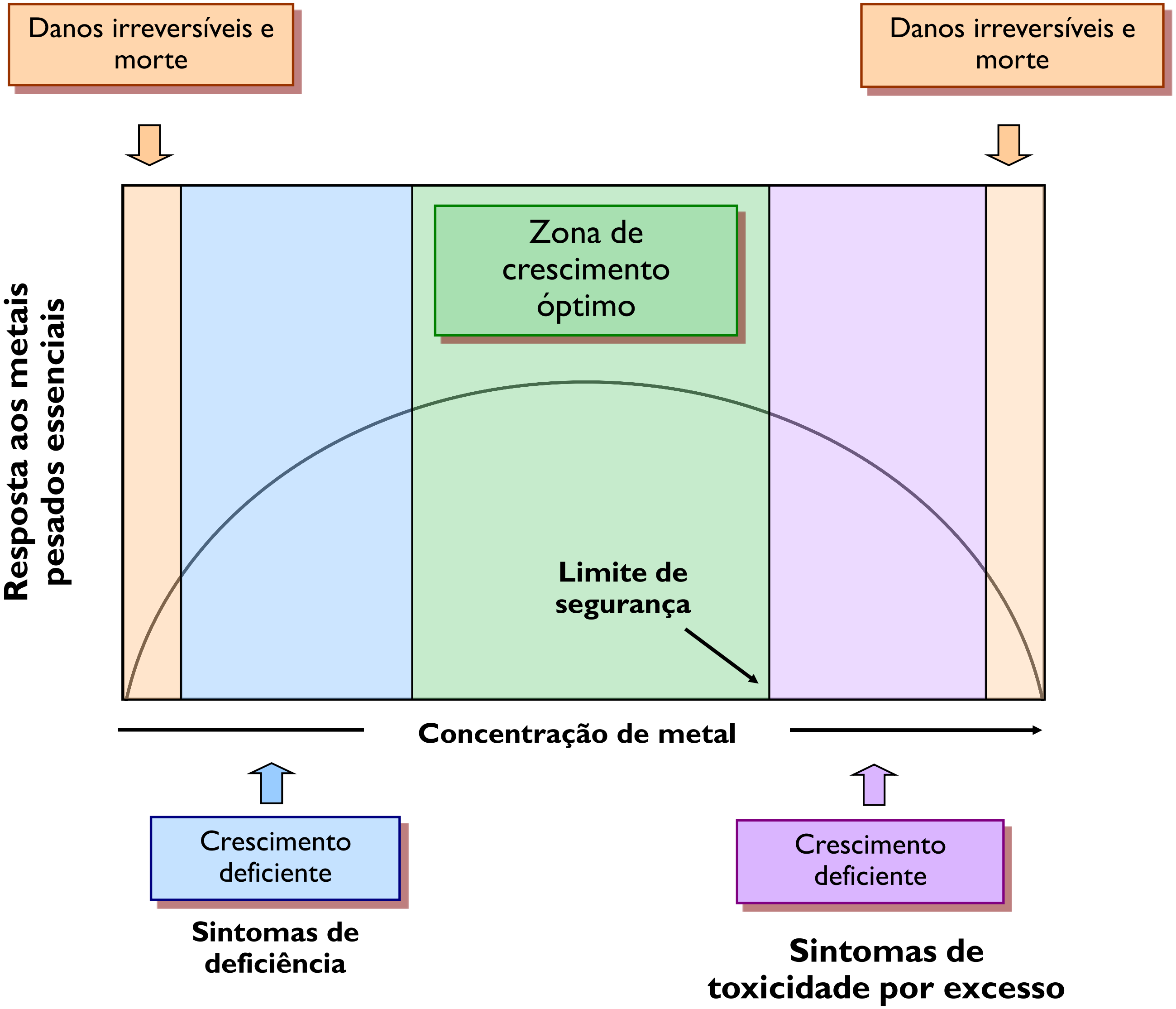
- **Other"elements":** (Prasad, 2004)

  Light-metal: Al (2.7) *Non-metal*: Se (4.8)
  Half-heavy metal: Sn (7.3) Metalóide: As (5.7)

- The concentration of these elements in the environment can increase through human activity: industy, mining, agriculture etc.

(use of fossil fuels, mines, use of contaminated sludges or organic residues in agriculture, fertilizers, pesticides, …)

Dose/response curve for an essential element for plants: Deficiency vs Toxicity Ex: zinc

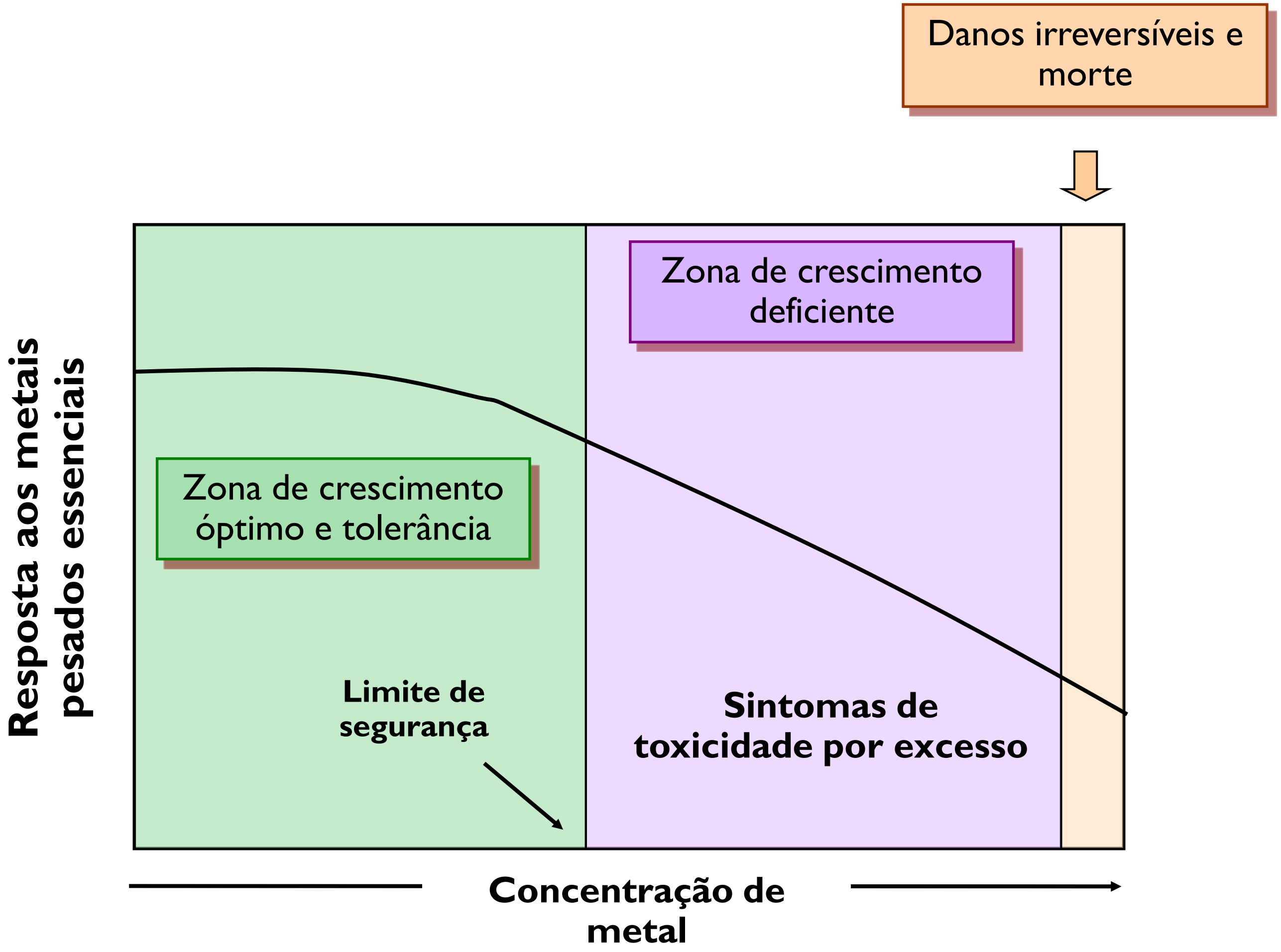


> "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous.“
> Paracelsus (1493-1541)

Dose/response curve for a non-essential element for plants:

Toxicity

Ex: Cd, Hg, Pb



Metals can bioaccumulate in food products, entering the food chain and accumulate in humans causing acute or chronic toxicity

Toxicity depends on:

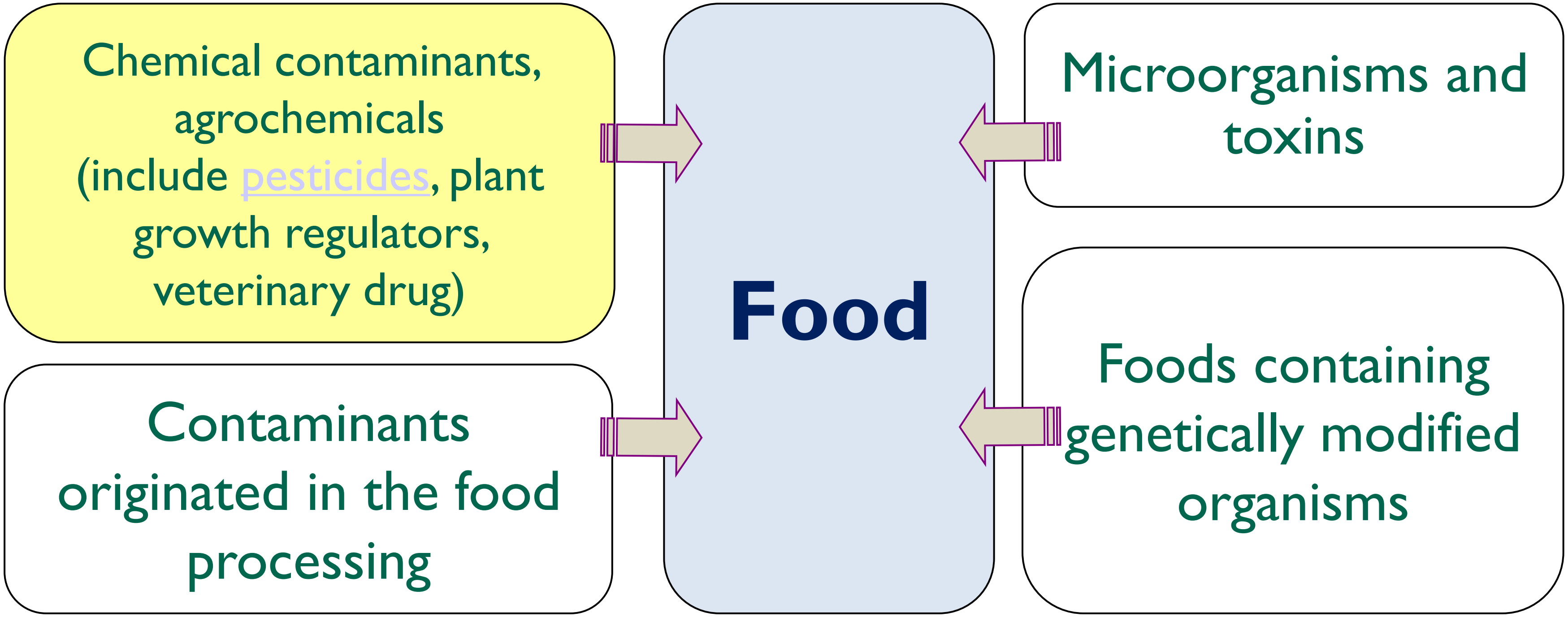
-type of metal
-chemical form
-bioavailability
-solubility
-mobility in the plant
-accumulation rate in living organisms
-accumulation location
-relative position of the organism in the food chain

Exposition degree and contamination of the food chain:

Types of contaminants:

according to the source of contamination and the mechanism by which they enter the food product

Chemical contaminants present in foods are often **unaffected** by thermal processing



Chemical contaminants, agrochemicals (include pesticides, plant growth regulators, veterinary drug)

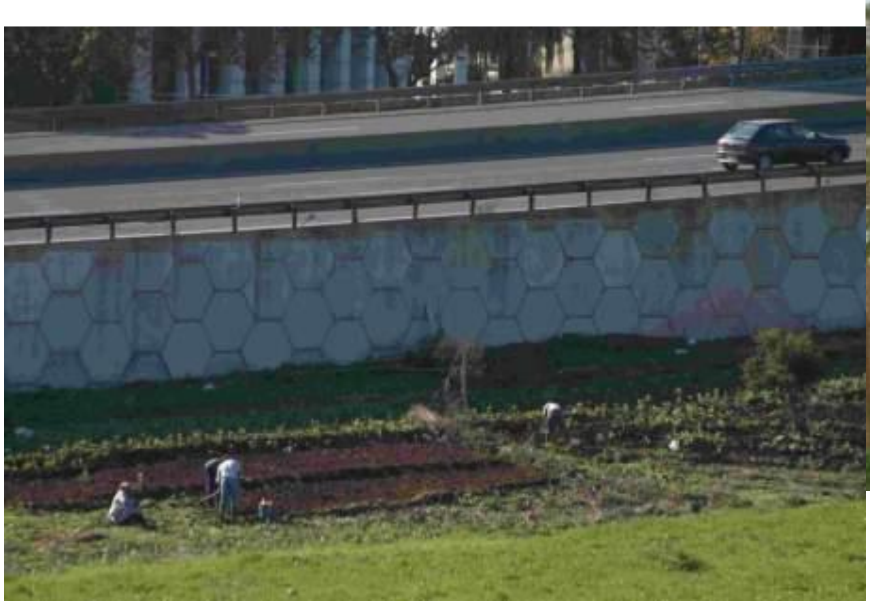
Contaminants originated in the food processing

Food

Microorganisms and toxins

Foods containing genetically modified organisms

Urban gardens / Hortas Urbanas



à roda das hortas

uma caminhada* revigorante à roda das hortas urbanas

* Cerca de 16 km.

24 de Fevereiro de 2013 (Domingo)
das 9h às 13h
Início: Quinta das Conchas
(junto à entrada mais próxima do Metro)

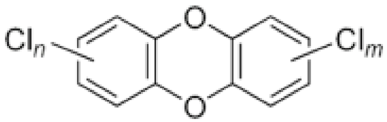
Caminharemos pelo Parque Agrícola da Alta de Lisboa e pela Horta Acessível para conhecer as hortas urbanas da zona norte de Lisboa. Venha daí!

AVAal

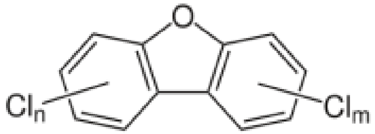
CONTAMINANTS

Organic

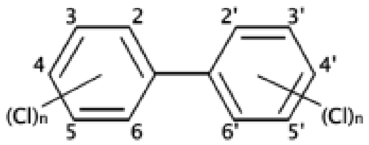
- *Dioxins* (Polychlorinated dibenzo-p-dioxins) - *In 1999, high levels of dioxins were found in poultry and eggs from Belgium!*



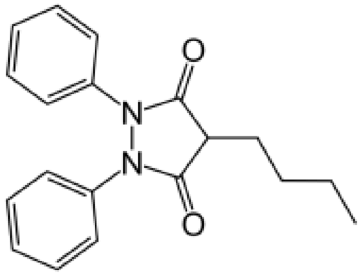
- Polychlorinated dibenzofurans



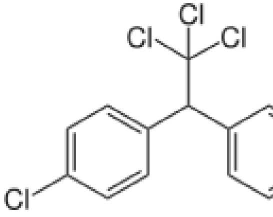
- Polychlorinated biphenyls (PCBs) - *Yusho disaster (Japan, 1968), Yu-Cheng disaster (Taiwan, 1978)*



- Veterinary drug residues - *Phenylbutazone or 'bute' – which is legally used to treat sore joints in horses but outlawed from entering the human food chain - was detected by the FSA in five cases at UK abattoirs in 2012.*

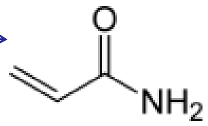


- Pesticides residues



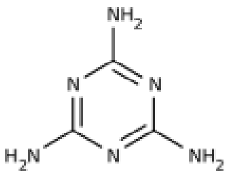
DDT

- Acrylamide →



- PAHs

-> Melamine (Melamine is sometimes unethically added to food products in order to increase the apparent protein content (ex. Kjeldahl method).



Inorganic

- Aluminium (Al)
- Arsenic (As)
- Cadmium (Cd)
- Copper (Cu)
- Iron (Fe)
- Lead (Pb)
- Mercury (Hg)
- Tin (Sn)
- Zinc (Zn)
- Nitrates (NO_3)
- …

Cadmium (Cd)

Can be present in food products. Tobacco smoke is an important Cd source for humans.

Arsenic (As)

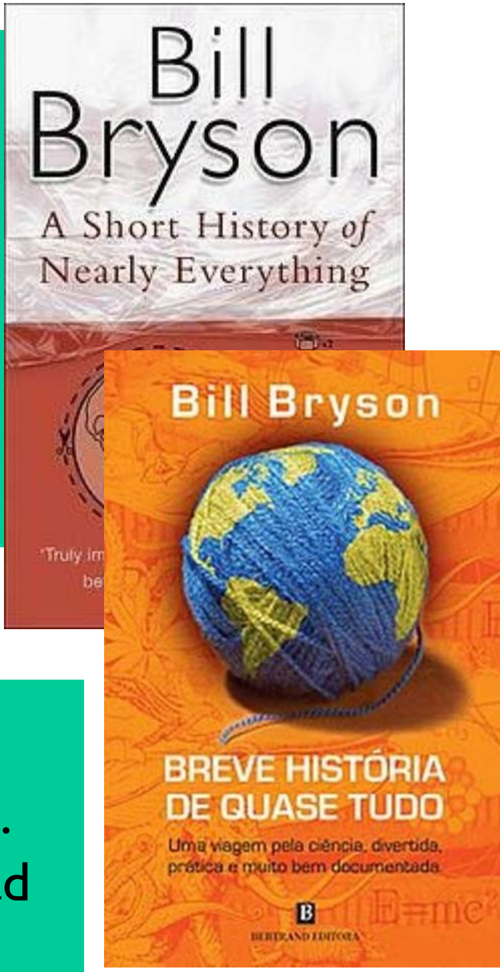
The "slow dead" mineral. Used frequently as a poison in the past.

Still used for example in pesticides and poultry food additives.

Lead (Pb)

Neurotoxic, was largely used in the 20th century (leaded gasoline, pesticides, paints, soldered food cans, water piping…)

Chapter 10 – Getting the Lead Out

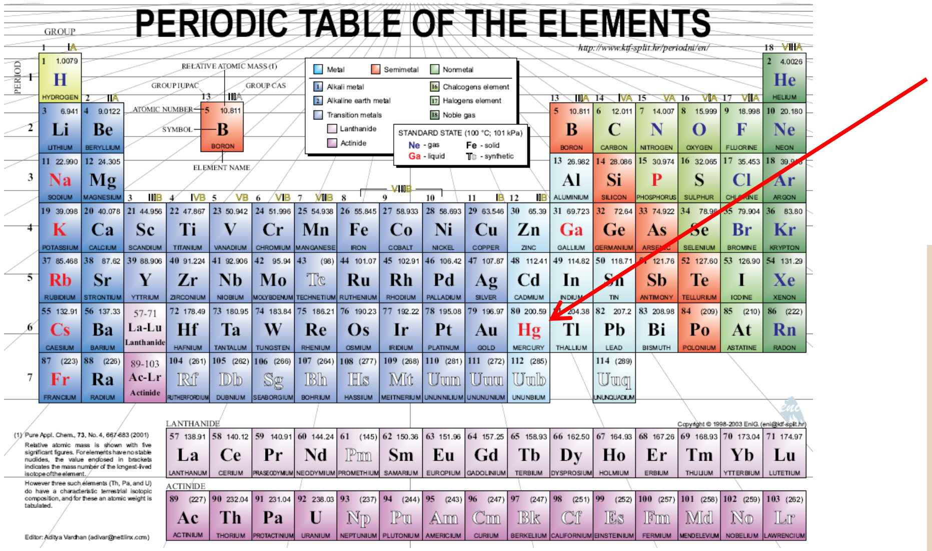


Mercury (Hg)

Frequently found in fish. Associated with the Mad Hatters disease in the 19th century (hence the expression "mad as a hatter").

Mercury (Hg^{2+}):

Minamata disease was first discovered in Minamata city in Japan in 1956. It was caused by the release of **methylmercury** in the industrial **wastewater** from the Chisso Corporation's chemical factory, which continued from 1932 to 1968. Chisso Co. began using Minamata's bay as a repository for its mercury waste, poisoning thousands of people, and killing hundreds.



Consumption of fish and waters contaminated with industrial residues containing Hg

Other examples of Hg contamination:

-Iraq (1971-1972), accidental cusumption of cereals (not supposed to be consumed by humans) treated with a mercury-based fungicide

-is **highly immobile in soil**, and so there is little absorption by plants; in the environment its levels have been stable for 50 years dimethylmercury $CH_3–Hg–CH_3$

methylmercury salts $CH_3–Hg–X$

phenylmercury $C_6H_5–Hg–X$

Most of the toxic effects result from the consumption of food products containing organomercury compounds

Allowed levels for a 70 kg adult:

0.35 mg Hg per week (0.2 as methylmercury)

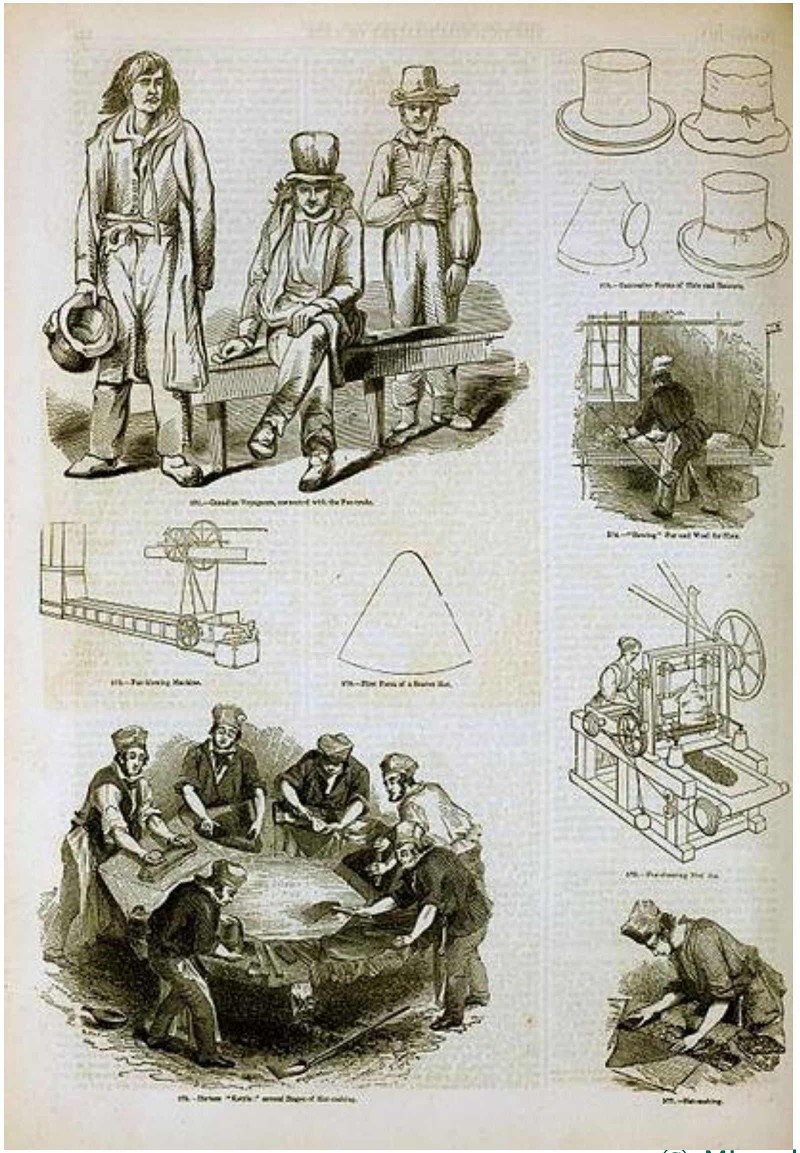
Most of the Hg intake comes from the ingestion of fish

Fish and shellfish concentrate mercury in their bodies, often in the form of methylmercury, a highly toxic organic compound of mercury.

Species of fish that are **high on the food chain**, such as shark, swordfish, king mackerel, albacore tuna, contain **higher concentrations of mercury than others,** stored in the muscle tissues of fish; when a predatory fish eats another fish, it assumes the entire body burden of mercury in the consumed fish.

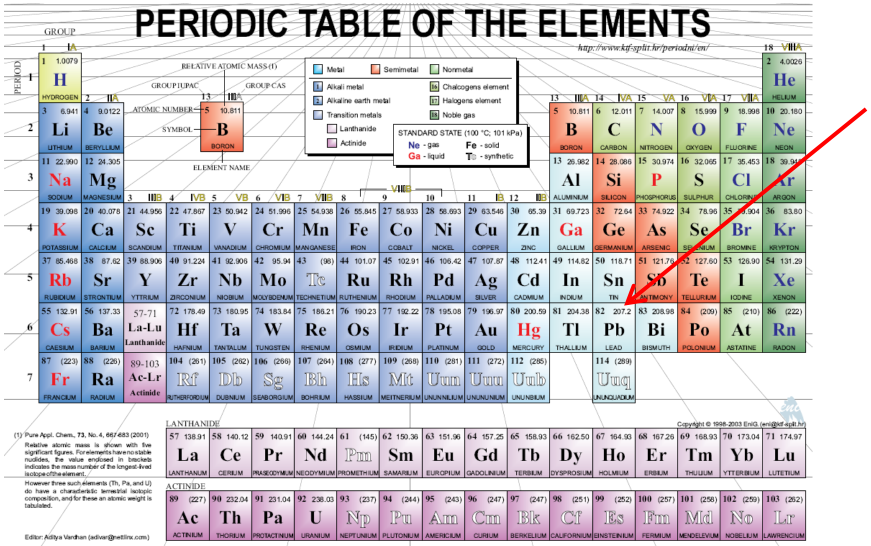
Recent tests indicate that up to 6 percent of canned light tuna may contain high Hg levels.

Mad hatter disease



Mad hatter disease, or mad hatter syndrome, is a commonly used name for occupational chronic mercury poisoning among hatmakers whose felting work involved prolonged exposure to **mercury** vapours. The neurotoxic effects included tremors, shyness and irritability.
It was due to the use of inorganic mercury in the form of mercuric nitrate ($Hg(NO_3)_2$) to treat the fur of small animals for the manufacture of felt hats, mainly up to the early 20th century. By the Victorian era the hatters' condition had become proverbial, as reflected in popular expressions like "mad as a hatter" and the hatters' shakes.

