MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

Assessment of the effectiveness of organic amendments for aided phytostabilization of mine soil using biomarkers

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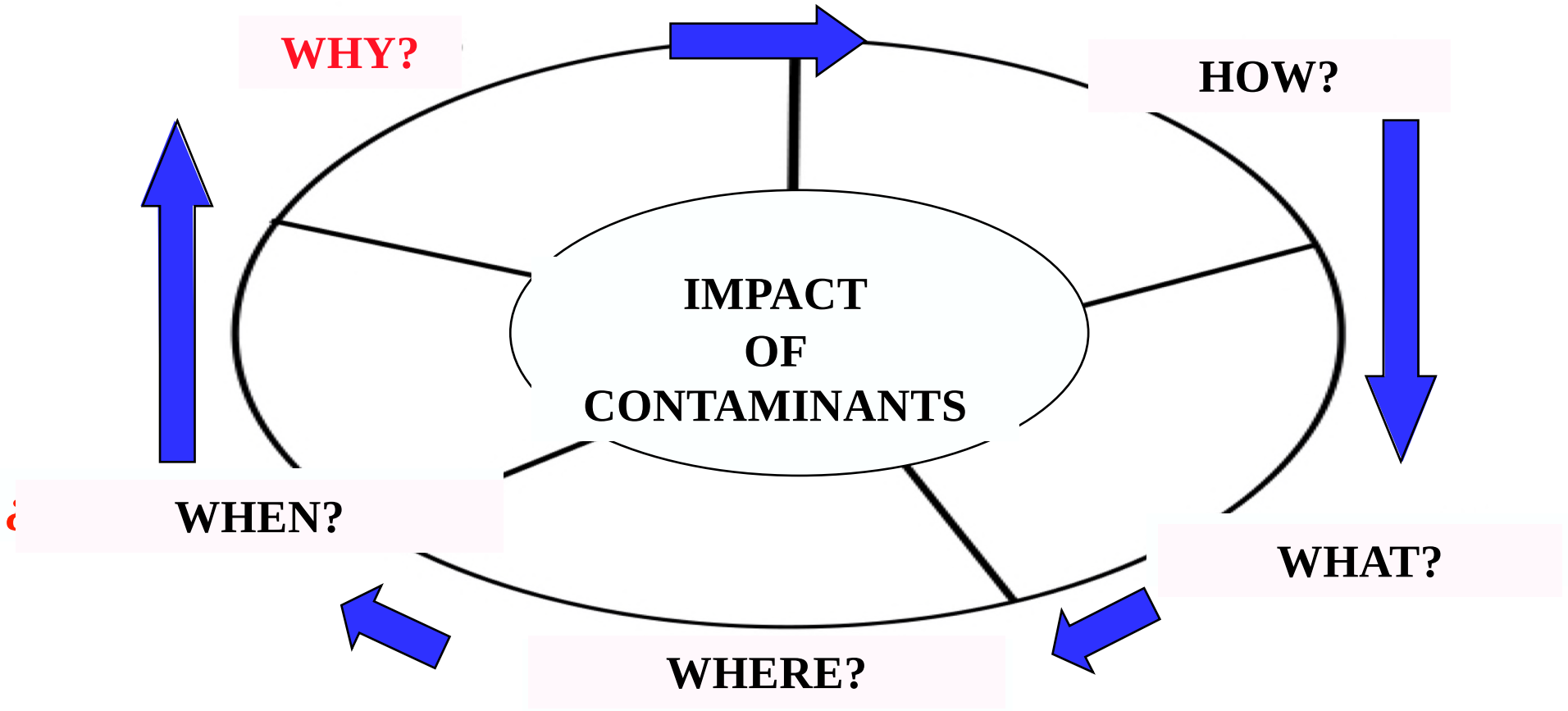
19-21 diciembre 2023

MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

the essential issues

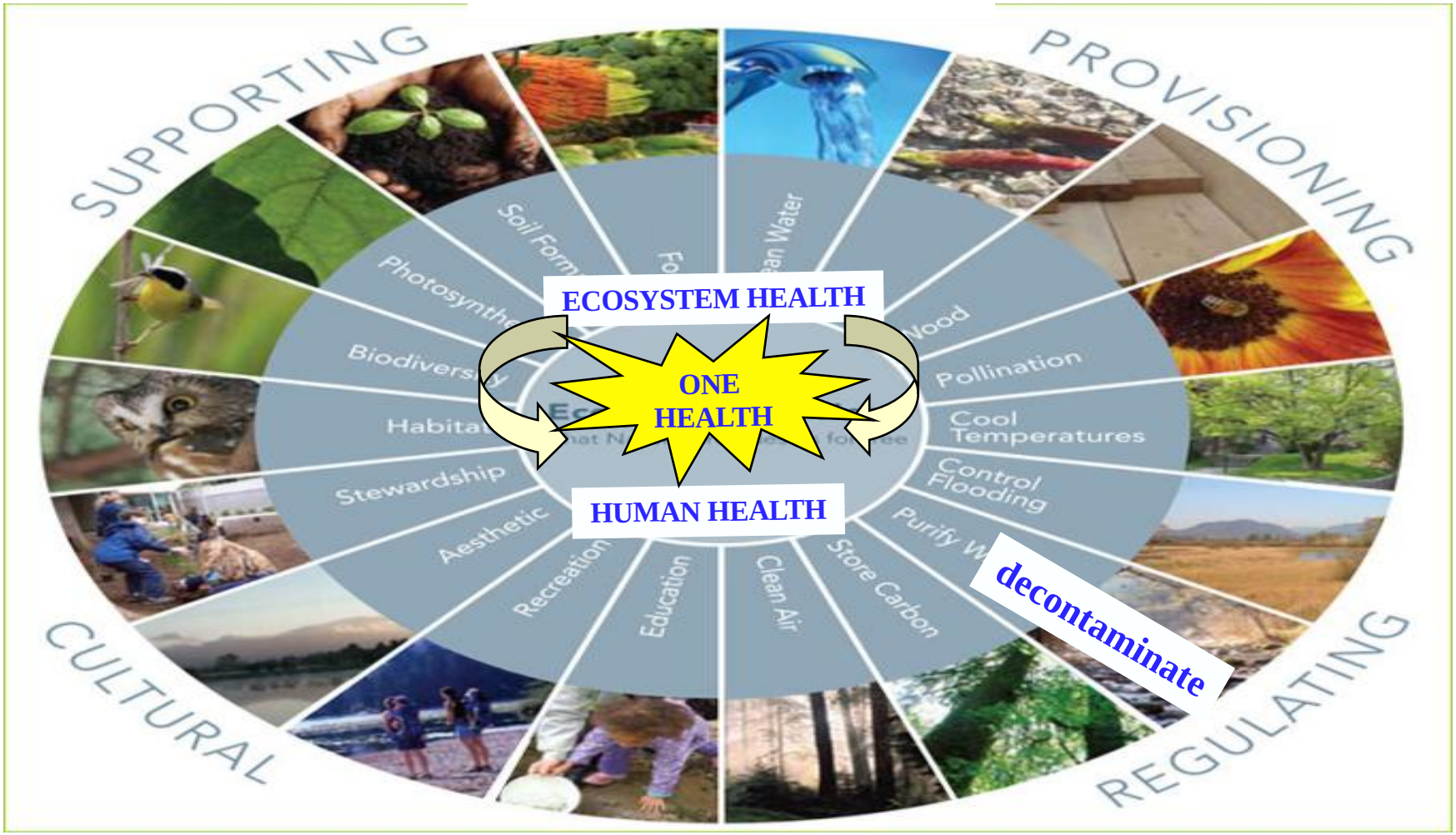


WHY?

Soil, organism, ecosystem health assessment

Soil Health : capacity of soil to function as a living system and carry out functions and services

ECOSYSTEM SERVICES



WHY?

There is a need for soil health assessment threatened by pollution

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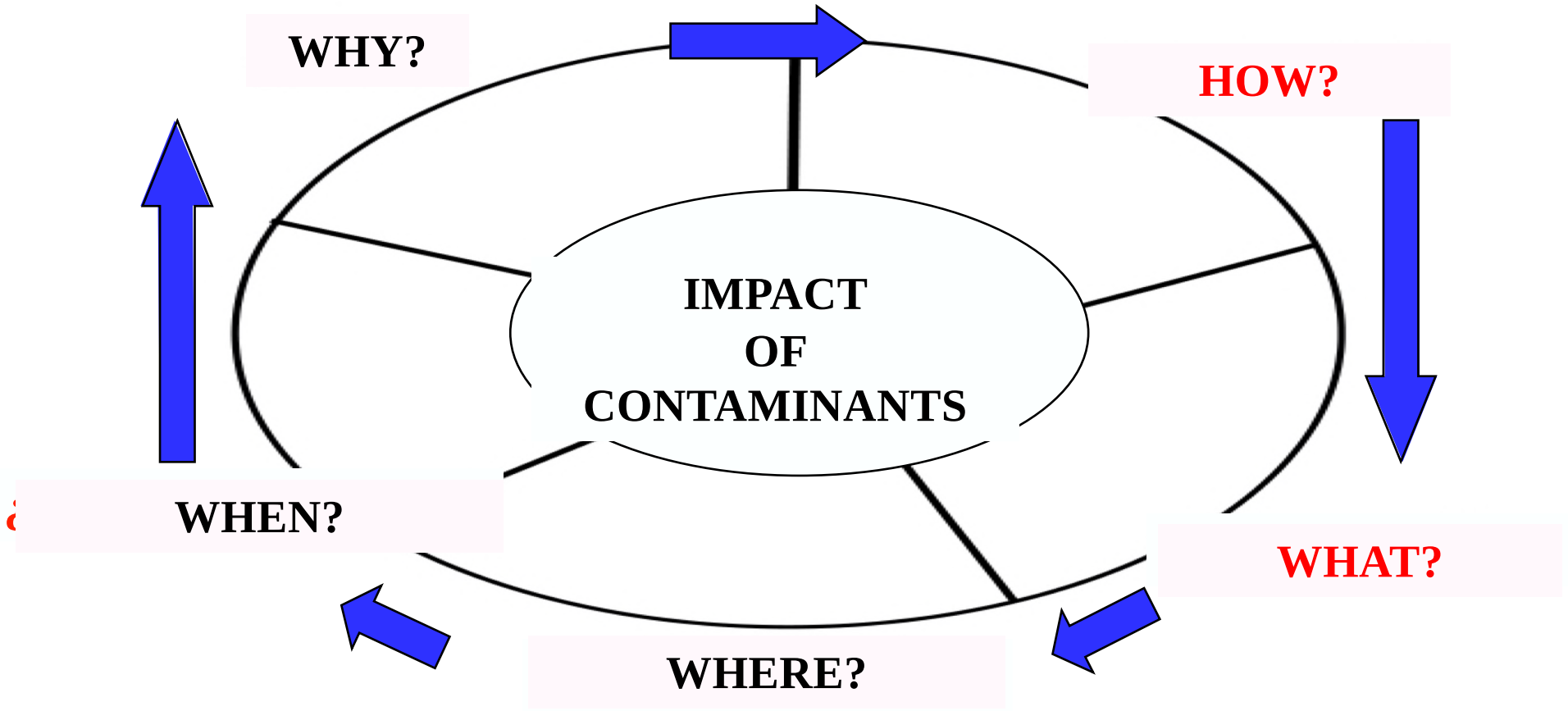
the essential issues



HOW?

DIAGNOSIS

Soil Health

capacity of soil to function as a vital living (eco)system

PHYSICO-CHEMICAL PARAMETERS

BIOLOGICAL PARAMETERS

Chemical Analysis

Who?
How much

Bioavailability Tests

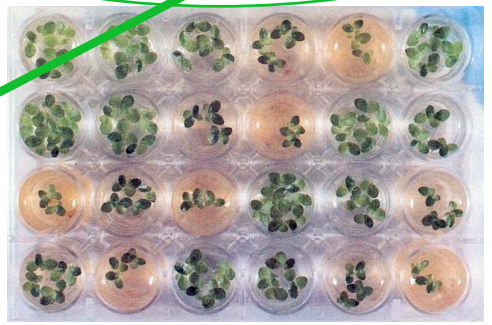
Bioaccesibility Tests

How much can impact?

Gastrointestinal simulation

Impact?

Ecotoxicity Tests



HOW?

DIAGNOSIS

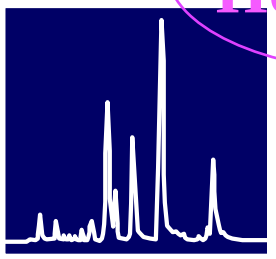
Soil Health

PHYSICO-CHEMICAL PARAMETERS

Chemical Analysis

Who?

How much?



