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Metabolomic and Biochemical analysis of Pb accumulation and tolerance in *Hirschfeldia incana*

Oumaima EL KHATTABI, Youssef LAMWATI, Fabrice COLIN, Abdelaziz SMOUNI and Mouna FAHR

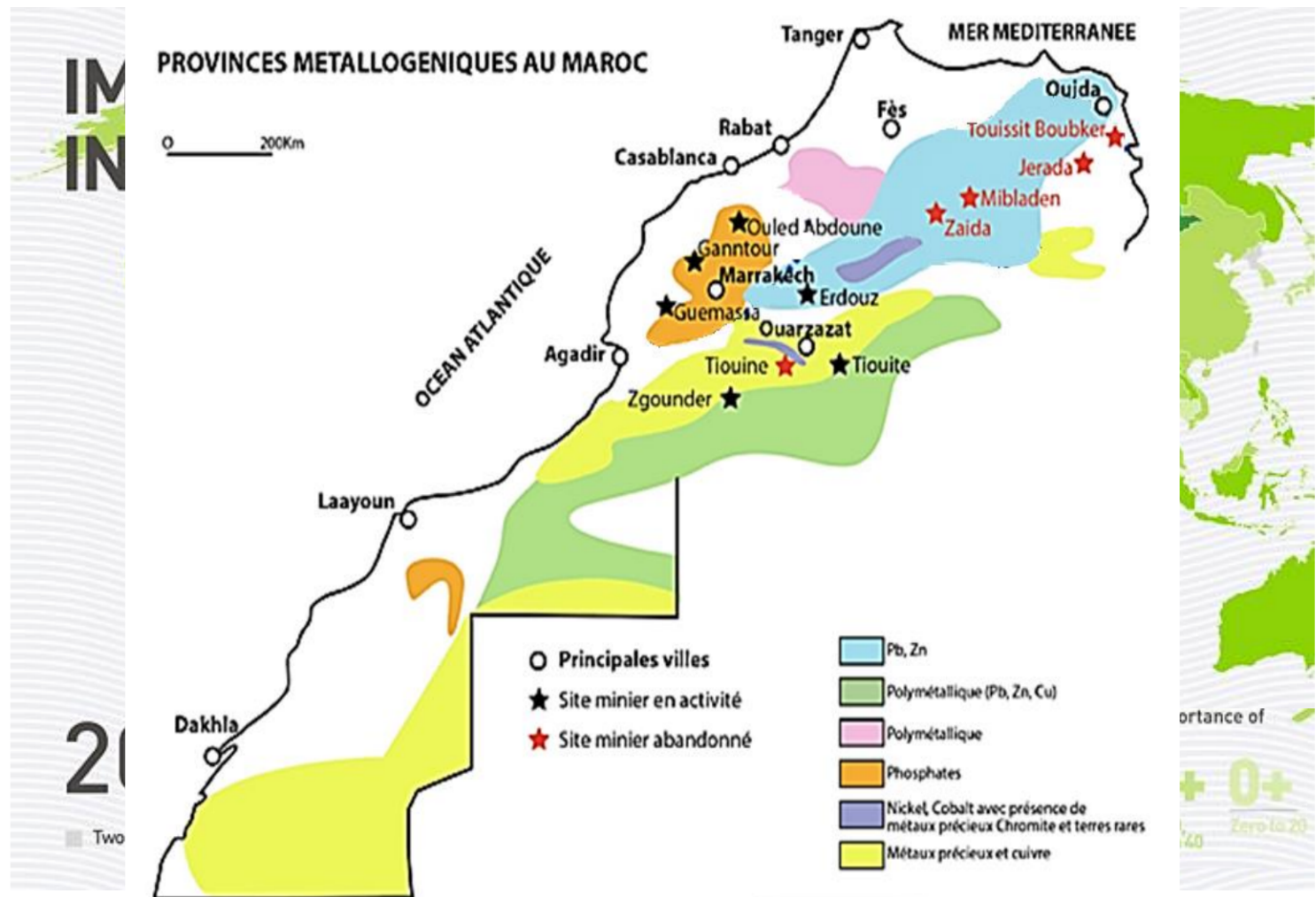


Mining Activities: Economic contribution

Morocco

Rich in mineral deposits

10% of national gross domestic product



Source: International council of mining and metals / Laboratoire Mixte International Activité Minière Responsable au Maroc (LMI-AMIR)

Mining Activities: principal source of pollution



Mining Pb/ Zn slag heaps at Touissit site – Eastern Morocco



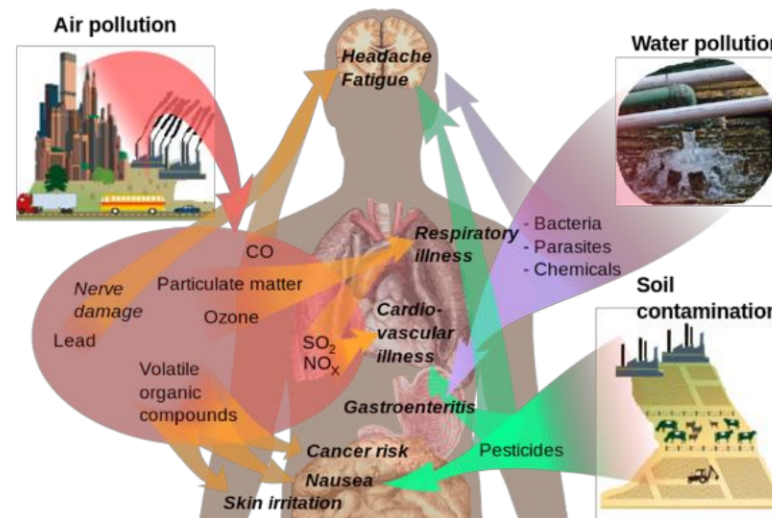
Scoria of Oued El Heimer Foundry - Eastern Morocco



