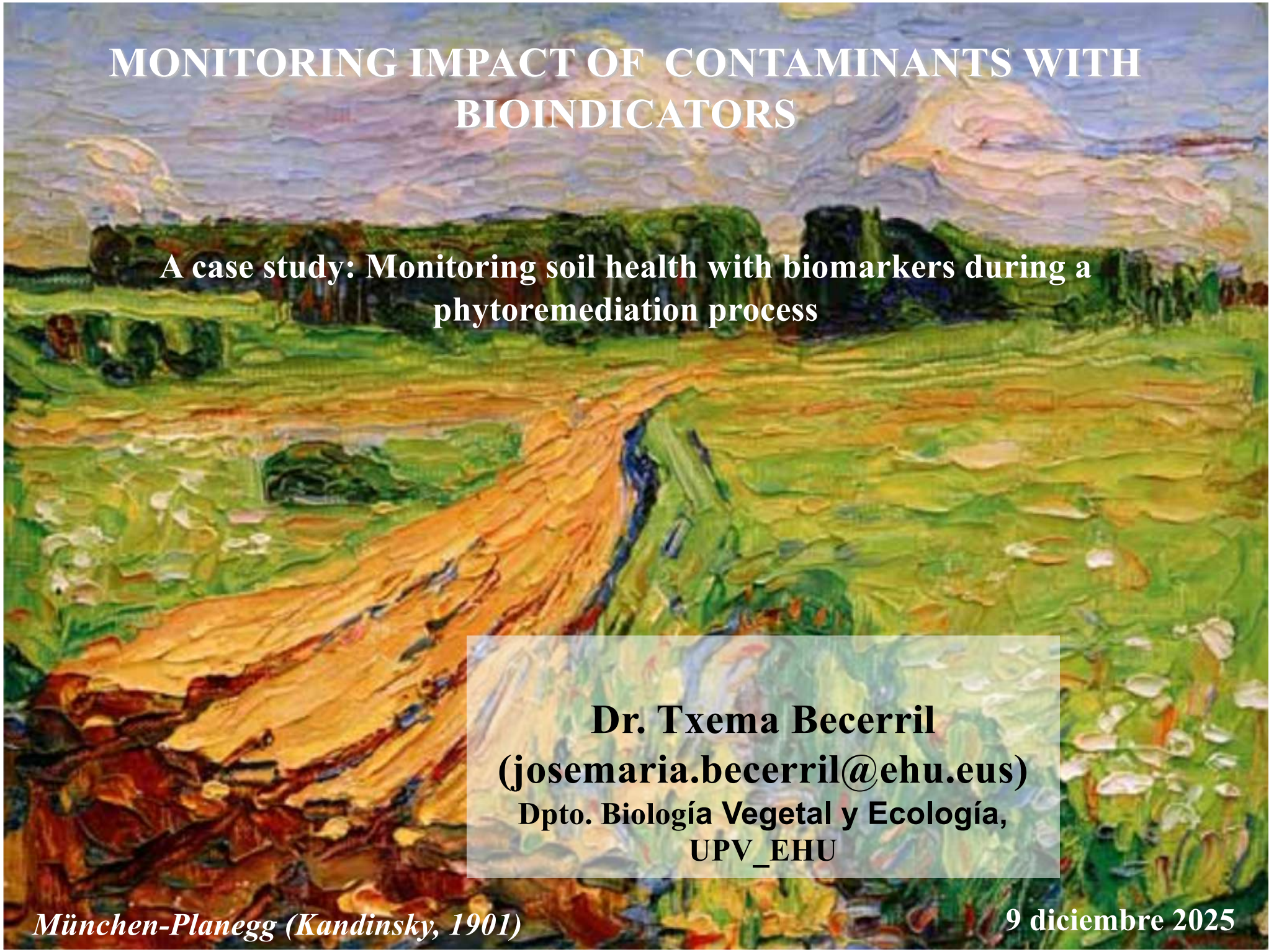

MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

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

Dr. Txema Becerril
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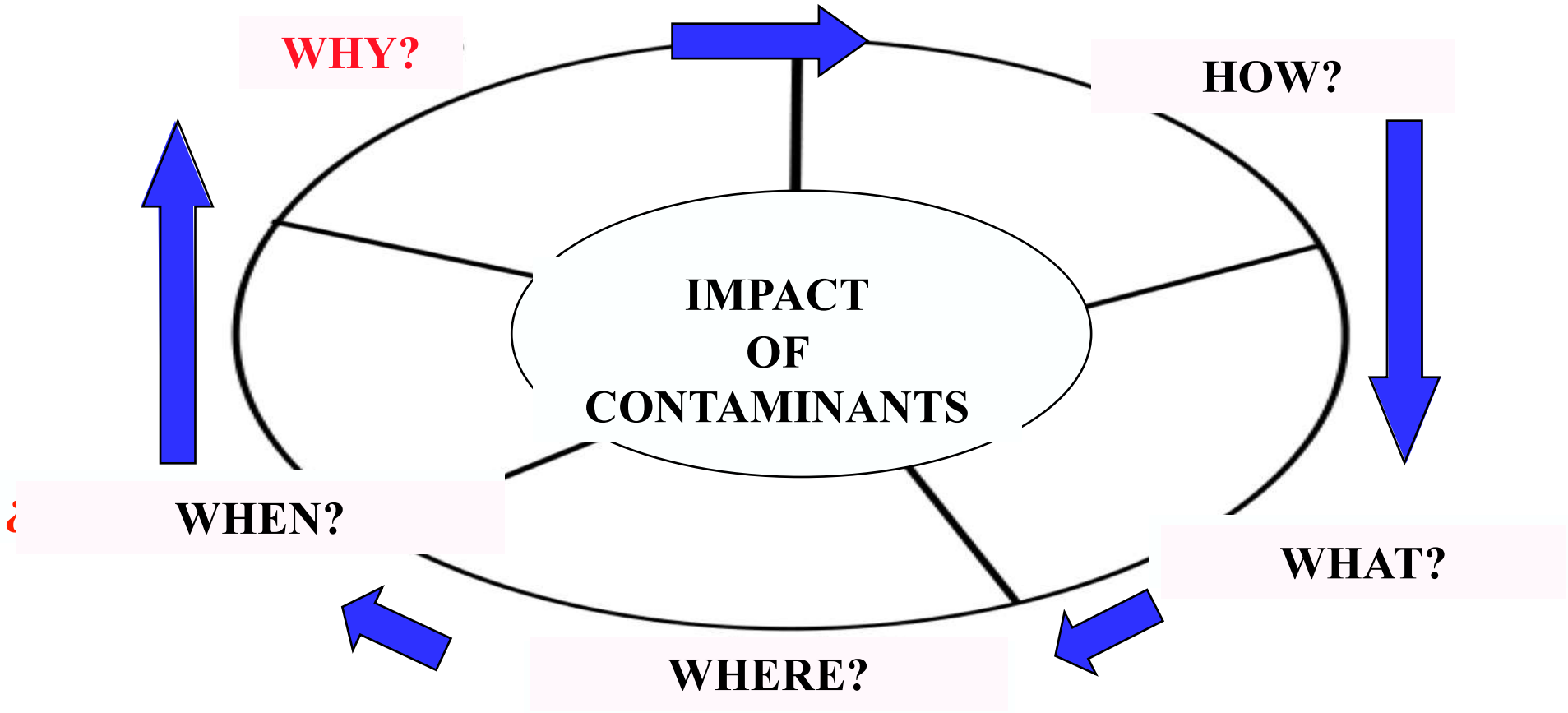
UPV_EHU