Lead (Pb^{2+}):



Toxic effect of Pb results from inhalation, oral and skin ingestion

Strong neurotoxic, accumulates in different tissues and bones and can lead to severe diseases or death

Allowed weekly dose for a 70 kg adult: 3.5 mg Pb per week

Currently the average ingestion levels does not present a serious risk to consumers (Soares, 2003)

Commission Regulation (CE) n° 466/2001, regulates maximum levels

The contamination do food products from soils is very low as lead is very immobile, and the uptake by plants is very small

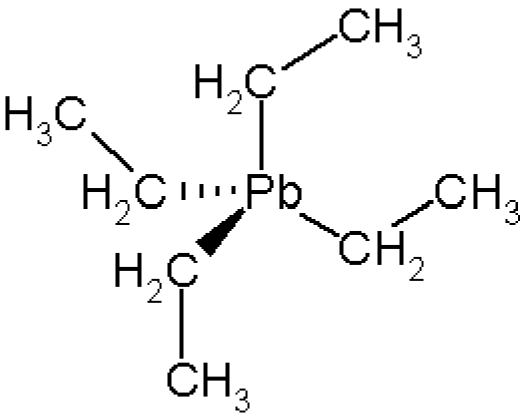
Lead contamination is not very significant today, more control of:

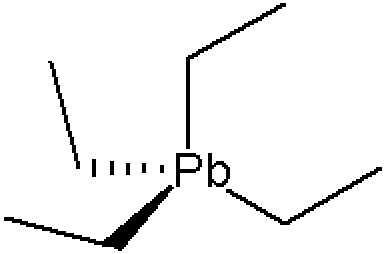
- Soldering, packaging, paints, cristals
- Water plumbing, kitchen equipment and cleaning products

Tetraethyl lead added to gasoline to increase engine performance during most of the 20th century:

$(CH_3CH_2)_4Pb$

-Pb in the environment increased with industrialization mainly due to the emissions from leaded gasoline:





The use of tetraethyl lead $(C_2H_5)_4Pb$ was used to allow greater engine compression and thus engine performance and fuel economy; it is converted to PbO, $PbCl_2$ and other inorganic lead compounds by the combustion process

-was found along roads at a distance up to 30 m;
-at 100 m from a road with heavy traffic the levels in soils and plants decreased up to 20 times

Cadmium (Cd^{2+}):

Carcinogenic, and accumulates in living organisms (half-life of 18-30 years)

Prolonged ingestion causes accumulation in kidneys and liver

Average lethal dose for man: 0.027 g/kg

Presently, the levels of ingestion do not present a serious danger for consumers

Around 50% of the intake of Cd is via the respiratory system and 5% orally

ex. tobacco smoke = 2-4 µg Cd

-is **highly soluble** and thus can be readly absorbed by plants and contaminate the food chain

Pathways for Cd contamination:

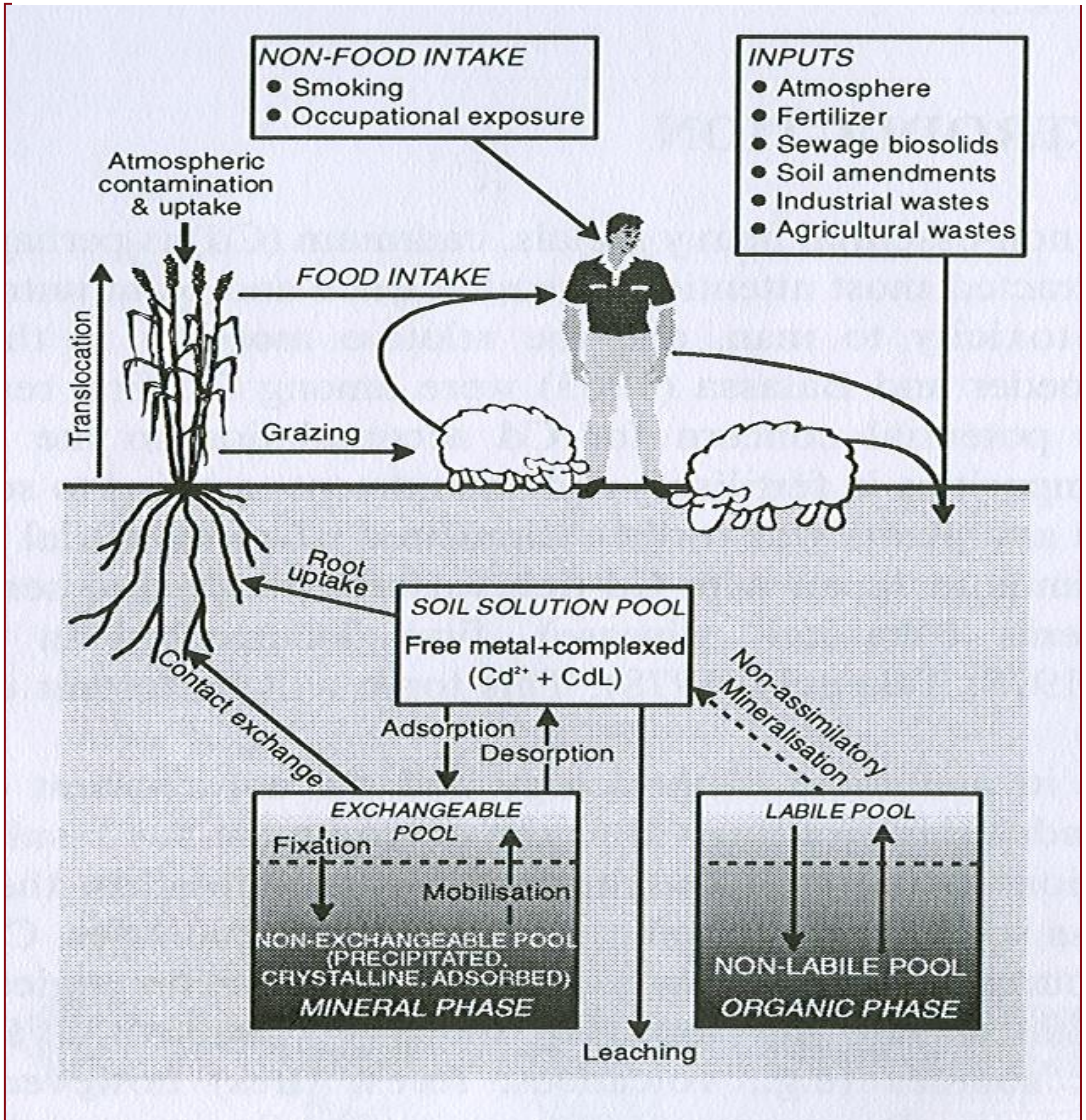


Figure 1.1 Fluxes of Cd in soils, plants and the food chain.

-Water and industrial residues, municipal solid wastes, wastewater sludges, batteries, fossil combustion processes

-Vegetable food products: uptake from soil (about 0.2-16 mg/kg) by plants and translocation to other plant parts

-Animal food products: milk, seafood, internal organs like liver and kidneys

INFLUÊNCIA DO CÁDMIO EM
PARÂMETROS BIOQUÍMICOS E
DE CRESCIMENTO DE
NABIÇAS (*Brassica rapa)*
Patricia Barbosa
Tese de mestrado, 6 Dezembro 2011

Controlo

10 µM de Cd

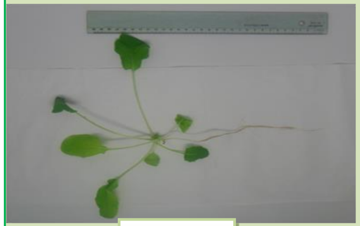
25 µM de Cd

Dia 0

Dia 4

Dia 4

Dia 4



Dia 6

Dia 6

Dia 6

Dia 10

Dia 10

Dia 10

Human health effects of continuous exposition to Cd:

Main organs affected: bones, lungs and respiratory system, kidneys, liver (detected in the urine)

Main effects: carcinogenic, teratogenic (malformations), mutagenic

Leads to several diseases in the liver, kidneys, bones and lungs

The rice absorbed all heavy metals, especially the cadmium, that accumulates in the people eating contaminated rice.

Itai-itai disease (イタイイタイ病 *itai-itai byō*), ("ouch-ouch" disease)

Caused by **cadmium poisoning** due to mining in Toyama; started in 1589 and continuing through 1945, cadmium was released in significant quantities by mining operations, and the disease first appeared around 1912.

The river *Jinzu* was used mainly for **irrigation** of rice fields, but also for **drinking water**, **washing**, **fishing**, and other uses by downstream populations.

http://en.wikipedia.org/wiki/Itai_itai

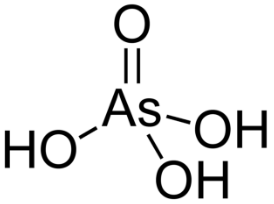
Arsenic: *The most toxic chemical forms contain oxygen, arsine* (AsH_3) *and arsenic trioxide* (As_2O_3) (carcinogenic)

Inorganic:

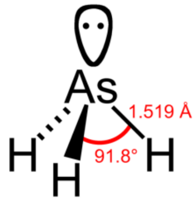
Arsenites (As^{3+}), As(III)
Arsenates (As^{5+}) As(V)

Organic:

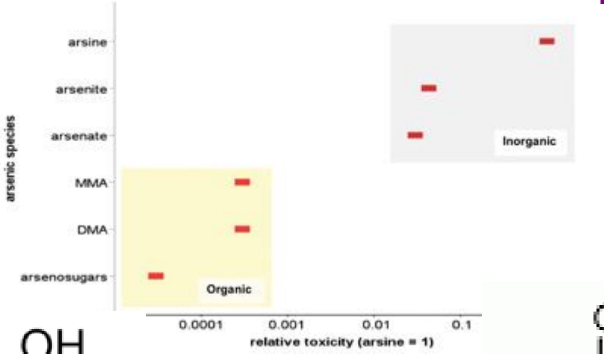
Arsenosugars, Dimethylarsenic acid,
Methylarsonic acid, etc.

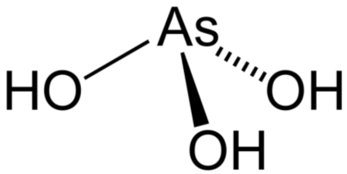


Arsenic acid (H_3AsO_4)
-arsenate-

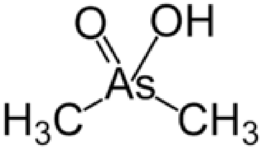


Arsine (AsH_3)

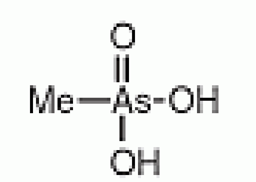




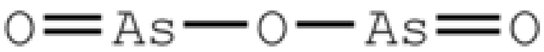
Arsenous acid (H_3AsO_3)
-arsenite-



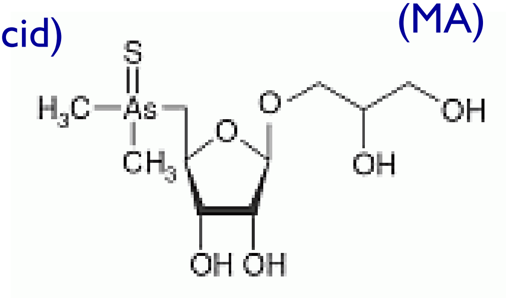
Dimethylarsenic acid (DMA) (cacodylic acid)



Methylarsonic acid (MA)

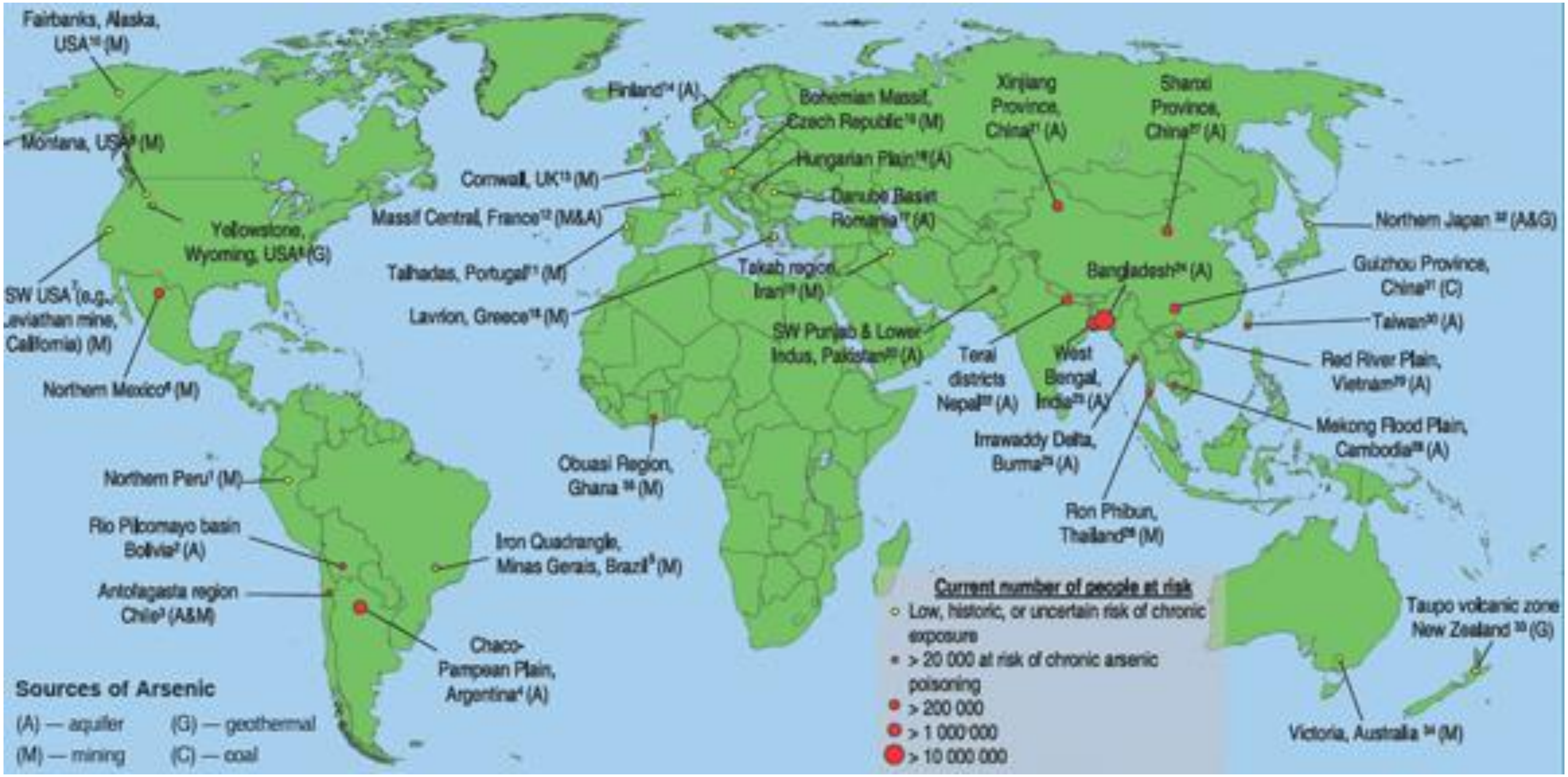


Arsenic trioxide (As_2O_3)



Arsenosugars

Contamination with arsenic



http://www.iupac.org/publications/ci/2008/3004/2_garelick.html

"Arsenic the king of poisons, has probably influenced human history more than other element or toxic compound"

"It was used to kill many aristocratic and noble gentlemen, terrorize others (...), employed by women to free themselves from tyrannical husbands and unwanted lovers" *Frankenberger, W.T. 2002*

Middle ages:

-Arsenic oxide is tasteless, odorless, cheap, powdery white

-Can be administered with sugar, does not diminuish appetite, is fatal in small doses,

*-The symptoms of **chronical and acute poisoning** mimic natural diseases obfuscating the true cause of death.*

There is a theory that **Napoleon Bonaparte** (1769–1821) suffered and died from arsenic poisoning during his imprisonment on the island of Saint Helena.

Forensic samples of his hair did show high levels, **13 times the normal amount of the element**, but at that time copper arsenite was used in several products as a **pigment in some wallpapers and inks**

Food contamination with As has anthropogenic origin and results in the industrial use and accidental contamination

Toxic effect by inhalation, ingestion and contact – rapid absorption and rapid excretion by urine (half-life of 1-2 days)

Continuous exposition can lead to retention of 30-40% in the bones, muscles, skin, hair and nails (keratin rich tissues)

-Pesticides, herbicides, insecticides (copper arsenate has high toxicity)

-Decolouring agents, preservers, pigments (paper, candles, decorative elements etc.)

-Pharmaceutic and veterinary products (feed additives): for long term treatment of skin deseases, leukemia, reumatism, sifilis, malaria and others *(Frankenberger, W.T. 2002)*

-Dangerous jobs (copper, gold and lead mining)

Accidental poisoning remains problematic:

-1955: drinking of contaminated **dry milk** results in chronic and acute poisoning of 13419 Japanese children, 839 have died

-of 5000 cases of heavy metal ingestion reported by American Association of Poison Control Centers in 1984, arsenic was found to be the most commonly involved (over 1200 incidents)

Contamination of the environment with ARSENIC tends to increase

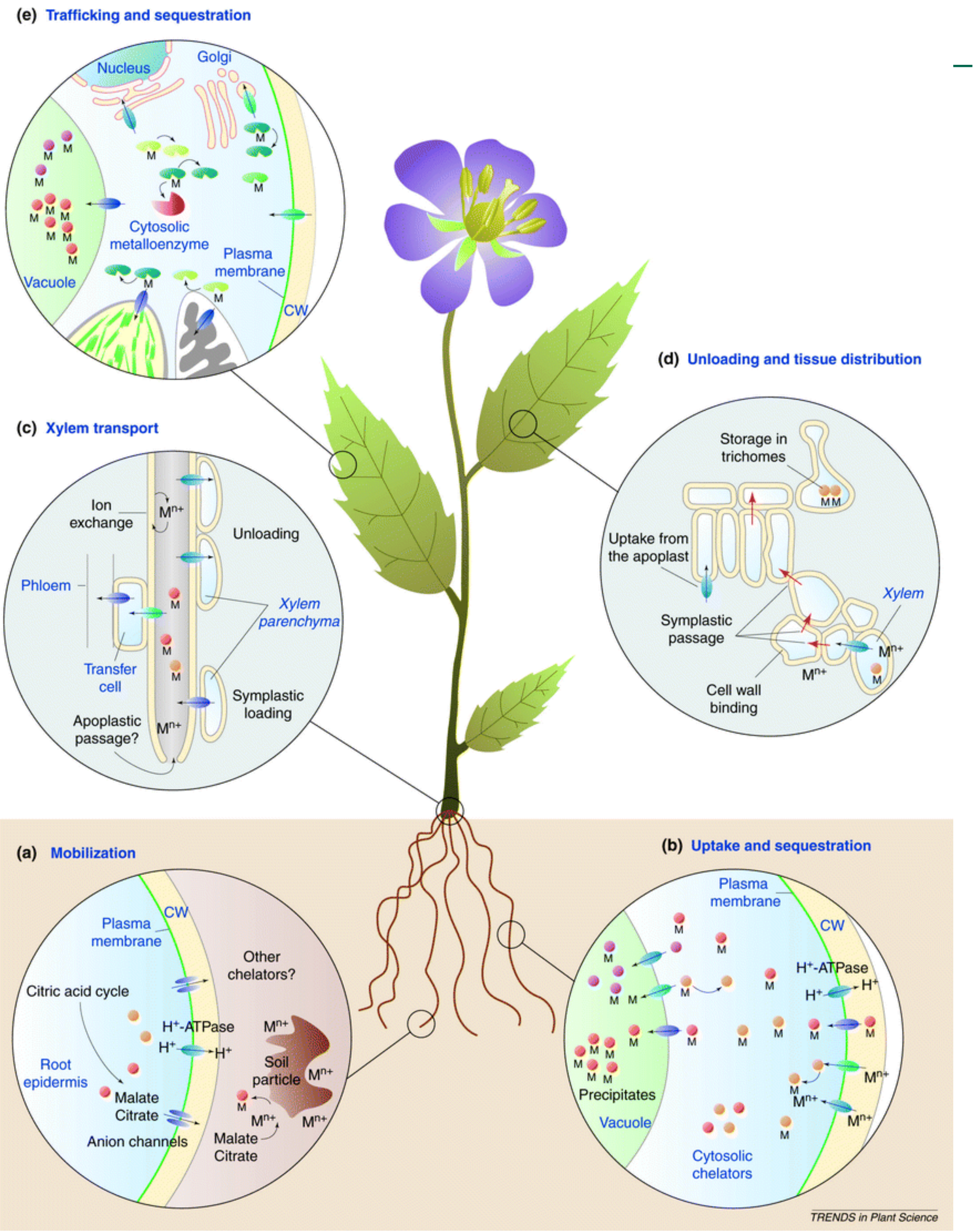
SOS ARSENIC POISONING IN BANGLADESH / INDIA.
THE WORLD'S POOREST POPULATION IN BANGLADESH, ARE SUFFERING FROM ARSENIC POISONING, SOCIAL AND ENVIRONMENT DEGRADATION

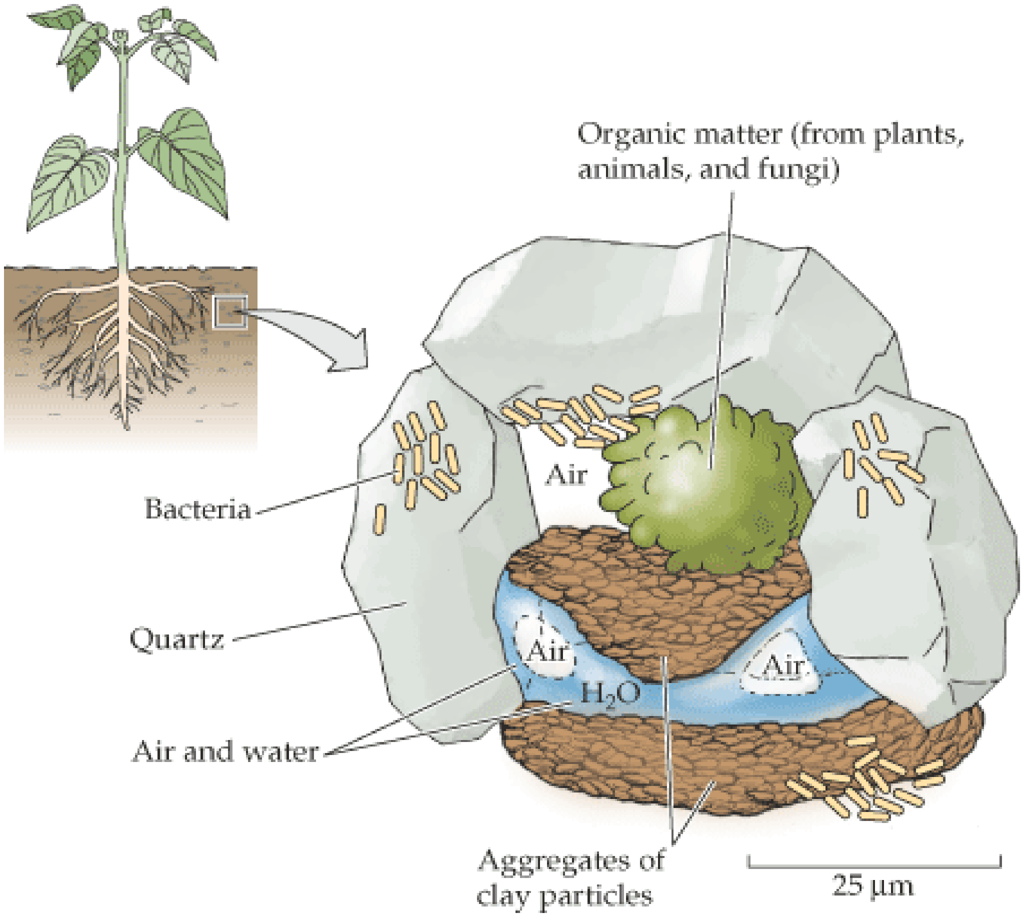
The exposure to arsenic, mainly through drinking water, increases the risk of different types of cancers (genetic, mutagenic and teratogenic effects).

Arsenic threatens the drinking water resources in many parts of the world, and the consumption of the water over the years must resulted in untold suffering by millions of people *Frankenberger, W.T.2002*

-high levels of arsenic in subterranean waters have been found in Thailand, China, India, Bangladesh, Taiwan, Chile, Argentina, Mexico, Canada and USA, mainly due to the use of products containing arsenic in agriculture.

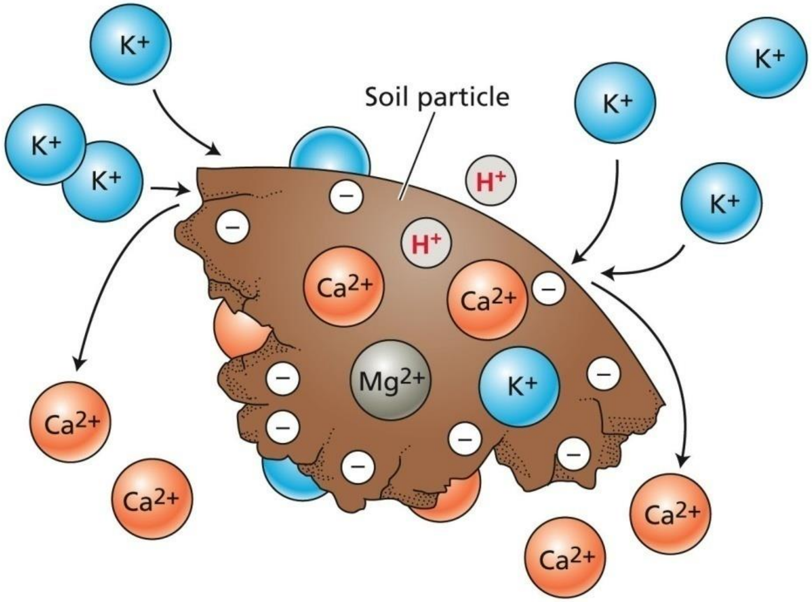
Plant response to the toxic effect of metals





Soil structure showing the presence of bacteria, organic and inorganic matter, water and air.

Imagem retirada de Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com)



PLANT PHYSIOLOGY, Fourth Edition, Figure 5.5 © 2006 Sinauer Associates, Inc.