AMD at Kettara mining site - northwest of Marrakech Morocco



Landscape change
Contamination of soil



Effects on Health



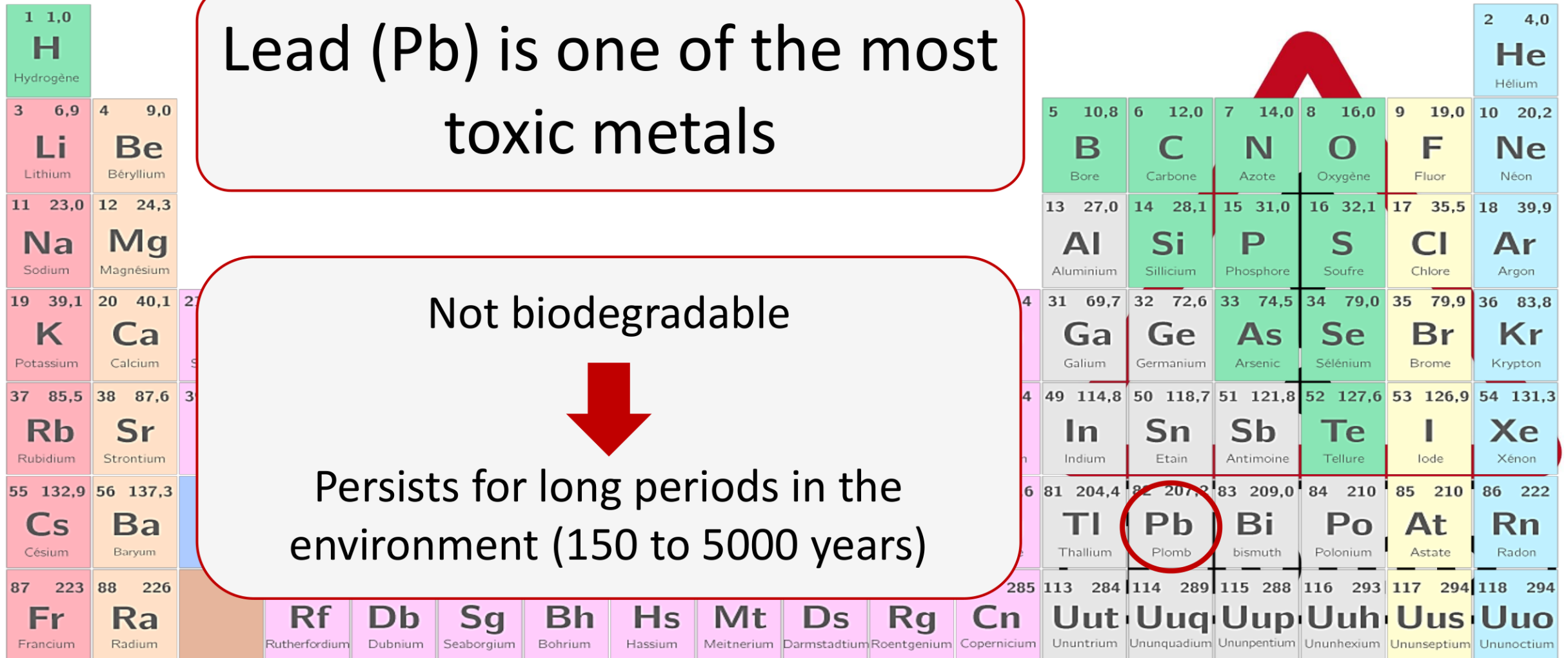
Contamination of
water resources

Mining Activities: Principal source of pollution

Lead (Pb) is one of the most toxic metals

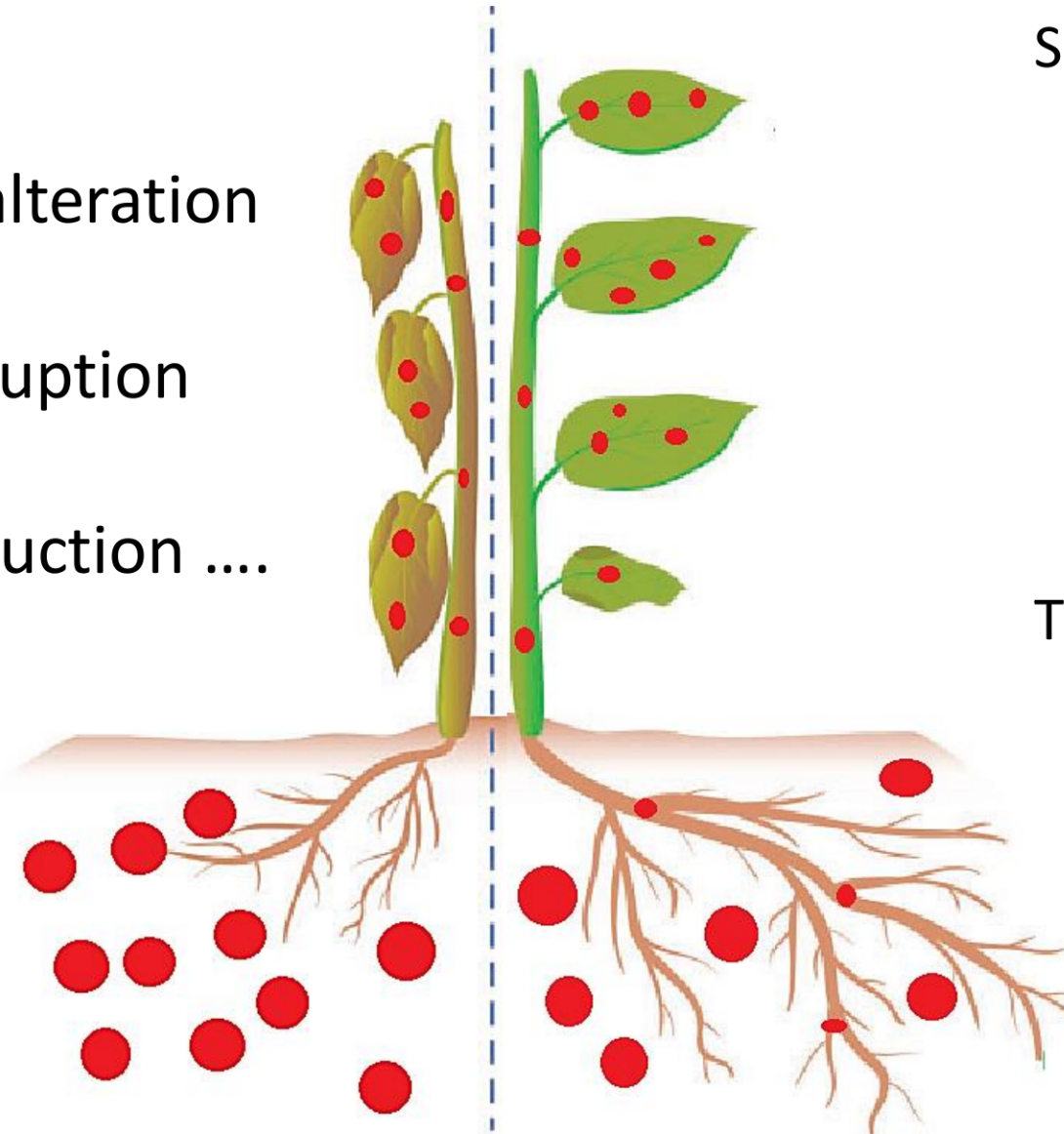
Not biodegradable

Persists for long periods in the environment (150 to 5000 years)



1 1,0 H Hydrogène																	2 4,0 He Hélium					
3 6,9 Li Lithium	4 9,0 Be Béryllium															5 10,8 B Bore	6 12,0 C Carbone	7 14,0 N Azote	8 16,0 O Oxygène	9 19,0 F Fluor	10 20,2 Ne Néon	
11 23,0 Na Sodium	12 24,3 Mg Magnésium															13 27,0 Al Aluminium	14 28,1 Si Silicium	15 31,0 P Phosphore	16 32,1 S Soufre	17 35,5 Cl Chlore	18 39,9 Ar Argon	
19 39,1 K Potassium	20 40,1 Ca Calcium	21 39,1 Sc Scandium															31 69,7 Ga Galium	32 72,6 Ge Germanium	33 74,5 As Arsenic	34 79,0 Se Sélénium	35 79,9 Br Brome	36 83,8 Kr Krypton