München-Planegg (Kandinsky, 1901)

9 diciembre 2025

MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

the essential issues

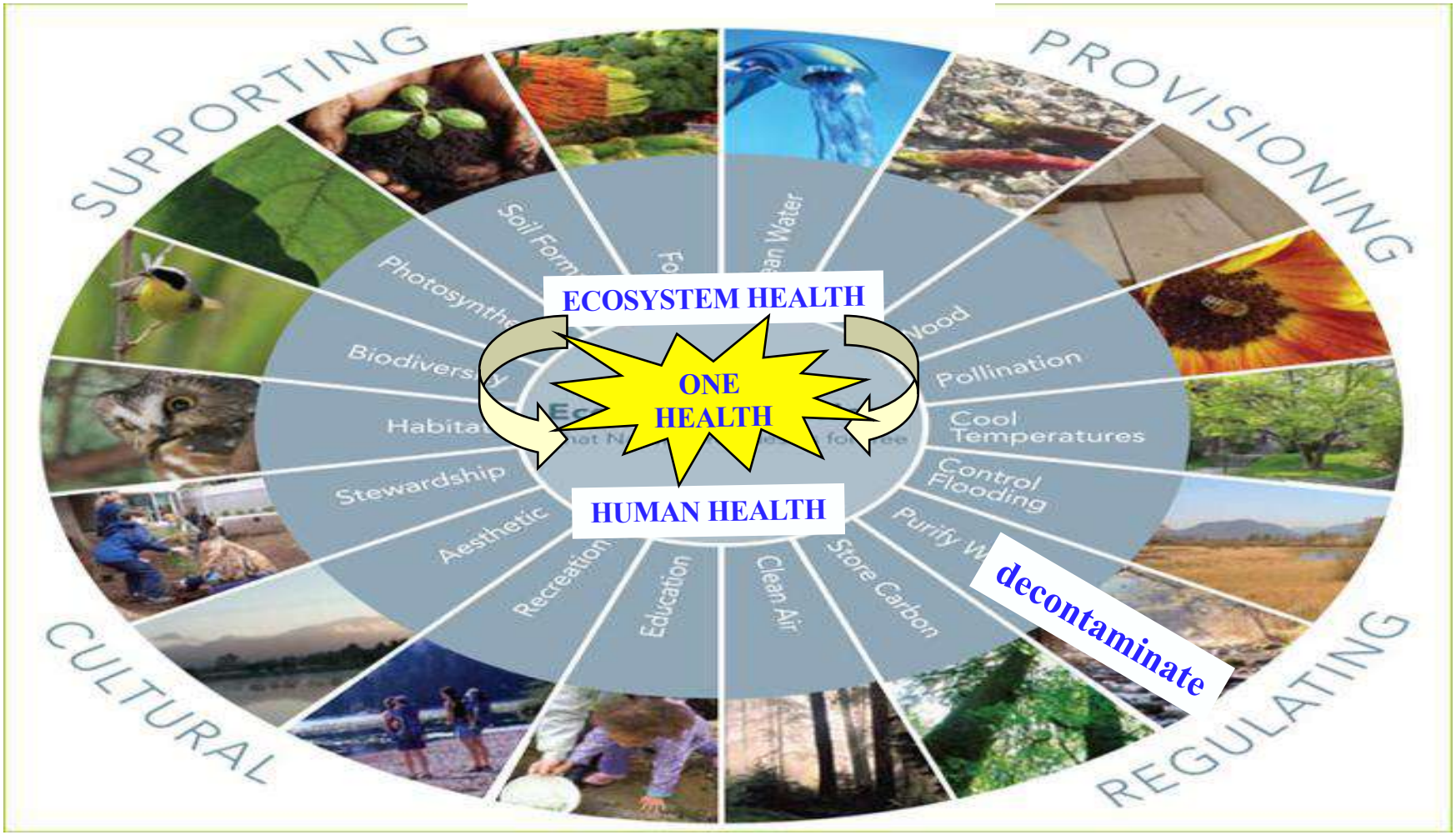


WHY?