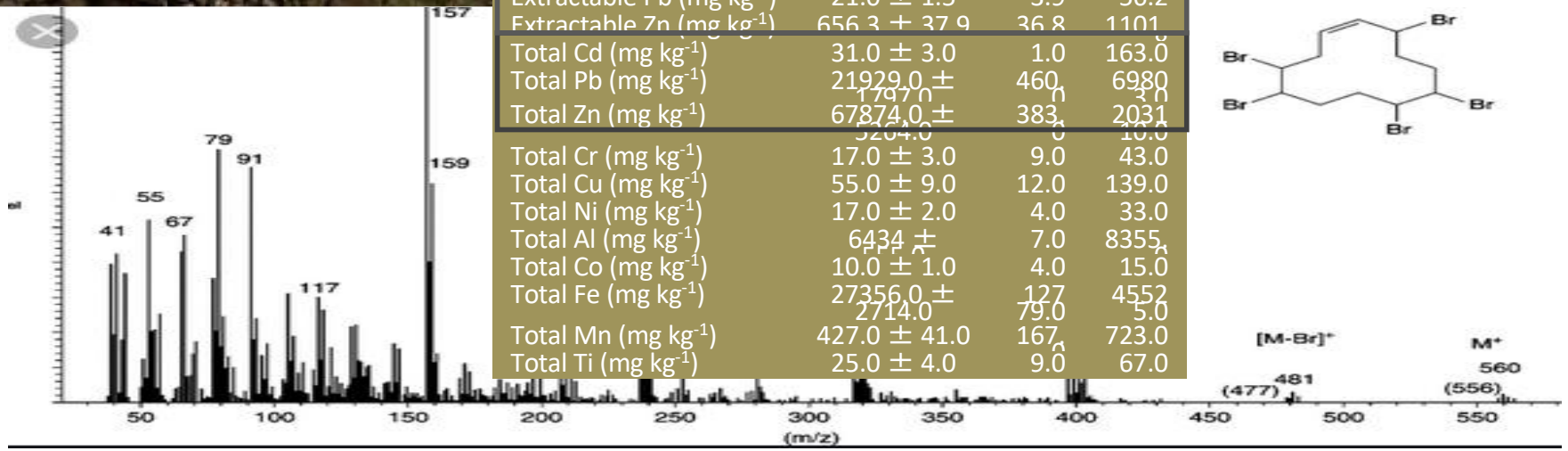
Soil Health Assesment **How? What?**

PHYSICOCHEMICAL INDICATORS of soil

Pollutants (Who? How much?),

structure, texture, pH, nutrients, organic matter, Cation Exchange Capacity (CEC), etc.

	Mean	Min.	Max.
pH	6.9 ± 0.1	6.7	7.1
OM (%)[a]	3.0 ± 1.0	0.9	4.8
Coarse sand, 1000-2000 µm (%)	26.1 ± 4.0	14.9	34.7
Fine sand, 50-1000 µm	49.9 ± 2.7	43.2	57.0
Lime (%)	12.1 ± 0.8	10.1	14.0
Clay (%)	11.9 ± 1.5	8.5	16.3
Total N (%)	0.15 ± 0.05	0.1	0.2
C/N	15.0 ± 2.0	9.7	18.4
WHC -33 kPa[b]	21.0 ± 0.8	19,8	22.1
Extractable NO_3 (mg kg^{-1})	8.0 ± 1.0	5.0	10.0
Extractable P (mg kg^{-1})	5.0 ± 1.0	4.0	8.0
Extractable Na (mg kg^{-1})	17.0 ± 3.0	12.0	23.0
Extractable K (mg kg^{-1})	24.0 ± 3.0	17.0	39.0
Extractable Ca (mg kg^{-1})	675.0 ±	338	913.0
Extractable Mg (mg kg^{-1})	88.0 ± 15.0	58.0	117.0
Extractable Cd (mg kg^{-1})	2.8 ± 0.2	0.7	6.9
Extractable Pb (mg kg^{-1})	21.0 ± 1.5	3.9	56.2
Extractable Zn (mg kg^{-1})	656.3 ± 37.9	36.8	1101
Total Cd (mg kg^{-1})	31.0 ± 3.0	1.0	163.0
Total Pb (mg kg^{-1})	21929.0 ±	460	6980
Total Zn (mg kg^{-1})	67874.0 ±	383	2031
Total Cr (mg kg^{-1})	17.0 ± 3.0	9.0	43.0
Total Cu (mg kg^{-1})	55.0 ± 9.0	12.0	139.0
Total Ni (mg kg^{-1})	17.0 ± 2.0	4.0	33.0
Total Al (mg kg^{-1})	6434 ±	7.0	8355
Total Co (mg kg^{-1})	10.0 ± 1.0	4.0	15.0
Total Fe (mg kg^{-1})	27356.0 ±	127	4552
Total Mn (mg kg^{-1})	427.0 ± 41.0	167	723.0
Total Ti (mg kg^{-1})	25.0 ± 4.0	9.0	67.0

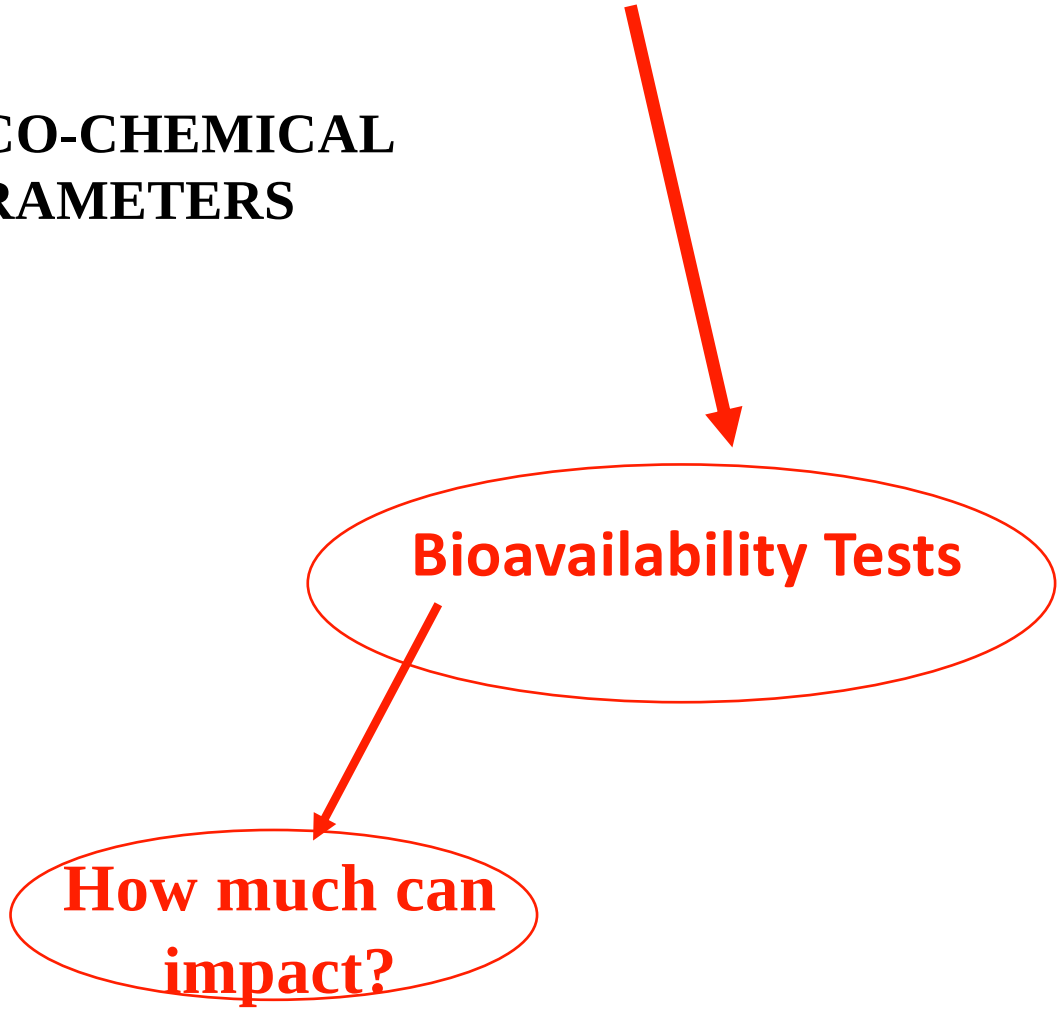


HOW?

DIAGNOSIS

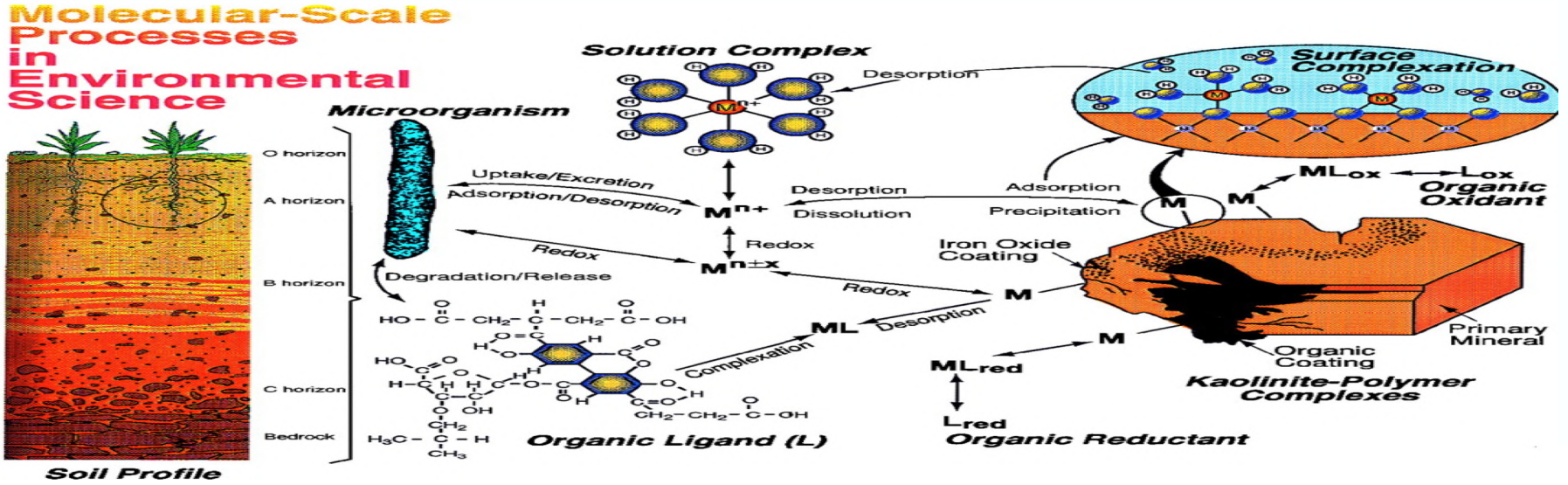
Soil Health

PHYSICO-CHEMICAL PARAMETERS



Bioavailability: ability of a chemical to enter in an organism

a crucial issue for soil health monitoring and soil remediation



How to determine metal exchangeable fraction (bioavailability)?

Houba et al. (2008). Commun. Soil Science & Plant Analysis

Ca Cl_2 0,01 M

10 ml

1g

2h, 20ºC
Filter and analize bioavailable elements in the solution

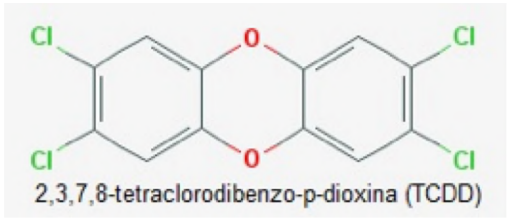
The three essential “B” in soil remediation

Bioavailability → **Bioaccumulation** → **Biomagnification**

Ability of a chemical to enter in an organism

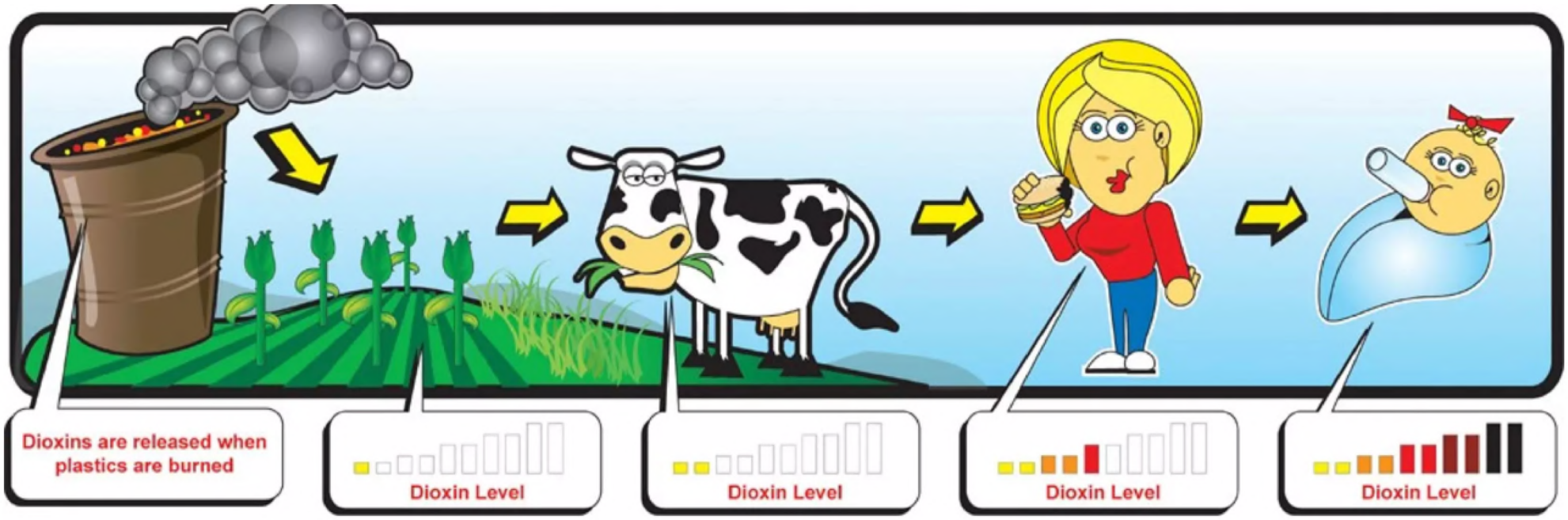
Concentration of a chemical in a living organism over time