Heavy metal *uptake*:
availability, absorption, transport and accumulation in plants

Bioavailability :
Metal is released from its bounded forms, and can become available to living organisms

Bioavailable fraction:
Fraction of the total metal content that plants can absorb

The different fractions of the metal in the soil are in a dynamic equilibrium

Different forms of the metals found in soils:

- **-Soluble in soil solution**

→ **The only soil fraction directly available for plant uptake**

- -Precipitated
- -Adsorbed (ex. in clays and organic matter)
- -In minerals

Lower bioavailable forms

Toxic metals only cause phytotoxicity **when they are bioavailable** in relatively high concentrations

The availability of metals in the soils depends on its type, properties and chemical characteristics

pH
Organic matter
cation-exchange capacity (CEC)
Ionic strength
Fe and Mn oxides
Redox potential
Texture
Clay content
Microorganisms

- Affect the partition of metals in the liquid and solid phases

- Affect the solubility and availability of metals to the plants

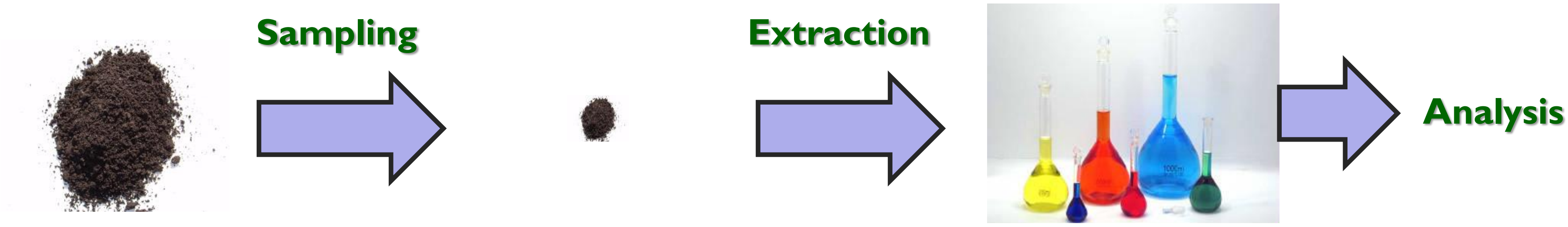
Some plants have developed specific mechanisms to partially regulate bioavailability

Experimental activity– soil characterization

- **pH and Conductivity**: 10 g of soil in 25 mL deionized water. Shake for 1 hour and measure.
- **P and K quantification**: Extraction with Egner-Rhiem solution. 2 g of soil in 40 ml solution, shake for 2 hours, filter (blue band filter). *To be analysed next week.*

Organic matter determination, P and K quantification, metal extraction

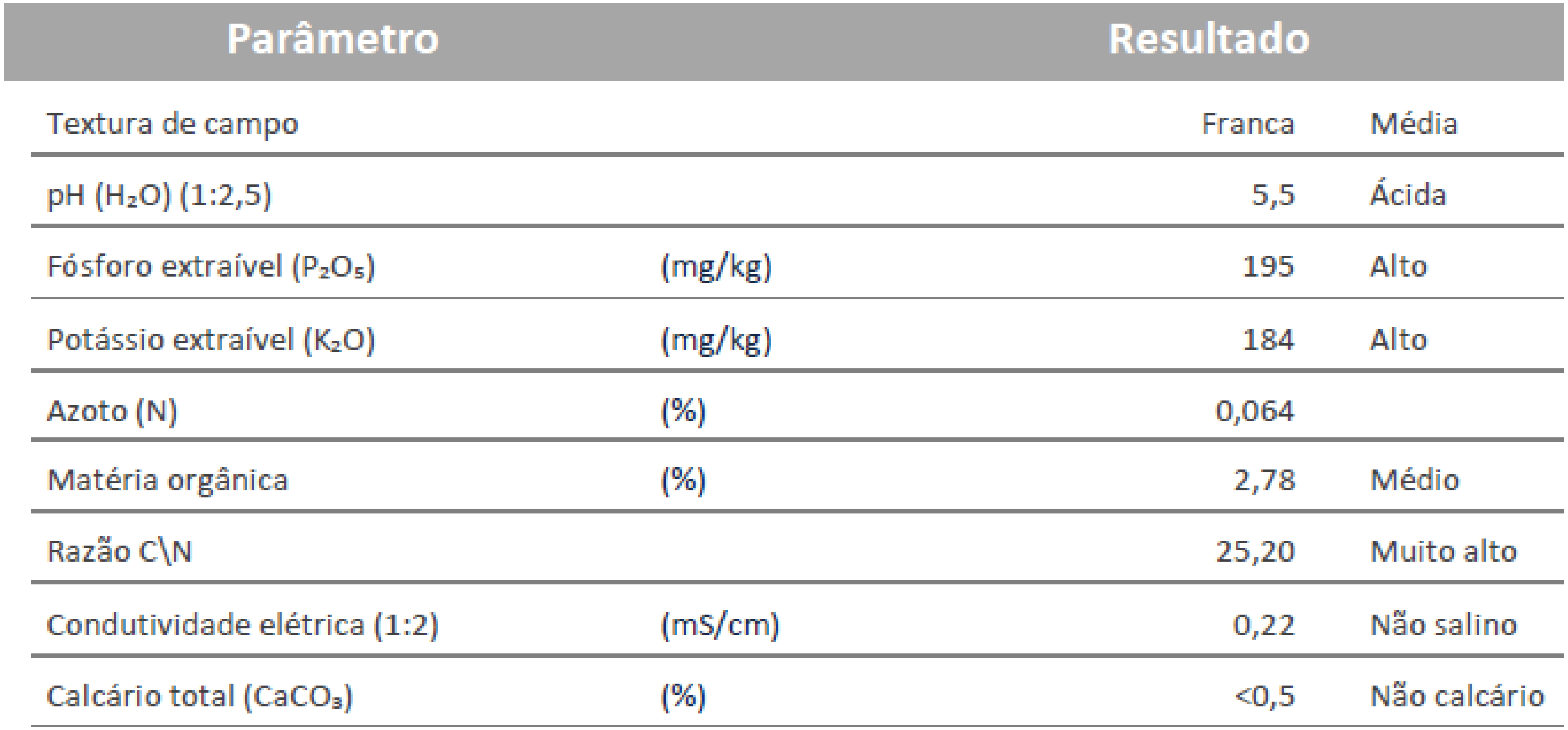
Soil collection and extraction, heavy metal determination



Sampling

Extraction

Analysis

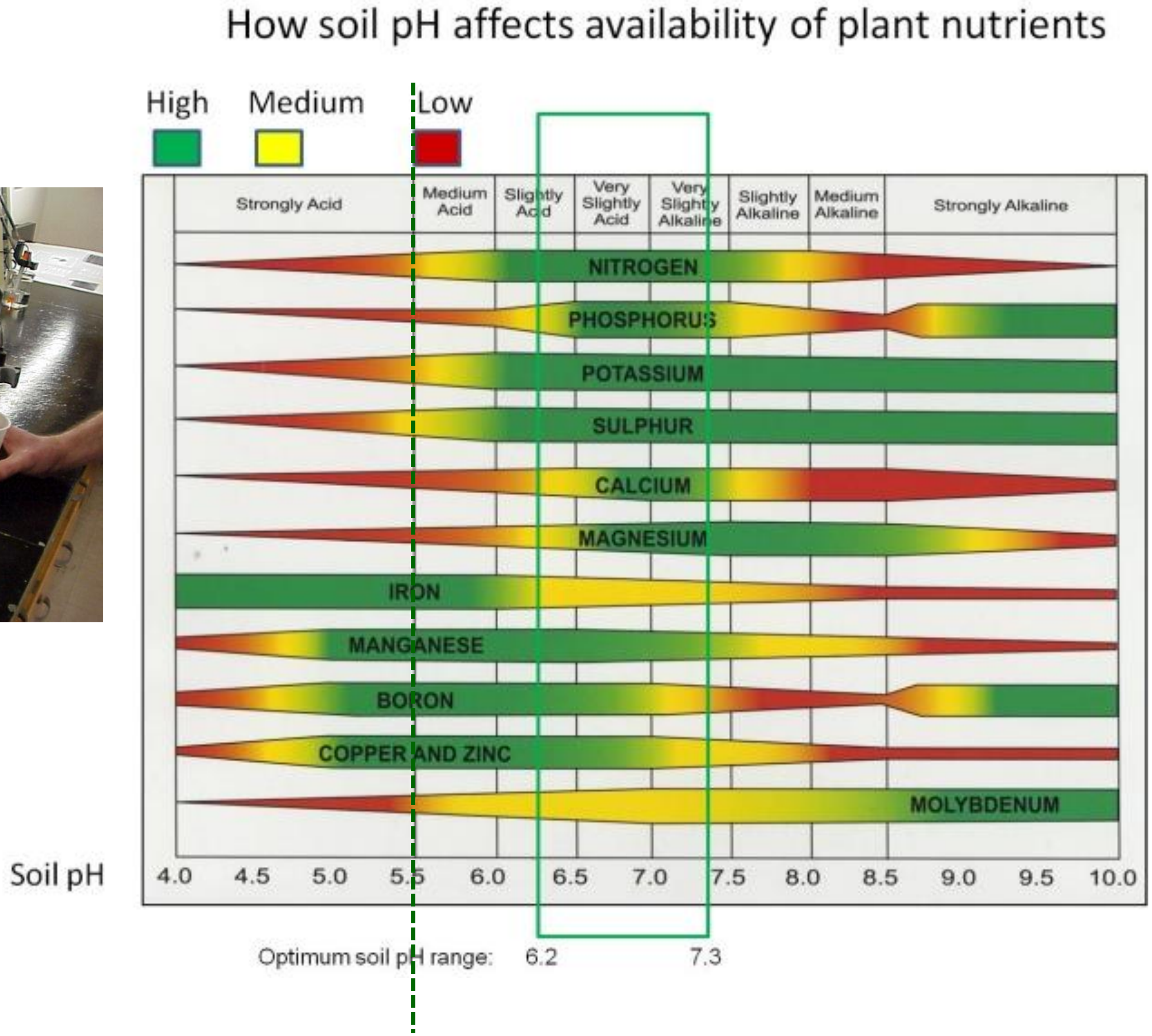


Parâmetro		Resultado	
Textura de campo		Franca	Média
pH (H_2O) (1:2,5)		5,5	Ácida
Fósforo extraível (P_2O_5)	(mg/kg)	195	Alto
Potássio extraível (K_2O)	(mg/kg)	184	Alto
Azoto (N)	(%)	0,064	
Matéria orgânica	(%)	2,78	Médio
Razão C\N		25,20	Muito alto
Condutividade elétrica (1:2)	(mS/cm)	0,22	Não salino
Calcário total ($CaCO_3$)	(%)	<0,5	Não calcário

Soil pH


accumet


6.3
bluelab


How soil pH affects availability of plant nutrients
High
Medium
Low
Strongly Acid
Medium Acid
Slightly Acid
Very Slightly Acid
Very Slightly Alkaline
Slightly Alkaline
Medium Alkaline
Strongly Alkaline
NITROGEN
PHOSPHORUS
POTASSIUM
SULPHUR
CALCIUM
MAGNESIUM
IRON
MANGANESE
BORON
COPPER AND ZINC
MOLYBDENUM
Soil pH
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
Optimum soil pH range: 6.2 7.3

Effect of pH on metal mobility/solubility

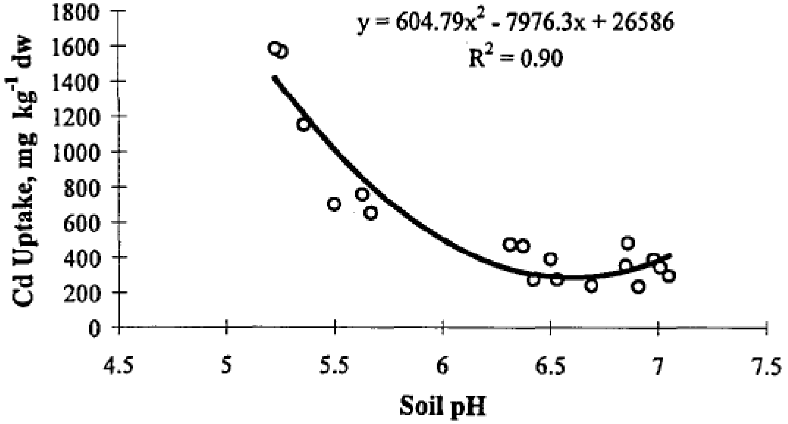
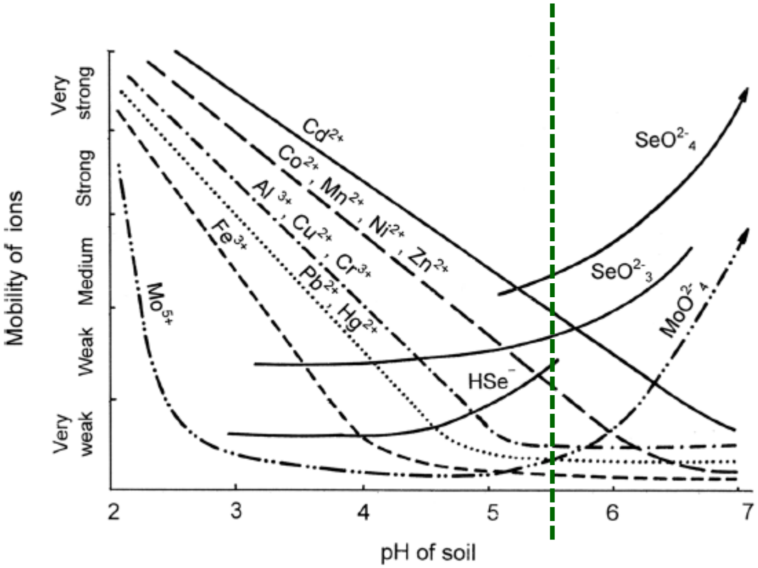
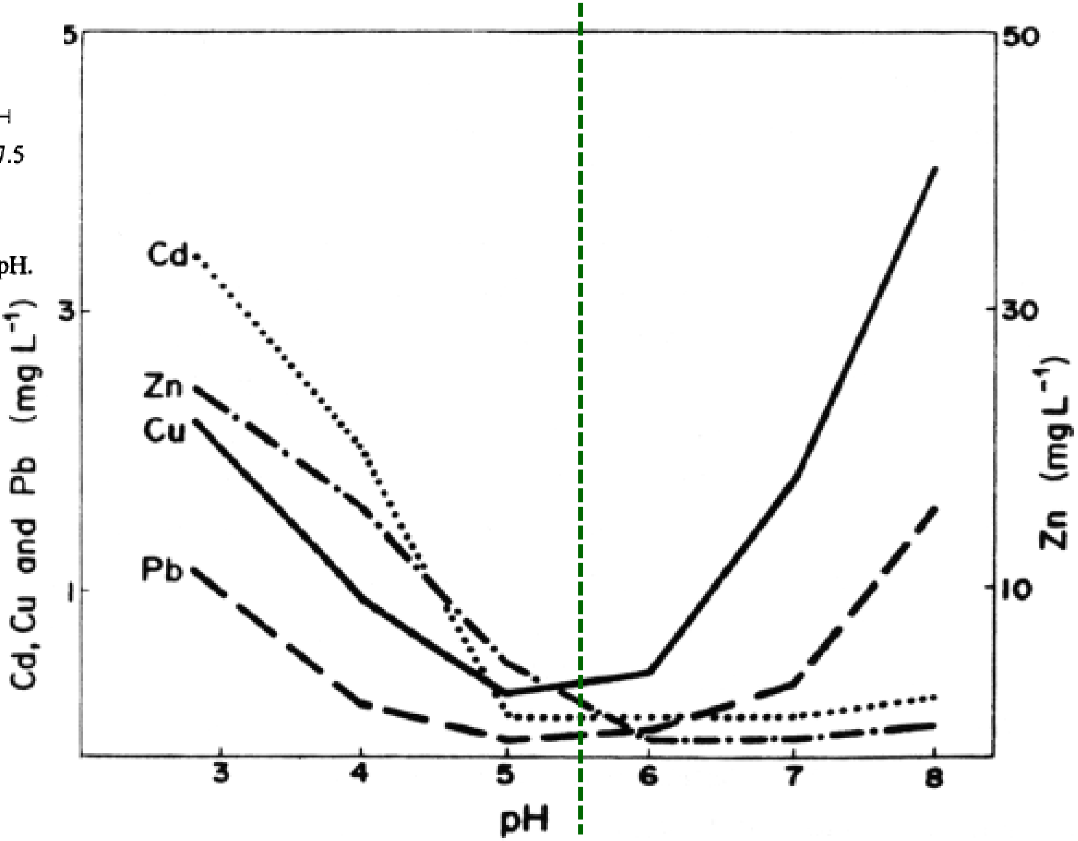


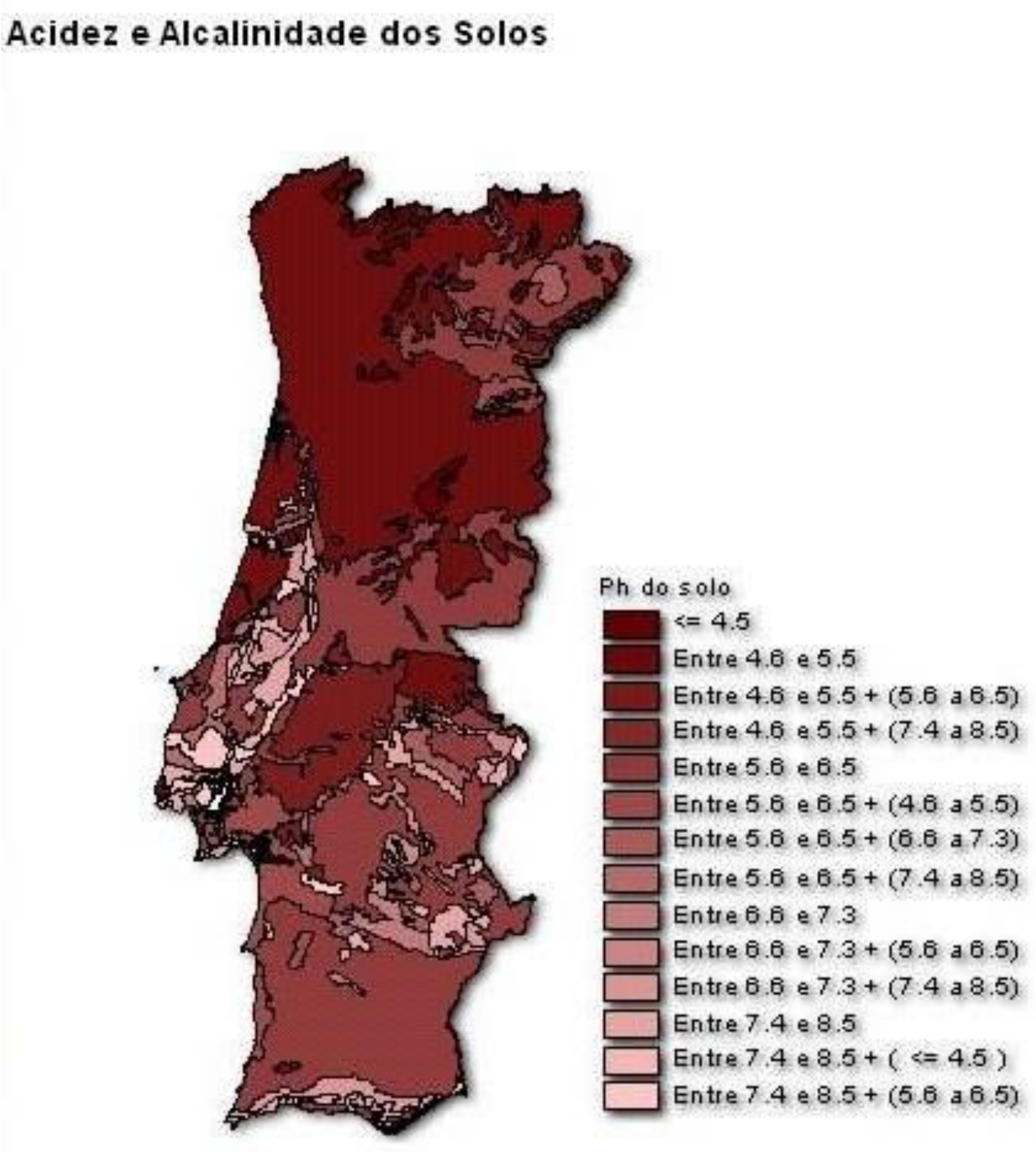
FIGURE 4. Relationship between cadmium uptake by tobacco and soil pH.

To cite this article: C. D. Tsadilas (2000) Soil pH influence on cadmium uptake by tobacco in high cadmium exposure, Journal of Plant Nutrition, 23:8, 1167-1178, DOI: 10.1080/01904160009382090



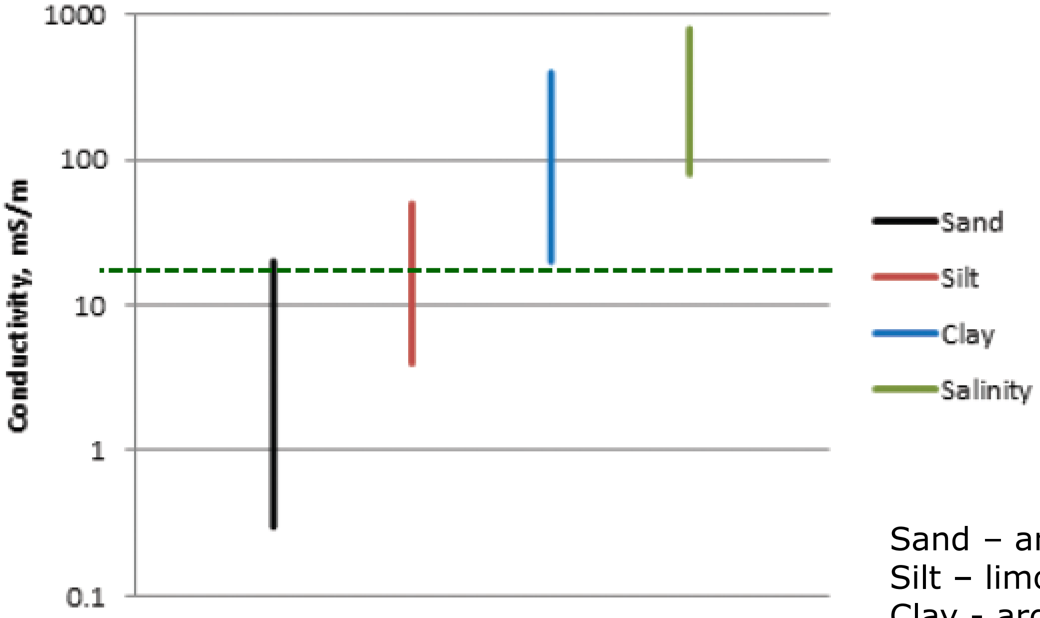


Concentrations of trace metals in equilibrium solutions of sandy gleyic podzol. (Modified from Brümmer and Herms.[956])


Acidez e Alcalinidade dos Solos
Ph do solo
<= 4.5
Entre 4.6 e 5.5
Entre 4.6 e 5.5 + (5.6 a 6.5)
Entre 4.6 e 5.5 + (7.4 a 8.5)
Entre 5.6 e 6.5
Entre 5.6 e 6.5 + (4.6 a 5.5)
Entre 5.6 e 6.5 + (6.6 a 7.3)
Entre 5.6 e 6.5 + (7.4 a 8.5)
Entre 6.6 e 7.3
Entre 6.6 e 7.3 + (5.6 a 6.5)
Entre 6.6 e 7.3 + (7.4 a 8.5)
Entre 7.4 e 8.5
Entre 7.4 e 8.5 + (<= 4.5)
Entre 7.4 e 8.5 + (5.6 a 6.5)

Soil conductivity

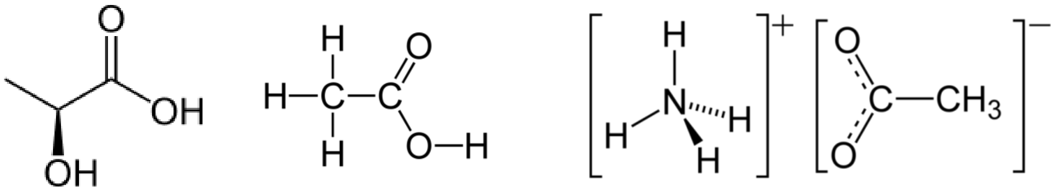
The soil electrical conductivity (EC) indicates the ability the soil water has to carry an electrical current. The EC levels of the soil water is a good indication of the amount of nutrients available for your crops to absorb. It also correlates with several soil properties, like particle size and soil texture and with water holding capacity and organic matter. Commonly expressed in mS/m (milisiemens per meter).



Sand – areia
Silt – limo
Clay - argila

Soil extraction (Egner-Rhiem solution)

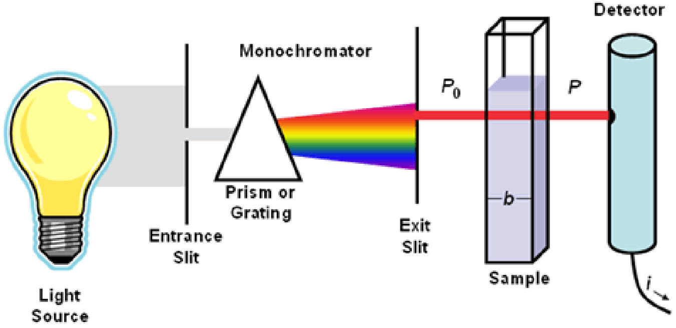
Lactic acid + acetic acid + ammonium acetate

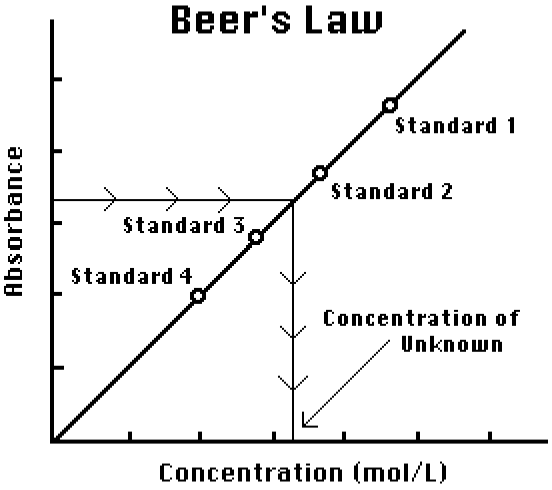


P determination → Molecular absorption spectrophotometry (775 nm)

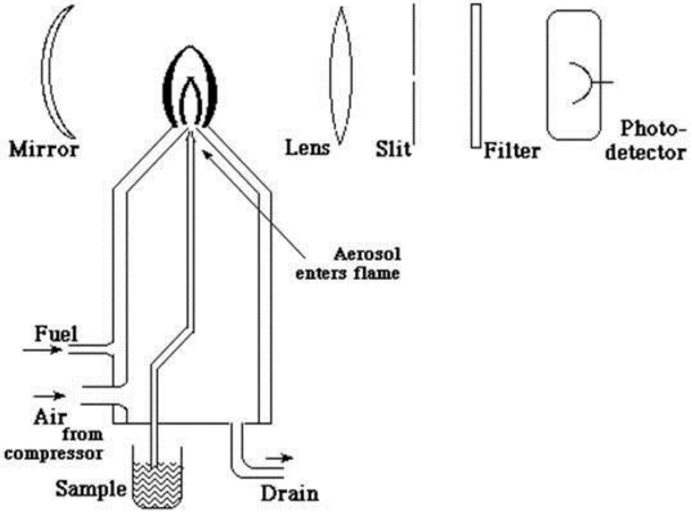
K determination → Flame photometry

Molecular absorption spectrophotometry (775 nm)



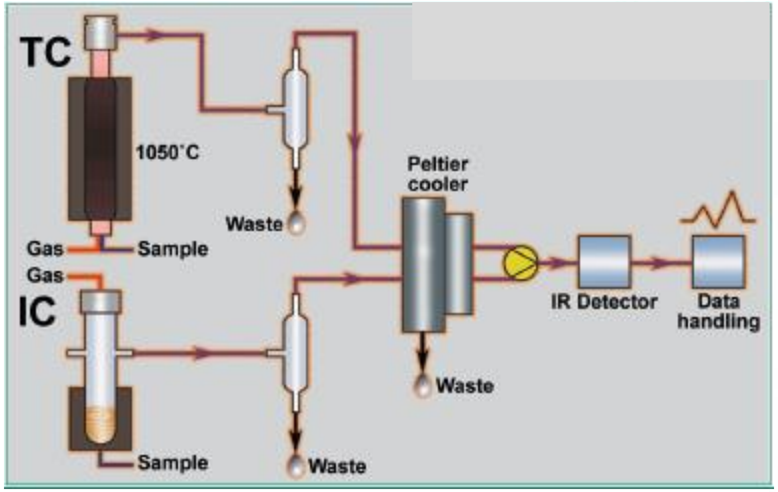


Flame photometry (atomic emission spectroscopy)

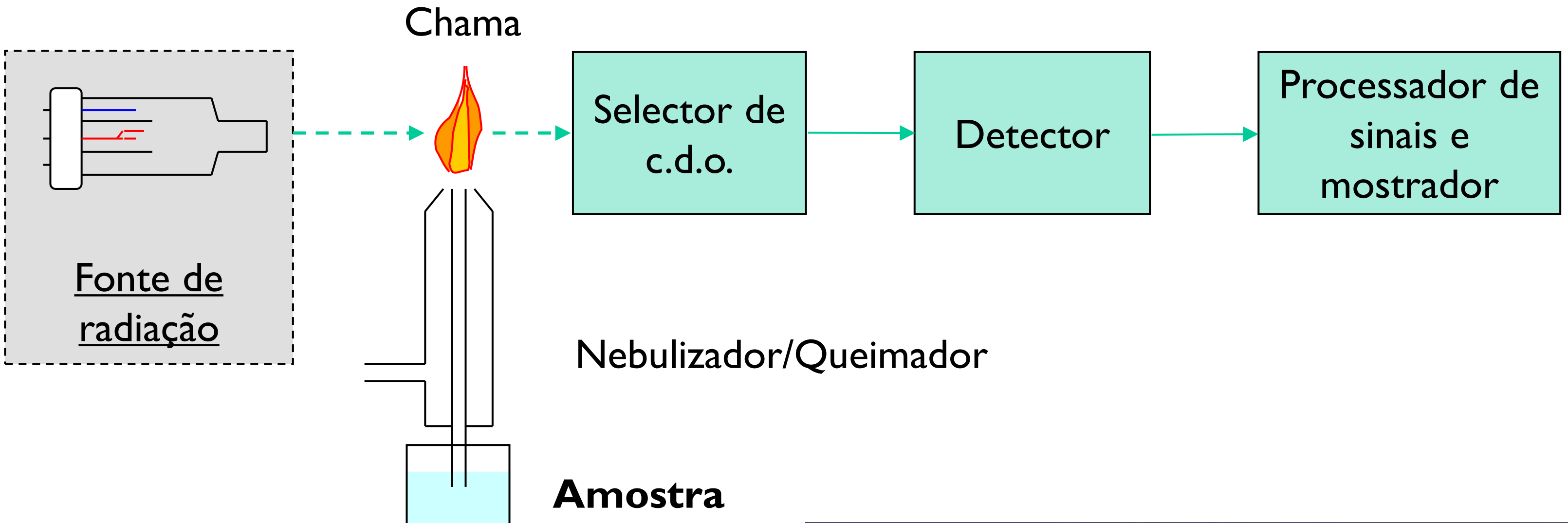


TOC (Total Organic Carbon) determination

The TC is analyzed by high temperature combustion at temperatures up to 1050 °C. The carbon present is oxidized to carbon dioxide (CO_2) and dispersed into the carrier gas. After passing a Peltier cooler for moisture removal and a scrubber system, the CO_2 is measured by Non Dispersive Infrared detection (NDIR).