37 85,5 Rb Rubidium	38 87,6 Sr Strontium	39 85,5 Y Yttrium															49 114,8 In Indium	50 118,7 Sn Etain	51 121,8 Sb Antimoine	52 127,6 Te Tellure	53 126,9 I Iode	54 131,3 Xe Xénon
55 132,9 Cs Césium	56 137,3 Ba Baryum	57 137,3 La Lanthane															61 204,4 Tl Thallium	62 207,2 Pb Plomb	63 209,0 Bi bismuth	64 210 Po Polonium	65 210 At Astate	66 222 Rn Radon
87 223 Fr Francium	88 226 Ra Radium	89 223 Ac Actinium	104 261 Rf Rutherfordium	105 262 Db Dubnium	106 263 Sg Seaborgium	107 263 Bh Bohrium	108 264 Hs Hassium	109 265 Mt Meitnerium	110 266 Ds Darmstadtium	111 267 Rg Roentgenium	112 268 Cn Copernicium	113 284 Uut Ununtrium	114 289 Uuq Ununquadium	115 288 Uup Ununpentium	116 293 Uuh Ununhexium	117 294 Uus Ununseptium	118 294 Uuo Ununoctium					

- Plant water status alteration
- Photosynthesis disruption
- Oxidative stress induction



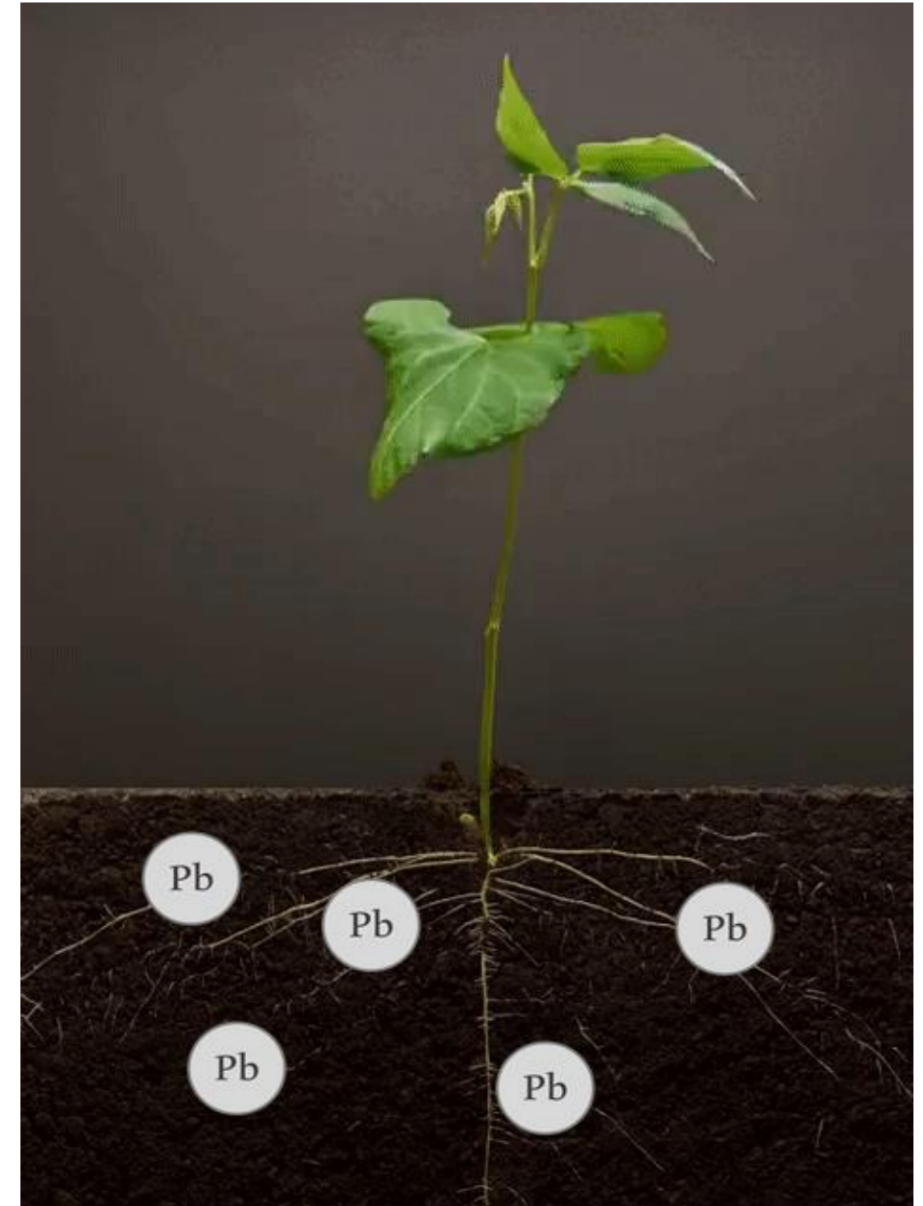
Some plant species develop effective strategies of protection against these elements