More concentration of a chemical through the food chain



2,3,7,8-tetraclorodibenzo-p-dioxina (TCDD)

Bioavailability, Bioaccumulation and Biomagnification of dioxins

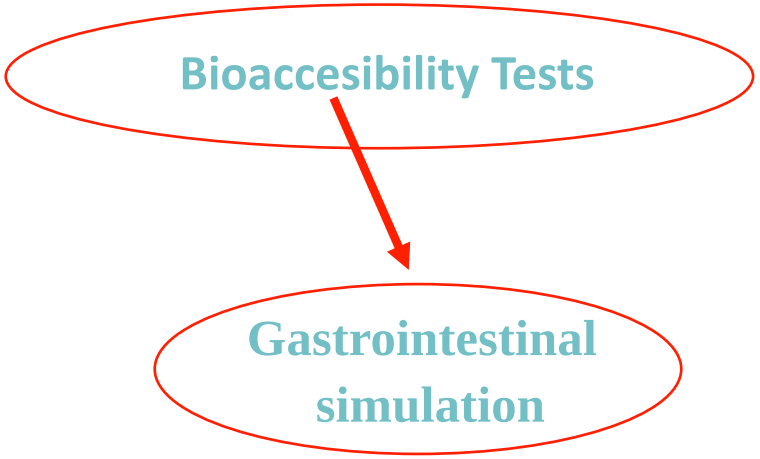


HOW?

DIAGNOSIS

Soil Health

PHYSICO-CHEMICAL PARAMETERS



Bioaccessibility:

concentration of pollutant that solubilizes in “simulated body fluids” to be predictive of human exposure

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Soil particle size fraction and potentially toxic elements bioaccessibility: A review

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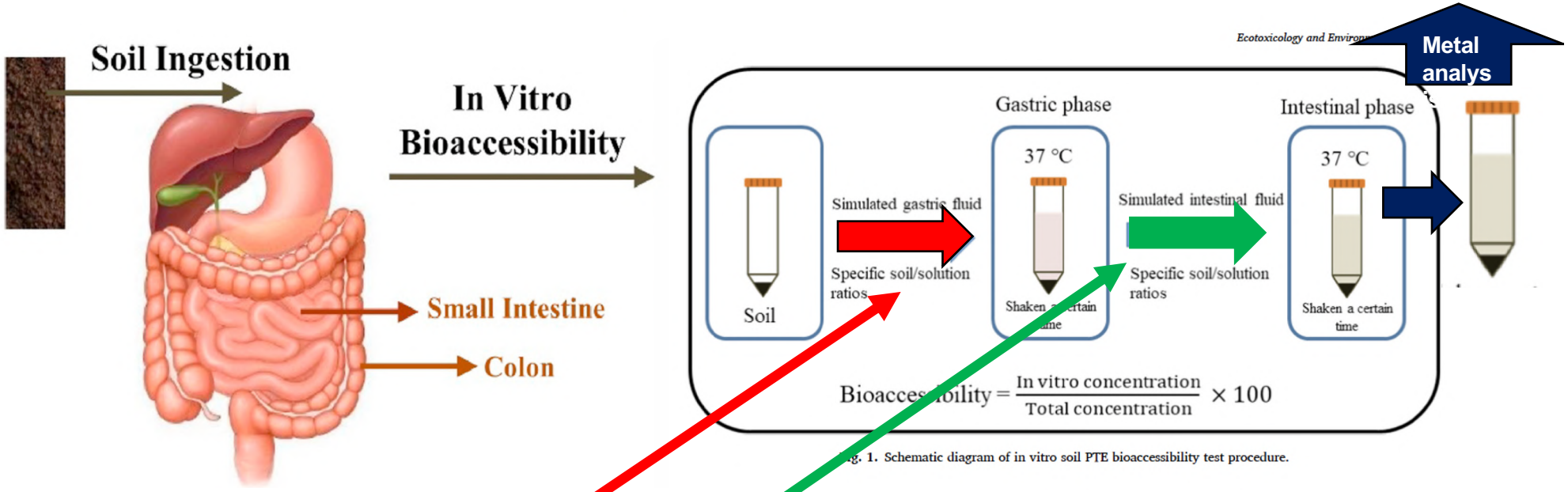


g. 1. Schematic diagram of in vitro soil PTE bioaccessibility test procedure.

pH

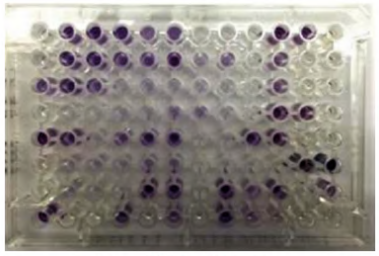
SBRC	Gastric	30.03 g glycine	1.5	1:100	1	Drexler and
	Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	1:100	4	Brattin (2007)

How? What?

DIAGNOSIS

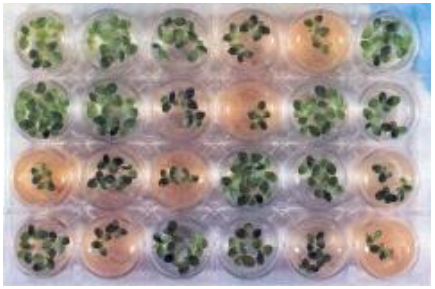
Soil Health

Biological parameters

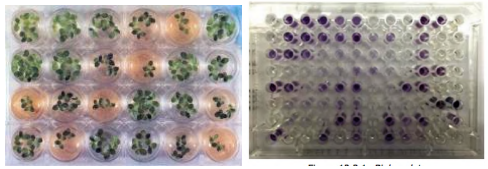


Ecotoxicity Tests

Biological impact?



How? What?



-. BIOLOGICAL INDICATORS (bioindicator)

Bioindicator: **Is a quantificable characteristic of biochemical, physiological, toxicological or ecological process or function that correlate to effects on health of organisms, populations, communities or ecosystems.**

Inform about a biological effect but also toxicological impact

e.i. root elongation → **Inhibition of root**

Soil toxicity (phytotoxicity)

Implementation on standardized Ecotoxicity Tests

Advantages

Rapid
Sensitive
Integration
Temporal integration

Root elongation bioassay

Control 0h

Control 72h

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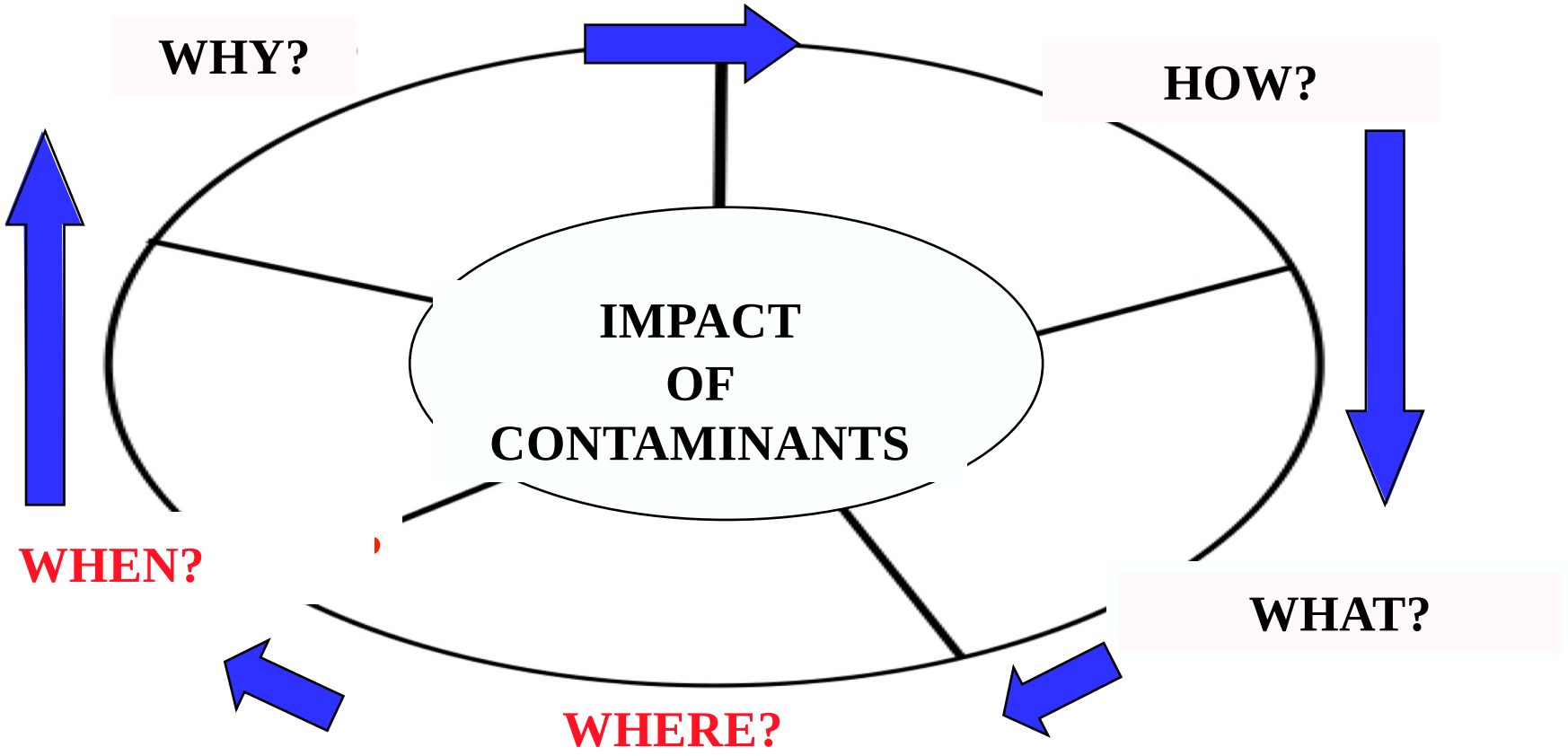
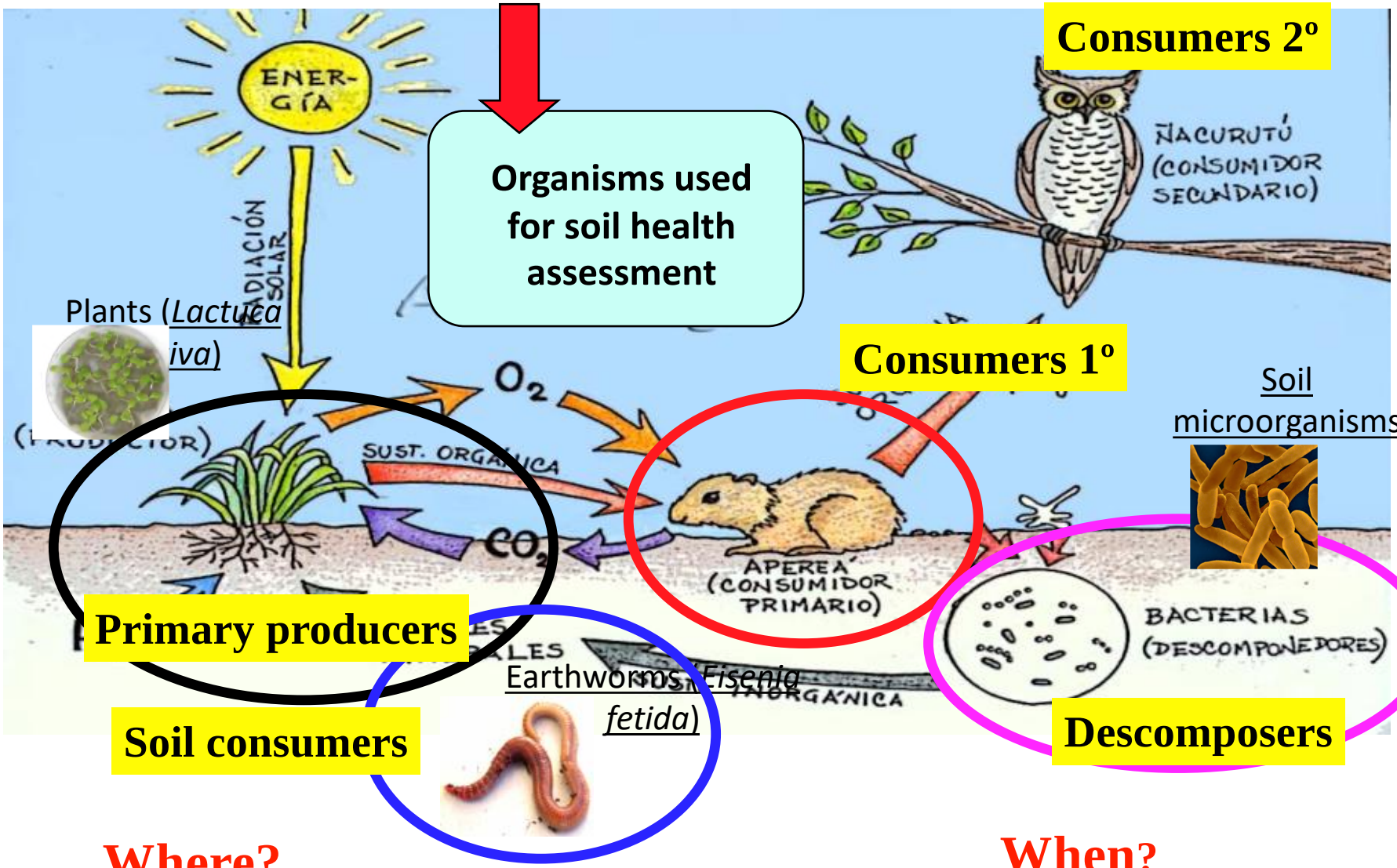
the essential issues





Where?