Espectrofotometria de absorção atómica



Chama

Selector de c.d.o.

Detector

Processador de sinais e mostrador

Fonte de radiação

Nebulizador/Queimador

Amostra

Mn

Cu

Cd

Zn

The stress effect depends on plant **tolerance**

Capacity the plants have to withstand unfavourable environmental conditions

Tolerant plants develop **physiological traits** that allow them to respond to the deleterious effects caused by stress

Development of responde strategies

-Controlling the ***uptake*** of heavy metals (external mechanisms)

-**Metabolic changes** (internal mechanisms)

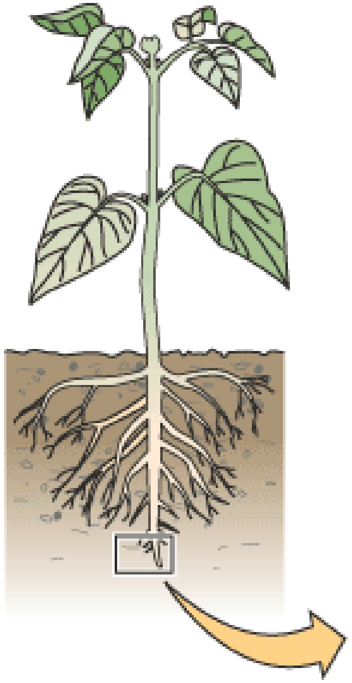
What is the effect of the metals in the plants? How does toxicity affect the plants

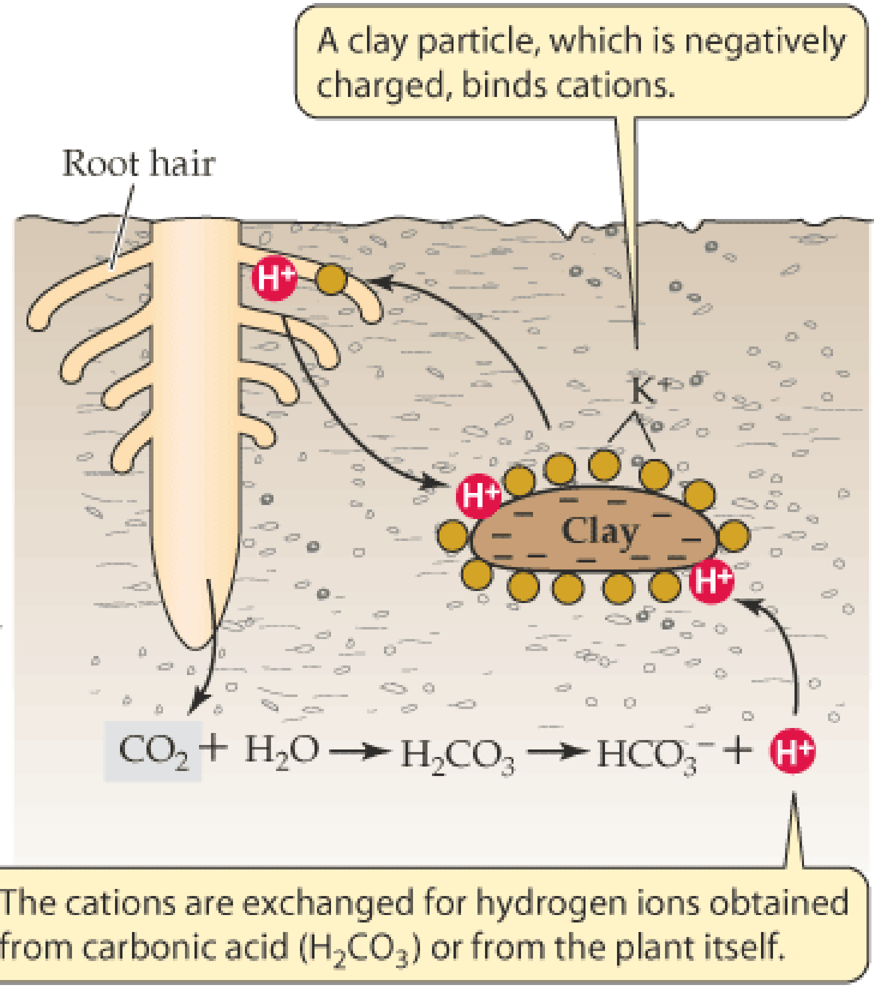
A few examples of our work in this area

Ex: Copper availability

Cu is absorbed by the plants mainly in the form Cu^{2+}

Only a small fraction of **total copper** in the soil is available to the plants, as it binds to organic matter. It is not very soluble in alkaline soils





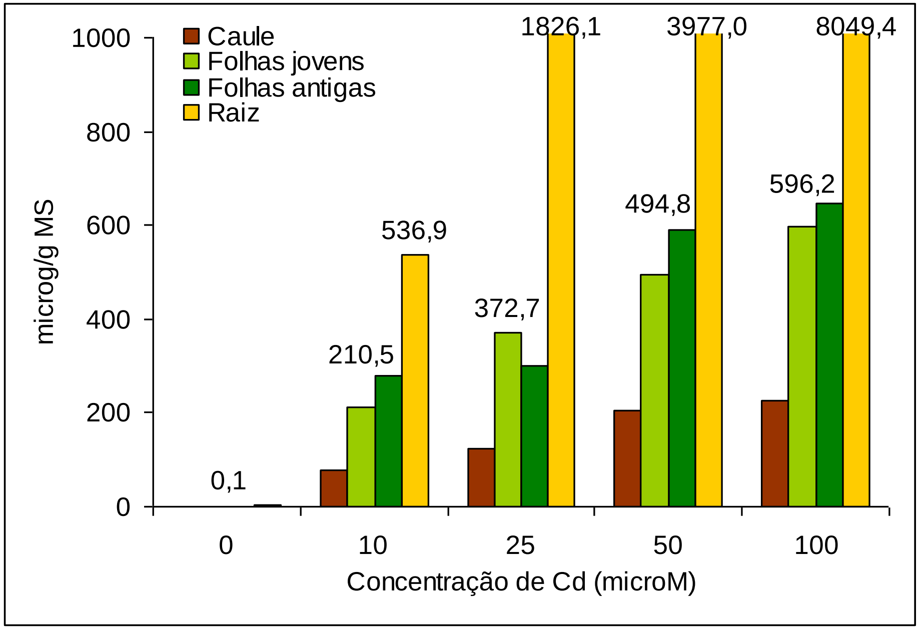
A clay particle, which is negatively charged, binds cations.

$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$

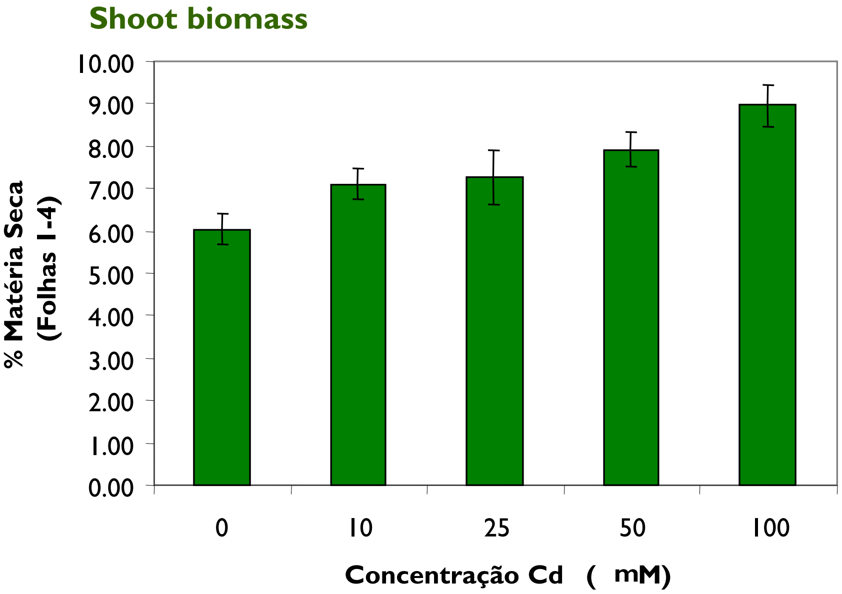
The cations are exchanged for hydrogen ions obtained from carbonic acid (H_2CO_3) or from the plant itself.

Example:

-Oxidative stress studies at UIQA (ISA) on tobacco plant, a known Cd accumulator



Plant continues to grow even with high levels of Cd in the leaves



-Visual effects of phytotoxicity:

-Root and shoot growth is affected, different visual effects in the leaves (ex: chlorosis)
-Symptoms that are dependent on the metal, plant, growth phase etc.

Are a consequence of:

- Metabolic effects of phytotoxicity :

Changes in the metabolism

-Formation of free radicals (ex: H_2O_2)
-Damage to proteins and membrane lipids (peroxidation index, MDA), changes in membrane permeability (K diffusion)
-Changes in chlorophyll, anthocyanins and carotenoids
-Changes in photosynthetic processes
-Protein synthesis
-Synthesis of specific metabolites (proline, organic acids, phytochelatins, etc.)

Visual effect of excess copper in lupins (*Lupinus luteus* L. cv Cardiga):

Licenciatura thesis in Agriculture, Ana Cristina Silva, 2004

FCT financed project POCTI/AGG/44895/2002, 2002 -2007

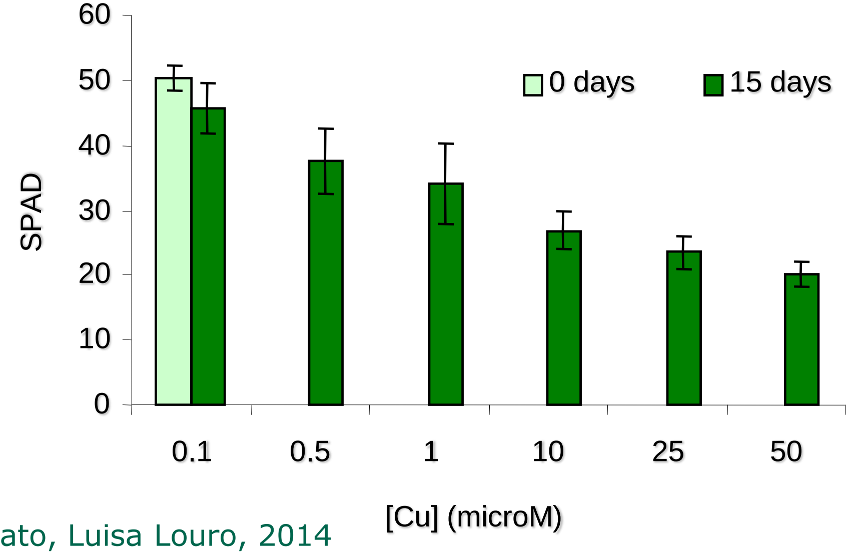
Plants growing under 25 µM Cu for 4 days

Plants growing under 50 µM Cu for 8 days

Yellow lupin

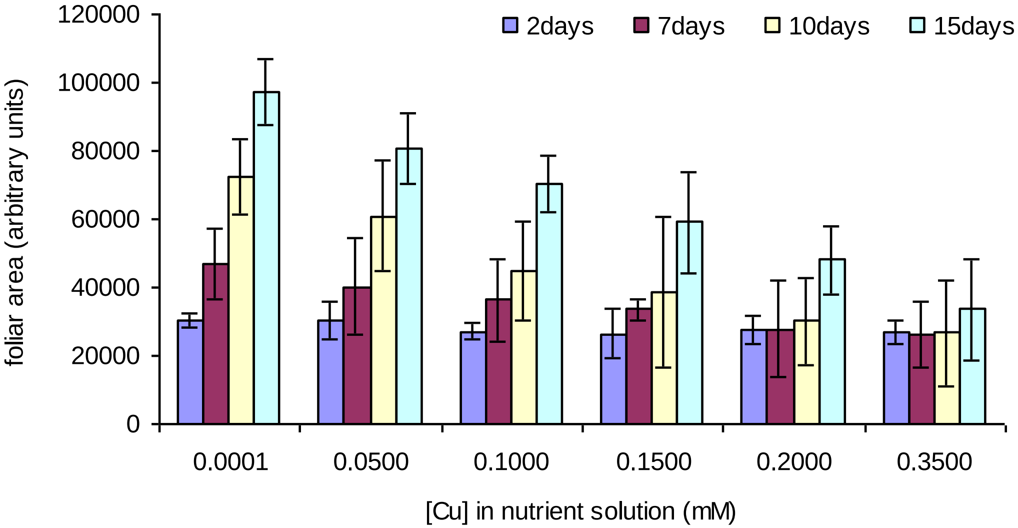
Leaf chlorosis

Chlorophyll levels in leaves after 15 days growing in excess copper



Changes in leaf area of tomato plants as a consequence of copper toxicity (*Licopersicum esculentum* M. cv Hymena):

Trabalho financiado pelo projecto POCTI/AGG/44895/2002



Chlorosis in young tomato plant leaves (*Licopersicum esculentum* M. cv Juncal):

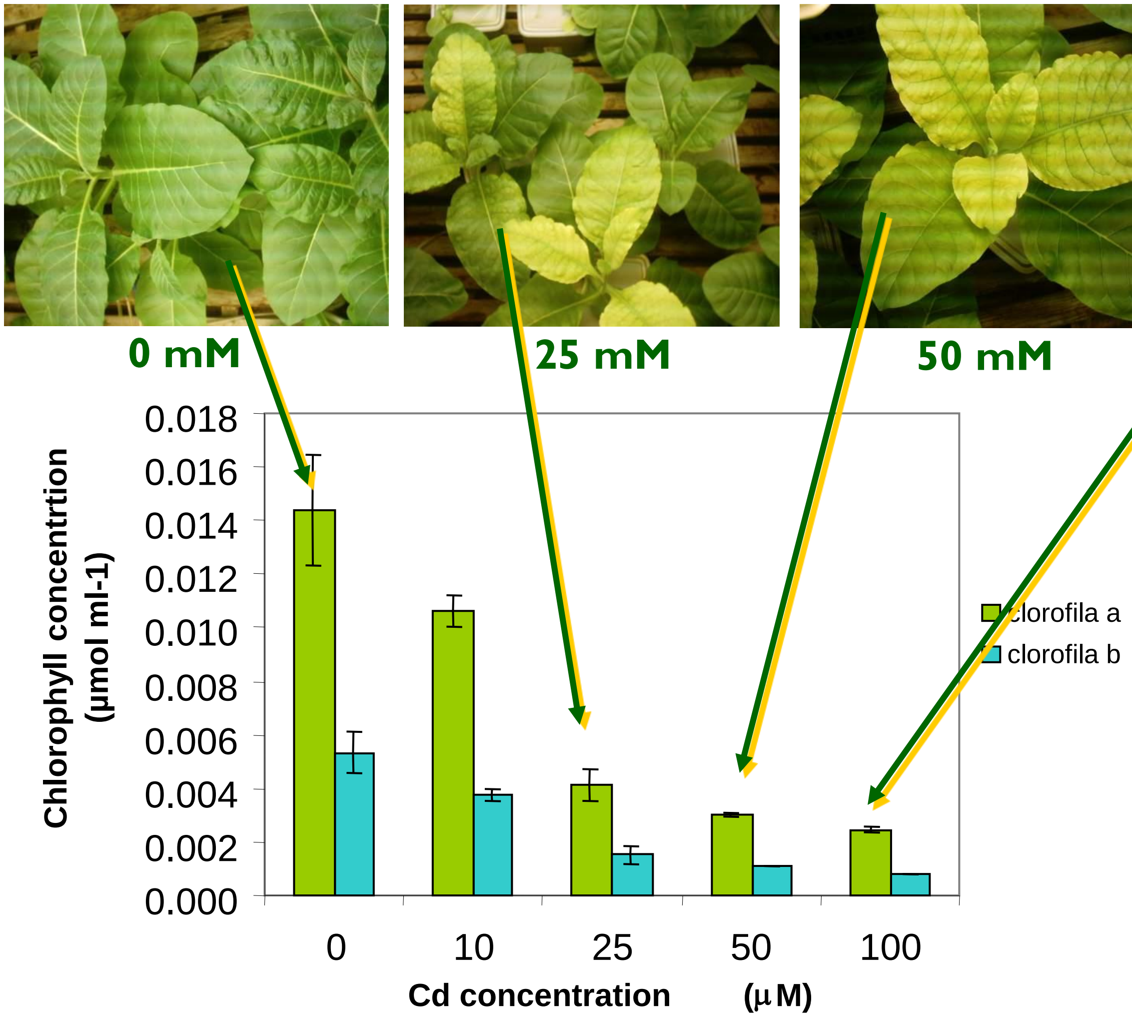
Control plants (0.1 µM)

100 µM

Copper toxicity is more apparent in young leaves that show chlorosis symptoms and reduced area, leading to stunted growth and reduced photosynthetic activity

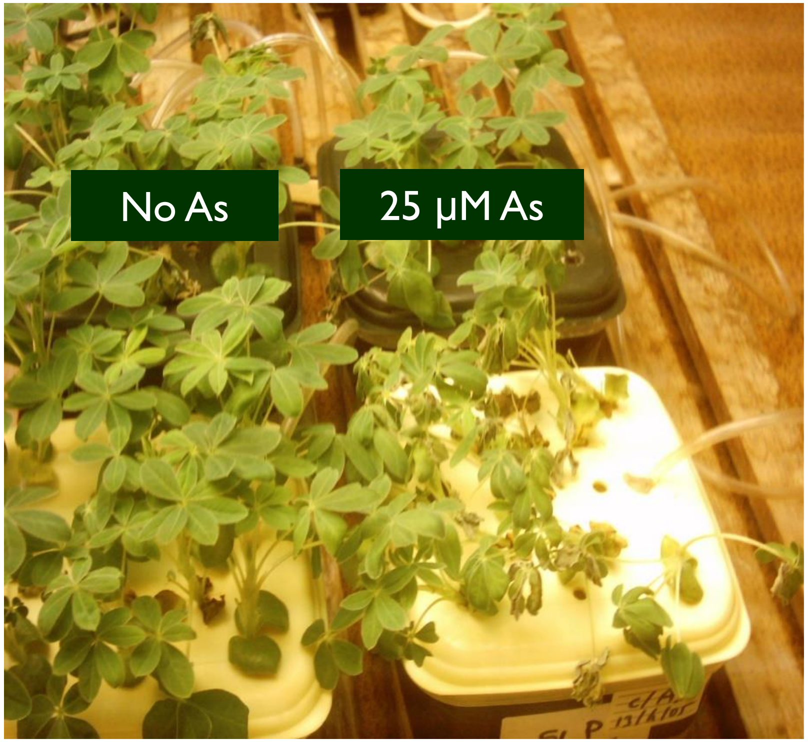
Effect of Cd in chlorophyll levels of tobacco plants (*Nicotiana tabacum* Virginia var. K 326)

Trabalho financiado pelo projecto POCI/AMB/55312/2004



Toxic effect of As in white lupin (*Lupinus albus*)

-Arsenic applied as sodium arsenate:



It was noticed early leaf senescence in plants after 7 days in the presence of 25 µM As

Trabalho de fim de curso de Engenharia Agronómica realizado por Vera Ferreira, 2006

Response (tolerance) mechanisms of plants to heavy metals toxicity:

Before plant uptake:

External (exclusion):

Restriction of uptake from soil and of transport into the plant

Excretion of chelating substances, **exclusion** of metals by selective absorption, **retention** of metals in the roots, avoiding its translocation to the shoots (fixation in root apoplast)

After plant uptake:

Internal:

-Compartmentation and complexation in the cell interior

-**Increase in the production of ligands** (complex the metal): organic acids, amino acids, peptides (Metalothioneins and phytochelatins)

-**Translocation** of the metal and accumulation in the vacuoles

-**Imobilization** of the metal by binding to the cell wall

-Changes in metabolism induced by oxidative stress

-**Activation of specific metabolic pathways** (enzymatic and non enzymatic mechanisms)

External mechanisms against toxic metals:

Exclusion, morfological changes, anatomical adaptations, accumulation in specific cell compartments

Before plant uptake:

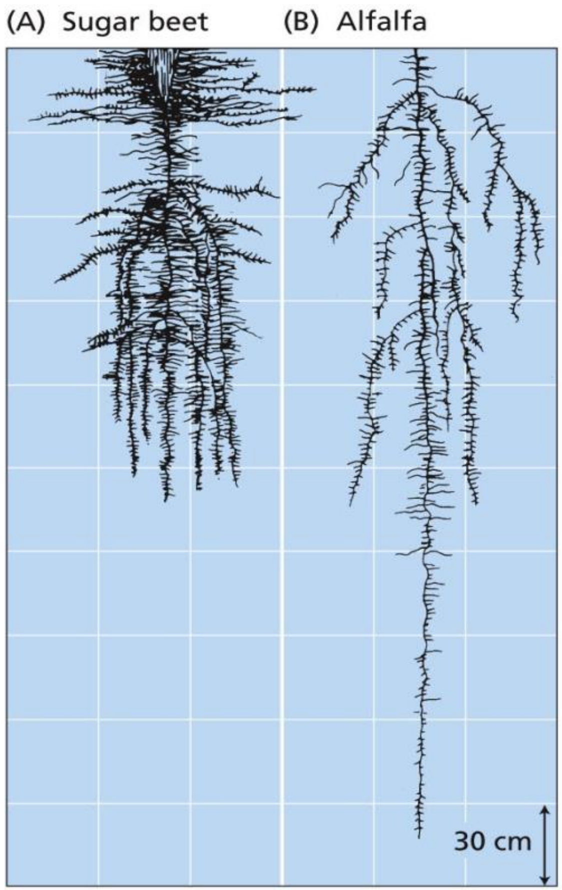
-Metal exclusion and uptake restriction

-*Roots strategies*

Excretion of chelating substances,
Exclusion of metals by selective absorption
Acidification of the rhizosphere: Organic acids (citric, malic) and **free amino acids** (histidine) are ligands used to chelate the metals

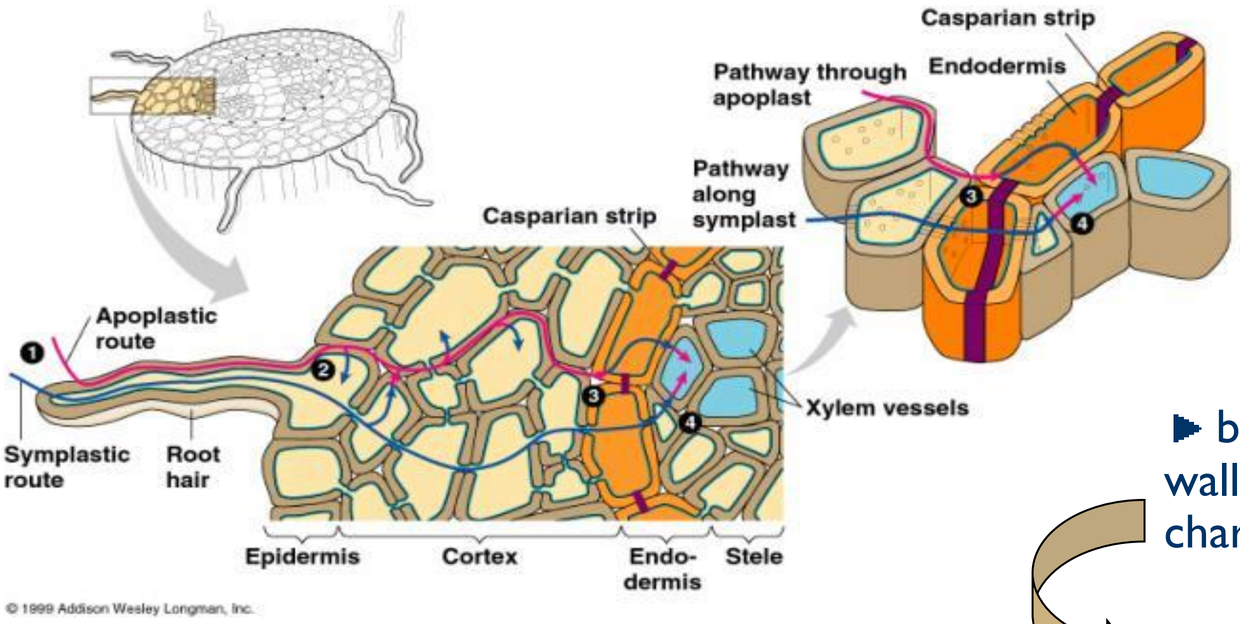
The movement of minerals to the root depends on the **absorption capacity** and the root system architecture

Root arquitecture:



PLANT PHYSIOLOGY, I

Absorption by the roots:



- The metals enter into the root cells
- movement via apoplast or simplast in to the interior of the cell and other regions
- binding to the cell walls via its negative charges
- Transport through the plasmatic membrane into the cell cytoplasm

Ex.
Hg and Pb bind preferably to cell walls reducing the mobile forms of these elements in the plants

Entry into the xylem, transport and distribution to the shoots, acumulation in cell compartments:

Transport of the metals from the root to the xylem, occurs mainly **in the cell interior (via symplast)** after sequestering by specific ligands

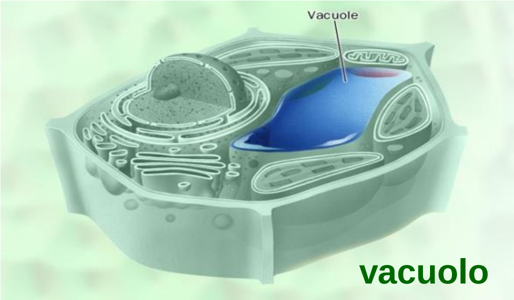
In the xylem the metals are present as hydrated ions or as complexes

Sequestering and storage in the shoots:
-cell compartmentalization

The storage of toxic substances can occur in the thrichomes

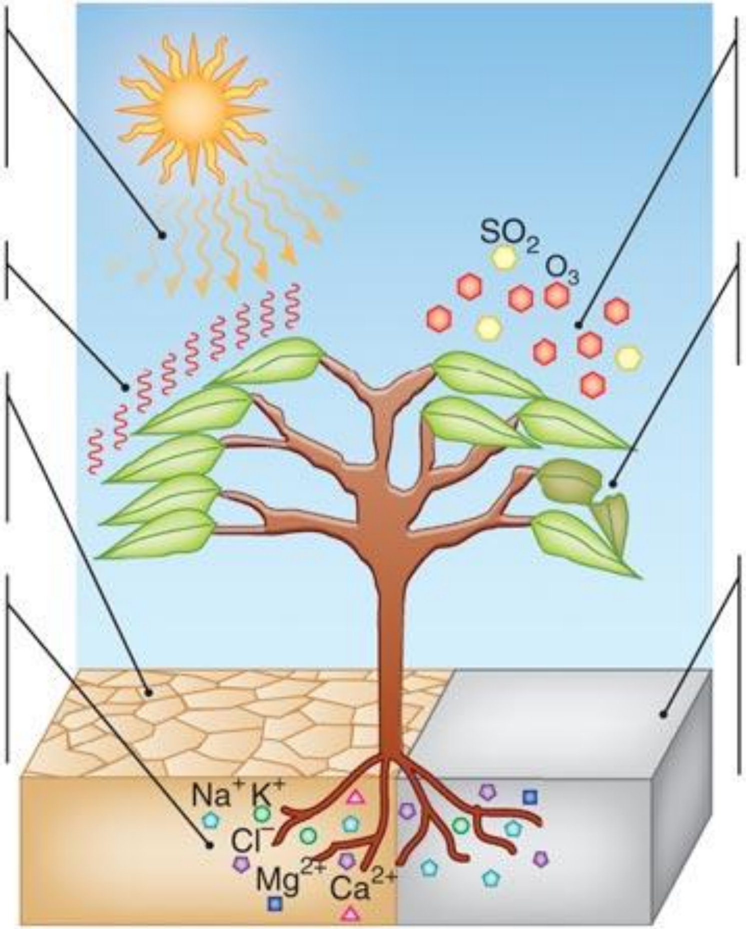
(http://bugs.bio.usyd.edu.au/2003A+Pmodules/module1/1AC1.html)

Excess heavy metals can be sequestered in the cell **vacuoles**



Abiotic Factors that Causes Abiotic Stress:

Temperature, salinity, alkalinity, acidity, drought, toxic metals, sunlight, environmental warming, water pollution, radiations and radioactivity etc., are the various abiotic factors that cause abiotic stresses.