Soil, organism, ecosystem health assessment

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

ECOSYSTEM SERVICES

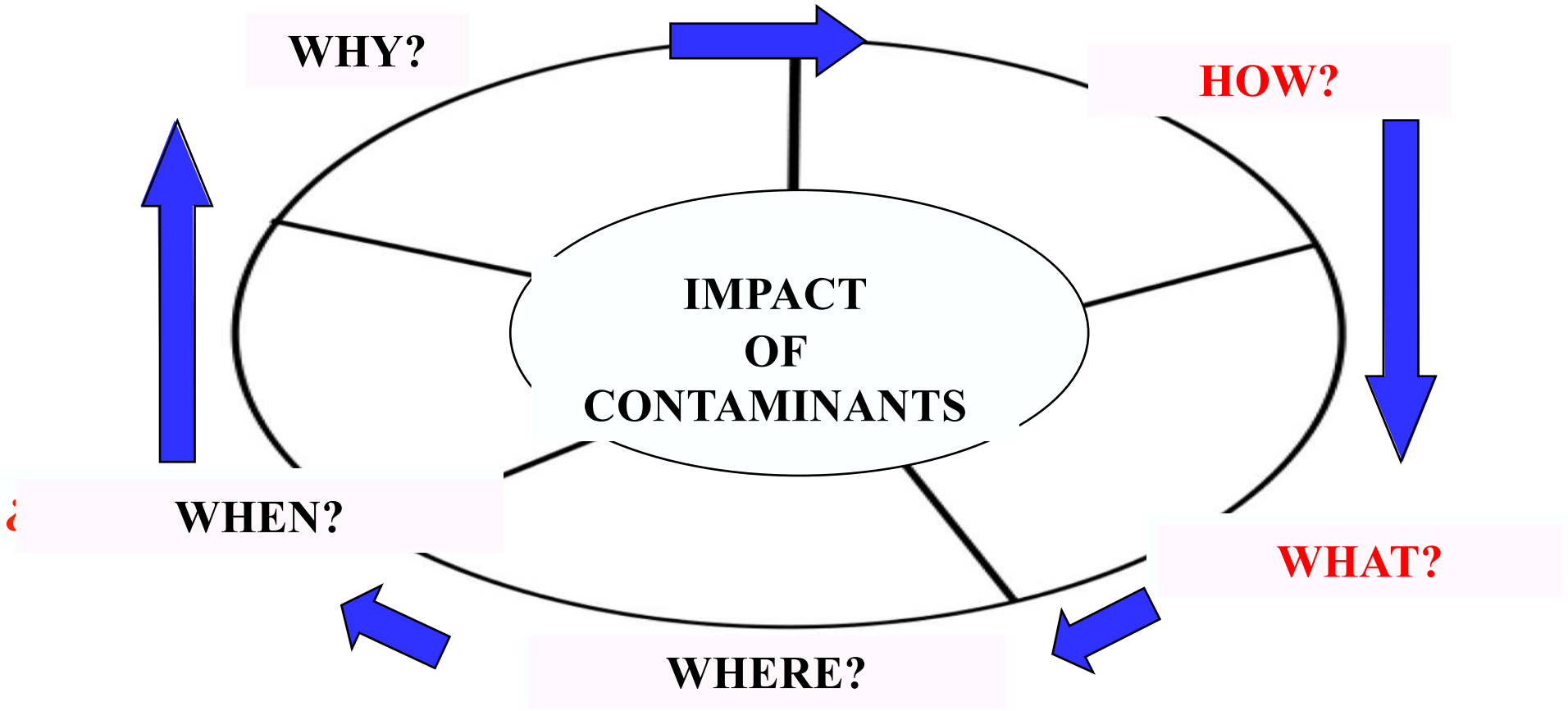


¿WHY?

Soil, organism, ecosystem health assessment

MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

the essential issues



HOW?

DIAGNOSIS

Soil Health

capacity of soil to function as a Living System (LIVING ENTITY)

PHYSICO-CHEMICAL PARAMETERS

BIOLOGICAL PARAMETERS

Chemical Analysis

Who?
How much

Bioavailability Tests

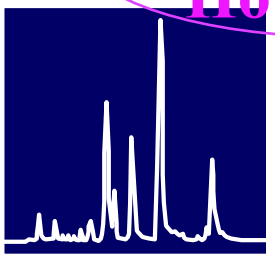
Bioaccesibility Tests

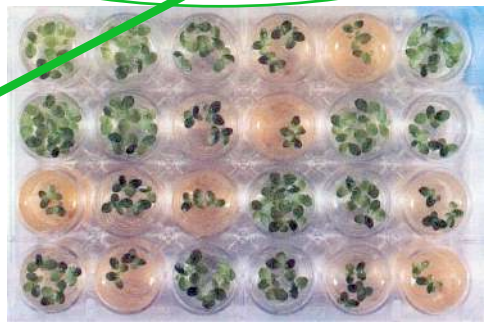
How much can impact?

Gastrointestinal simulation

Ecotoxicity Tests

Impact?





HOW?

DIAGNOSIS

Soil Health

PHYSICO-CHEMICAL PARAMETERS

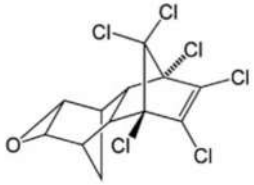
Chemical Analysis

Who?

How much?