Receptors

-. Relevant
-. Representative
-. Final

When?

-. Life cycle
-. Seassons
- Events (regular, eventual)

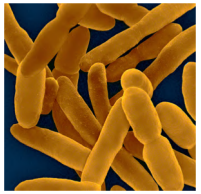
Organisms used for soil health assessment in bioassays

Primary producers

Plants (*Lactuca sativa*)

- Bio-metric parameters
- Physiological and biochemical parameters

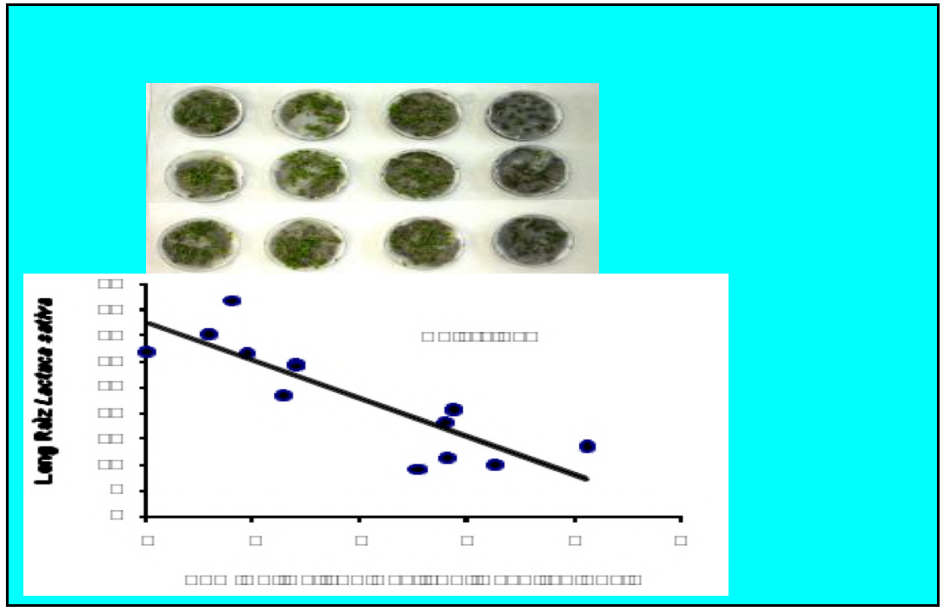
Descomposers

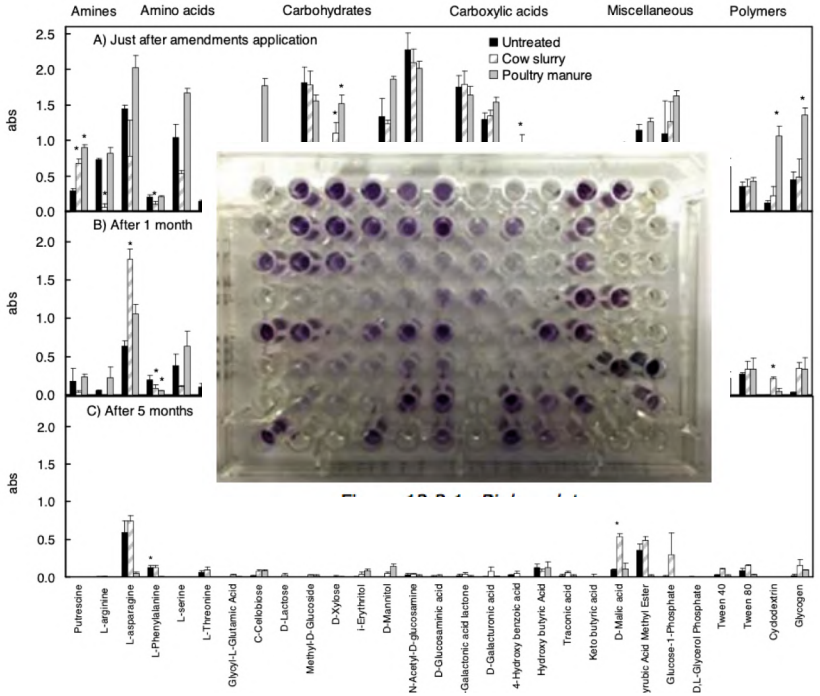


Soil microorganisms

- Basal respiration
- Induced Respiration
- Diversity

BIOASSAYS OF PHYTOTOXICITY ON SOILS





Where? When?

Organisms used for soil health assessment: bioassay

Plants (*Lactuca sativa*)

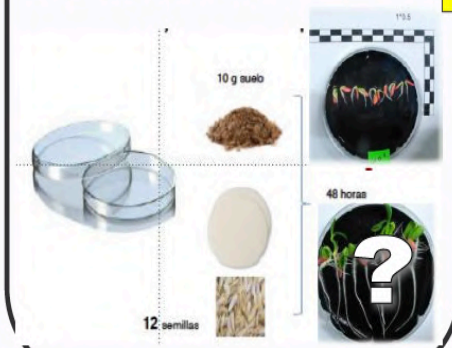
- Root elongation bioassay

Root elongation bioassay

ELONGACIÓN RADICAL

Preparación

-. Pesar 10 gramos de suelo fresco.
-. Colocar en la placa petri.
-. Poner un papel de filtro.
-. Colocar en la parte superior 8 semillas de pepino pregerminadas en fila.
-. Colocar la placa con una escala exterior y fotografiar la placa.
-. Dejar crecer durante 48 h.

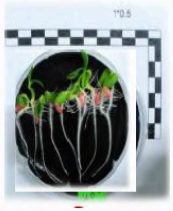


48 h

ELONGACIÓN RADICAL

Determinación

-. Colocar la placa con una escala exterior y fotografiar la placa.

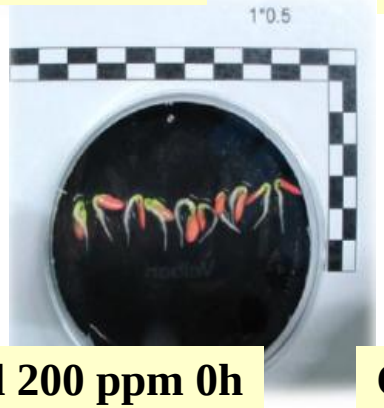


Parámetros biométricos

-. Cosechar las plantas.
-. Poner en un sobre, pesar y anotar.

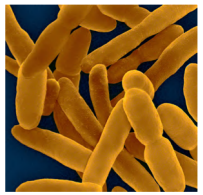
Control 0h

Control 72h



Cd 200 ppm 0h

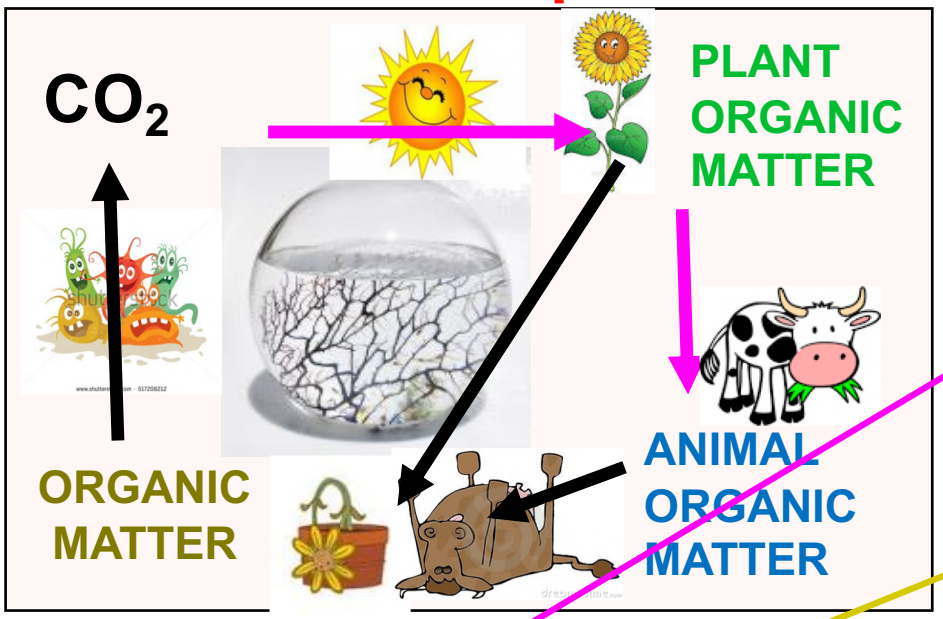
Cd 200 ppm 72h

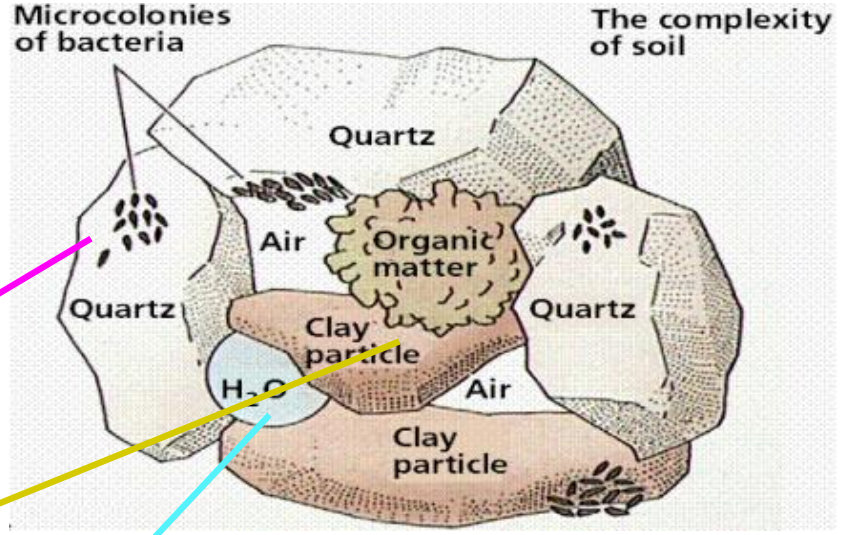


Soil microorganisms

- Basal respiration = Microbial activity (organic matter present in soils)
- Induced Respiration= Microbial Biomass (glucose added to soils)

Essential processes in soils: Cicle of C





ORGANIC MATTER

Microbial respiration

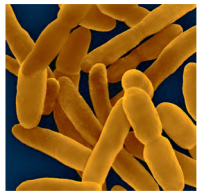
CO_2 + H_2O → H_2CO_3

C-CO_2 equivalents
0,5x12 (g/eq)