High light causes production of excess excitation energy in the photosynthetic reaction centers, resulting in direct accumulation of a variety of reactive oxygen species.

High temperature stress denatures proteins and causes lipid peroxidation.

Water deficit, or drought, interferes with metabolism. ROS produced under drought conditions trigger signaling pathways that generate defense responses.

Soil salinity is usually caused by excess salts of chloride and sulfate. Salinity results in ion cytotoxicity and osmotic stress, and decreases uptake of nutrients. Resulting metabolic imbalances lead to oxidative stress.

Air pollution with oxidizing species (including ozone and sulfuric acid) causes direct oxidative damage to tissues. Local and systemic signaling responses also occur.

Mechanical damage—both biotic (e.g., from insect feeding) and abiotic (e.g., from wind damage)—triggers expression of defense-related genes.

Cold stress interferes with metabolic processes (particularly enzyme activity) and alters membrane properties. Frosting can severely damage tissues when ice forms. Extracellular ice formation also causes intracellular water deficit.

http://www.tutorvista.com
/biology/abiotic-stresses

What is stress...

Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant
(Taiz & Zeiger, 2002, Cap 25-Stress Physiology)

ABIOTIC STRESS can be defined as:

- The **negative impact** of various abiotic (non-living) factors on the living organism in a particular habitat
- The various abiotic factors makes tremendous impact on the organism and its performance. Abiotic stress can affect both plants and animals

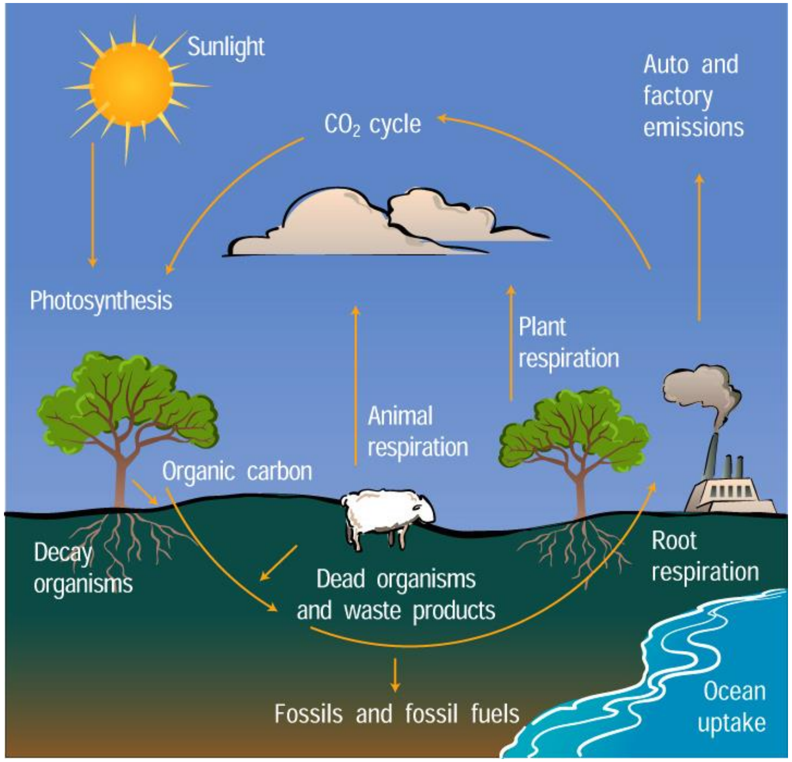
What is oxidative stress...

-Is a consequence of intracellular increase of ROS (reactive oxygen species)

-Toxic effect of some substances is due to induction of oxidative stress

Living with oxygen...

Did you know that the air you are breathing now was in someone else's lungs earlier?



http://feedfury.com/content/12190121-plant_bamboo.html

BUT...

-ROS are naturally formed as a consequence of aerobic metabolism...

-Signaling and regulation role, controlling signal metabolite production (H_2O_2, GSH, GSSG, ASC...)

Toxic metals like Cu and Cd induce oxidative stress by specific reactions and induces **higher HO• formation**

Fenton reactions
(ex. Fe, Cu)
and
Haber-Weiss reactions
(ex. Cd)

$$\underset{(Fe^{2+}/Cu^{+})}{H_2O_2} \longrightarrow \underset{(Fe^{3+}/Cu^{2+})}{OH^- + HO^\bullet}$$

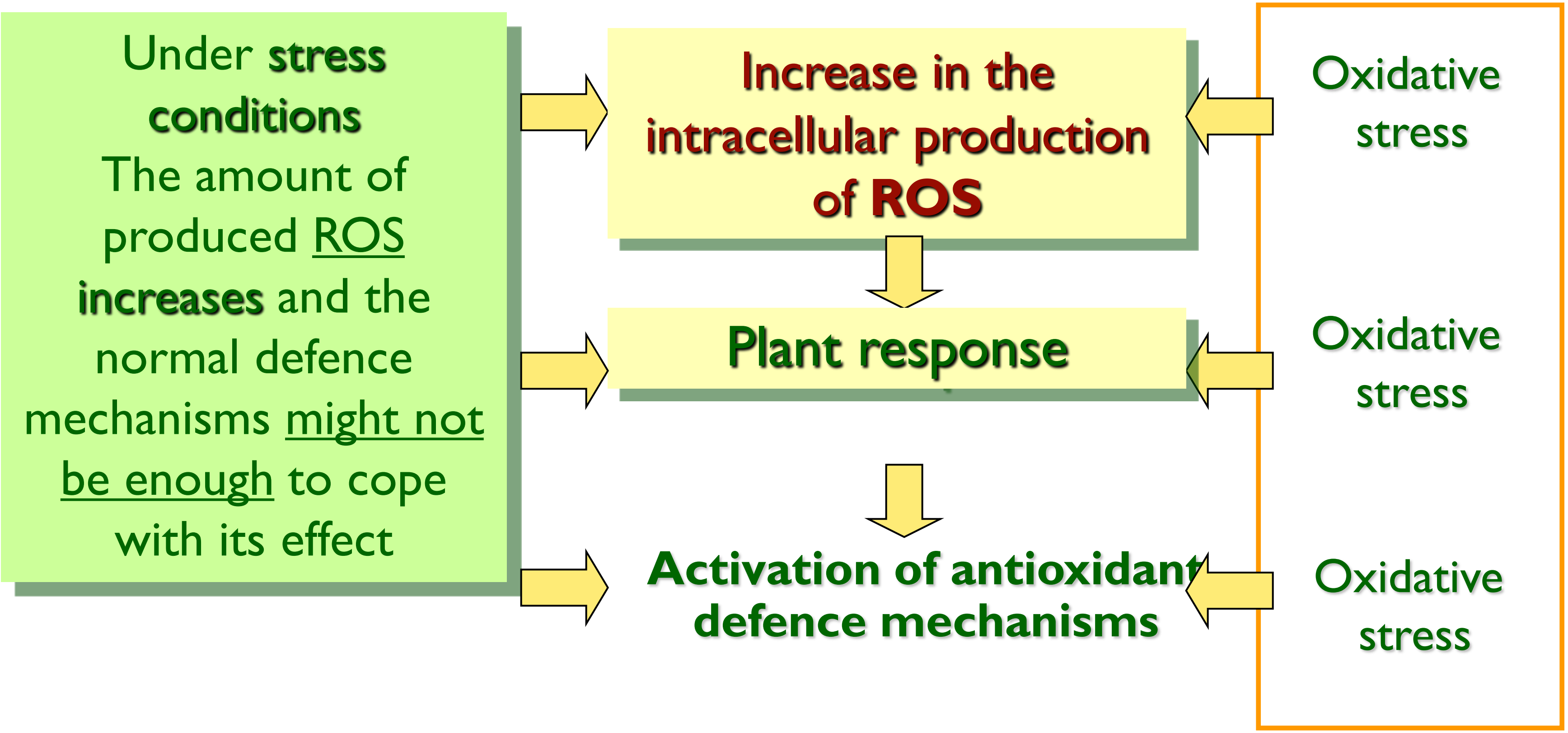
$$\underset{(Fe^{2+}/Cu^{+})}{O_2} \longrightarrow \underset{(Fe^{3+}/Cu^{2+})}{O_2^{\bullet -}}$$

$$H_2O_2 + O_2^{\bullet -} \longrightarrow O_2 + OH^- + HO^\bullet$$

$$2O_2^{\bullet -} + 2H^+ \longrightarrow O_2 + 2H_2O_2$$

The formation of $OH^\bullet$ from H_2O_2 (by Fenton and Haber-Weiss reactions) can be reduced by keeping the concentration of metals in the cell under control

Møler IM et al. 2007



Under **stress conditions**
The amount of produced ROS **increases** and the normal defence mechanisms might not be enough to cope with its effect

Increase in the intracellular production of ROS

Plant response

Activation of antioxidant defence mechanisms

Oxidative stress

Oxidative stress

Oxidative stress

In living cells ROS plays a key role in signaling but these compounds can also damage macromolecules

What are ROS?

ROS, Reactive Oxygen Species
(*AOS-Active Oxygen Species)

Life with oxygen:

↓

Production of ROS

↓

In excess can be toxic

-ROS are naturally formed as a sub-product of the aerobic metabolism, in the mitochondria, chloroplasts and peroxisomes, as a result of those metabolic processes.

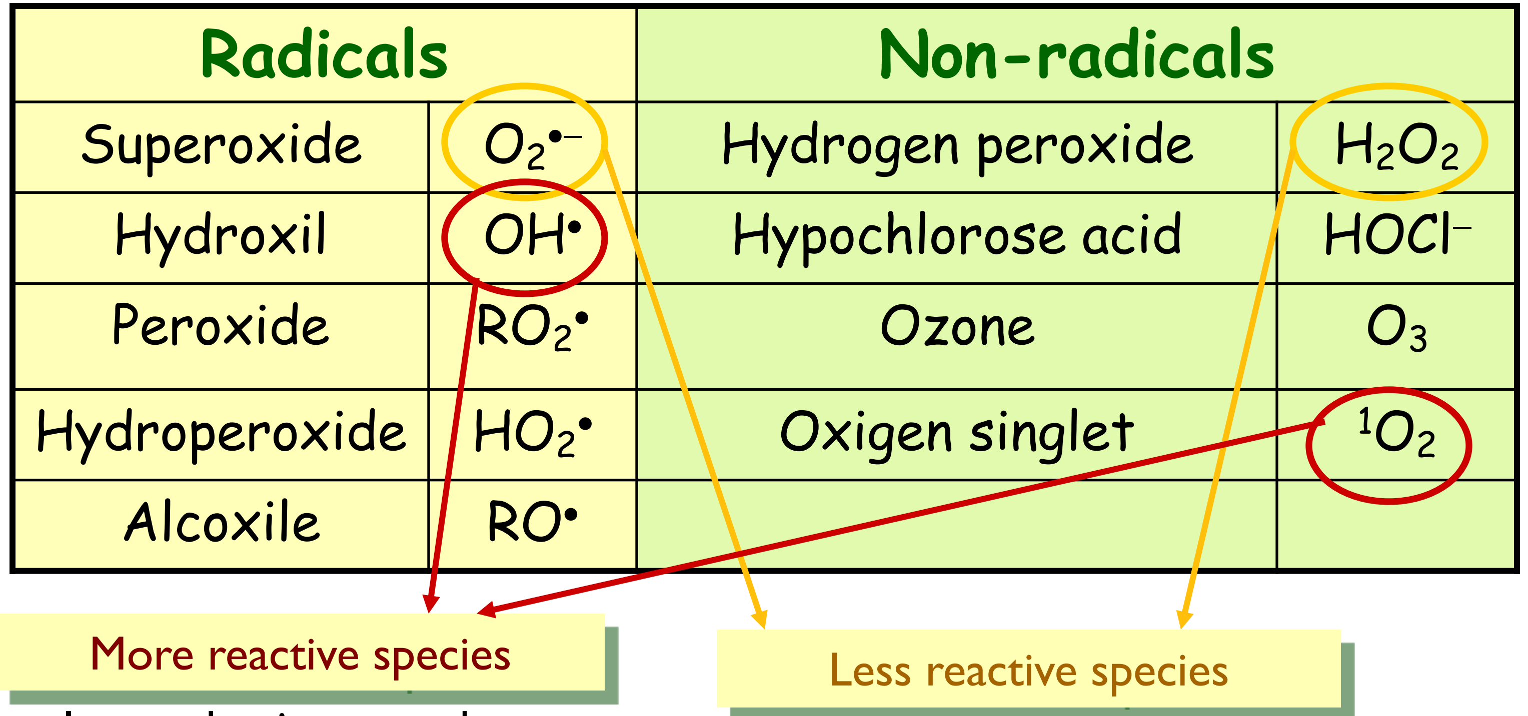
-cellular respiration, ETC
-photorespiration
-photosynthesis
-lipid oxidation

ROS production and ROS-induced damage **increase** during abiotic and biotic stress

-H_2O_2 and $O_2^{\bullet-}$ are alwas formed even in normal metabolic conditions

-$OH^{\bullet}$ and $^1O_2$ are usually formed under stress conditions

ROS types



Radicals		Non-radicals	
Superoxide	$O_2^{\bullet -}$	Hydrogen peroxide	H_2O_2
Hydroxil	$OH^{\bullet}$	Hypochlorose acid	$HOCl^-$
Peroxide	$RO_2^{\bullet}$	Ozone	O_3
Hydroperoxide	$HO_2^{\bullet}$	Oxigen singlet	$^1O_2$
Alcoxile	$RO^{\bullet}$		

More reactive species

Less reactive species

Its production must be minimized!

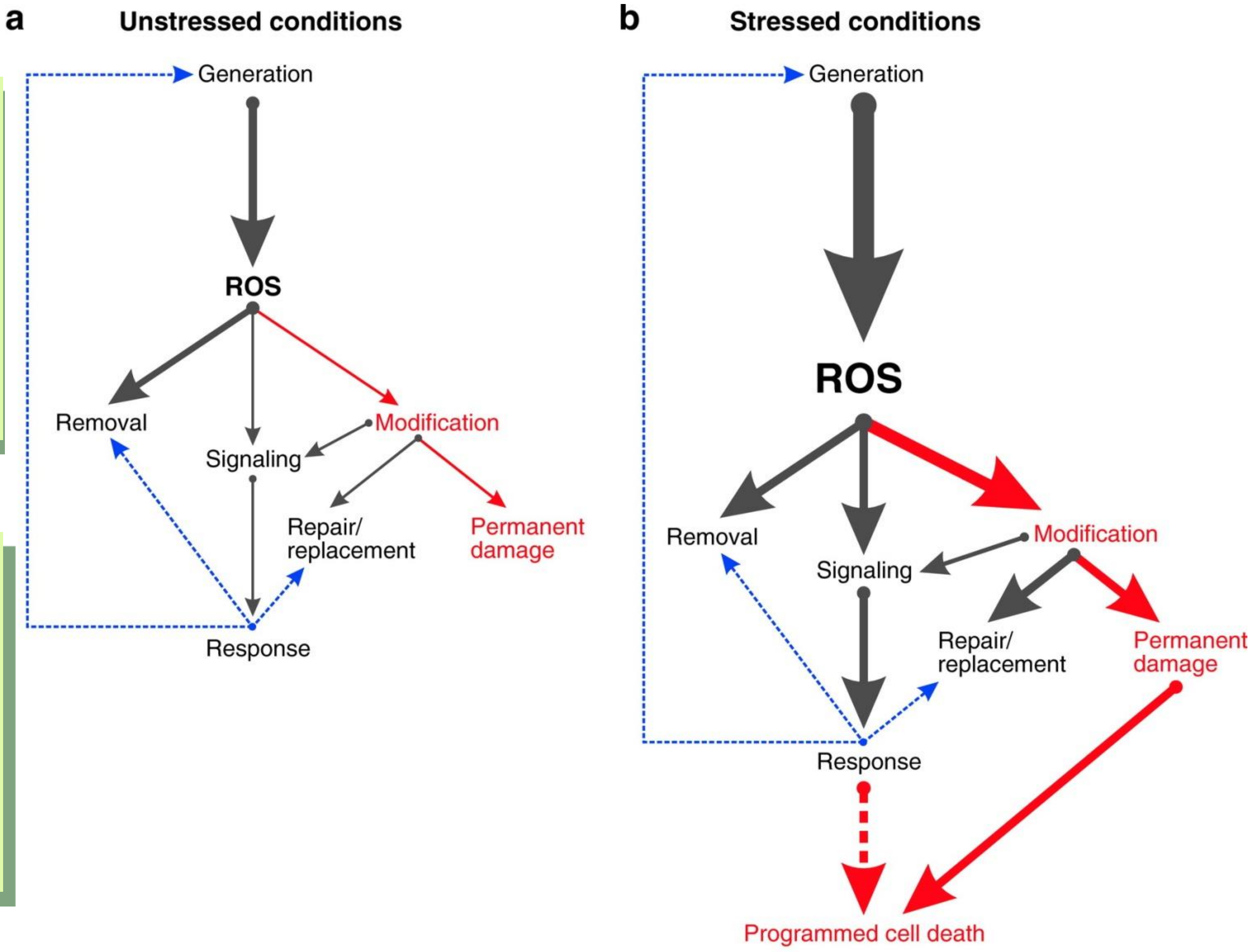
Free radicals

-species that contain **1 or more unpaired electrons**, very mobile in the cells and unstable (very reactive)

-they form in the cells, in the aerobic metabolism by the transfer of one electron only

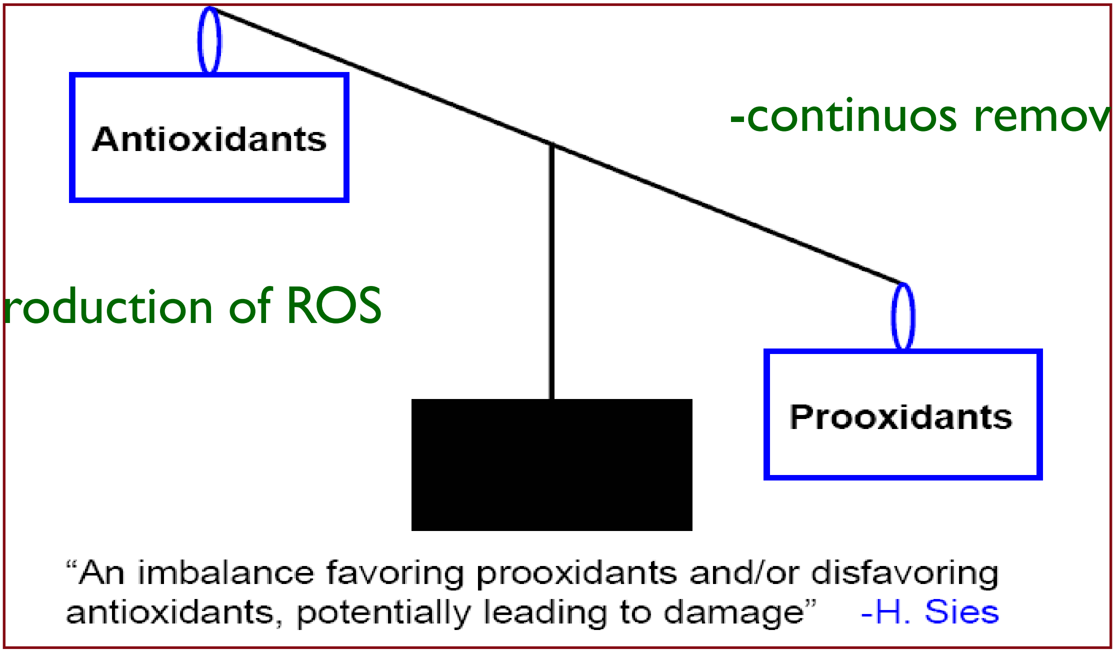
More damage is observed under stress conditions when the ROS levels are increased

The oxidized products can be important secondary signalling molecules



Møller IM, et al. 2007.
Annu. Rev. Plant Biol. 58:459–81

In the normal AEROBIC METABOLISM the production and removal of ROS is in equilibrium



-continuos removal of ROS

-continuous production of ROS

"An imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage" -H. Sies

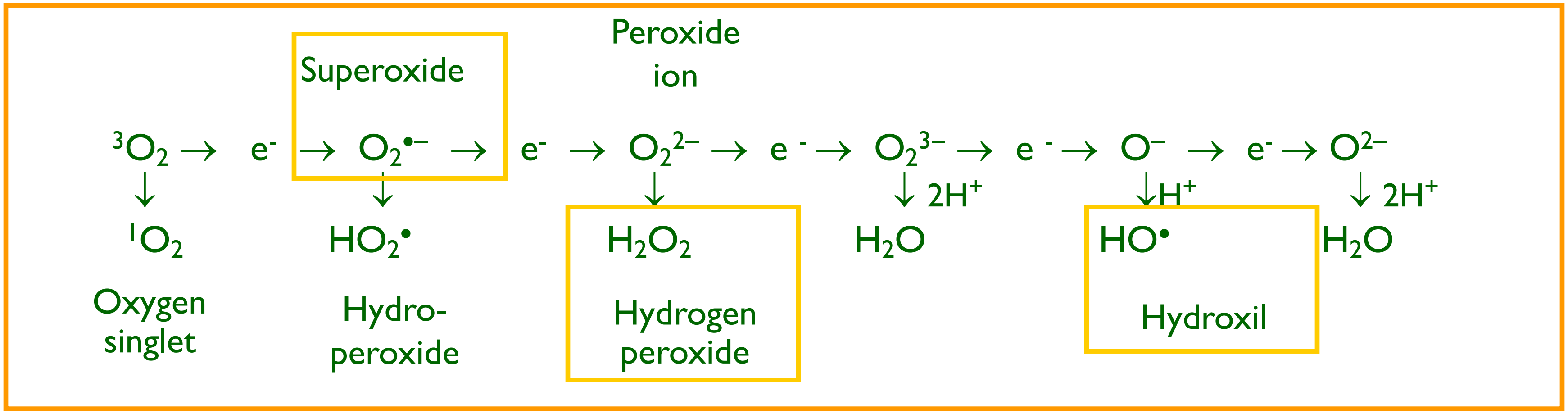
In normal physiological conditions ROS are **sequestred** and/or **accumulated** in certain cellular compartments

In each compartment of the plant cell, ROS formation and removal are tightly regulated

ROS formation mechanisms

They form through sequential reactions from oxygen in the ground state by spin inversion of one electron – **oxygen singlet,** or by transfer of one electron leading to the successive formation of superoxide, hydrogen peroxide and hydroxyl radical

(Apel and Hirt, 2004)



Superoxide

Peroxide ion

$^3O_2 \rightarrow e^- \rightarrow O_2^{\bullet-} \rightarrow e^- \rightarrow O_2^{2-} \rightarrow e^- \rightarrow O_2^{3-} \rightarrow e^- \rightarrow O^- \rightarrow e^- \rightarrow O^{2-}$

$^3O_2 \downarrow$ $^1O_2$ Oxygen singlet

$O_2^{\bullet-} \downarrow$ $HO_2^\bullet$ Hydro-peroxide

$O_2^{2-} \downarrow$ H_2O_2 Hydrogen peroxide

$O_2^{3-} \downarrow 2H^+$ H_2O

$O^- \downarrow H^+$ $HO^\bullet$ Hydroxil

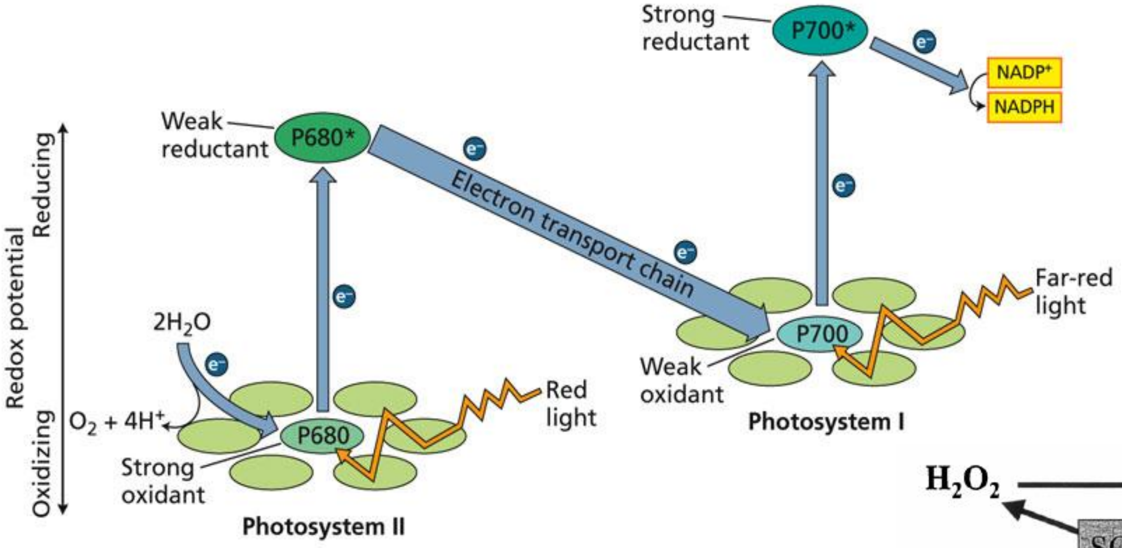
$O^{2-} \downarrow 2H^+$ H_2O

There are no known sequesters for $HO^\bullet$

The reactions that lead to $HO^\bullet$ formation must be stopped to prevent its oxidative damage

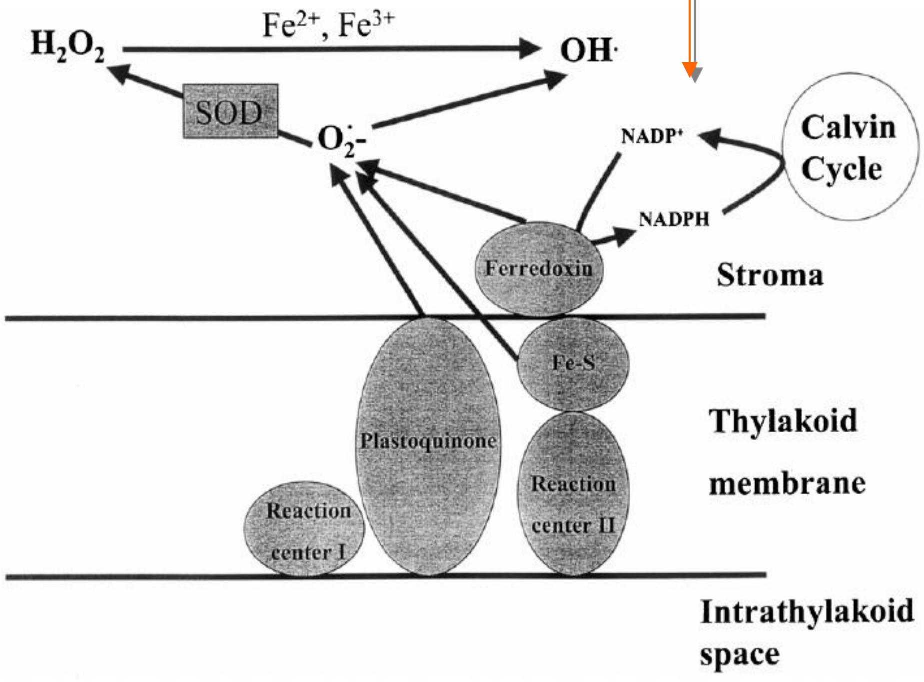
Controlling the reactive species that originate $HO^\bullet$