Basque Reference Threshold for Polluted Soils

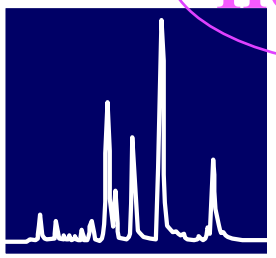
Compound		Basque Thresholds
Cadmium	mg/kgdw	**5**
Chromium	mg/kgdw	**200**
Lead	mg/kgdw	**120**
Nickel	mg/kgdw	**110**
BaP	mg/kgdw	**0.02**
Dieldrin	µg/kgdw	**10**



Reference Threshold levels for Soils

(VIE-A, VIE-B, VIE-C)

Law 4/2015; Decree 209/2019

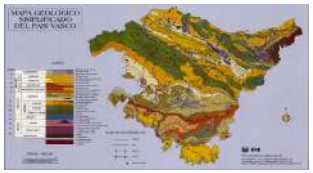


(https://www.euskadi.eus/legislacion-sobre-suelos-contaminados/web01-a2inglur/es/)

Calidad del Suelo : Niveles de Riesgo para los Ecosistemas

Valores Indicativos de Evaluación

Ihobe; RD 9/2005 (BOE 18 de Enero 2005)



VIE-C

Riesgo inaceptable

VIE-B

Incertidumbre de Riesgo Aceptable según uso

VIE-A

Riesgo aceptable

Concentración de contaminante en el suelo

Cd
< 0,8 ppm

Cd
Area juego infantil= <5 ppm
Huerta = <5 ppm
Residencial = <8 pm
Parque publico = <25 ppm
Industrial = <50 ppm

Cd
Area juego infantil= >5 ppm
Huerta = >5 ppm
Residencial = >8 pm
Parque publico = >25 ppm
Industrial = >50 ppm

Nivel de riesgo

¿?

ANLISIS DEL RIESGO
PROYECTO RECUPERACIÓN

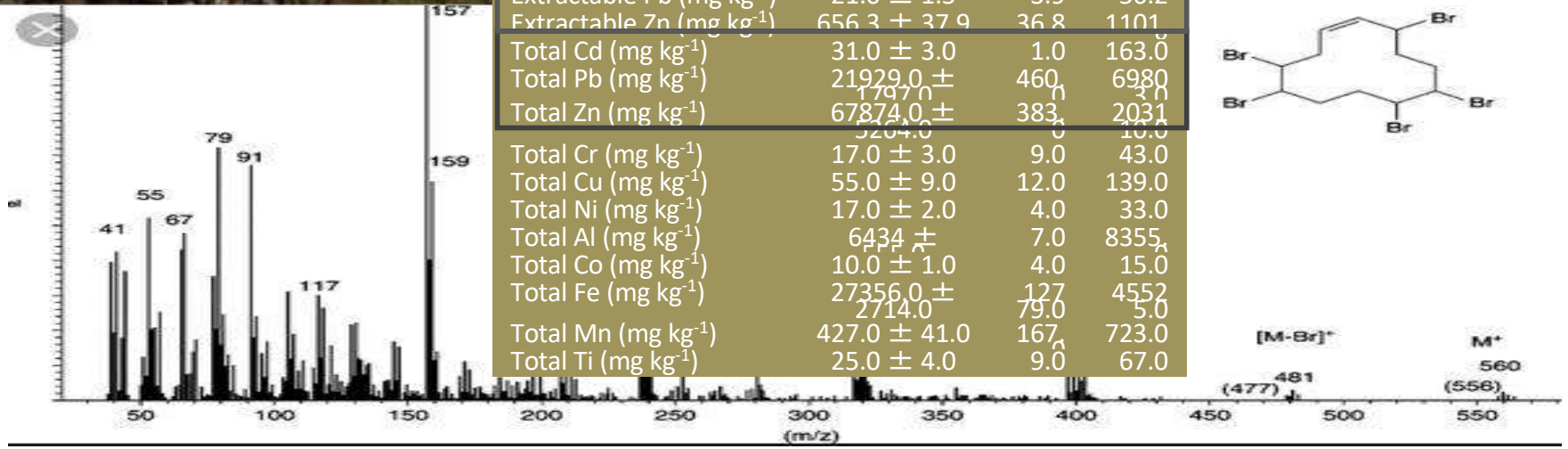
Soil Health Assesment **How? What?**

PHYSICOCHEMICAL INDICATORS of soil

Pollutants (Who? How much?),

structure, texture, pH, nutrients, organic matter, Cation Exchange Capacity (CEC), redox potential, 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 ± 2714.0	127 79.0	4552 5.0
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

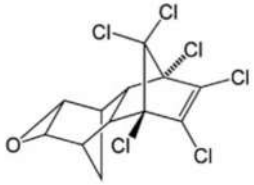
Chemical Analysis

Who?

How much?

Basque Reference Threshold for Polluted Soils

Compound		Basque Thresholds	B Pre	B Post
Cadmium	mg/kgdw	5	11.73 ± 3.4	9.7 ± 4.2
Chromium	mg/kgdw	200	140 ± 10	131.9 ± 49.5
Lead	mg/kgdw	120	86.33 ± 3.2	73.6 ± 19.7
Nickel	mg/kgdw	110	60.66 ± 4.7	46 ± 12.4
BaP	mg/kgdw	0.02	0.08 ± 0.01	0.06 ± 0.03
Dieldrin	µg/kgdw	10	13.26 ± 13.2	5 ± 5.1



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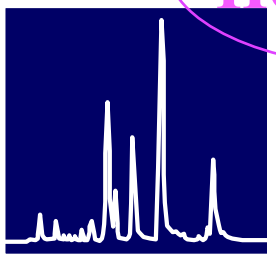