$2Na\,OH + H_2CO_3 \rightarrow Na_2CO_3 + 2H_2O$

Titration of non neutralized NaOH

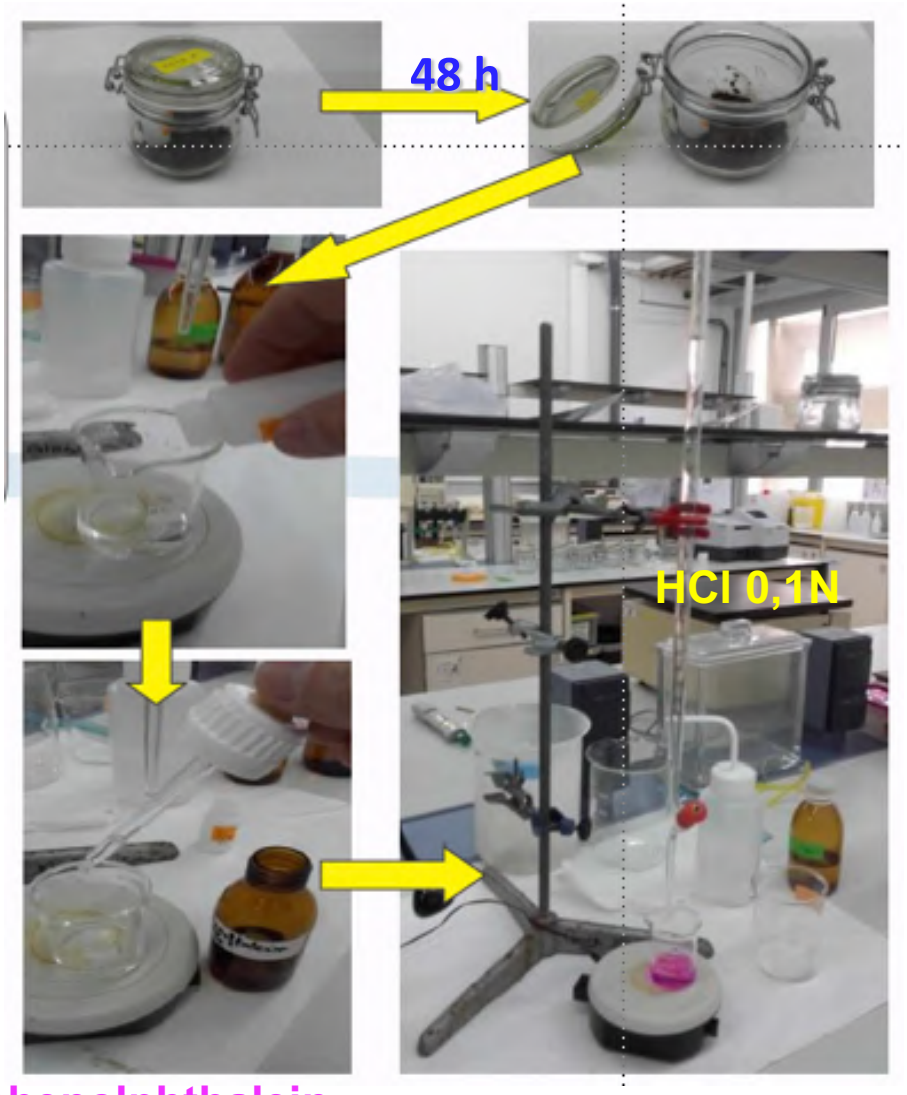
$Na\,OH + HCl \rightarrow NaCl + H_2O$



Soil microorganisms

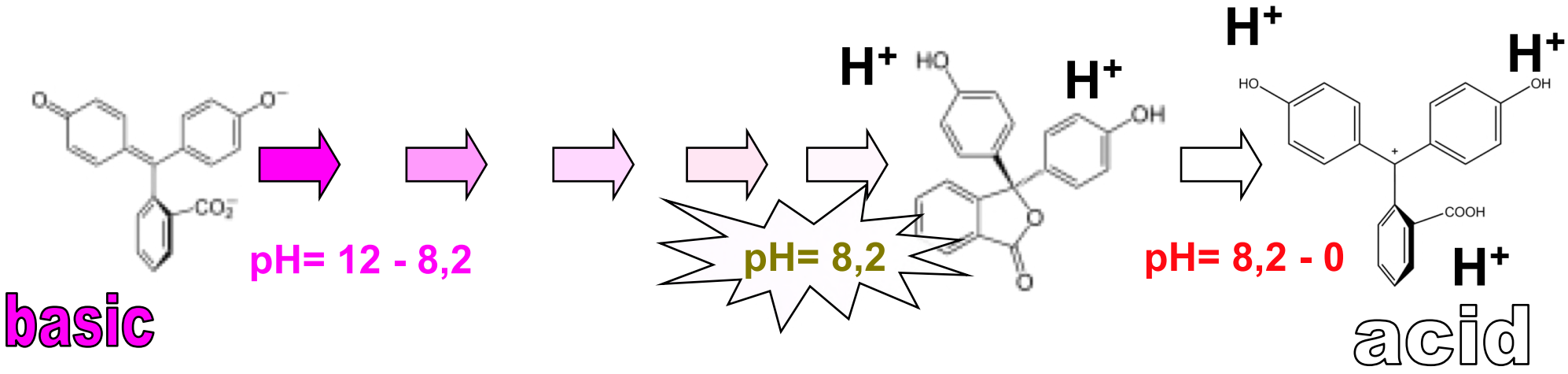
- Basal respiration = Microbial activity (organic matter present in soils)

Basal Respiration (microbial activity)



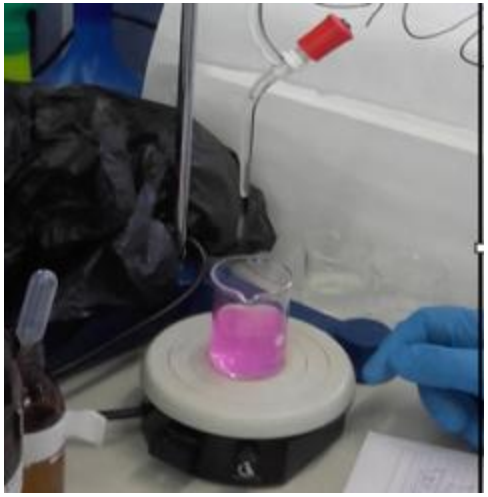
phenolphthalein

¿WHY DOES PHENOLPHTHALEIN CHANGE COLOR?



H^+ Cl^-

$$NaOH + HCl \longrightarrow NaCl + H_2O$$



A case study: Monitoring soil health with biomarkers during a phytoremediation process

Objetives

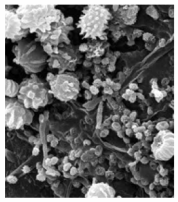
- Use a **ecofriendly technology** for soil remediation

PHYTOREMEDIATION

- Use a innovative technique for **soil health** and **toxicity assessm**

BIOLOGICAL INDICATORS

Soil microorganisms

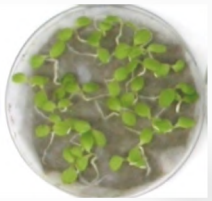


- Soil enzyme activities
- CLPPs

Earthworms (*Eisenia fetida*)

- Survival
- Weight
- NRU

Plants (*Lactuca sativa*)



- Bio-metric parameters
- Antioxidant response

A case study: Monitoring soil health with biomarkers during a phytoremediation process

Objetives

- Use a **ecofriendly technology** for soil remediation

PHYTOREMEDIATION

Phytoremediation is a set of technologies that use green plants and associated microbes to remove contaminants from soils, sediments and water.

Buscando herramientas verdes para la fitorremediación

Tipos de especies en función de la acumulación

Especies hiperacumuladoras
(Noccaea caerulescens)

Fig. 3. Roots of *Thlaspi caerulescens* on the soil surface of rhizoboxes containing (a) homogeneous Zn-enriched

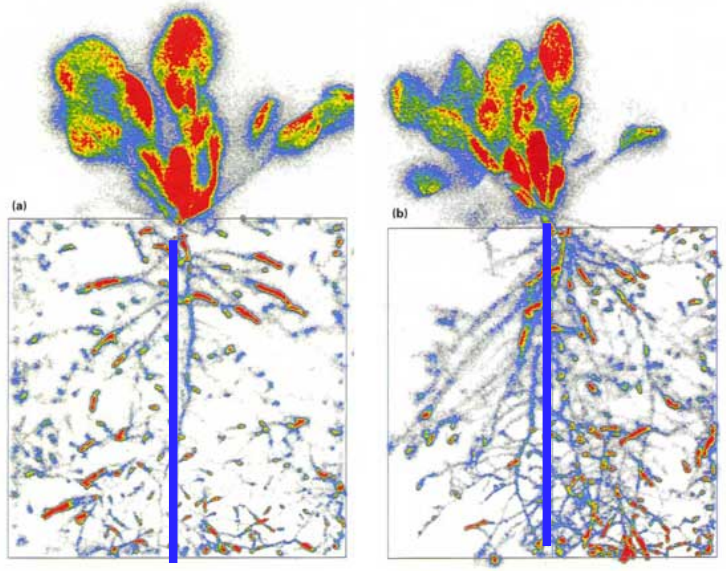
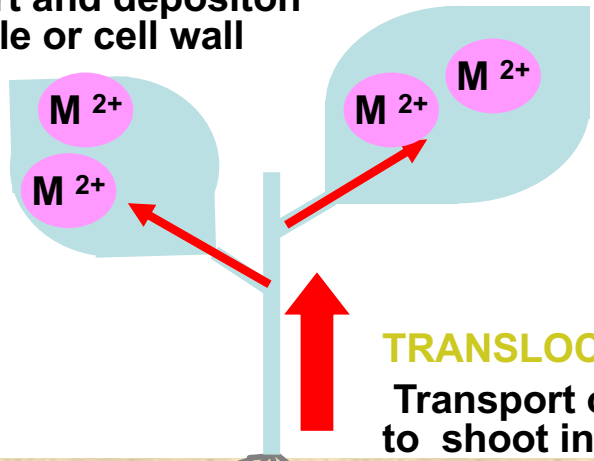


Fig. 6. The effect of localized Zn-enrichment on the allocation of ^{11}C within the roots of *Thlaspi caerulescens* in treatment 1000/1000 (a) and treatment C/1000 (b). Radiographic images were taken 48 h after labelling. The red threshold (points with highest ^{11}C activity) was set at 50 counts.

Key Processes in Metal Hyperaccumulation

SHOOT ACCUMULATION

Transport and depositon in vacuole or cell wall

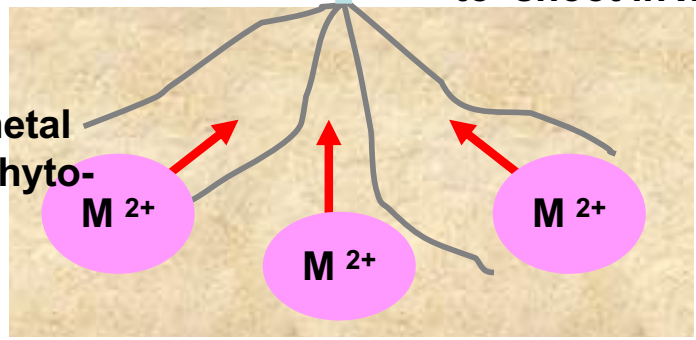


TRANSLOCATION

Transport of metal to shoot in xylem

CHELATION

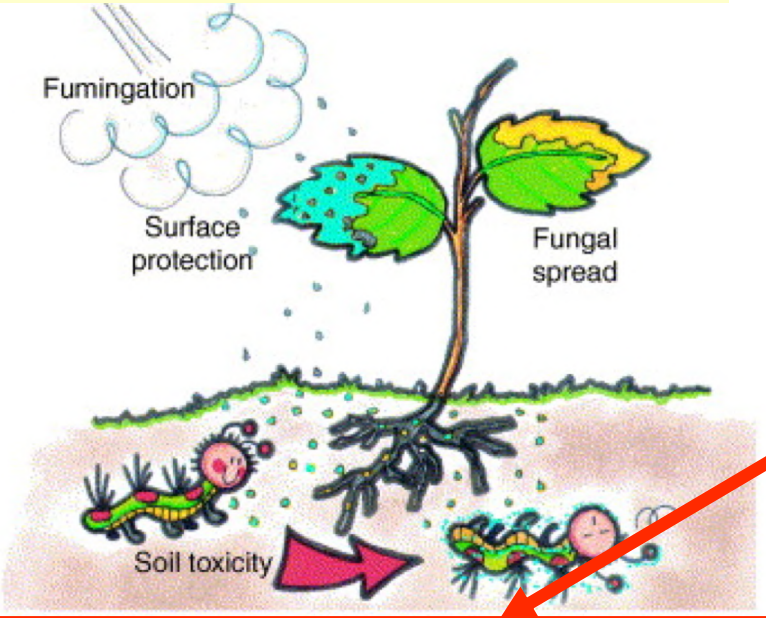
Ligands to chelate metal in root to minimize phyto-toxicity



METAL ACQUISITION

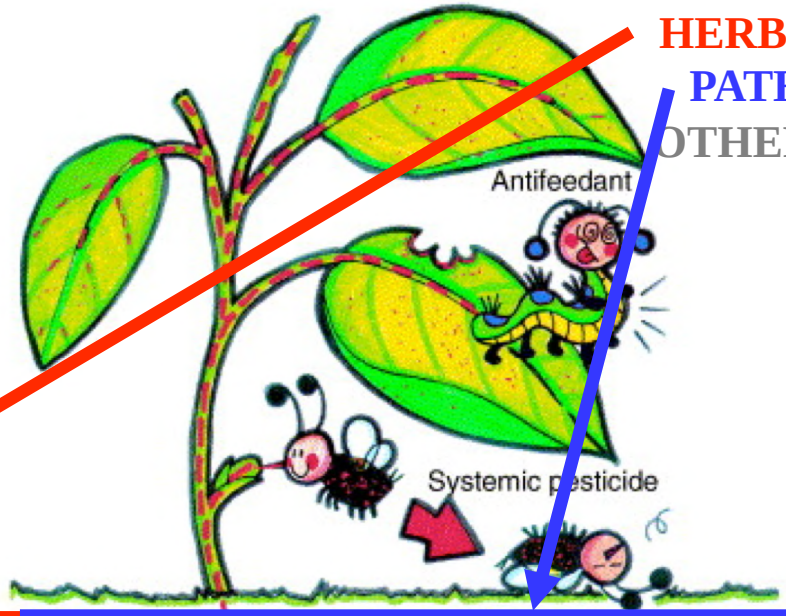
Effective dissolution and uptake of specific metals