Produção de ROS nos cloroplastos



PLANT PHYSIOLOGY, Fourth Editi

Eliminação dos ROS pelo potencial redutor da célula:
utilização do NADPH



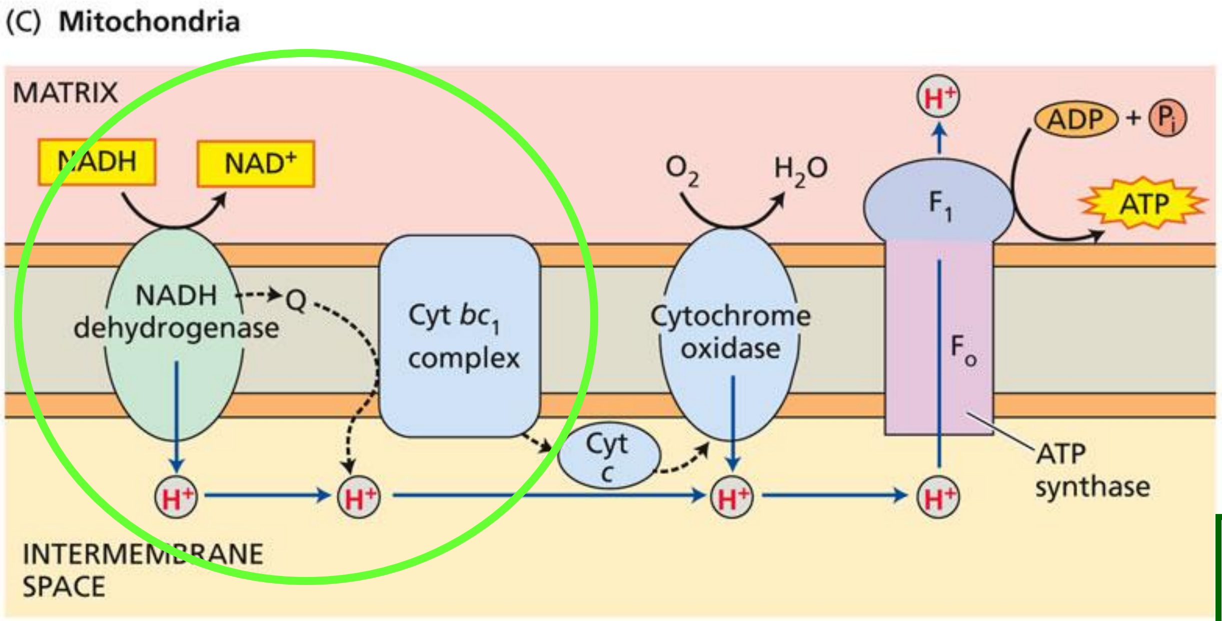
Os cloroplastos produzem como sub-produtos:

$^1O_2$ (singleto de oxigénio) no PSII

$O_2^{\bullet-}$ (superoxido) no PSI e PSII
(nos aceitadores de e-, núcleos de Fe-S, ferredoxina)

© Miguel Mour

Produção de ROS na mitocondria



A respiração produz como sub-produtos:

$O_2^{\bullet-}$ (superóxido) nos complexos I e III

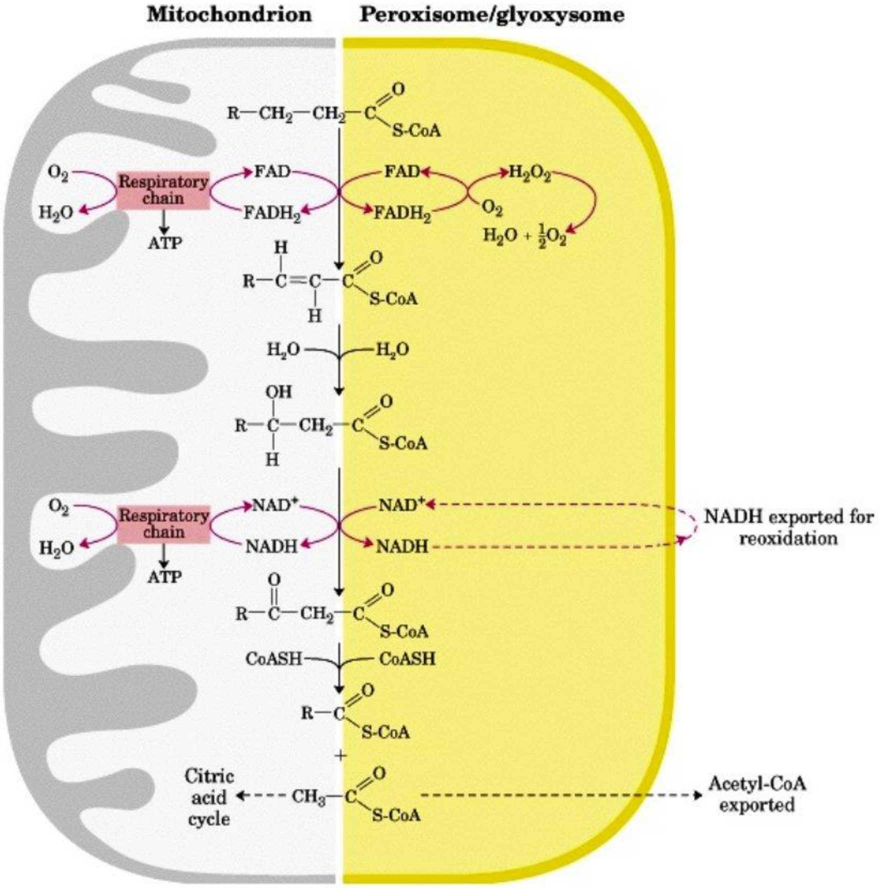
Estima-se que 1-5 % do O_2 consumido na mitocondria é convertido em ROS

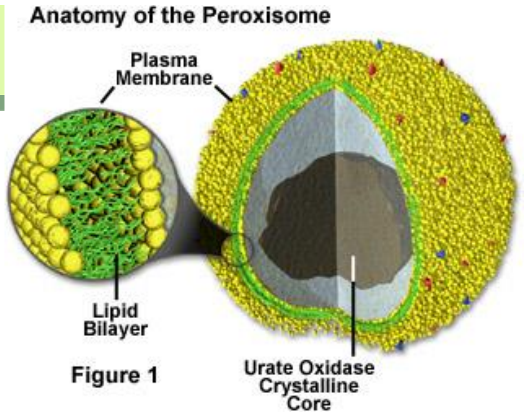
Eliminação do superóxido é catalisada por enzimas:

Dismutação: $O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$

Redução do H_2O_2: $H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$

Produção de ROS nos peroxissomas





O peroxissoma (nos eucariotas) produz como sub-produtos:

- $O_2^{\bullet -}$ (superóxido)
- H_2O_2

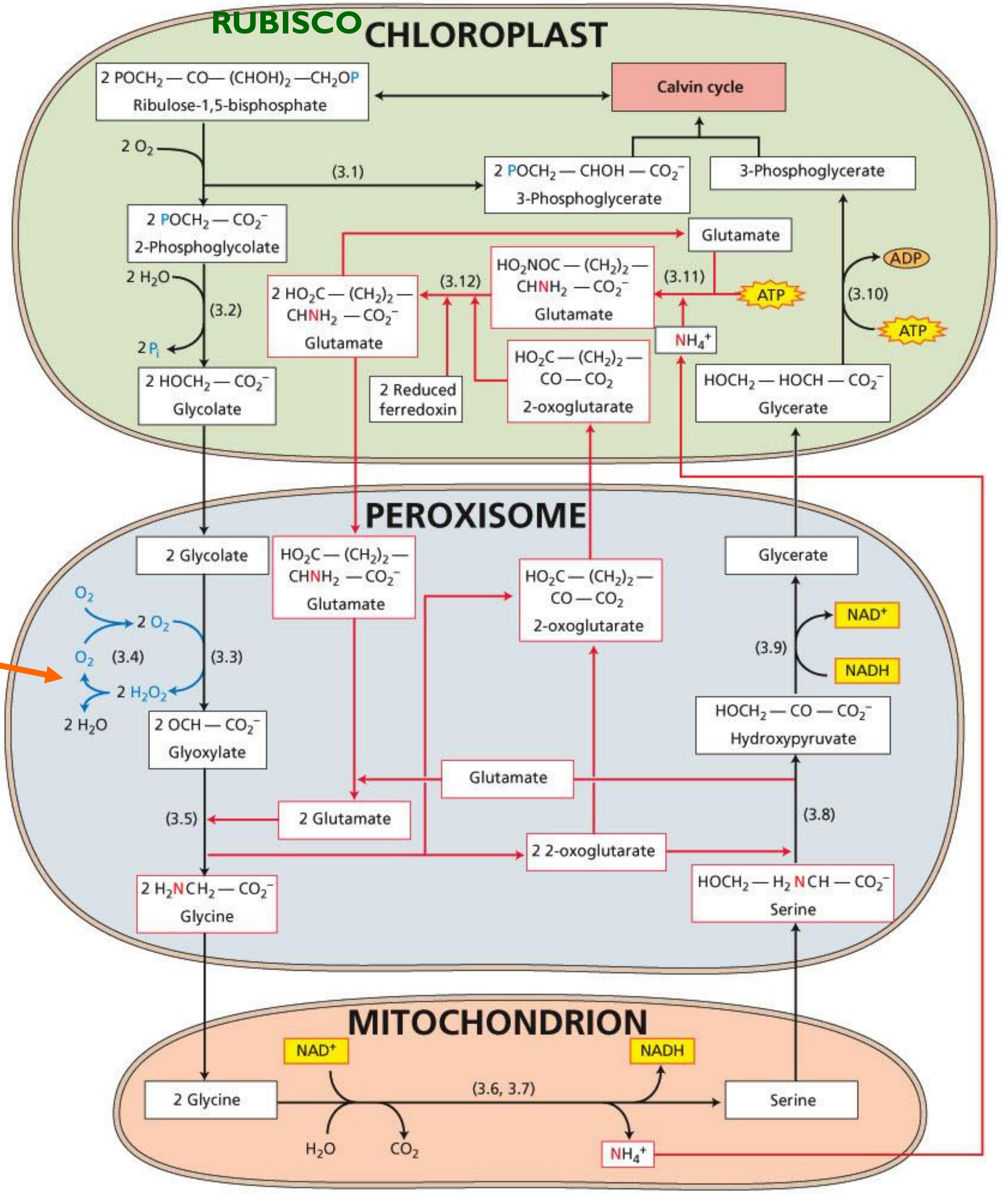
Resultantes de diversas reacções da β-oxidação dos ácidos gordos, e reacções enzimáticas das membranas celulares (oxidases e peroxidases)

É nos peroxissomas que se localiza a CATALASE

A regeneração do NADH pela fotorrespiração parece ser importante para diminuir a formação de ROS resultantes da fotossintese

Møler IM et al. 2007

Produção de H_2O_2 ocorre na oxidação do glicolato



PLANT PHYSIOLOGY, Fourth Edition, Figu

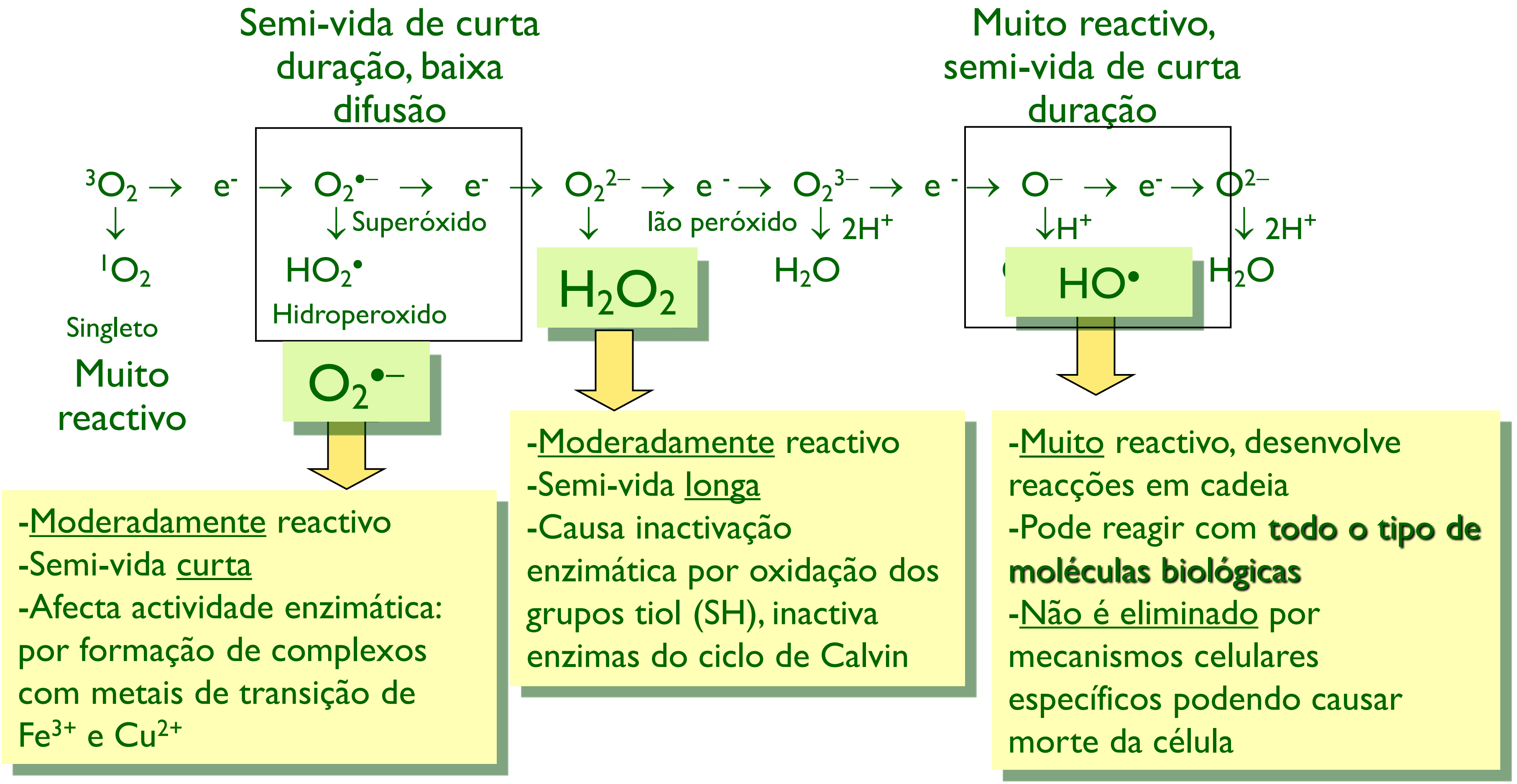
Actividade oxigenásica da RUBISCO (formação de gliceraldeido-3P e glicolato)

-ocorre para níveis elevados de O_2

-envolve 3 compartimentos celulares: cloroplastos, peroxissomas e mitocondrias

-produz NADH na mitocondria (consome 2 ATP e 1 NADH no peroxissoma por cada G3P formado)

The different ROS have very different properties:



Semi-vida de curta duração, baixa difusão

Muito reactivo, semi-vida de curta duração

$^3O_2$ → e^- → $O_2^{\bullet-}$ → e^- → O_2^{2-} → e^- → O_2^{3-} → e^- → O^- → e^- → O^{2-}

↓ $^1O_2$

Singleto

Muito reactivo

↓ Superóxido

$HO_2^\bullet$

Hidroperoxido

$O_2^{\bullet-}$

↓

H_2O_2

Ião peróxido ↓ $2H^+$

H_2O

↓H^+

$HO^\bullet$

↓ $2H^+$

H_2O

-<u>Moderadamente</u> reactivo
-Semi-vida <u>curta</u>
-Afecta actividade enzimática: por formação de complexos com metais de transição de Fe^{3+} e Cu^{2+}

-<u>Moderadamente</u> reactivo
-Semi-vida <u>longa</u>
-Causa inactivação enzimática por oxidação dos grupos tiol (SH), inactiva enzimas do ciclo de Calvin

-<u>Muito</u> reactivo, desenvolve reacções em cadeia
-Pode reagir com **todo o tipo de moléculas biológicas**
-<u>Não é eliminado</u> por mecanismos celulares específicos podendo causar morte da célula

Todos os ROS podem reagir com DNA, proteínas e lípidos, embora os diferentes tipos tenham alvos preferenciais

The different ROS have very different properties

$$\begin{tikzcd}
{}^3O_2 \arrow[r] \arrow[d] & e^- \arrow[r] & O_2^{\bullet -} \arrow[r] \arrow[d] & e^- \arrow[r] & O_2^{2-} \arrow[r] \arrow[d] & O_2^{3-} \arrow[r] & e^- \arrow[r] & O^- \arrow[d, "H^+"] \\
{}^1O_2 & & HO_2^{\bullet} & & H_2O_2 & & & HO^{\bullet}
\end{tikzcd}$$

Property	**Singlet oxigen ($^1O_2$)**	**Superoxide ($O_2^{\bullet -}$)**	**Hydrogen peroxide (H_2O_2)**	**Hydroxyl radical ($HO^{\bullet}$)**
Half-life in biol. systems	1 μs	1 μs	1 ms	1 ns
Distance traveled	30 nm	30 nm	1 μm	1 nm
Cellular conc.	?	?	μM-mM	?
Reacts with: **Lipids**	**PUFA**	HARDLY	HARDLY	**RAPIDLY**
DNA	Mainly guanine	No	No	**RAPIDLY**
Carbohydrates	No	No	No	**RAPIDLY**
Proteins	Trp, His, Tyr, Met, Cys	Fe-S centers	Cysteins	**RAPIDLY**

Os ROS desencadeiam reacções de oxidação que causam danos em biomoléculas:
Lípidos, aminoácidos, proteínas, glúcidos, ácidos nucleicos

Alterações em membranas celulares:

-alterações em **proteínas** de membrana (por oxidação de grupos tiol)

-os ROS iniciam **reacções de peroxidação de lípidos insaturados**; causa destabilização das membranas, perda da compartimentação celular, aumento da permeabilidade a iões e solutos

-os ROS causam alterações nas membranas dos cloroplastos, afectam os teores de **clorofila** total e a **capacidade fotossintética** (Reichman, 2002)

Inibição de enzimas de determinadas vias metabólicas (ex: fotossíntese, ciclo de Calvin) e **activação** de enzimas de outras vias

Danos oxidativos estão relacionados com o desenvolvimento de doenças como o cancro, arteriosclerose, Alzheimer e em processos inflamatórios (Grassman *et al.* 2002, Halliwell, 2006)

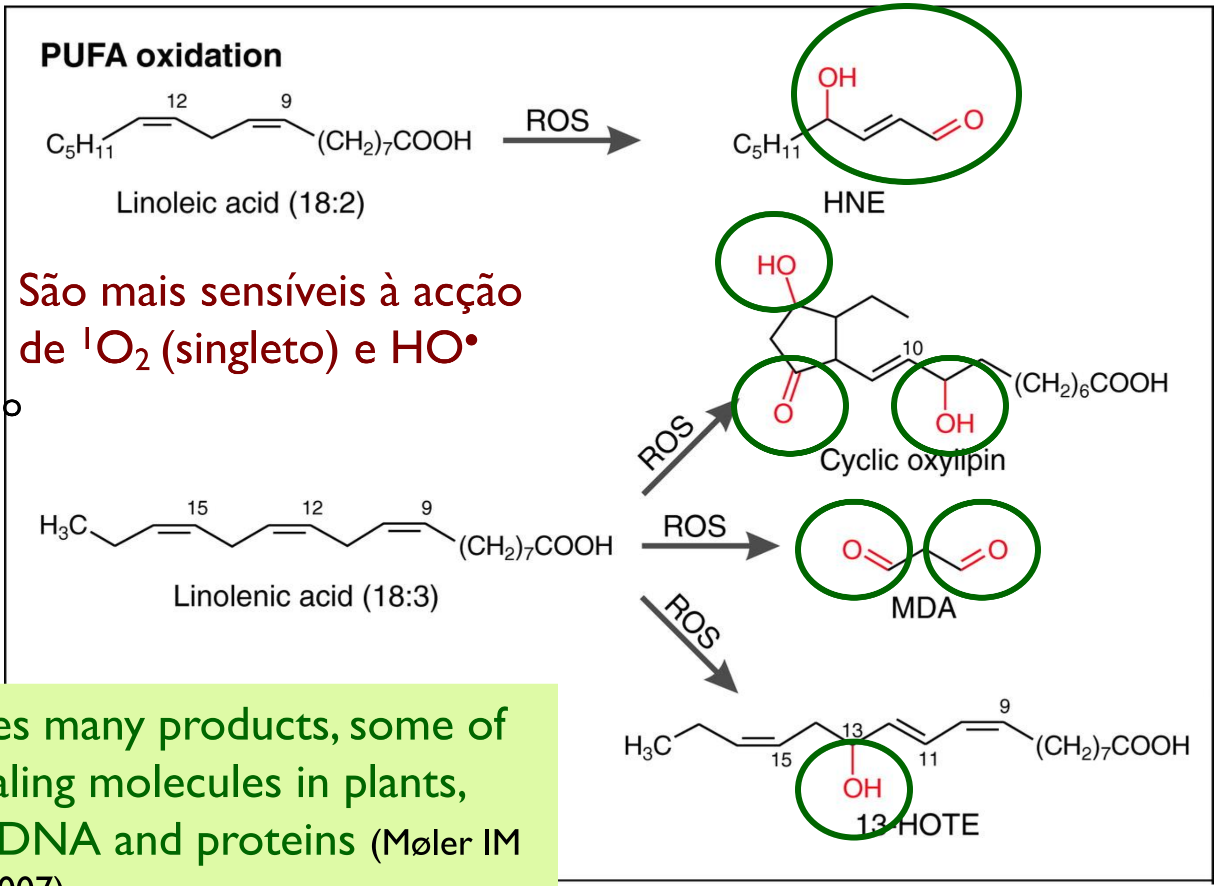
Reacções nocivas causados pelos ROS em ácidos gordos PUFA:

Os PUFA são os principais constituintes de lípidos de membranas, e são danificados tanto na forma livre como em fosfolípidos e galactolípidos:

Formação de hidroperóxidos diversos a partir dos acidos linoleico e linolénico:

-Aldeídos:
4-Hidroxinonenal (HNE), malondialdeido (MDA)

-Hidroxilos e cetoácidos
(oxilipinas cíclicas, ácido 13-hidroxioctadecatrienoico (HOTE)



São mais sensíveis à acção de $^{1}O_2$ (singleto) e HO•

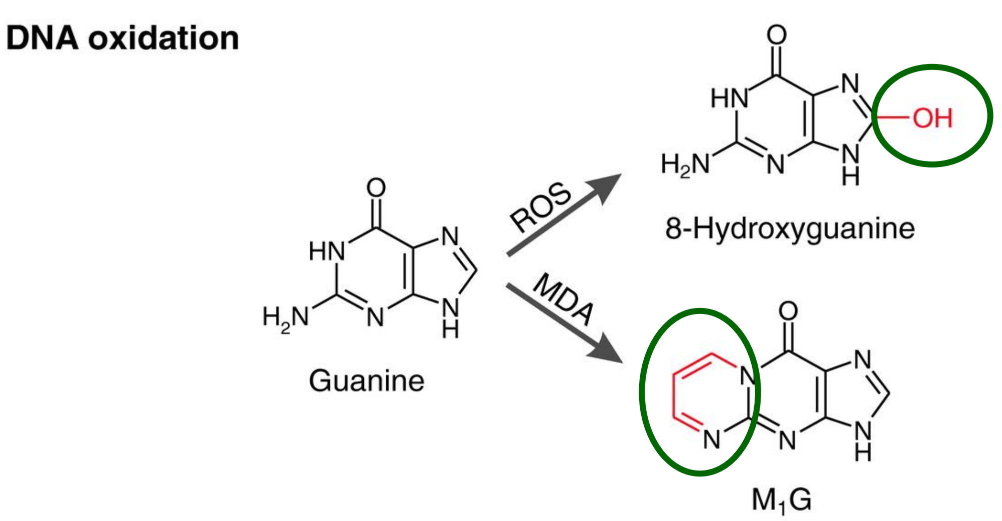
Oxidation of PUFA generates many products, some of which are secondary signaling molecules in plants, whereas others can damage DNA and proteins (Møler IM et al. 2007)

Reacções nocivas causados pelos ROS em DNA:

O DNA pode ser danificado por acção dos ROS nas bases azotadas

-Oxidação de Guanina e formação da hidroxiguanina

H_2O_2 e $O_2^{\bullet -}$ provocam menores danos no DNA



HO• é o mais reactivo
$^1O_2$ (singleto) ataca principalmente a guanina

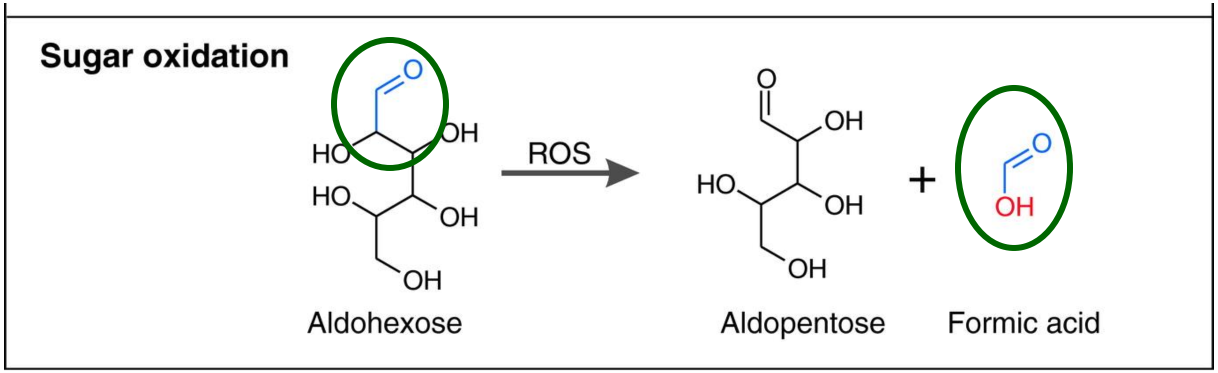
Mecanismos de reparação do DNA podem diminuir estes efeitos, no núcleo e nas mitocondrias (ex: substituição da base)

Uma produção excessiva de ROS supera a capacidade de reparação; algumas doenças estão associadas ao aparecimento de DNA danificado

Møller IM, et al. 2007.
Annu. Rev. Plant Biol. 58:459–81

Reacções nocivas causados pelos ROS em Glúcidos:

-Oxidação de glúcidos livres e polióis



Møller IM, et al. 2007.
Annu. Rev. Plant Biol. 58:459–81

Ex:
Tabaco transgénico que acumula manitol nos cloroplastos apresenta maior resistência ao stresse oxidativo

→

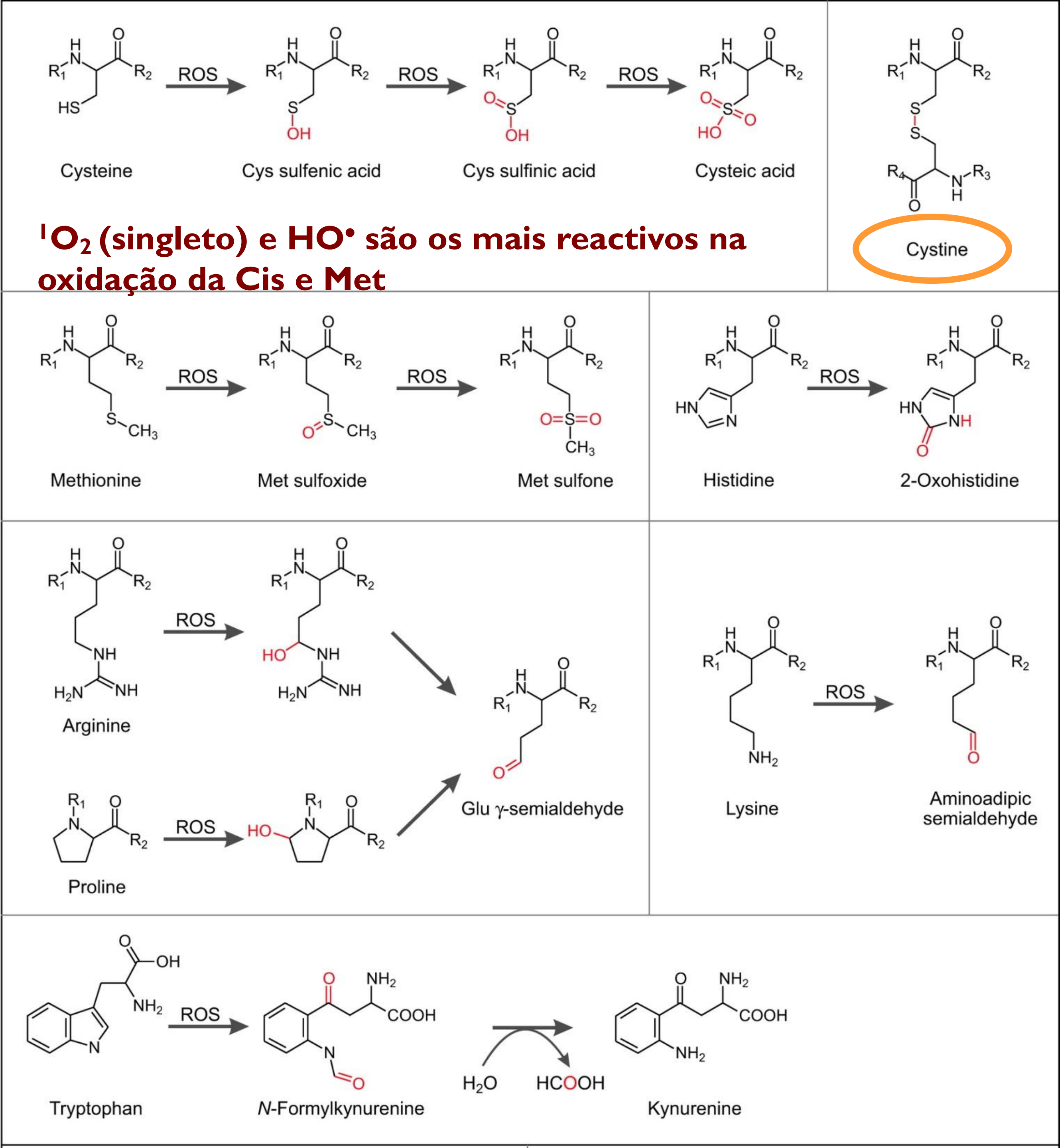
O manitol remove $HO^{\bullet}$ rapidamente, impedindo a sua acção nociva em outros componentes celulares