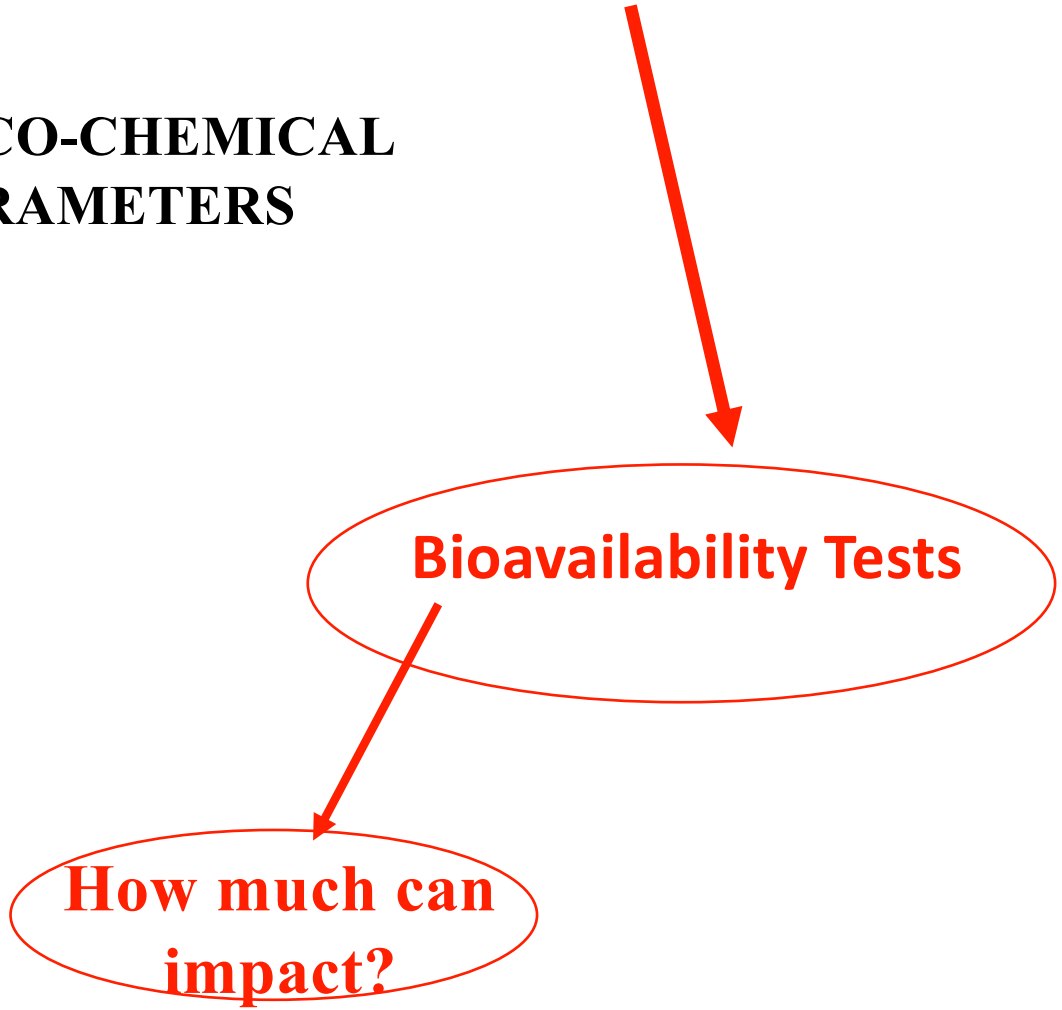
(https://www.euskadi.eus/legislacion-sobre-suelos-contaminados/web01-a2inglur/es/)

HOW?

DIAGNOSIS

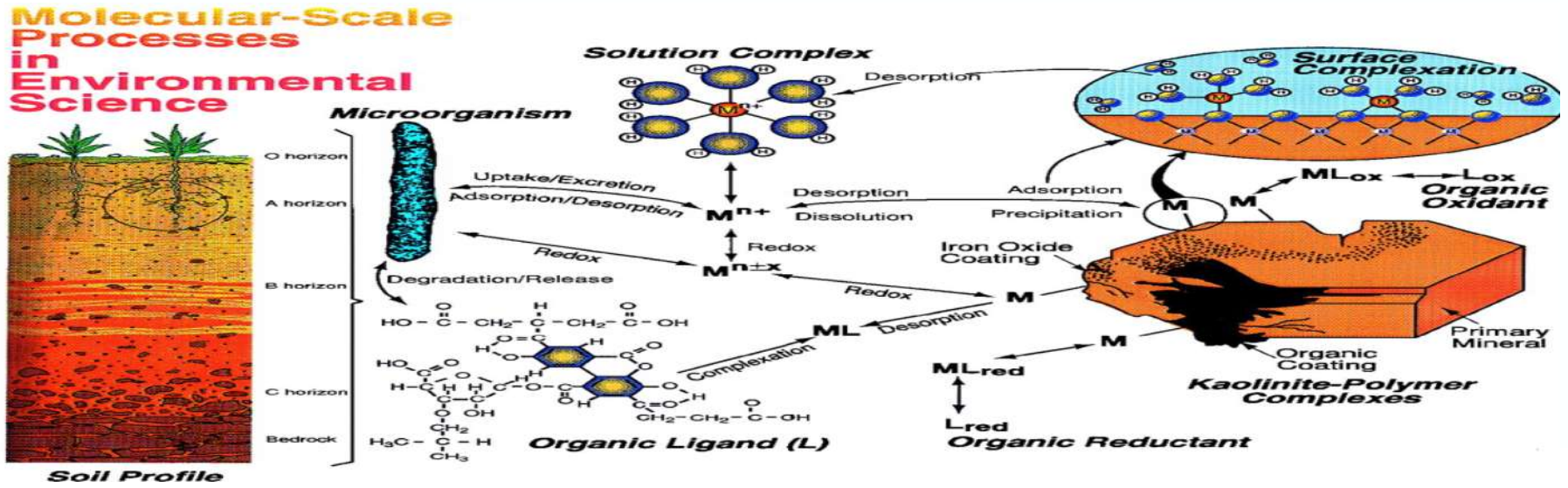
Soil Health

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 four essential “B

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

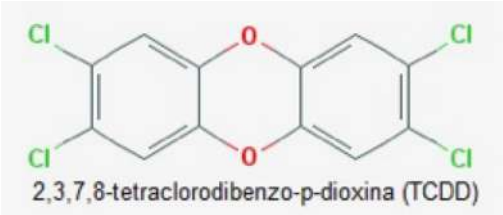
Ability of a chemical to enter in an organism