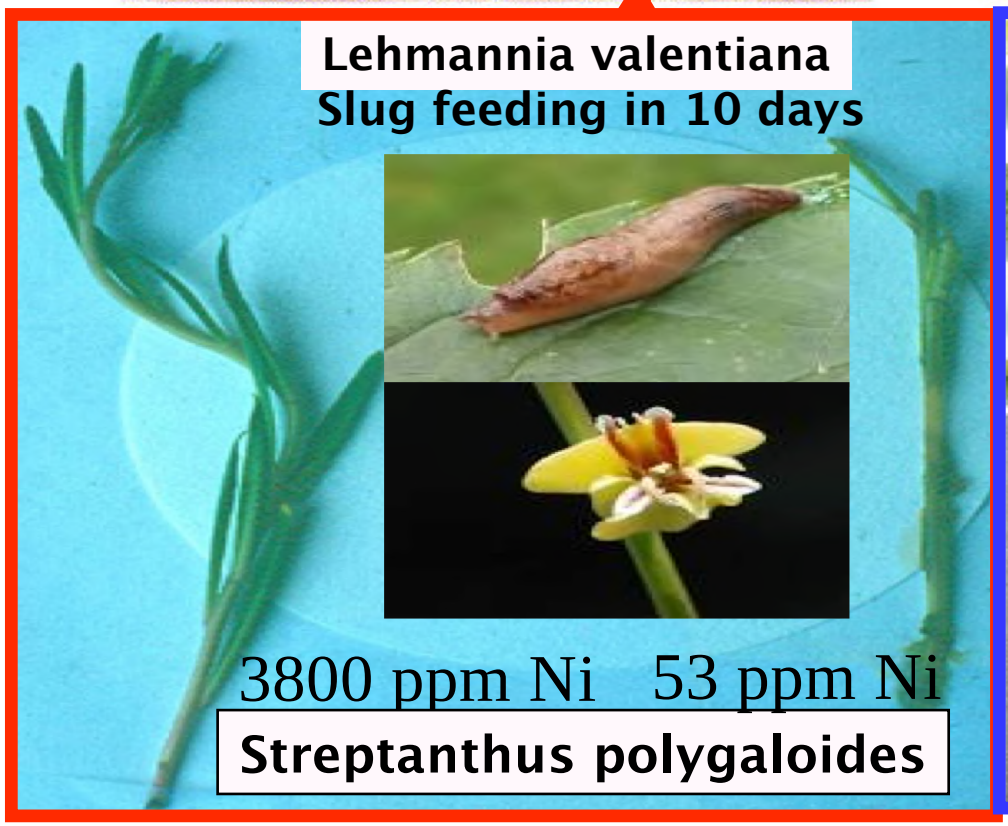
HUMAN ARTIFICAL DEFENSE

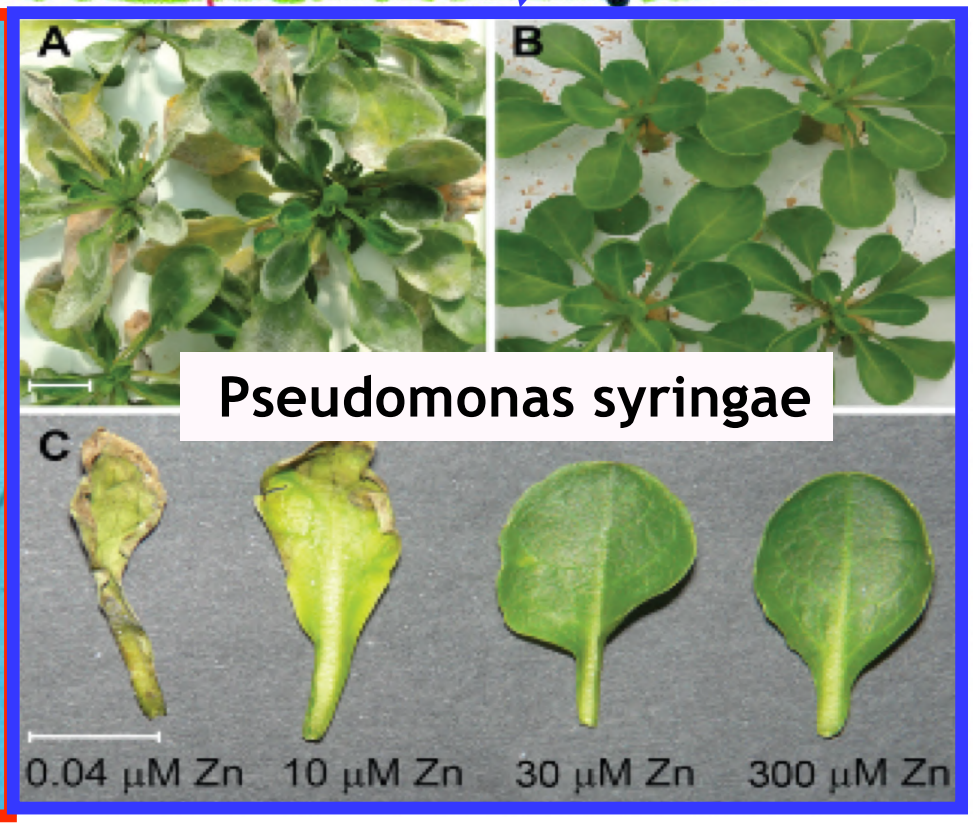


NATURAL METAL DEFENSE

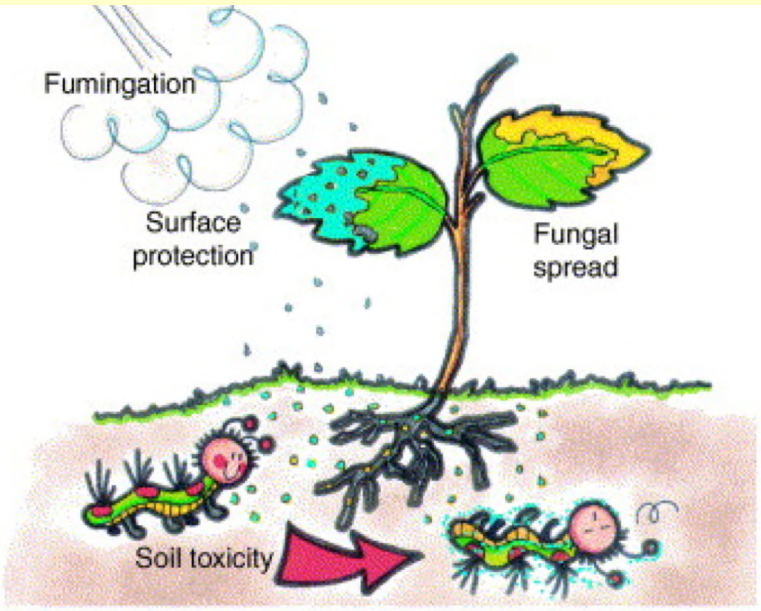
HERBIVOROUS
PATHOGENS
OTHER PLANTS



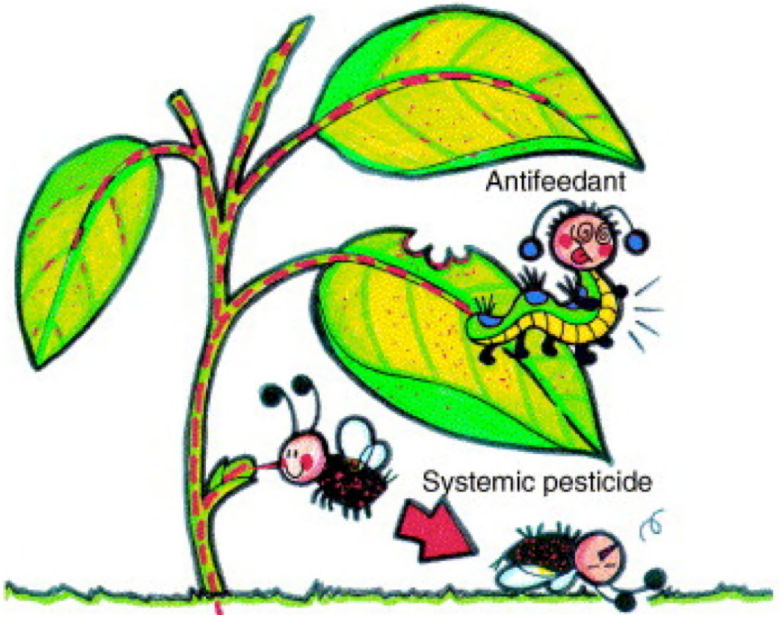


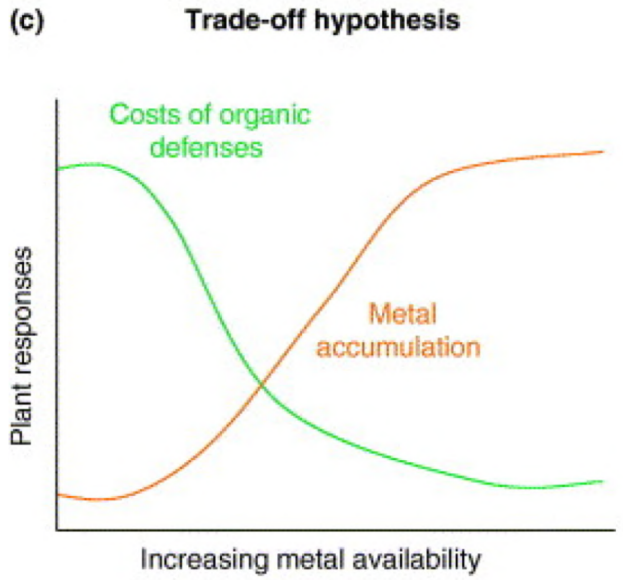


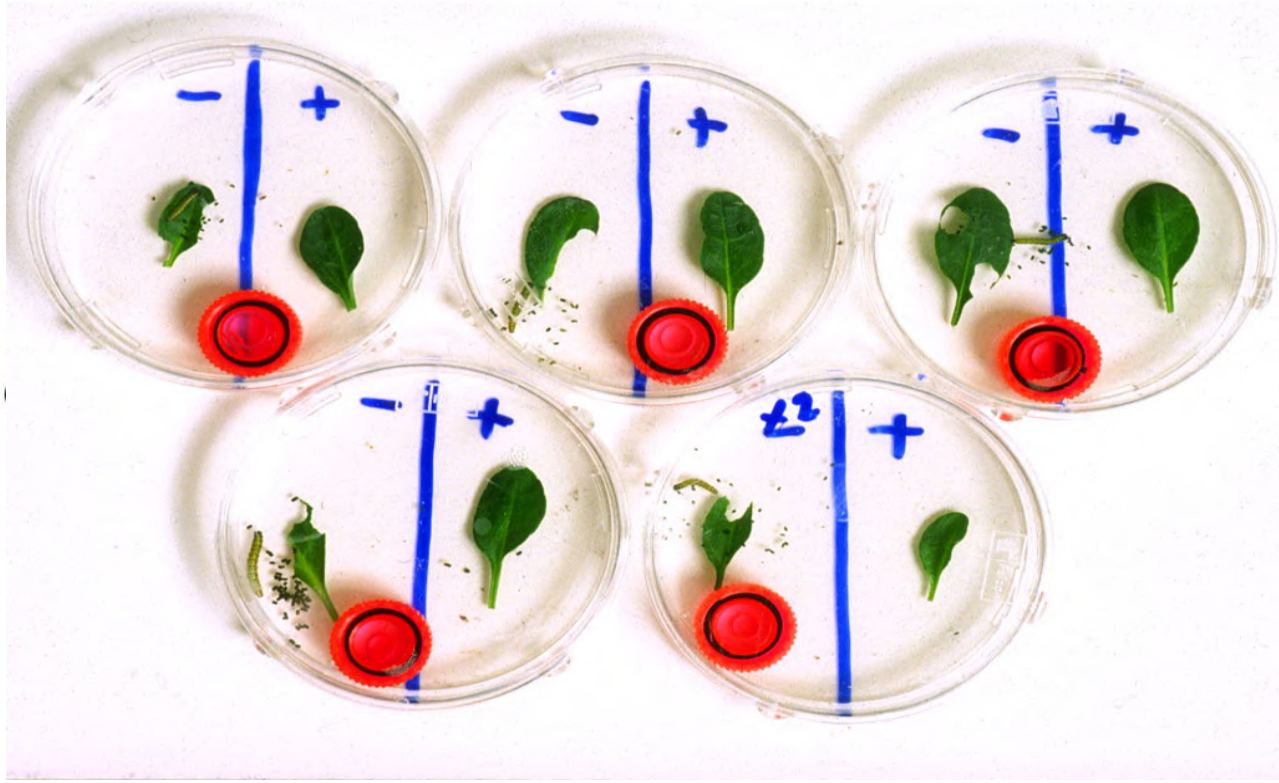
HUMAN ARTIFICAL DEFENSE



NATURAL METAL DEFENSE







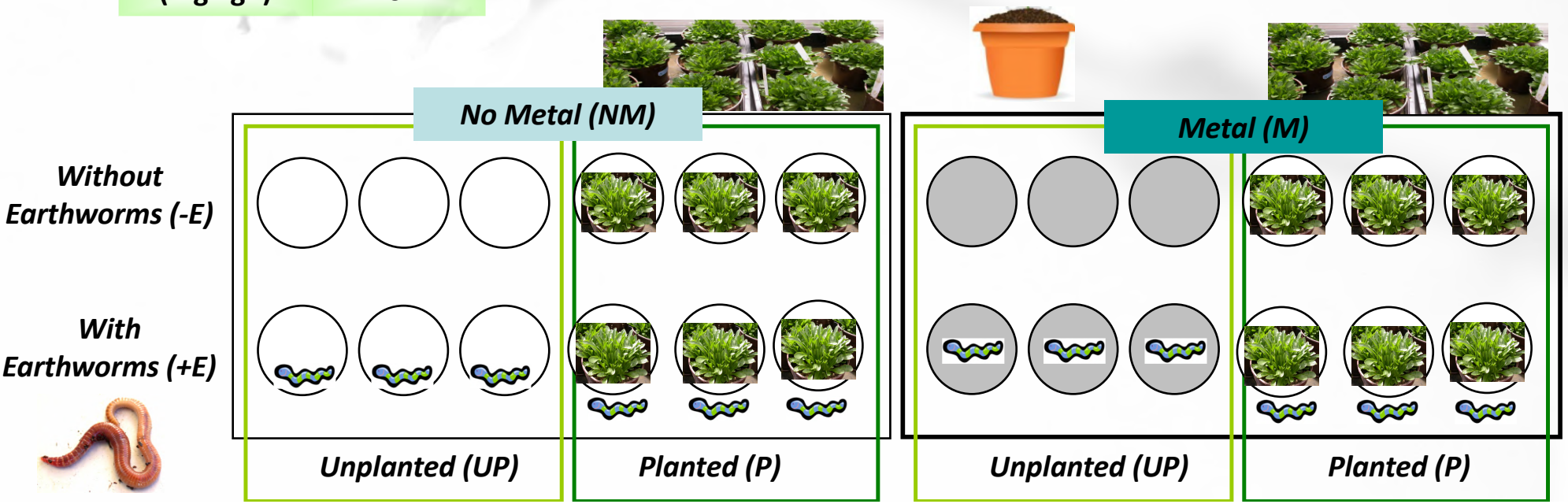
A case study: Monitoring soil health with biomarkers during a phytoremediation process

Experimental design of the Microcosm study

Physicochemical characterization of the soil used for the study

Texture	**Clay loam**
pH	**5.2**
OM (%)	**4.12**
Total N (%)	**0.23**
C/N	**10.4**
EC (dS m^{-1})	**0.08**
P (mg kg^{-1})	**26.4**

- Greenhouse conditions
- **5 months**
- 1.5 kg DW pot^{-1}
- Metal treatment: **1000 mg Zn, 500 mg Pb and 250 mg Cd kg^{-1} DW soil** (metal nitrate) / Control pots supplemented with 100 mg Zn kg^{-1} DW
- Plant treatment: **3 plants pot^{-1}** (***T. caerulescens*** Lanestosa ecotype)
- Earthworm treatment: 15 earthworms pot^{-1} (***Eisenia fetida***)



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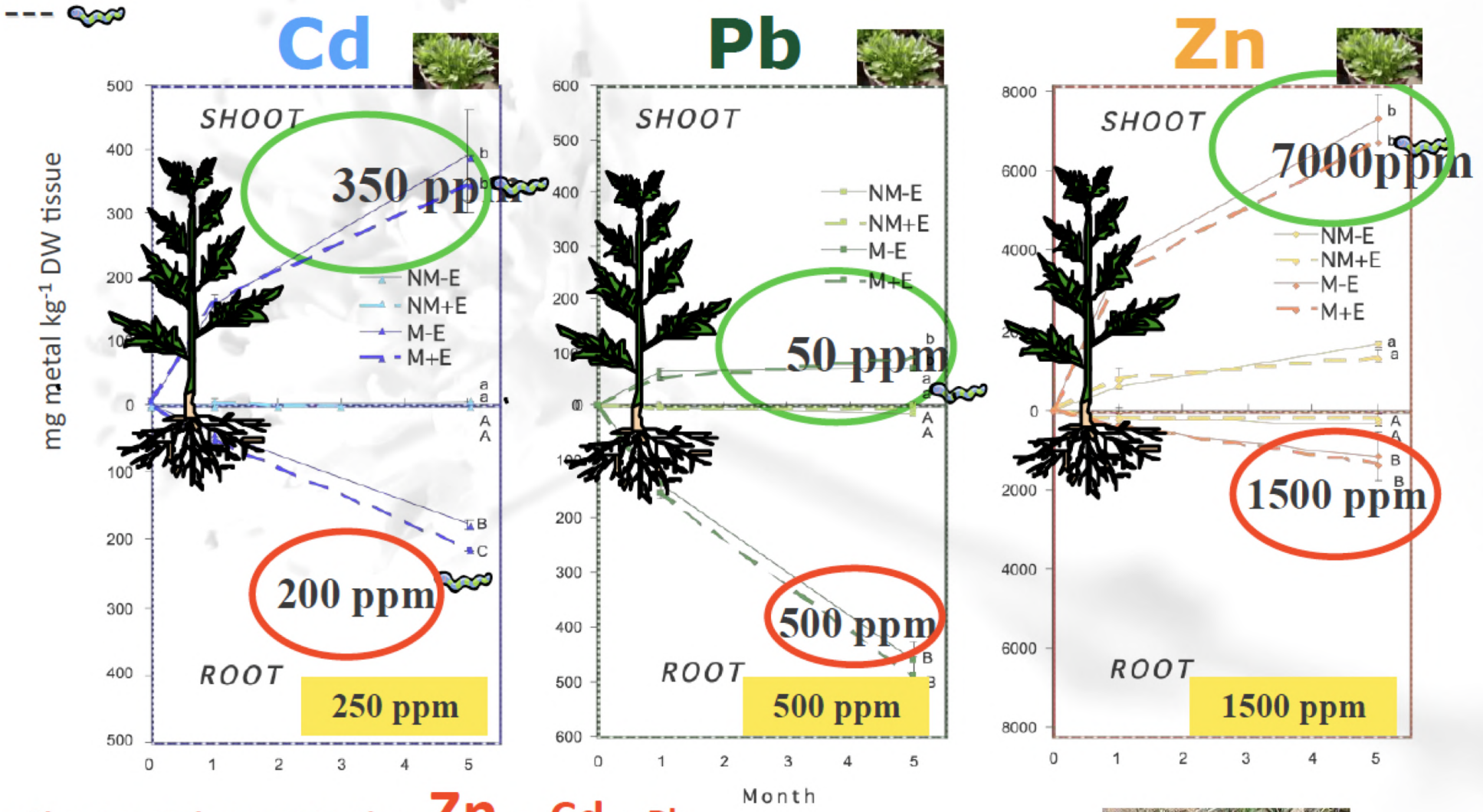
Metal concentration in plant tissues



- Shoot metal concentration: **Zn** >> **Cd** > **Pb**