Reacções nocivas causados pelos ROS em Proteínas:

A oxidação das proteínas é um conjunto de modificações covalentes induzidas pelos ROS ou por produtos resultantes da sua acção

-são muito diversas, variando com o AA envolvido

-a maioria são reacções <u>irreversíveis</u> (excepto com o tiol)



$^1O_2$ (singleto) e HO• são os mais reactivos na oxidação da Cis e Met

Mecanismos de defesa contra o stress oxidativo induzido por metais pesados: enzimáticos e não-enzimáticos

Mecanismos internos: enzimáticos e não-enzimáticos

Os mecanismos **antioxidantes** (enzimáticos ou não-enzimáticos) actuam na interrupção dos processos de **oxidação** desencadeados pelos ROS

mecanismos não-enzimáticos

compostos antioxidantes

Compostos capazes de sequestrar os ROS sem formação de novas espécies reactivas (são oxidados)

Ex: ascorbato, glutationa, carotenoides

mecanismos enzimáticos

enzimas antioxidantes

Enzimas que catalisam reacções de sequestração dos ROS ou que estão envolvidas directamente na sua remoção

Ex: peroxidases, superoxido-dismutase, catalase

Mecanismos não-enzimáticos:

-Síntese *de novo* de proteínas

(produção de 'heat-shock proteins', HSP, com função protectora e de reparação)

-Produção de sequestrantes específicos:

ligandos de complexação (ácidos orgânicos, aminoácidos livres, ex: prolina) e péptidos de ligação (a metais): fitoquelatinas, metalotioneinas

-Produção de anti-oxidantes não enzimáticos:

ascorbato, **glutationa (GSH)**, carotenoides, flavonoides, antocianinas e outros polifenóis, tocoferóis, alcaloides, terpenóides

São produzidos pelas plantas (alguns pelo metabolismo secundário)

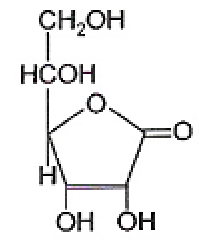
Mecanismo antioxidante:

Redução do $O_2^{\bullet -}$ e H_2O_2 em H_2O por compostos redutores

Sequestrantes de espécies radicais

Estrutura dos principais antioxidantes não-enzimáticos

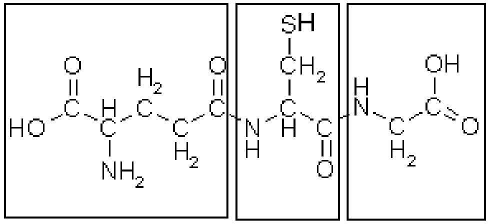
Ascorbato
(Vitamina C)



L-ascorbic acid

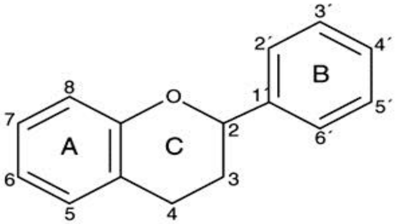
Glutationa
(tiol não
proteico)

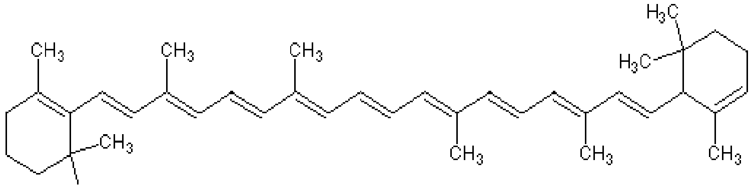
γ-Glu-Cys-Gly
SH



Flavonoides
(polifenóis com
estrutura cíclica)

Figure 1: Basic Structure and Numbering System of Flavonoids

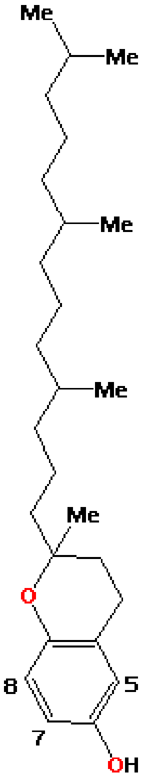




α-caroteno

Carotenoides:
Sistema conjugado de ligações duplas

α-tocoferol
(vitamina E)
Antioxidante de
fases não
aquosas



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Os antioxidantes não-enzimáticos são protectores das células contra os ROS

Redutores e sequestrantes de espécies radicais

Ascorbato
Glutationa
Alcalóides
α-tocoferol

Reagem directamente com O_2^-, H_2O_2 or $OH^\bullet$

Carotenoides
Flavonoides

Reagem directamente com o singleto de oxigénio $^1O_2$

Ainda não é bem conhecido o modo de acção de todos os antioxidantes nos mecanismos de defesa das plantas

-Não é bem conhecido o papel dos **flavonoides** e **carotenoides** na destoxificação dos ROS (Apel & Hirt, 2004, Gratão *et al.* 2005)

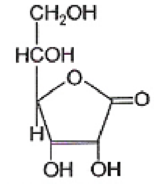
-**β-caroteno** parece aumentar a quantidade de xantofila nos cloroplastos, resultando uma maior tolerância ao stress oxidativo;

-pode estar envolvido na regeneração do ascorbato (Apel & Hirt, 2004)

Outras funções do ascorbato (ASC):

No metabolismo celular:

Principal antioxidante primário



L-ascorbic acid

-protecção de enzimas que contém iões metálicos como grupo prostético

-mecanismos de **protecção** e regulação da fotossintese (participa na regulação da captação da luz para a fotossintese, compensa a ausência de catalase nos cloroplastos)

ascorbato (reduzido)

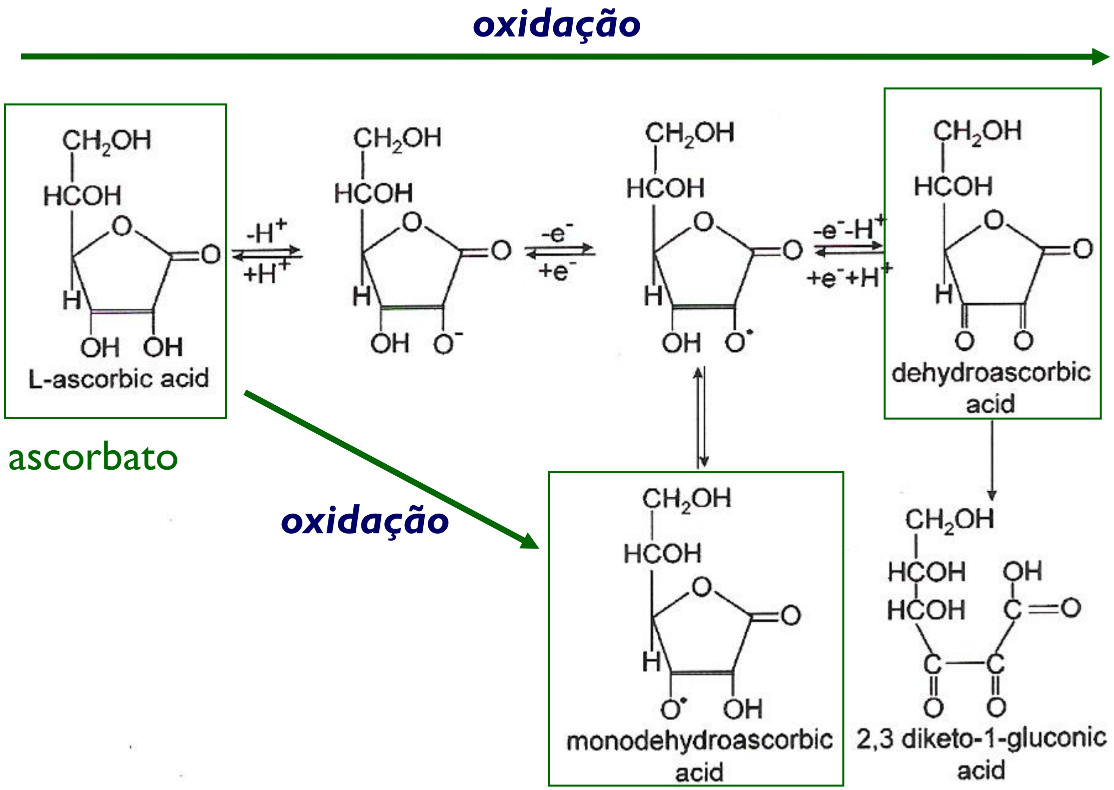
desidroascorbato (oxidado)

Nos mecanismos de defesa:

Importante antioxidante secundário

-regeneração de outros antioxidantes (como o α-tocoferol)

-regulação: a diminuição do ASC no stress oxidativo causa paragem da divisão celular prevenindo erros de replicação do DNA



ascorbato

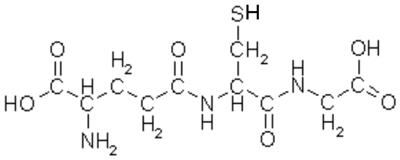
Desidroascorbato
DHA

Monodesidroascorbato
MDHA

Outras funções da glutationa (GSH):

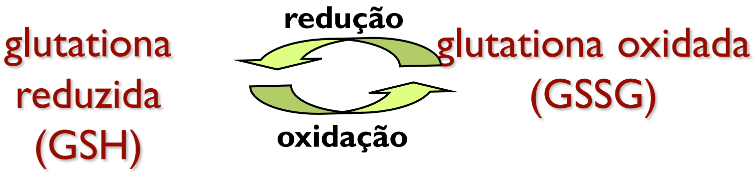
No metabolismo celular:

Antioxidante e regulador redox



-protecção de enzimas que contêm grupos tiol (–SH) evitando a oxidação (ex: enzimas do ciclo de Calvin)

-interconvertivel em GSSG em processos de oxidação-redução:



Nos mecanismos de defesa:

Protector e percursor de biomoléculas

-**regeneração de ascorbato** (reduzido) a partir do desidroascorbato formado na eliminação de ROS (ex: nos cloroplastos)

-percursor de fitoquelatinas (ligantes de metais pesados, regulando a sua concentração celular)

Mecanismos enzimáticos:

Activação da **via da glutationa-ascorbato** (Asada-Foyer-Halliwell):

Ascorbato peroxidase APOD (EC 1.11.1.11)

Monodesidroascorbato-redutase MDHAR (EC 1.6.5.4)

Desidroascorbato-redutase DHAR (EC 1.8.5.1)

Glutationa-redutase GR (EC 1.6.4.2)

Indução de **enzimas antioxidantes**:

Superoxido-dismutase SOD (EC 1.15.1.1)

Catalase CAT (EC 1.11.1.6)

Glutationa-peroxidase Glu-POD (EC 1.11.1.9)

Guaiacol-peroxidases GPOD (EC 1.11.1.7)

Remoção dos ROS:

Mecanismo antioxidante:

Efectua a redução do $O_2^{\bullet-}$ e H_2O_2 em H_2O usando electrões do NADPH

Mecanismo antioxidante:

Catalisa a redução enzimática do $O_2^{\bullet-}$ e H_2O_2 em H_2O usando agentes redutores específicos

O sistema antioxidante SOD, CAT, POD é muito eficiente na remoção do $O_2^{\bullet-}$ e H_2O_2 formados:

A eliminação do superóxido pela SOD é a primeira via de defesa contra os ROS

CAT e PODs actuam na eliminação do H_2O_2

*PODs são mais eficientes na remoção do H_2O_2

Superóxido-dismutase SOD

Catalisa a dismutação de duas moléculas de superóxido:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$$

Catalase CAT

Catalisa a redução do H_2O_2:

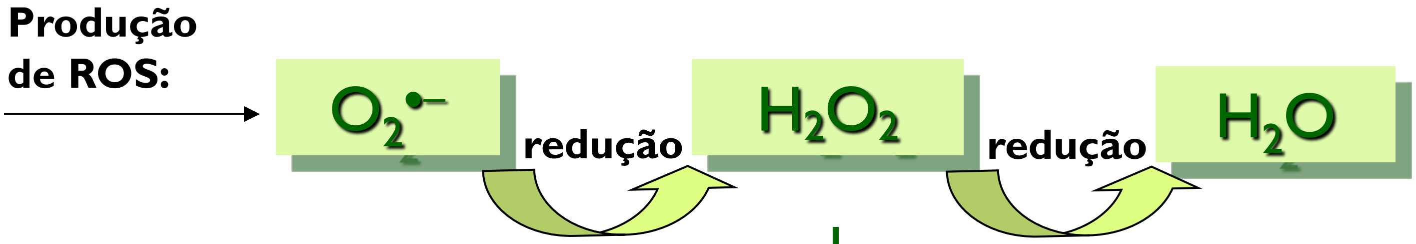
$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

Peroxidases PODs

Catalisam a redução do H_2O_2 utilizando um substrato diferente como agente redutor:

$$H_2O_2 + R(OH)_2 \rightarrow 2H_2O + RO_2$$

- Glutationa-peroxidase Glu-POD: utiliza a GSH
- Guaiacol-peroxidases GPOD: utiliza fenóis aromáticos
- Ascorbato-peroxidases APOD: utiliza ácido ascórbico



Produção de ROS:

$O_2^{\bullet -}$ redução H_2O_2 redução H_2O

SOD

O radical superóxido $O_2^{\bullet -}$ formado é convertido em H_2O_2

Os cloroplastos contêm geralmente elevada concentração de <u>ascorbato</u>, pelo que o $O_2^{\bullet -}$ é reduzido rapidamente em H_2O_2

CAT $2H_2O_2 \rightarrow 2H_2O + O_2$

PODs Usam redutor diferente do H_2O_2

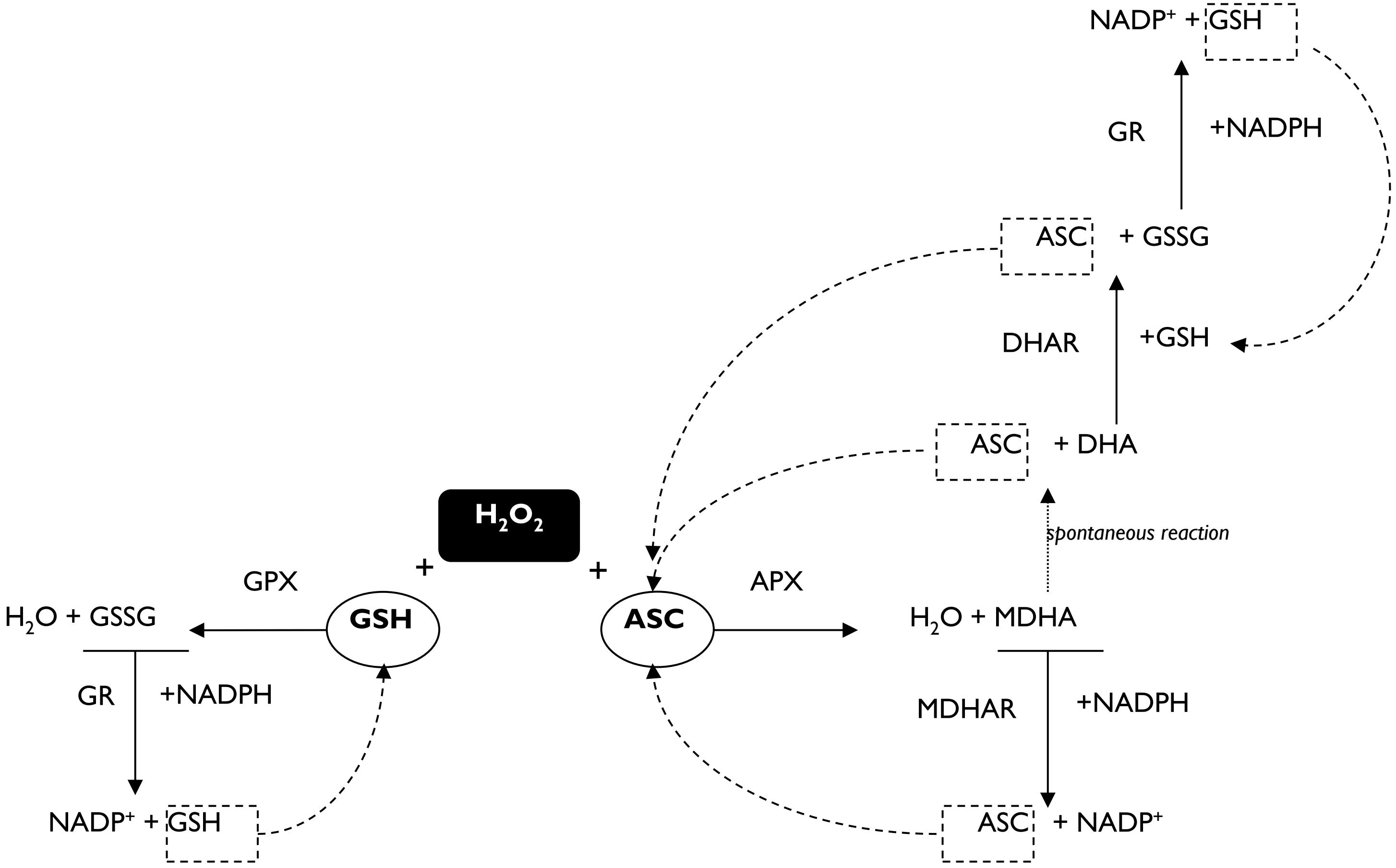
Glu-POD

$H_2O_2 + GSH \rightarrow H_2O + GSSG$

APOD

$H_2O_2 + ASC \rightarrow H_2O + MDA$

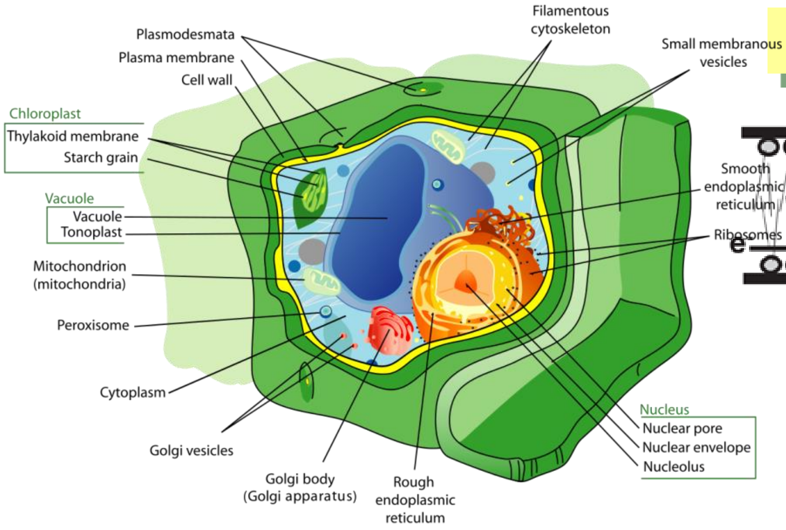
Via da glutationa-ascorbato

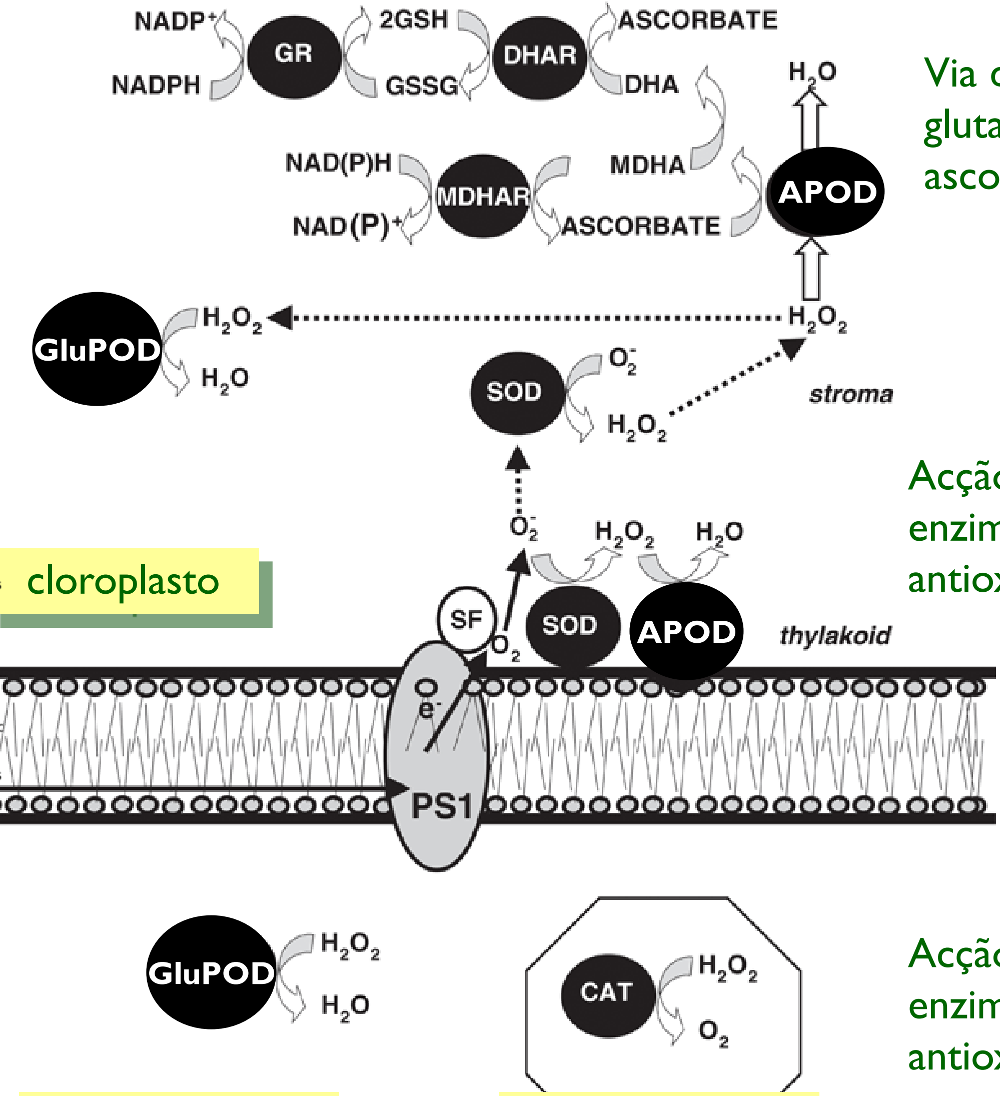


Enzimas da **via da glutationa-ascorbato:**

APOD	Catalisa a redução do H_2O_2 em H_2O utilizando **ascorbato** como redutor: $H_2O_2 + \text{ascorbato} \rightarrow H_2O + \text{monodesidroascorbato (MDHA)}$
MDHAR	Catalisa a redução do MDA em **ascorbato** utilizando o NADPH como redutor: $\text{MDA} + \text{NADPH} \rightarrow \text{ascorbato} + \text{NADP}^+$
DHAR	Catalisa a redução do DHA (obtido a partir do MDA) em **ascorbato**, utilizando a **glutationa** como redutor: $\text{Desidroascorbato (DHA)} + \text{GSH} \rightarrow \text{ascorbato} + \text{GSSG}$
GR	Catalisa a regeneração da **glutationa** (GSH) a partir da **glutationa oxidada** GSSG, utilizando o NADPH como redutor: $\text{GSSG} + \text{NADPH} \rightarrow \text{GSH} + \text{NADP}^+$

Mecanismos enzimáticos em células da parte aérea de plantas (Gratão *et al.* 2005)





cloroplasto

citosol

peroxisoma

Via da glutationa-ascorbato

Acção de enzimas antioxidantes

Acção de enzimas antioxidantes

Alguns exemplos de respostas de plantas ao stress por metais pesados

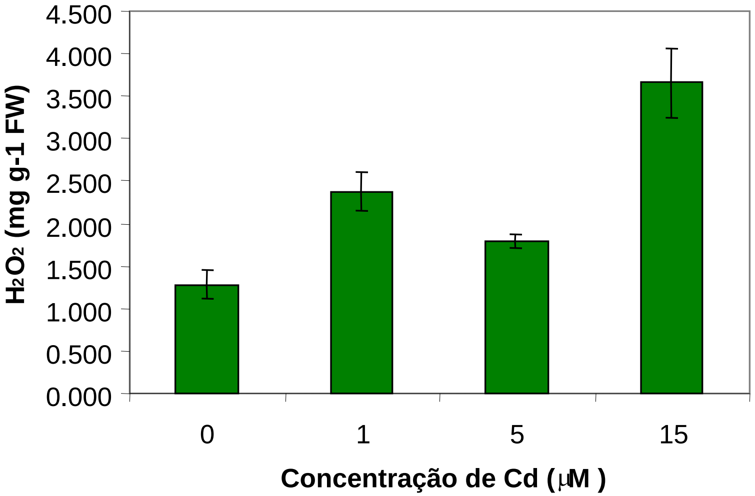
Toxicidade do Cd em sorgo (hibrido *Sorghum bicolor* (L.) *Moench. x Sorghum sudanense* var. Speedfeed)

Trabalho financiado pelo projecto POCI/AMB/55312/2004 "*Transferência solo/planta de elementos vestigiários. Um estudo de fitoremediação*", inicio 2006

0 μM (controlo)

Após 7 dias da aplicação de 15 μM de $CdCl_2$

Peróxido de hidrogénio em folhas de tabaco

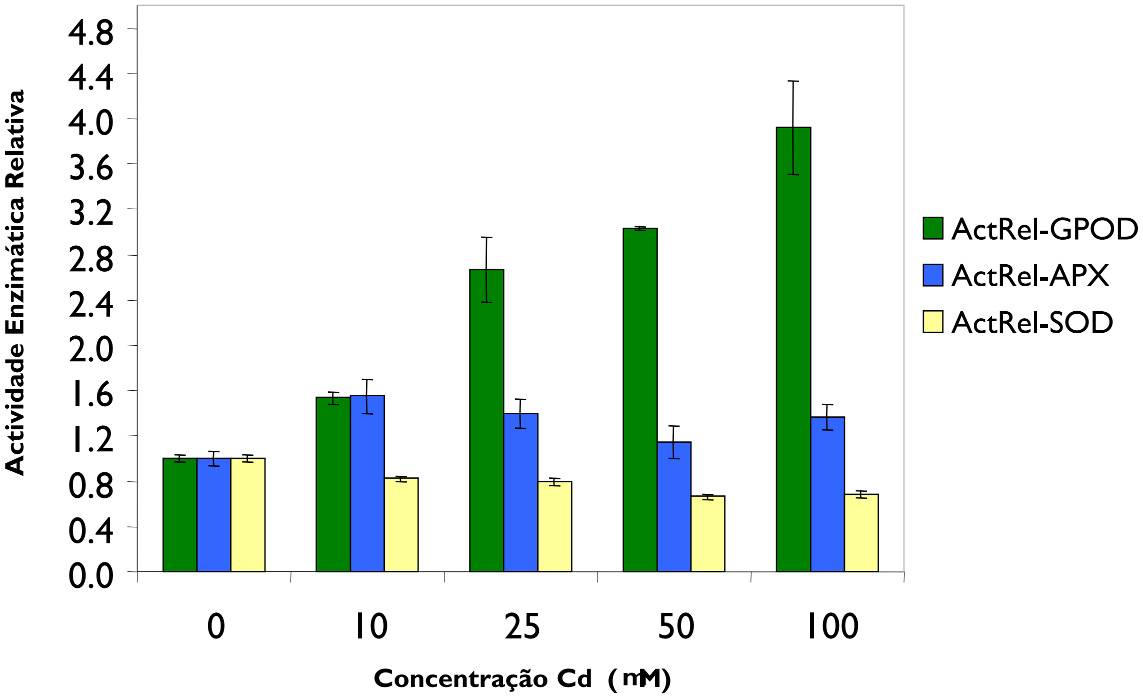


O Cd induz stress oxidativo que se pode avaliar pelo aumento do teor de peróxido de hidrogénio nas folhas

Efeito do Cd na actividade enzimática de folhas de planta de tabaco (*Nicotiana tabacum* Virginia var. K 326)

Trabalho financiado pelo projecto POCI/AMB/55312/2004 "*Transferência solo/planta de elementos vestigiários. Um estudo de fitoremediação*", inicio 2006

Níveis de actividade enzimática da GPOD, APX e SOD, em folhas jovens, após 11 dias da aplicação de 50 e 100 µM de $CdCl_2$



A alteração da actividade de enzimas especificas indica alterações do metabolismo associadas a uma resposta/tolerância de determninadas plantas aos metais pesados

Utilização de plantas para remediação de solos

Remediação do solo

Métodos e processos usados para tratar os contaminantes presentes no solo de modo a contê-los, removê-los, degradá-los ou torná-los menos prejudiciais

Fitorremediação:
Remediação do solo por acção das plantas

(A.Varennes 2003. Produtividade dos Solos e Ambiente, Escolar Editora)

The use of plants and their associated microbes for environmental cleanup
(Pilon-Smits, E. 2005, Annu.Rev.Plant Biol. 56:15-39)

-as plantas podem ser utilizadas para extracção, estabilização, degradação, volatilização, absorção e chelatação de poluentes

-podem usar-se diferentes técnicas de fitorremediação consoante o tipo de poluente, orgânico ou inorgânico

Selecção de diferentes espécies de plantas para situações específicas (Pilon-Smits, 2005)

Quando é necessário fazer remediação do solo?