Concentration of a chemical in a living organism over time

More concentration of a chemical through the food web

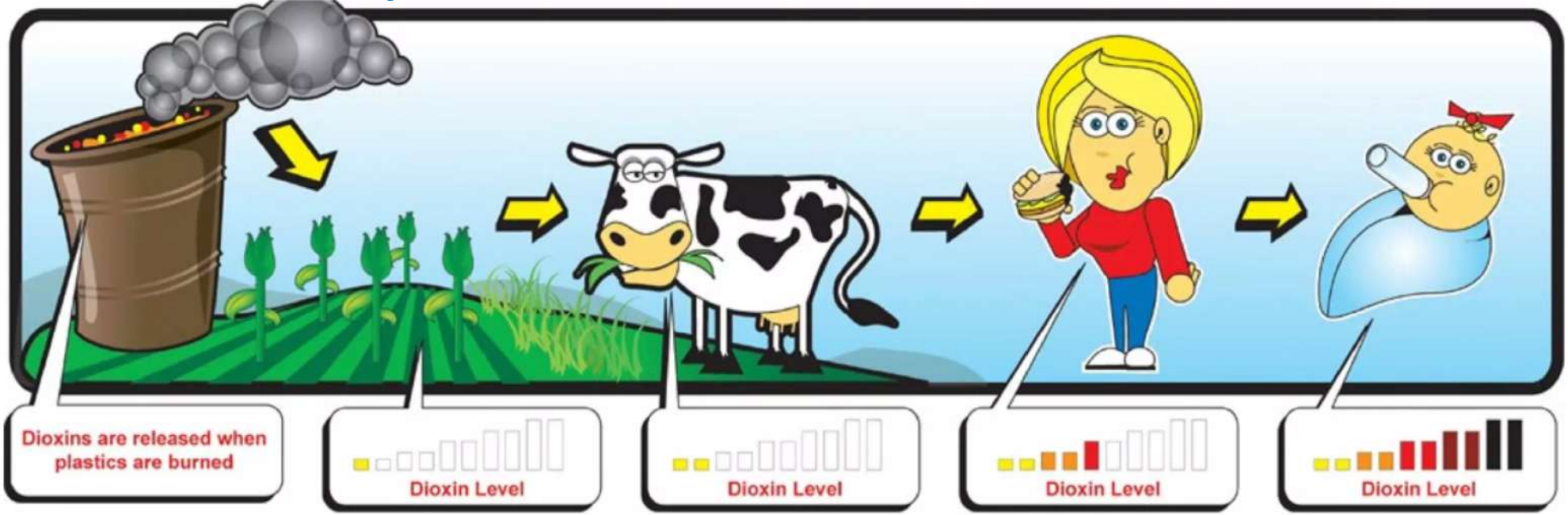
Bioaccessibility:

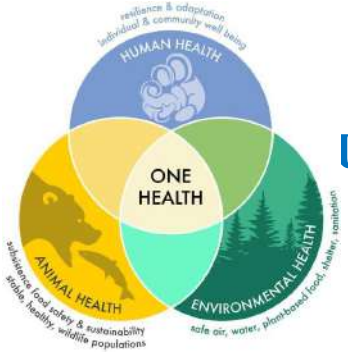
Ability of a chemical to become accessible for human absorption



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

Bioavailability, Bioaccumulation and Biomagnification of dioxins Bioaccessibility