These natural properties are exploited in the field of **Phytoremediation**

Phytoremediation

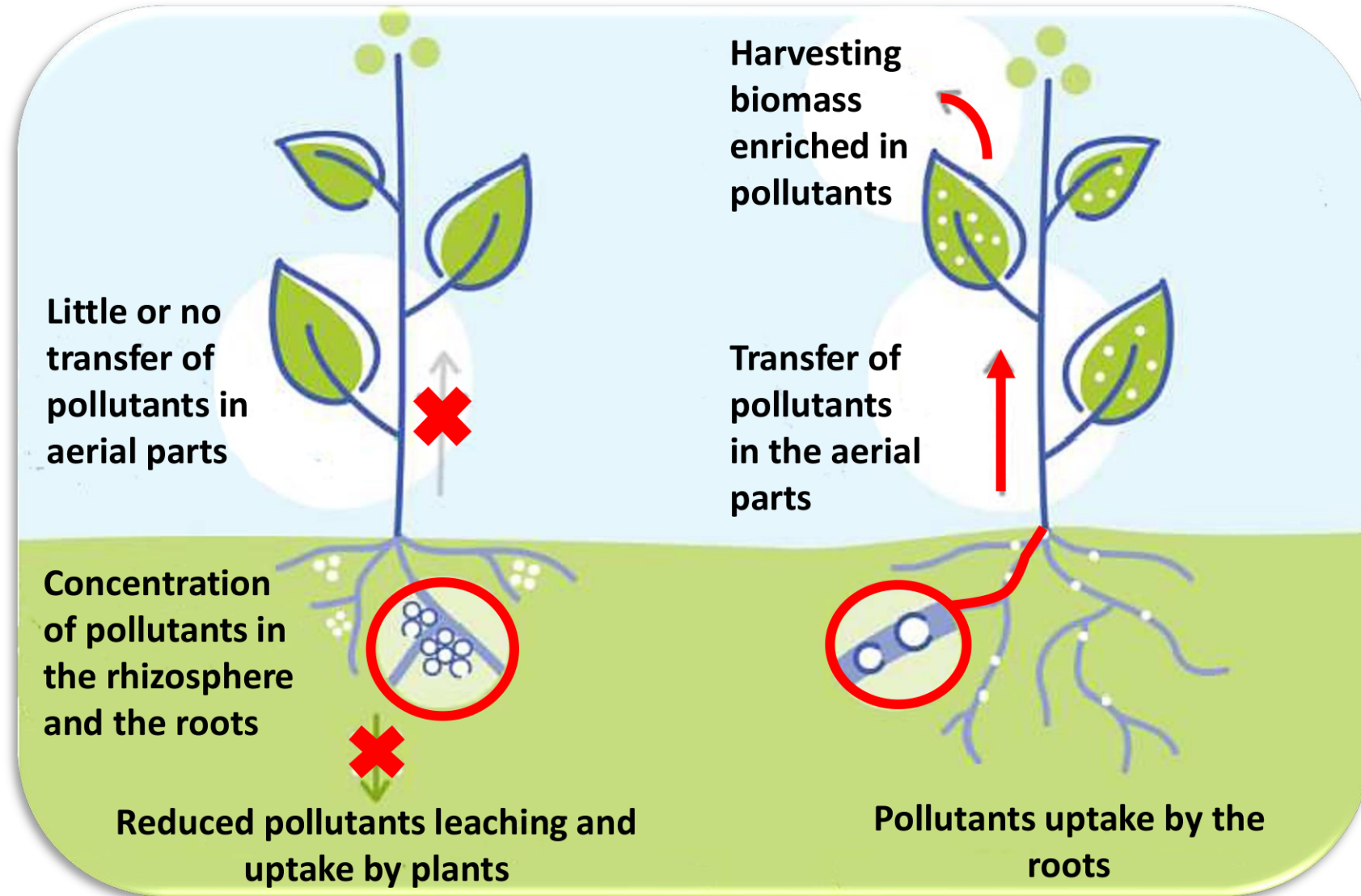
- A biological Method based on the ability of plants to extract or block pollutants from contaminated soil or water
- More efficient
- Less expensive



PHYTOSTABILISATION

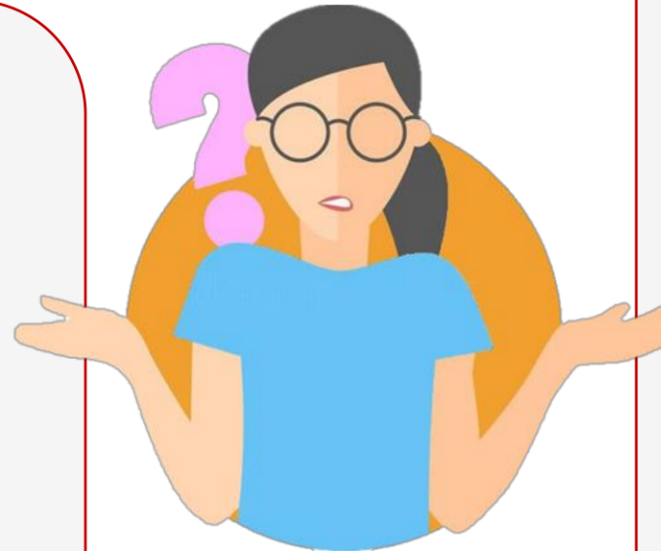
PHYTOEXTRACTION

**Phytoremediation
strategies**



Although phytoremediation offers several advantages :