- Shoot to Root Translocation Factor for Cd and Zn > 1
- Earthworms presence did not affect metal phytoextraction

A case study: Monitoring soil health with biomarkers during a phytoremediation process

Soil health recovery assessment at the end of the experiment

•CHEMICAL INDICATORS

• Metal concentration in polluted soils after 5 months assay

	TOTAL			BIOAVAILABLE		
	Cd	Pb	Zn	Cd	Pb	Zn
NM-UP	0.8^{a}	47^{a}	77^{a}	0.27^{a}	2.8^{a}	63^{a}
NM-P	0.8^{a}	25^{a}	119^{a}	0.39^{a}	2.9^{ab}	44^{a}
M-UP	101.0^{c}	591^{c}	1060^{c}	77.6^{b}	47.1^{c}	572^{c}
M-P	68.3^{b}	344^{b}	672^{b}	79.9^{b}	34.2^{b}	495^{b}

33% 42% 37% 27% 13%

•Phytoextraction of metals by plants reduced extractable and total metal content

A case study: Monitoring soil health with biomarkers during a phytoremediation process

Soil health recovery assessment at the end of the experiment

- **BIOLOGICAL INDICATORS**

Organisms used for soil health assessment

Soil microorganisms

- Soil enzyme activities
- CLPPs

Earthworms (*Eisenia fetida*)

- Survival
- Biomass
- NRU

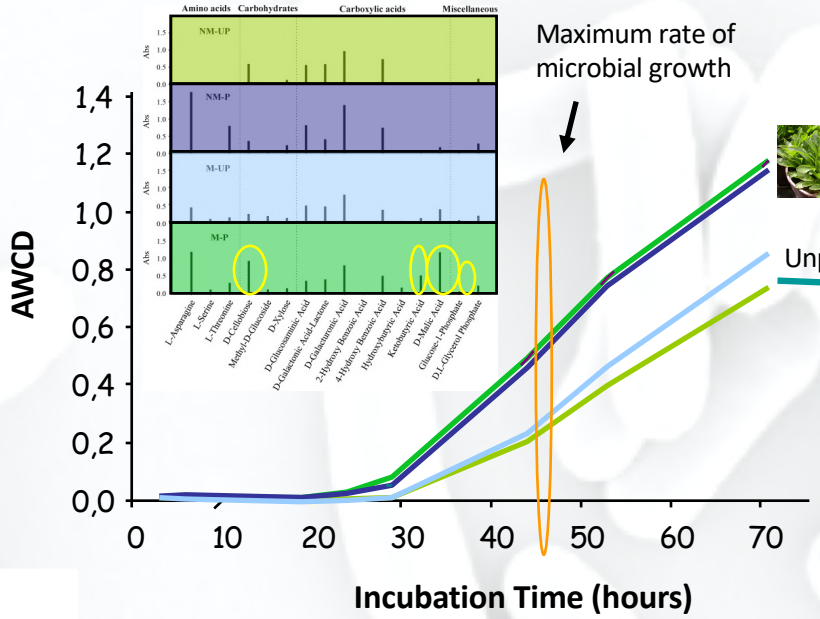
Plants (*Lactuca sativa*)

- Bio-metric parameters
- Antioxidant response

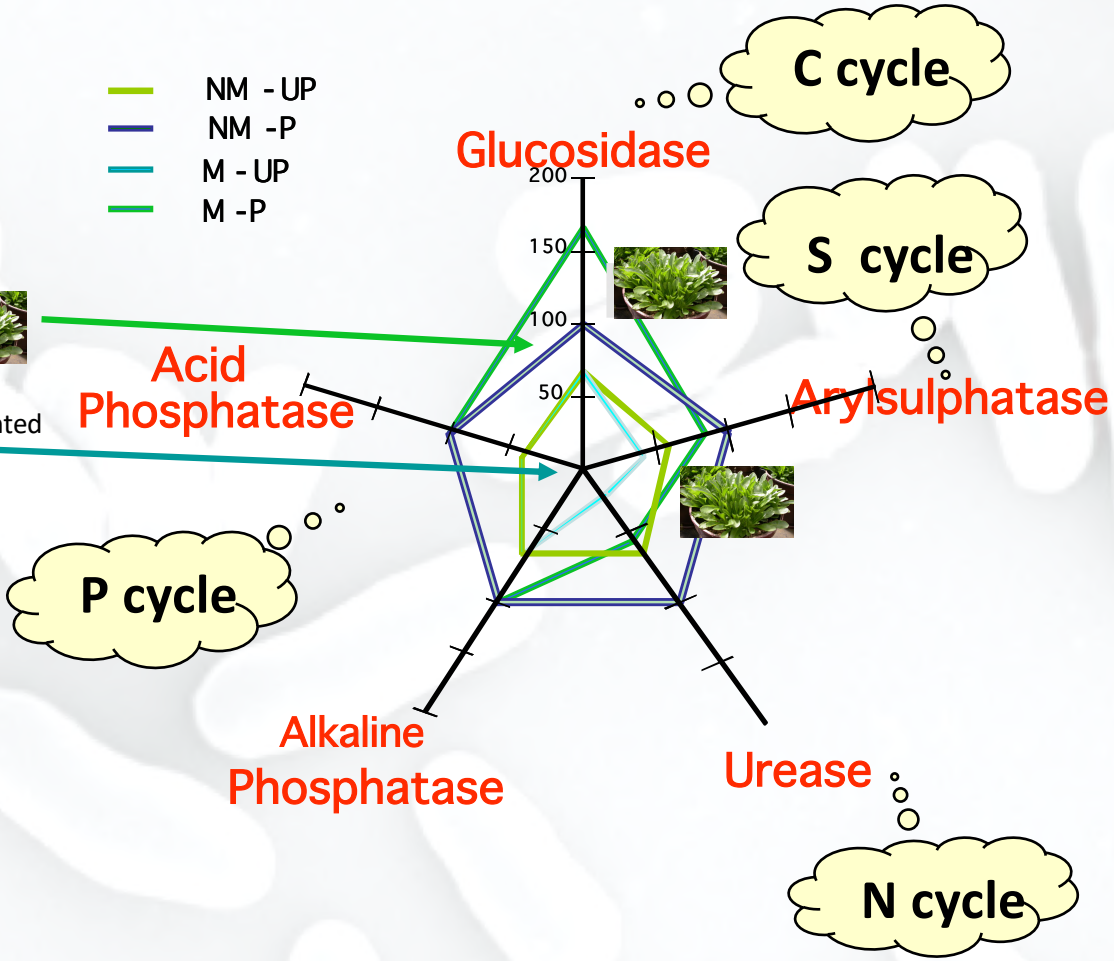
A case study: Monitoring soil health with biomarkers during a phytoremediation process