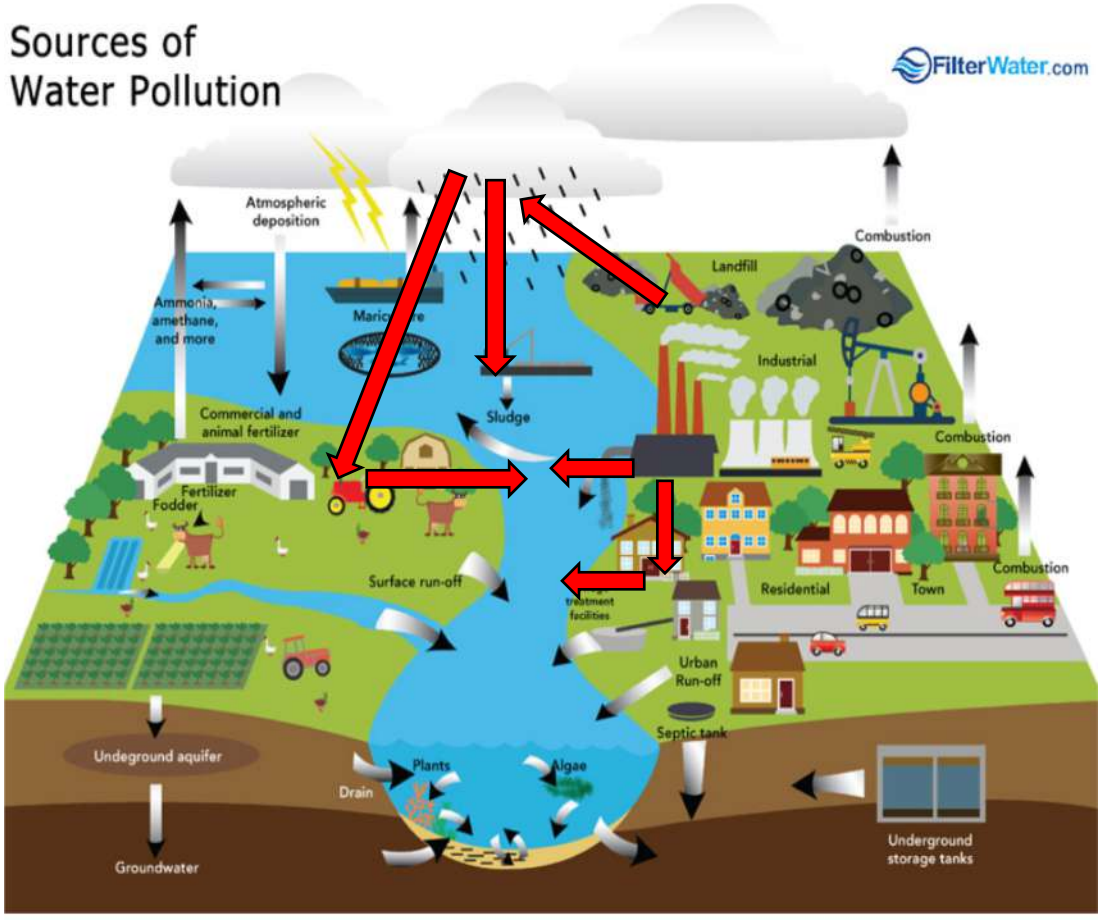


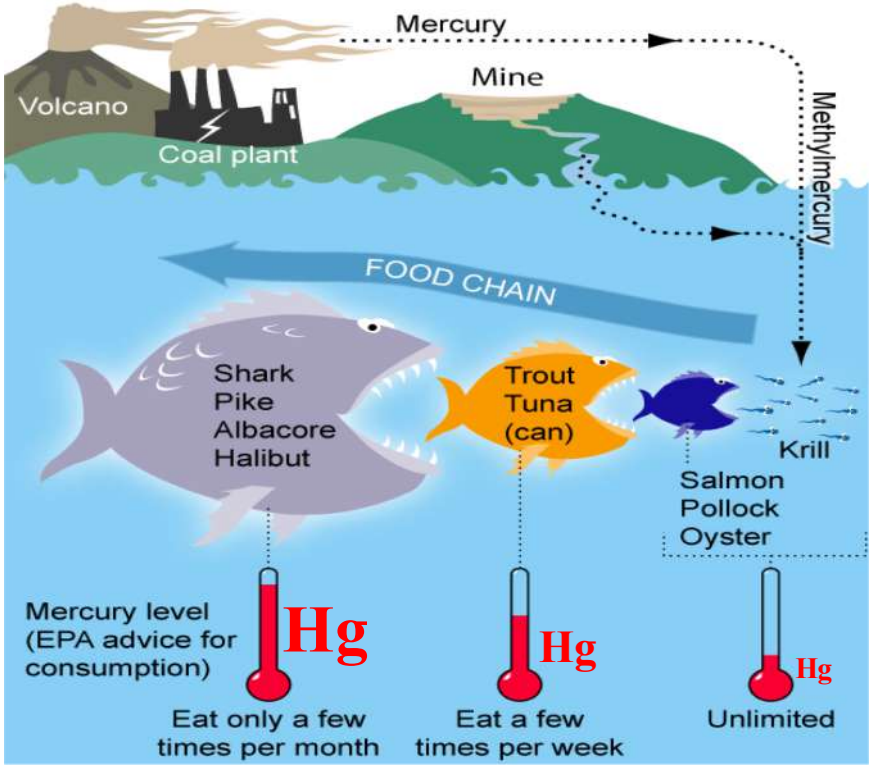


The ONE paradigm: UN PLANETA

UNA SALUD: la salud de todos y de todo son interdependientes

UNA CONTAMINACIÓN
vision holística del problema de la contaminación del suelo



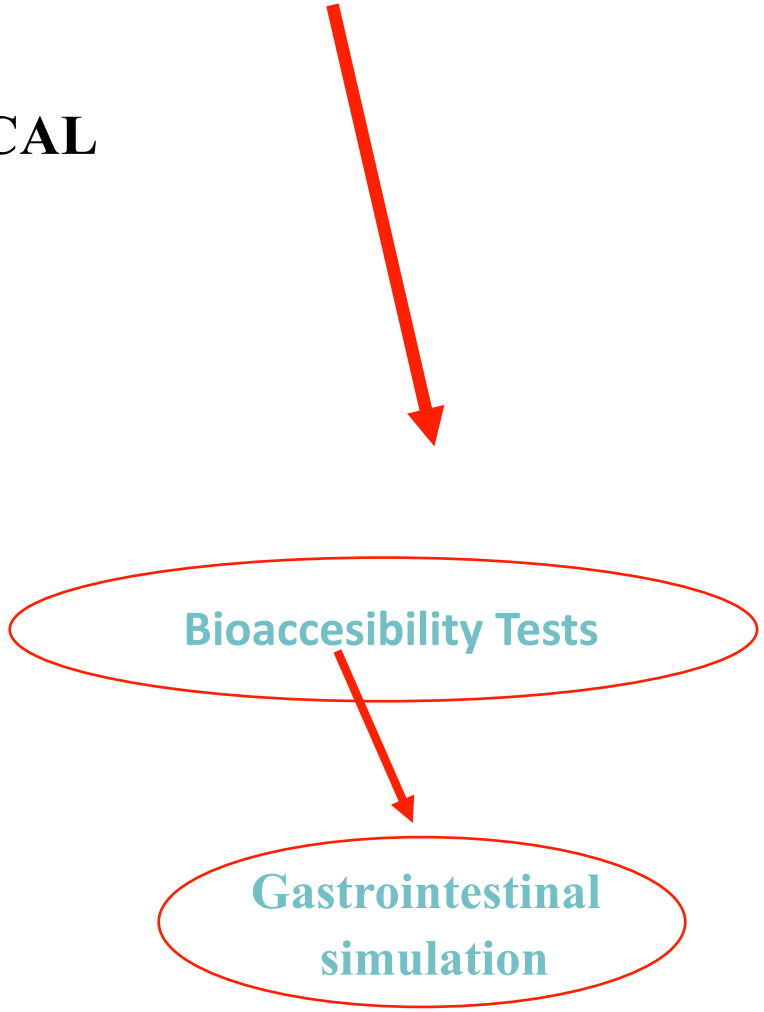


HOW?