Economic
Ecological
Aesthetic



Phytoremediation limits

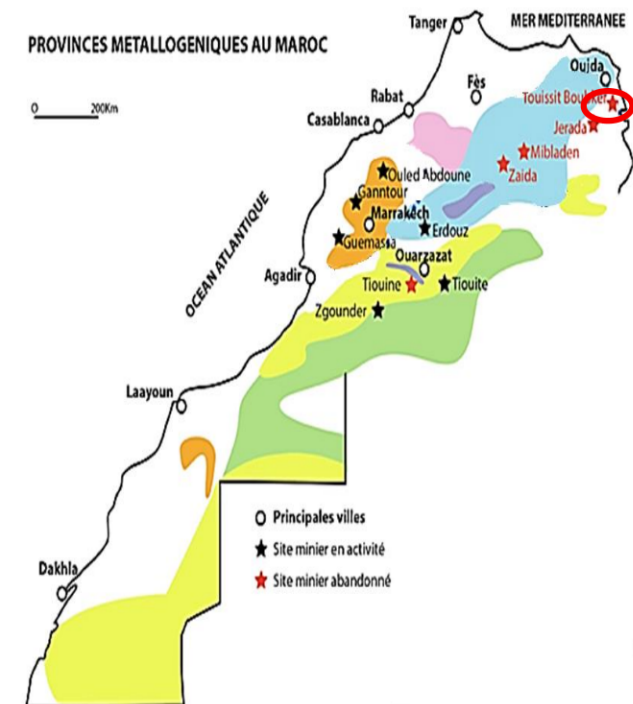
Slow decontamination process

Accumulator plants have :

- Slow growth rate
- Low biomass production

With the aim of optimizing the use of plants in the rehabilitation of soils polluted by Pb

- *Hirschfeldia incana*
- Oued el heimer-touissit sites

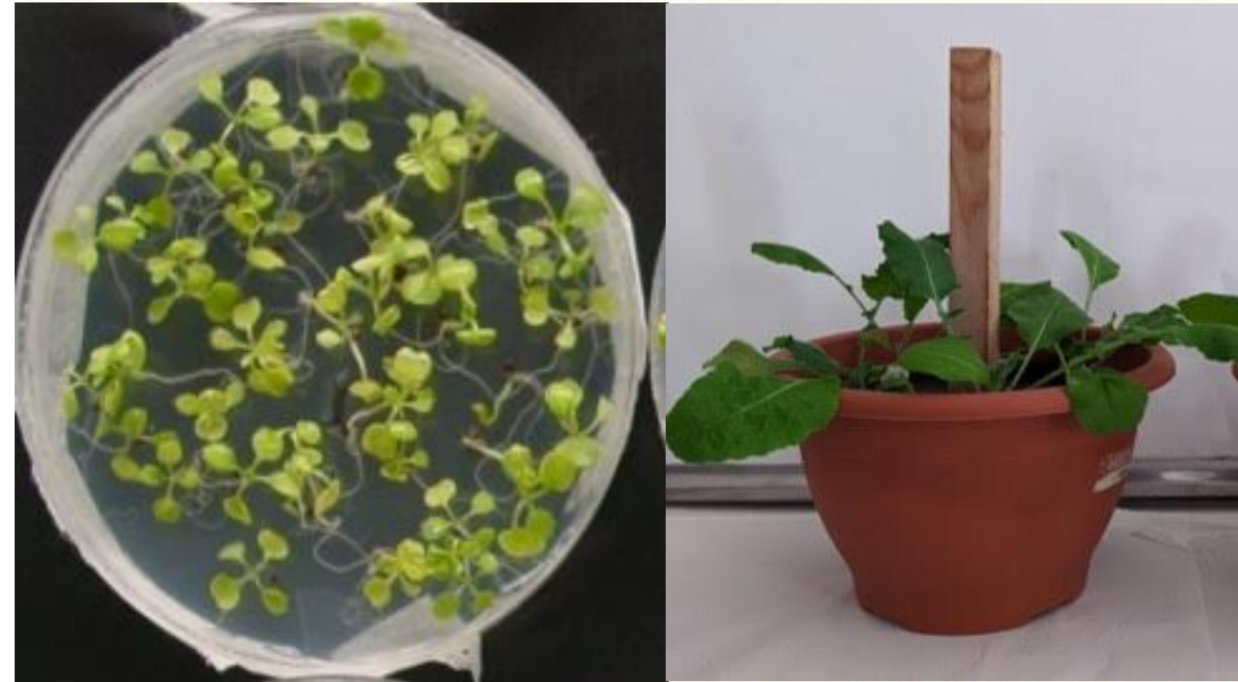


Hirschfeldia incana growing on lead slag from the site of Oued El Heimer near Oujda in Morocco

With the aim of optimizing the use of plants in the rehabilitation of soils polluted by Pb

Hirschfeldia incana

- Accumulates in its aerial parts up to 1400 mg of Pb/Kg of dry matter
- Short vegetative cycle (2 months)
- Rapid growth
- Easy to handle in the laboratory
- Its transcriptom has just been sequenced



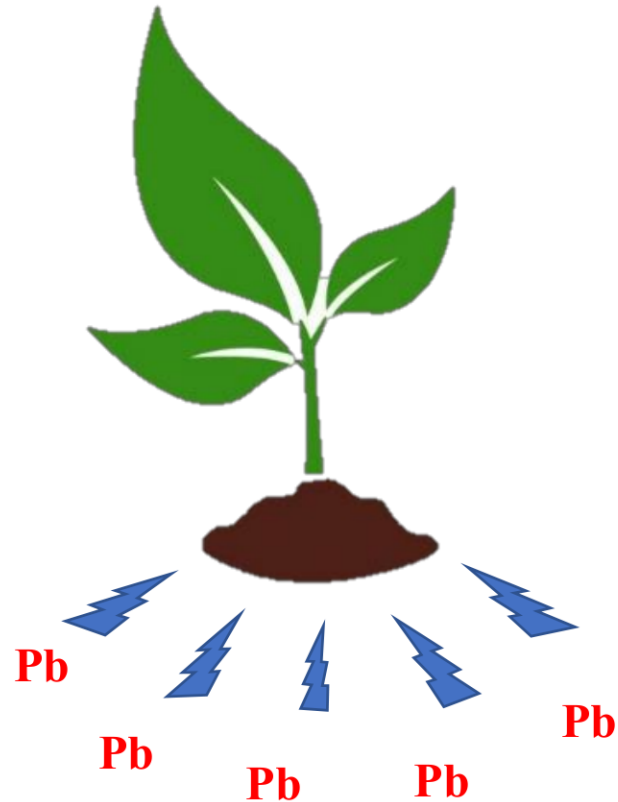
Hirschfeldia incana growing on lead slag from the site of Oued El Heimer near Oujda in Morocco