-quando a contaminação causa danos ecológicos, ou prejudica a saúde de animais e plantas

-quando há violação dos limites legais estabelecidos para um dado contaminante

A escolha do tipo de remediação depende:

-da localização do solo
-da área atingida
-do grau de contaminação e risco que representa
-da função e uso futuro do local
-do custo dos métodos disponíveis

Remediação *ex situ*

-tratamentos que envolvem a remoção física do solo e tratamento noutro local

-para pequenas áreas e para alguns tipos de substancias (lavagem, separação de partículas, volatilização, migração, etc.)

Remediação *in situ*

-tratamentos efectuados no solo no próprio local

-geralmente para casos de contaminação menos grave, adição de MO, etc., inclui a

Fitorremediação

Aplicações de fitorremediação

Pode efectuar-se fitorremediação em substratos sólidos, líquidos e gasosos (Pilon-Smits, 2005)

substratos sólidos	Utilização em solos contaminados de plantas específicas para sequestrar, remover e acumular compostos (metais e elementos minerais, compostos orgânicos)
substratos líquidos	Utilização de plantas em águas poluídas -Remoção de metais e compostos orgânicos de esgotos e resíduos orgânicos municipais -Remoção de metais e compostos orgânicos em águas contaminadas pela agricultura (adubos, fertilizantes, metais, pesticidas orgânicos e herbicidas)
substratos gasosos	Utilização de plantas para filtração do ar em interiores e exteriores (remoção de CO_2, poeiras, hidrocarbonetos halogenados voláteis)

Técnicas de fitorremediação:

Adaptado de Pilon-Smits, 2005, Annu. Rev. Plant Biol, 56:15-39

-com aplicação a águas, solos e ar contaminados

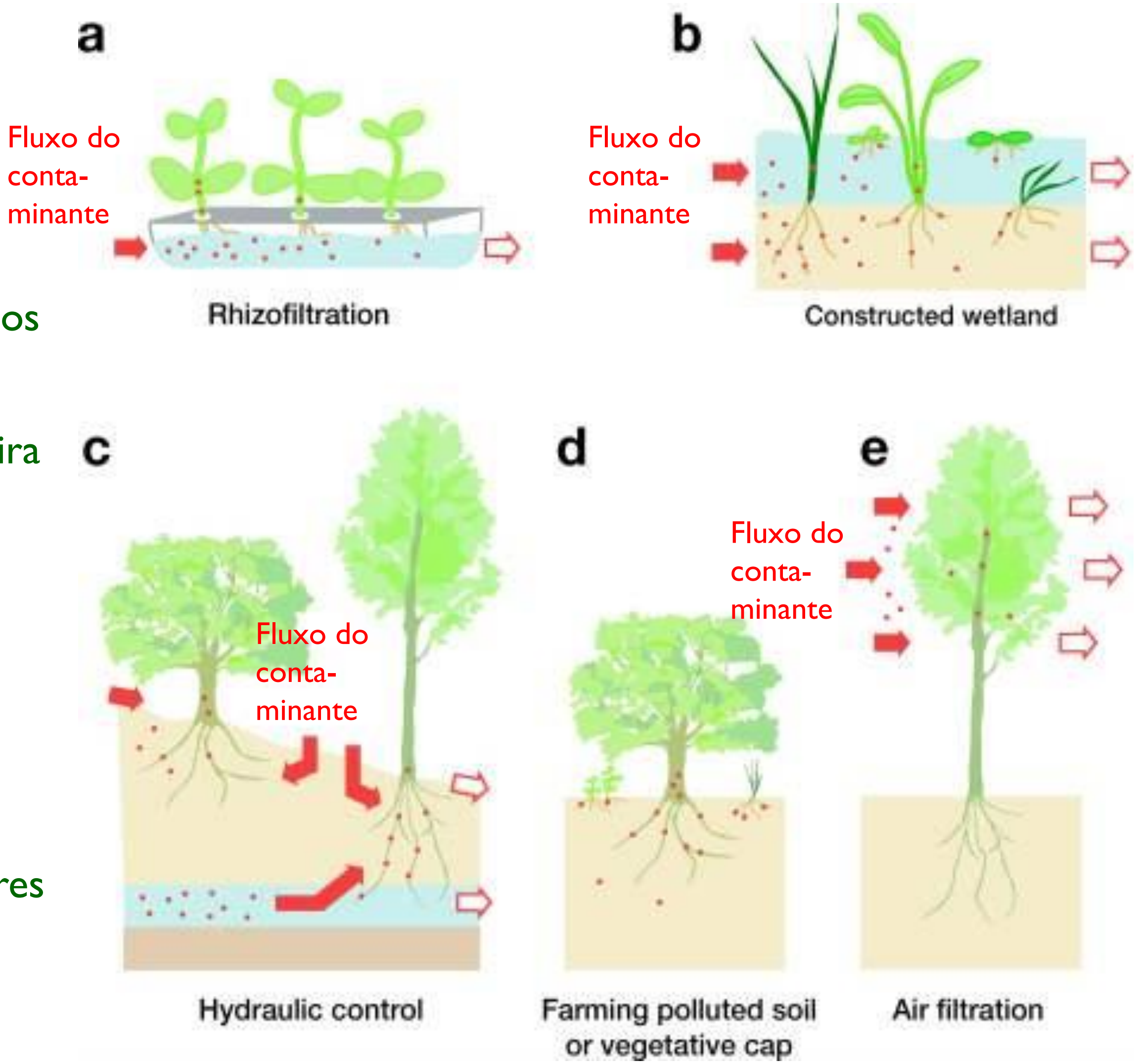
a) Rizofiltração
(aplicação em hidroponia)

b) Leitos de crescimento (em terrenos pantanosos)

c) Utilização de árvores como barreira hidráulica
(recorrendo às características do sistema radicular)

d) Utilização de árvores e plantas herbáceas
(recorrendo às características do sistema radicular)

e) Filtração do ar: utilização de árvores
(recorrendo às características da copa)

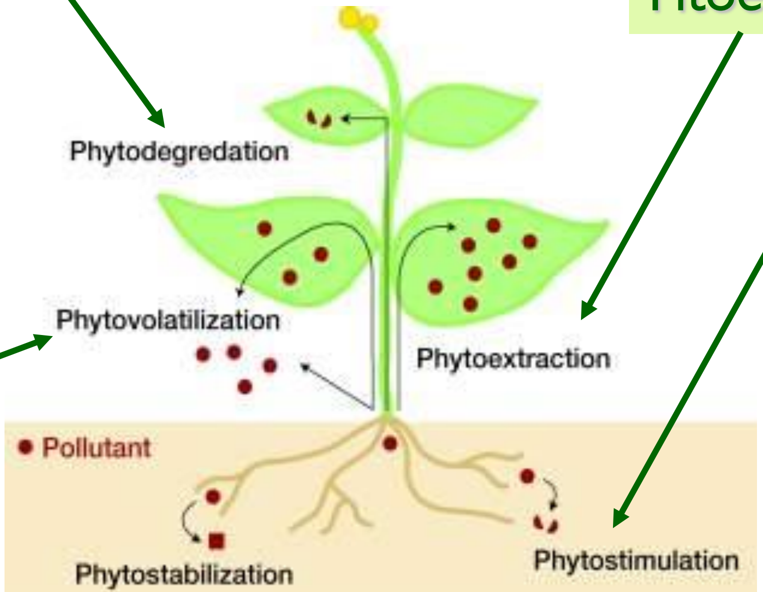


Processos (mecanismos) de Fitorremediação:

-Fitodegradação (ou Fitotransformação):

o sistema enzimático das plantas utiliza os contaminantes no seu metabolismo, inclui-os em estruturas vegetais (lenhificação)

Fitoextracção



-Fitoestimulação ou rizodegradação:

Plantas estimulam a biodegradação de compostos, porque absorvem água, provocam o movimento da solução para as raízes e favorece a degradação pelos microrganismos da rizosfera

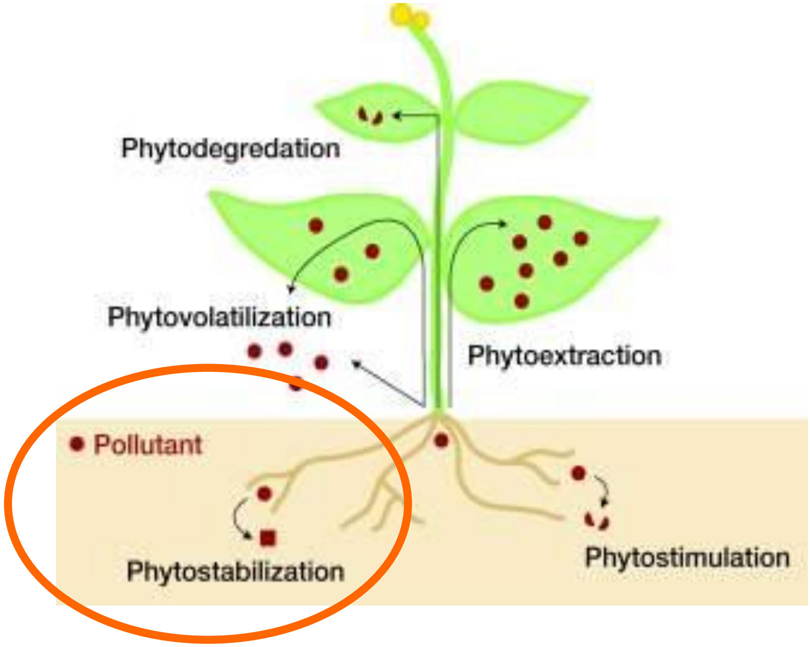
-Fitovolatilização (ou Fitotransformação):

os contaminantes são metabolizados em produtos voláteis e libertados para a atmosfera

Podem ser aplicados a **contaminantes orgânicos e inorgânicos**; a utilização é limitada a determinado tipo de compostos

Fitoestabilização

Fitoestabilização: Uso das plantas para estabilizar o contaminante no solo, recorrendo a uma cobertura vegetal



-reduz a erosão hídrica e eólica (há crescimento da planta)

-reduz perdas por lixiviação, reduzindo a contaminação de camadas inferiores de solo e de águas subterrâneas

-favorece a formação de formas de menor biodisponibilidade (precipitação na rizosfera)

Ex. monocotiledóneas e árvores

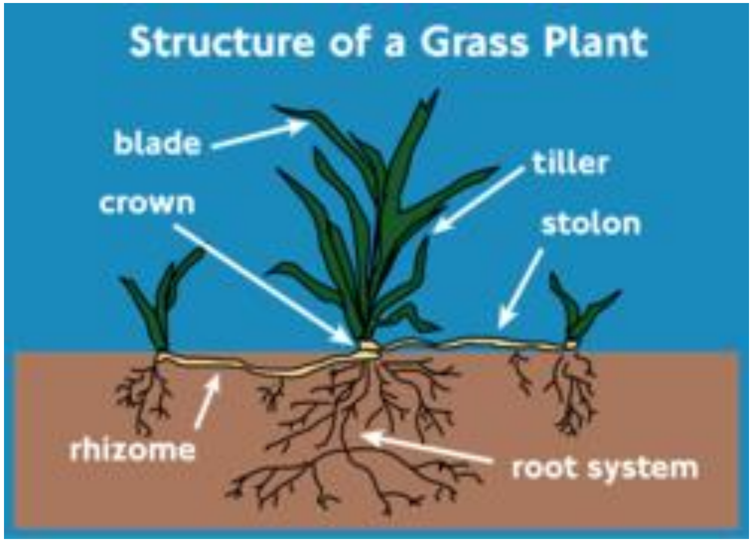
Pode ser aplicado a contaminantes inorgânicos (caso dos metais)

Não permite descontaminação mas destina-se a reduzir a contaminação produzida a partir deste solo

Exemplos de plantas para Fitoestabilização:

Populus

São **arvores** da espécie do choupo, que têm raízes profundas e taxas de transpiração elevadas, mantém o fluxo ascendente muito elevado, pelo que reduz as perdas por lixiviação e reduz a contaminação de camadas inferiores de solo e de águas subterrâneas



Monocotiledóneas

Acumulam pouco os contaminantes inorgânicos na parte aérea (ao contrario das dicotiledóneas) e portanto fornecem uma cobertura vegetal que previne a erosão do solo contaminado sem prejudicar os animais que as utilizam na sua alimentação

Exemplos de utilização de árvores para combater a poluição:

Asian Dust (also yellow dust, yellow sand, yellow wind or China dust storms) is a seasonal meteorological phenomenon which affects much of East Asia sporadically during the springtime months

In the last decade or so, it has become a serious problem due to the increase of industrial pollutants contained in the dust and intensified desertification in China causing longer and more frequent occurrences, as well as in the last few decades when the Aral Sea of Kazakhstan and Uzbekistan started drying up due to the diversion of the Amu River and Syr River following a Soviet agricultural program to irrigate Central Asian deserts, mainly for cotton plantations.

http://en.wikipedia.org/wiki/Asian_Dust

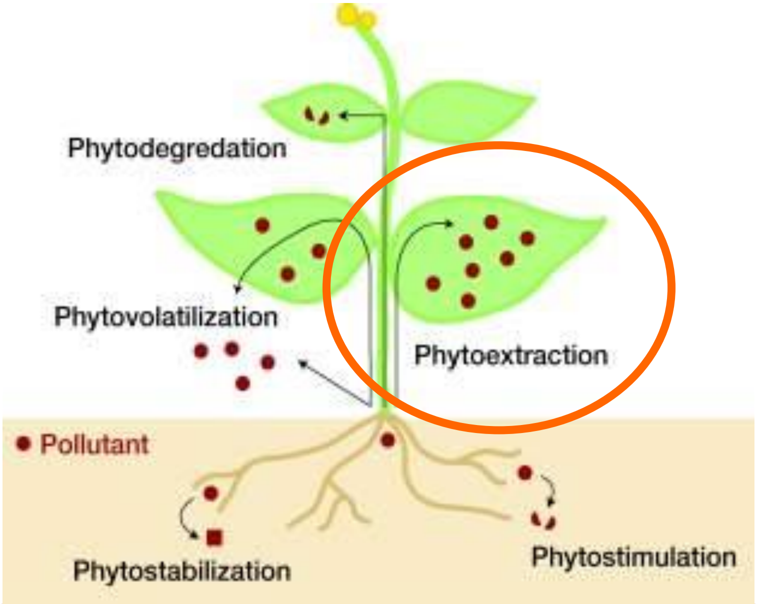
Exemplos de utilização de árvores para combater a poluição:

Sulfur (an acid rain component), soot, ash, carbon monoxide, and other toxic pollutants including heavy metals (such as mercury, cadmium, chromium, arsenic, lead, zinc, copper) and other carcinogens, often accompany the dust storms, as well as viruses, bacteria, fungi, pesticides, antibiotics, asbestos, herbicides, plastic ingredients, combustion products as well as hormone mimicking phthalates.

In recent years, South Korea and the People's Republic of China have participated in reforestation efforts in the source region. In 2007, South Korea sent several thousand trees to help block the migration of the yellow dust.

Fitoextracção

Utilização das plantas para remover os elementos do solo acumulando-os nos tecidos



Pode ser aplicado a contaminantes inorgânicos (caso dos metais)

Utiliza plantas para fins não alimentares

-podem ser utilizadas plantas hiperacumuladoras (absorvem grandes quantidades de alguns elementos como o Ni, Co, Cu, Pb, U, Zn, Se; geralmente o crescimento é lento e produzem pouca biomassa)

-podem ser utilizadas plantas de crescimento rápido, que acumulam menos metal mas produzem muita biomassa

-o material vegetal é depois recolhido e incinerado (o elemento mineral é recolhido na cinza)

Exemplos de plantas para Fitoextracção:

Brassica juncea (mostarda da India)

-usada para remover metais pesados de solos contaminados devido à sua elevada tolerância à toxicidade

-é hiperacumuladora de Cd e outros elementos vestigiais; pode por isso ser usada na alimentação para suplemento de Se, Cr, Fe, Zn

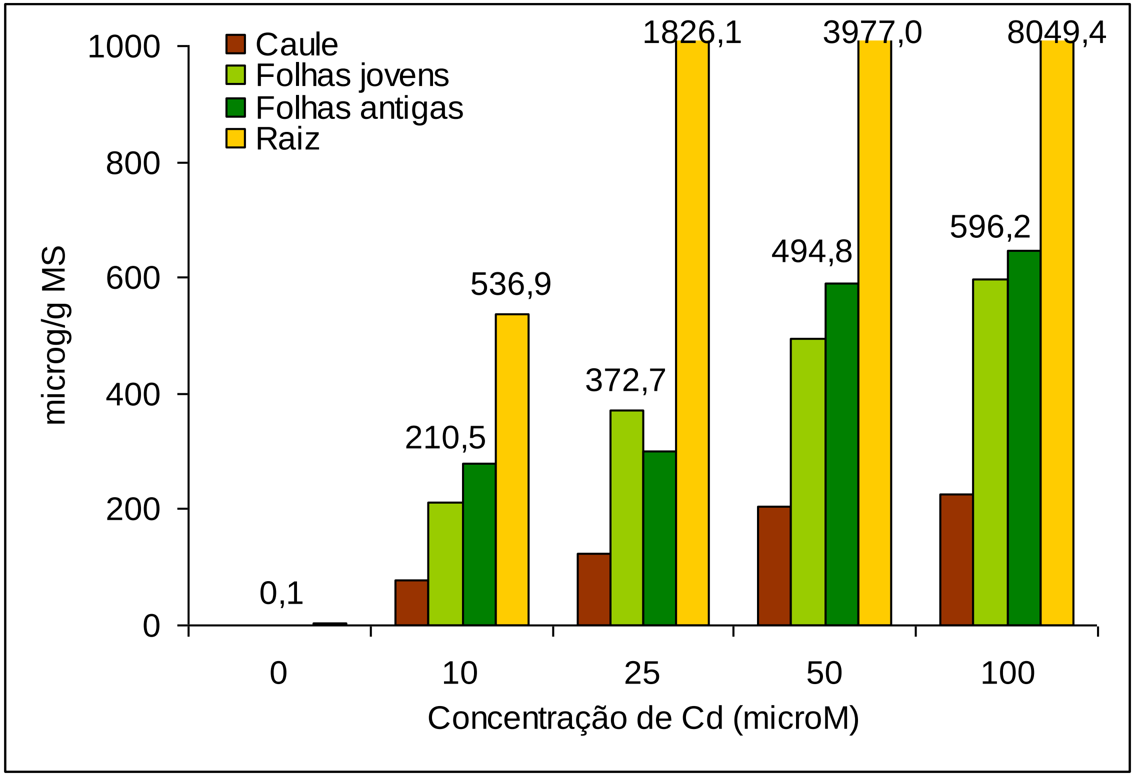
Estudos recentes concluem que são necessários **vários anos** para se conseguir uma fitorremediação satisfatória de um solo contaminado mesmo utilizando espécies de plantas hiperacumuladoras (Hernandez-Allica et al. 2008)

-planta que pertence à família das Brassicas, que inclui espécies de grande importância na alimentação, como couves, nabos, rabanetes, colza, mostarda, couve-flor

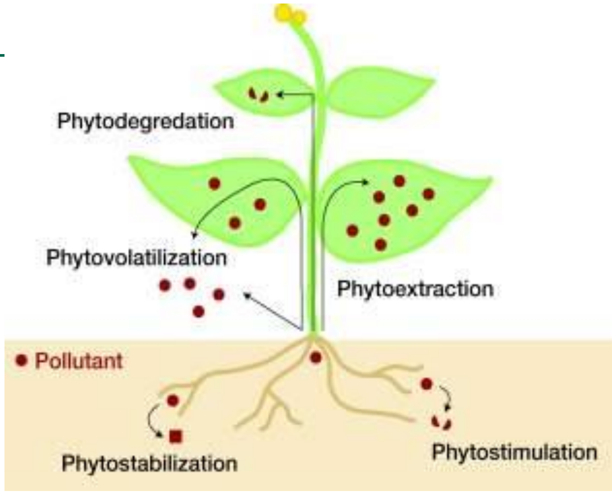
Exemplo:

-Estudos realizados no ISA baseiam-se no conhecimento de que a planta do tabaco é uma planta acumuladora de Cd

-Será que tem potencial para ser usada em fitoextracção?



Há aumento da biomassa da parte aérea mesmo para niveis de Cd elevados



As aplicações das técnicas de fitorremediação são diferenciadas:

-podem actuar várias simultaneamente

-dependem do tipo de contaminantes

-depende do custo

-dos volumes (de água ou solo) a tratar

Fitoextracção: principalmente usada para remoção de metais tóxicos e compostos inorgânicos

Fitoestabilização: para evitar o arrastamento de solo contaminado de antigas minas, para evitar lixiviação de contaminantes inorgânicos e orgânicos

Fitodegradação: usada para metabolização de compostos orgânicos que são moveis nas plantas, como por exemplo herbicidas

Fitovolatilização: usada para VOC (compostos orgânicos voláteis)

Limitações da fitorremediação

As plantas têm que se desenvolver de forma razoável na presença do contaminante

-biodisponibilidade dos poluentes (diferentes formas químicas têm diferente solubilidade)

-níveis de toxicidade existentes (diferentes formas químicas têm diferente toxicidade)

As plantas devem retirar do solo os contaminantes acumulando-os preferencialmente na sua parte aérea

-propriedades do solo (capacidade de ligação aos poluentes)

-clima

-profundidade que a raíz alcança (raízes de 50 cm em herbaceas, 3 m em árvores)

A fitorremediação através da acumulação na planta pode demorar vários anos

-a persistência dos contaminantes (resistência à decomposição) e o tempo de residência dos contaminantes (deslocação no solo)

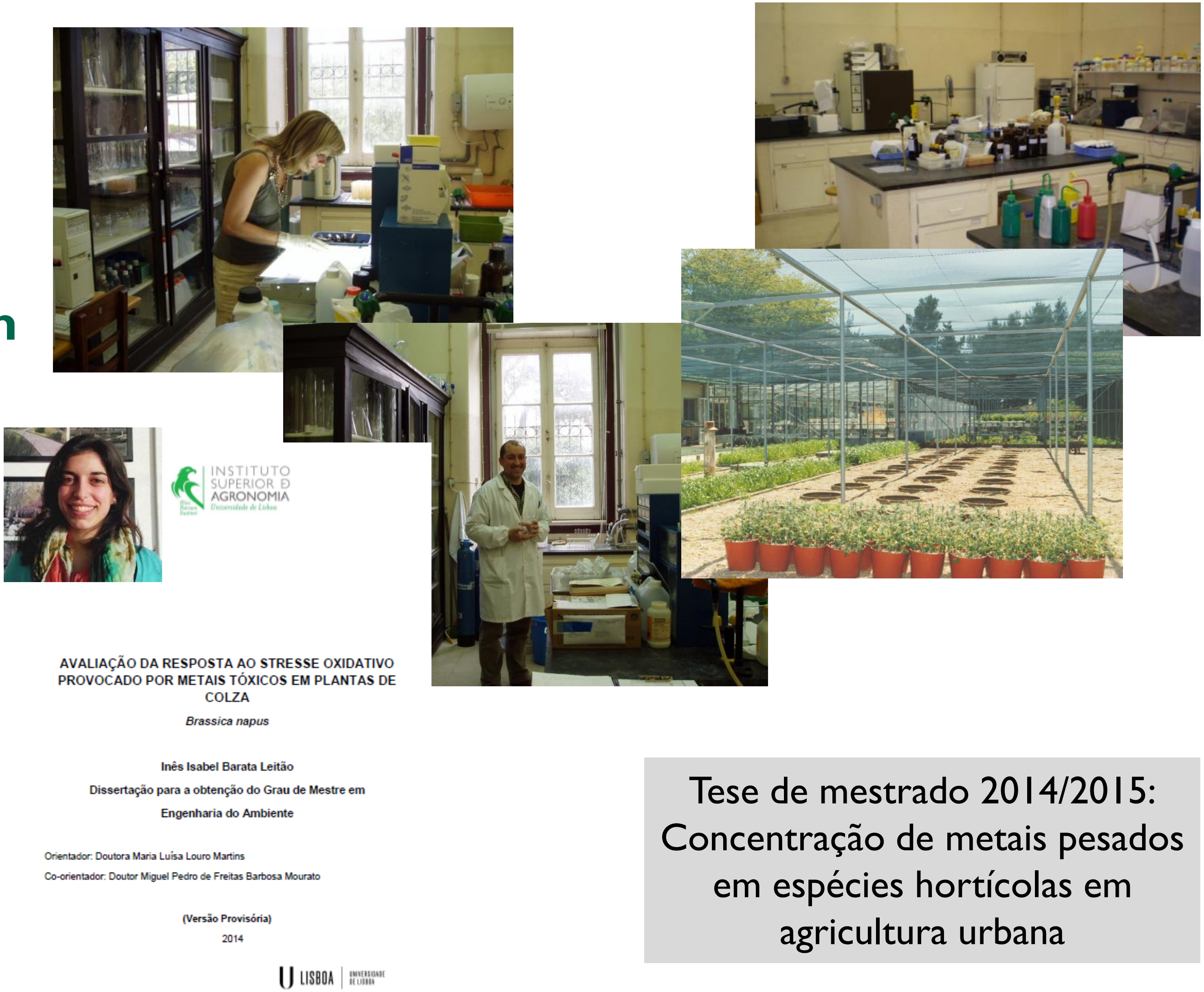
Características das plantas para serem usadas em fitorremediação:

A planta ideal para fitorremediação	-crescimento rápido -formação de muita biomassa -competitiva -resistente -tolerante ao contaminante
A planta ideal para fitodegradação	-area foliar grande (parte aérea) -sistema radicular denso -teores elevados de enzimas

-A planta ideal existe?

-Há uma planta ideal para todas as situações?

Se gostam destes temas, *venham trabalhar connosco!*



INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

AVALIAÇÃO DA RESPOSTA AO STRESSE OXIDATIVO PROVOCADO POR METAIS TÓXICOS EM PLANTAS DE COLZA

Brassica napus

Inês Isabel Barata Leitão

Dissertação para a obtenção do Grau de Mestre em

Engenharia do Ambiente

Orientador: Doutora Maria Luísa Louro Martins

Co-orientador: Doutor Miguel Pedro de Freitas Barbosa Mourato

(Versão Provisória)

2014

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Tese de mestrado 2014/2015: Concentração de metais pesados em espécies hortícolas em agricultura urbana

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