Soil microbial biomarkers

Microbial Functional diversity



Key Soil Enzyme Activities



Decrease of **extractable and total metal content, and presence of *T. caerulescens* plants had the major effect on soil microbial parameters**

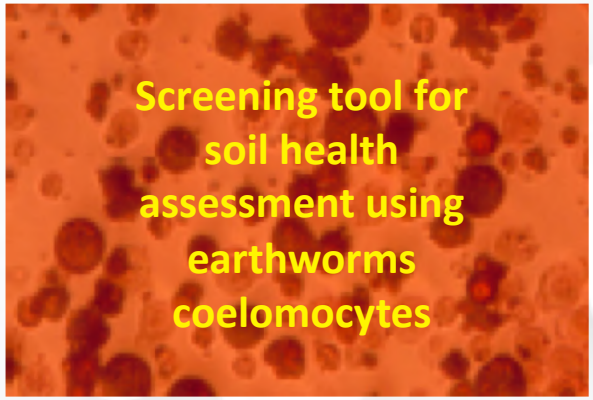
A case study: Monitoring soil health with biomarkers during a phytoremediation process

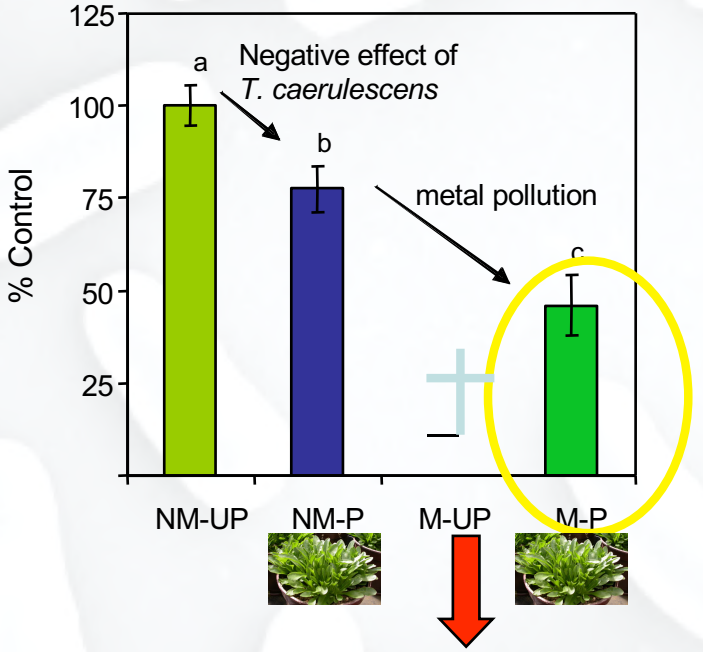
Soil health recovery assessment at the end of the experiment

Animal (earthworm) biomarkers

	Survival (%)	Weight (mg)
NM-UP	100	238
NM-P	100	252
M-UP	16.6	220
M-P	83.3	238

Cellular integrity

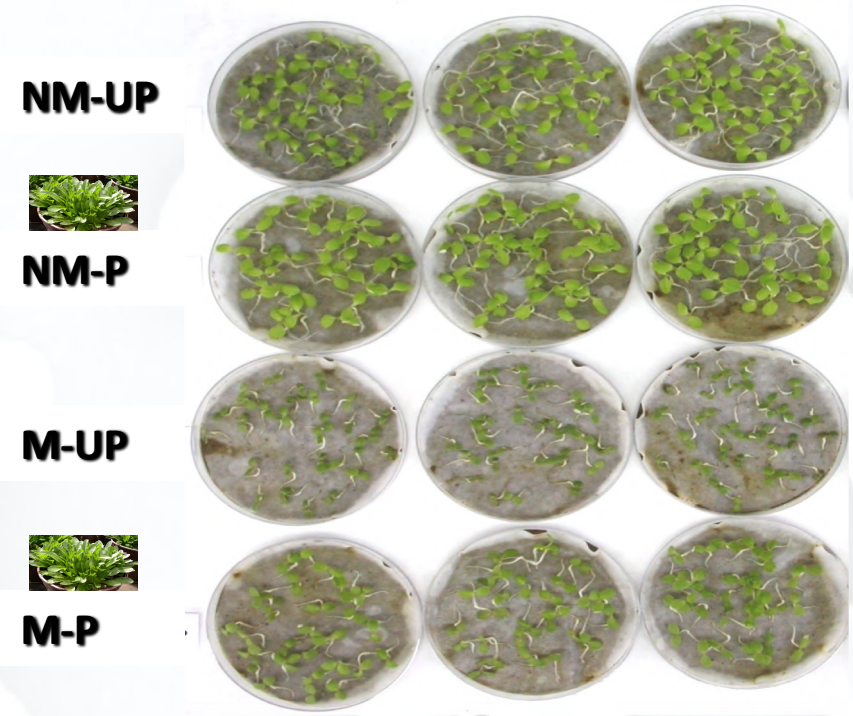




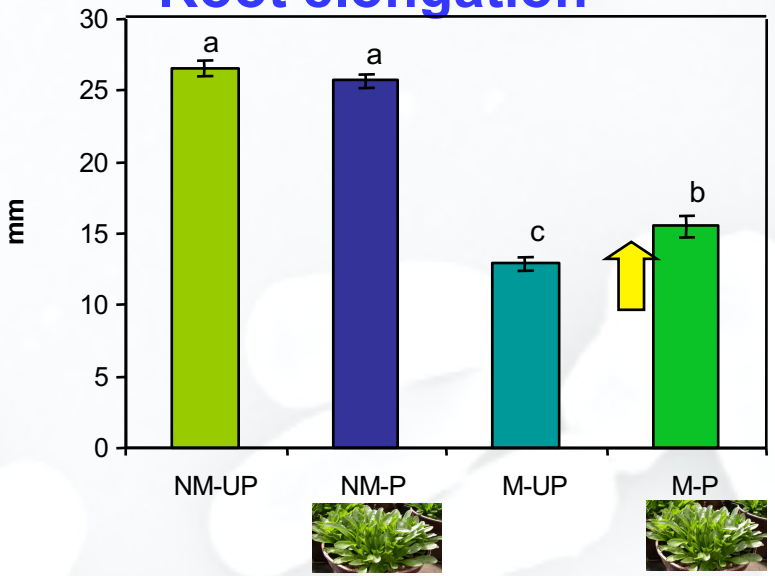
A case study: Monitoring soil health with biomarkers during a phytoremediation process

Plant (L. sativa) biomarkers

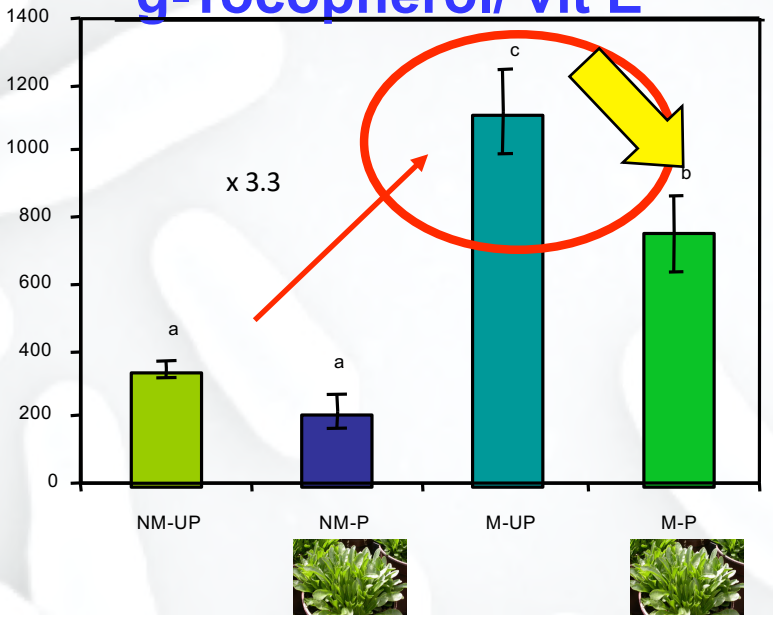
3 days exposure

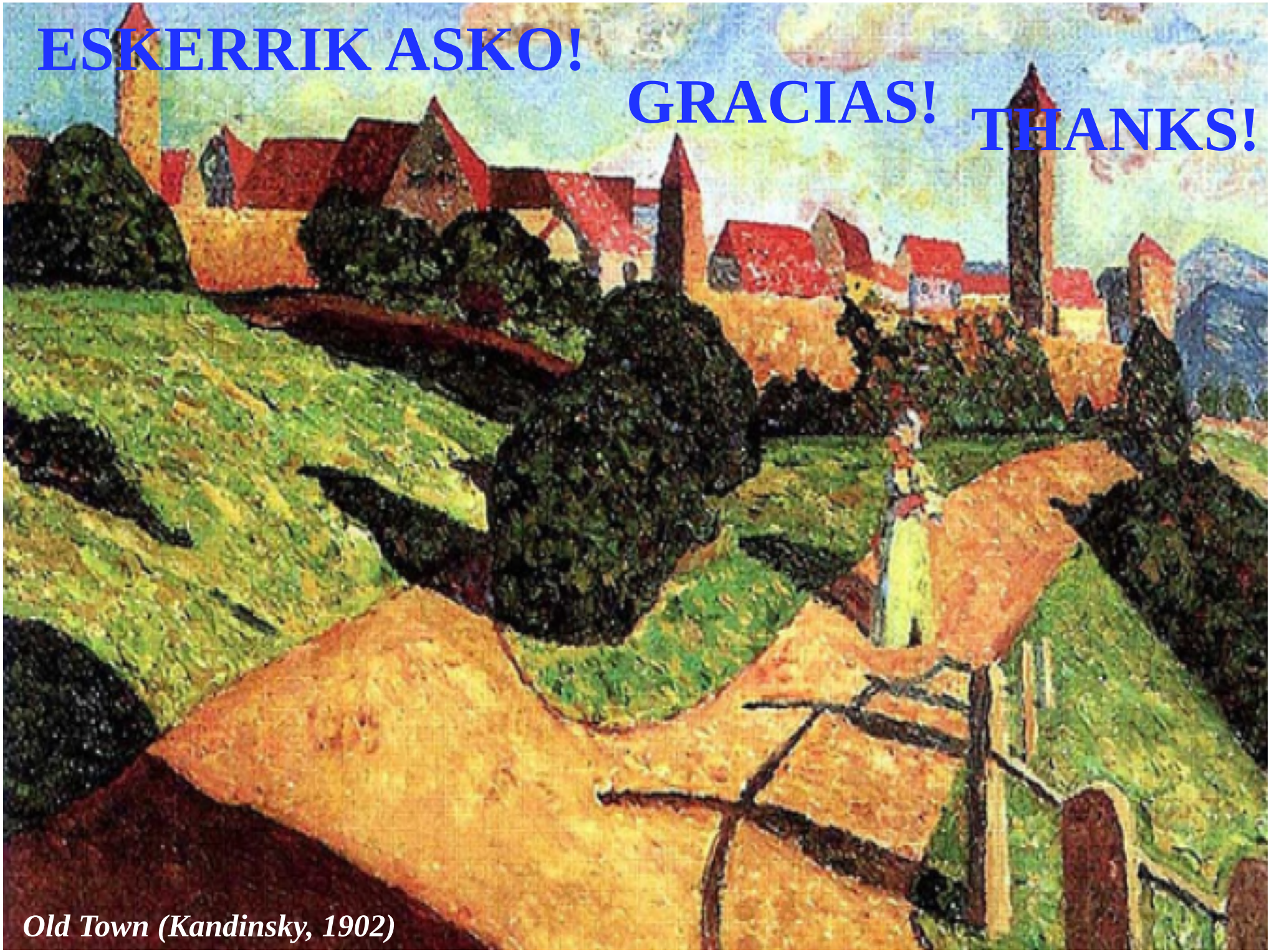


Root elongation



g-Tocopherol/ vit E





Old Town (Kandinsky, 1902)