A good model to study Pb tolerance and accumulation mechanisms

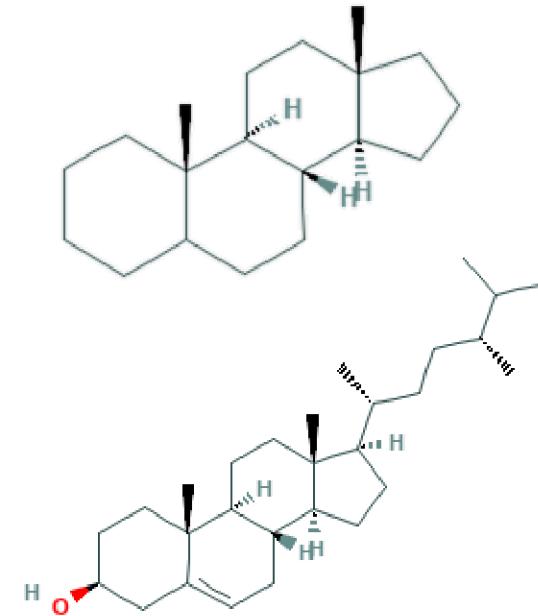
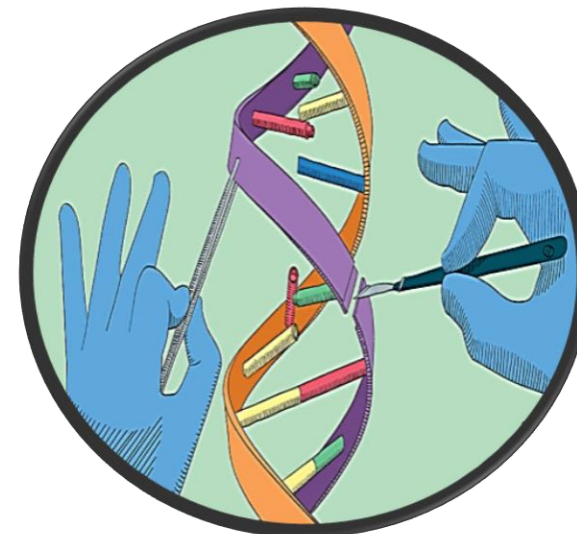
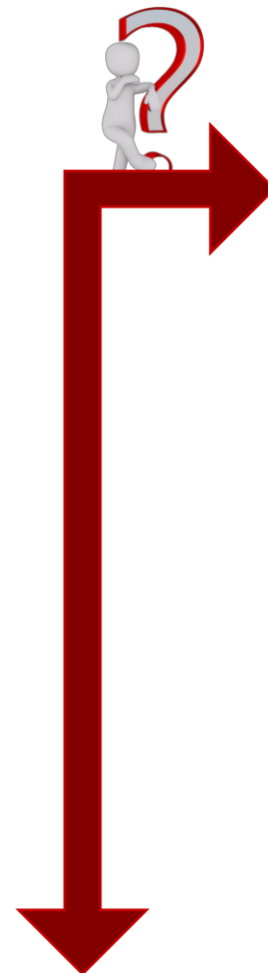
Molecular changes



Physiological changes
Metabolites variation



Molecular and metabolomic data on heavy metal accumulating plants are limited

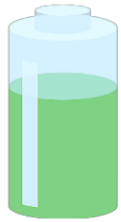




Metabolomic analysis of Pb accumulation and tolerance in *Hirschfeldia incana*

Biochemical analysis of Pb accumulation and tolerance in *Hirschfeldia incana*

Metabolites extraction



Plants grinding in liquid nitrogen

Enzymes extraction



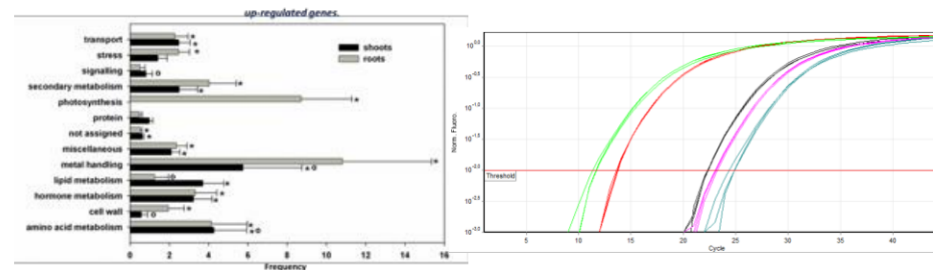
Transesterification

Analyse via GC-MS



Molecular analysis of Pb accumulation and tolerance in *Hirschfeldia incana*

Dosage by spectrophotometry



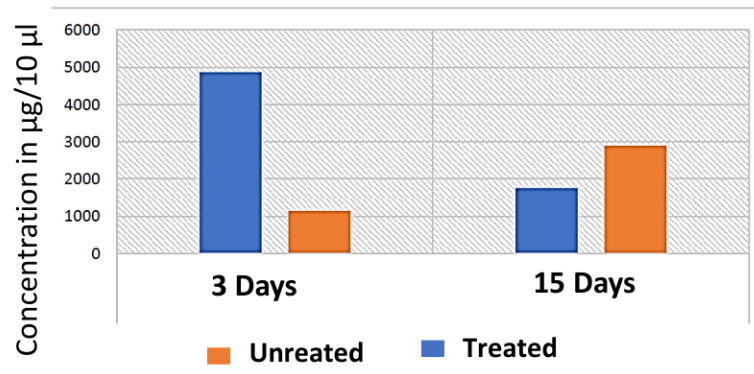
Genes implicated in metabolomic pathways

Metabolites implicated in Pb tolerance and accumulation

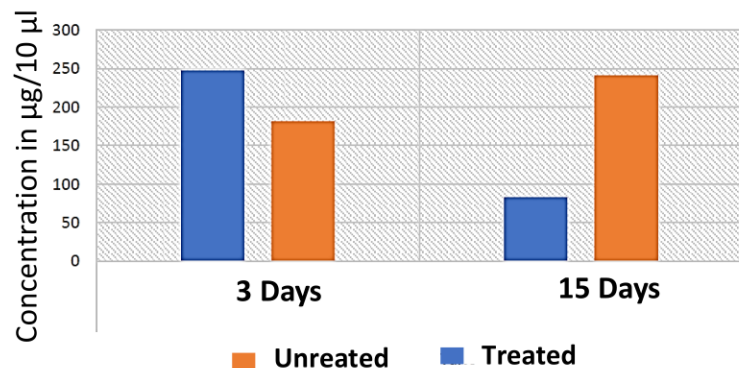
Enzymes and molecules implicated in Pb tolerance and detoxification

Metabolomic characterization

Variation in fatty acids composition



Variation in steroids composition



Plants require a period for the modulation of their metabolism to overcome Pb stress

Lipids after 15 days of Pb treatment

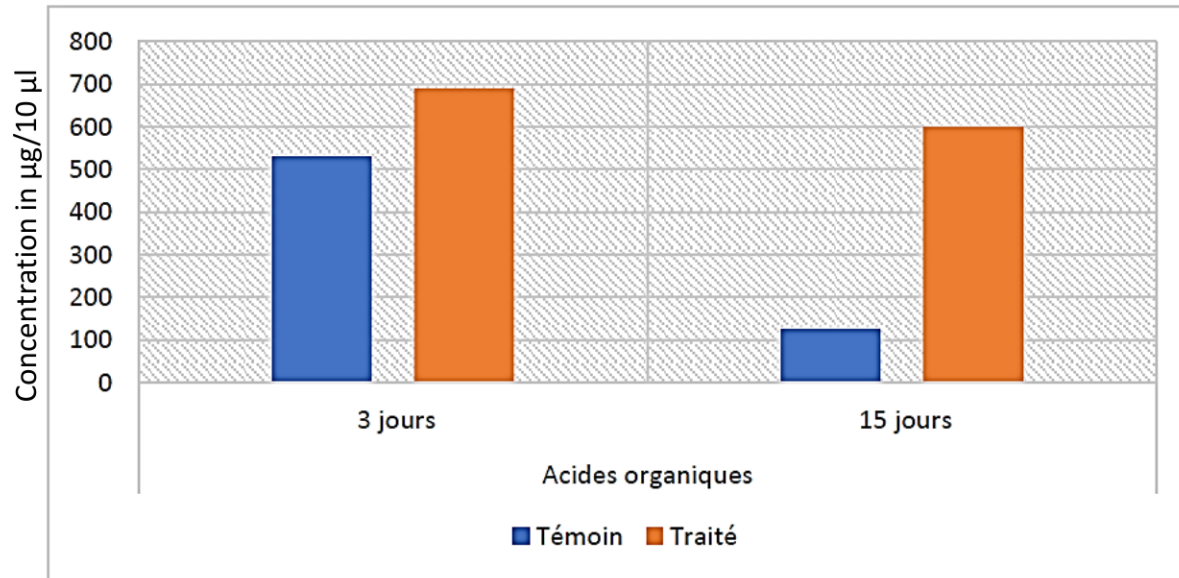
	metabolites	TEMOIN vs TRAITÉ%
Acides gras insaturés	7,10,13-Hexadecatrienoic acid,	-11.87
	9,12,15-Octadecatrienoic acid,	-18.7
	6,9,12,15-Docosatetraenoic acid,	-68.76
	1-(4-Fluorophenyl)-2-[(4-hydroxy-	-5.88
Acides gras saturés	Hexadecanoic acid, methyl ester	79.52
	Methyl stearate	19.96
	Ethyl 3-hydroxyicosanoate	+
	Tetracosanoic acid, methyl ester	41.31
	Hexacosanoic acid, methyl ester	58.5
	Methyl 18-methylnonadecanoate	+
	Octacosanoic acid, methyl ester	+

Steroids after 15 days of Pb treatment

metabolites	TEMOIN vs TRAITÉ%
ethyl iso-allocholate	153.81
campesterol	79.83
5-cholesten-3-ol 24-methyl-	+
gamma.-Sitosterol	151.54
Androstan-17-one, 3-ethyl-3-hydroxy-	+

Metabolomic characterization

Variation in organic acids composition



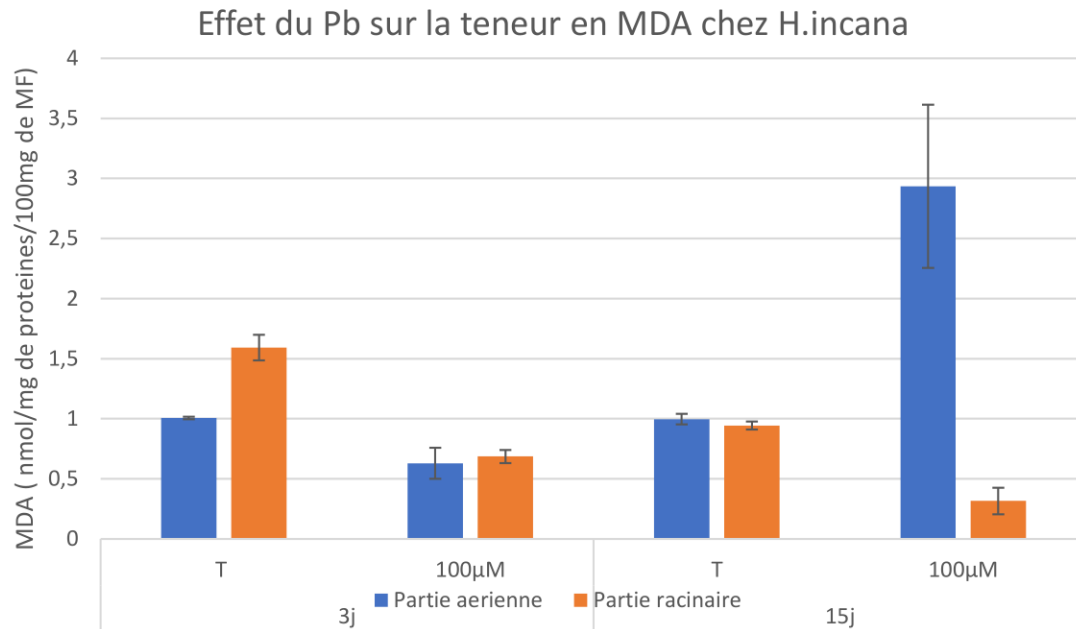
	metabolites	TEMOIN vs TRAITE%
Phenoles	2,4-Di-tert-butylphenol	-4.51
	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	+
Acides carboxyliques	1,4-Benzenedicarboxylic acid, dimethyl ester	+
	1,3-Benzenedicarboxylic acid, dimethyl ester	+
	Succinic acid, tridec-2-yn-1-yl 3-methylpentyl ester	20.93
	Succinic acid, cyclohexylmethyl 3-chlorophenyl ester	20.93
	propanoic acid	+

Organic acids after 15 days of Pb treatment

- **Increase in organic acids concentration (succinic acid)**
 - Succinic acid is involved in the signaling of salicylic acid (mitigate Pb stress effects)

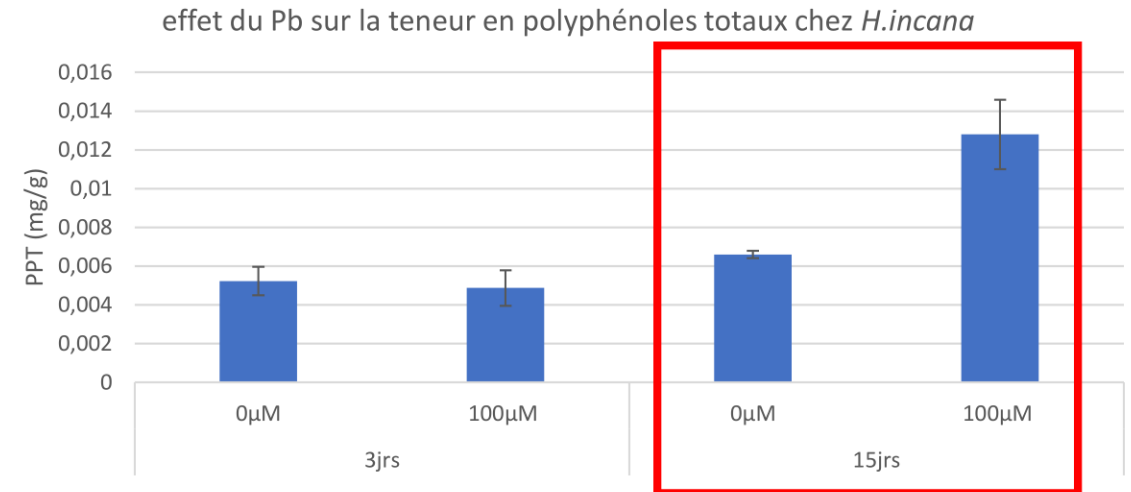
Biochemical characterization

Malondialdehyde (MDA) content



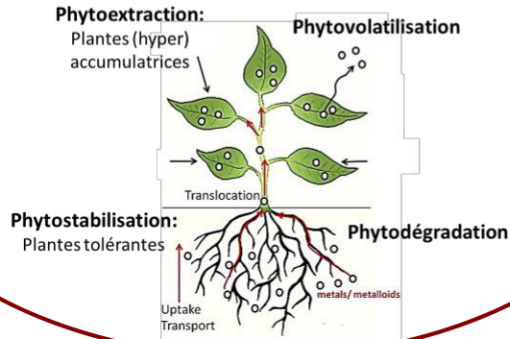
Pb induces oxydative stress → Lipid peroxydation → Increase in MDA

Polyphenol content



Implication of polyphenols in Pb detoxification and Pb-induced oxydative stress attenuation

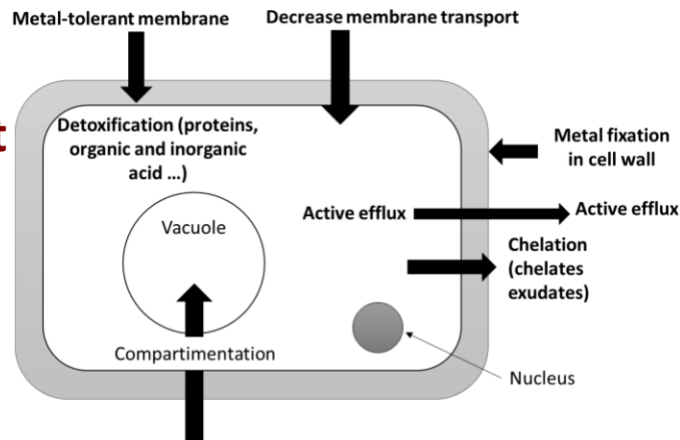
Phytoremediation



Fundamental aspect

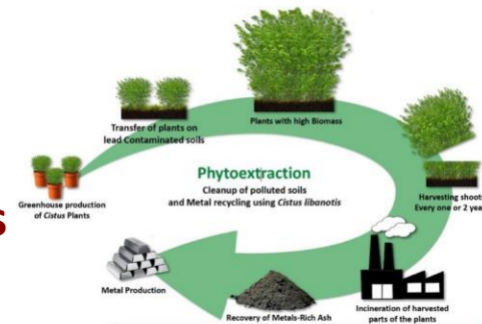
Applied aspect

Enrich knowledge about the fundamental aspects of Pb tolerance and accumulation



Use of the identified molecules :

- Boosting plants response to Pb
- Selection of candidate plants for phytoremediation programs
- Extraction of high value-added metal



Thank you for your attention



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