DIAGNOSIS

Soil Health

PHYSICO-CHEMICAL PARAMETERS



Bioaccessibility:

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

Ecotoxicology and Environmental Safety 209 (2021) 111806

Contents lists available at ScienceDirect

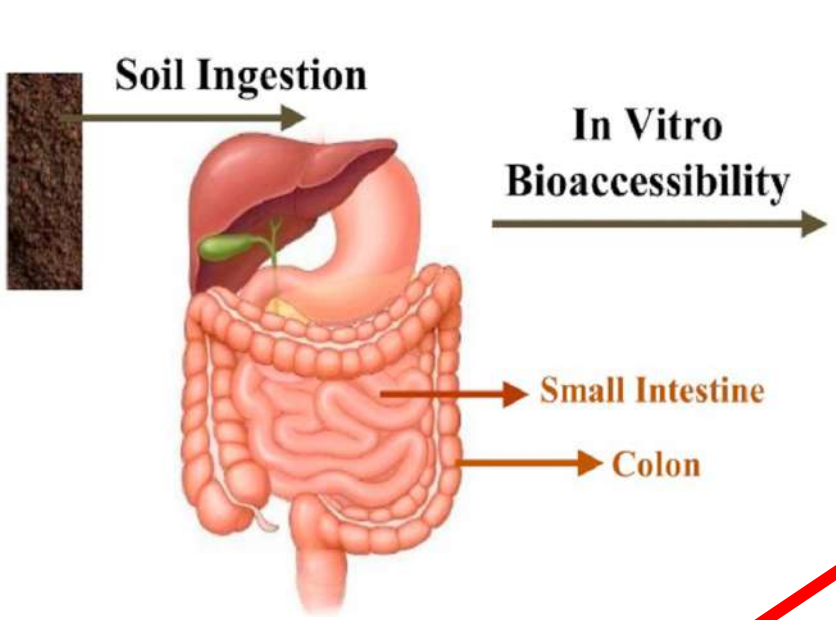
Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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

Yan Li, Elio Padoan *, Franco Ajmone-Marsan

University of Turin, Department of Agricultural, Forest and Food Sciences, Largo Paolo Braccini 2, Grugliasco, Torino 10095, Italy



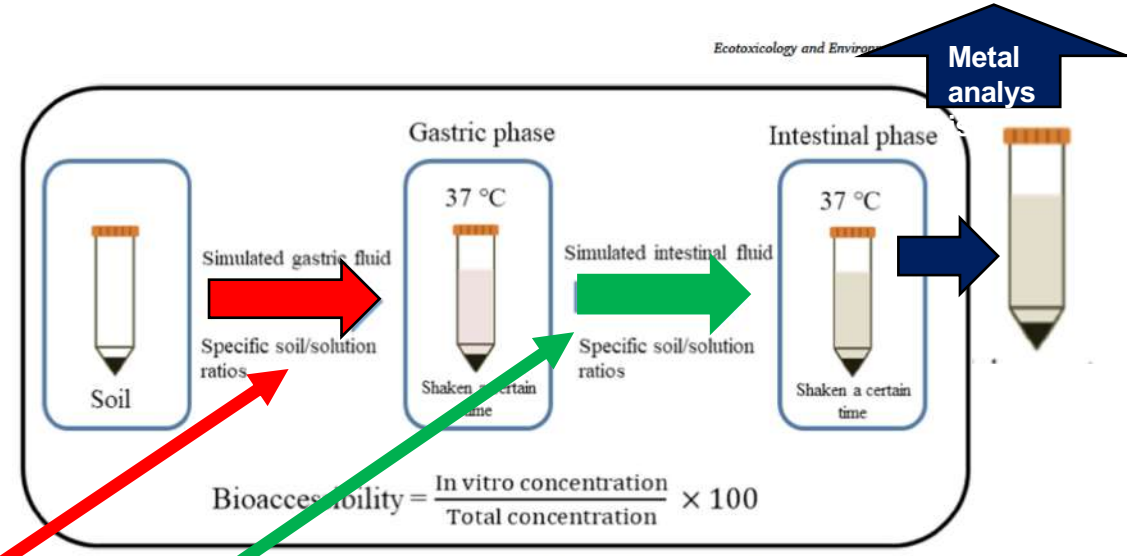


Fig. 1. Schematic diagram of in vitro soil PTE bioaccessibility test procedure.

pH

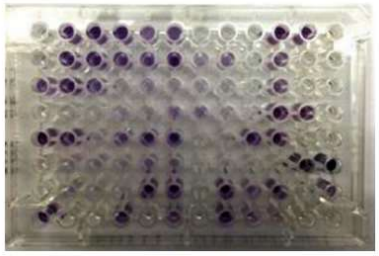
SBRC	Gastric	30.03 g glycine	1.5	1:100	1	Drexler and
	Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	1:100	4	Brattin (2007)

How? What?

DIAGNOSIS

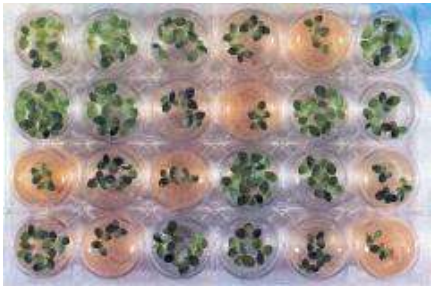
Soil Health

Biological parameters

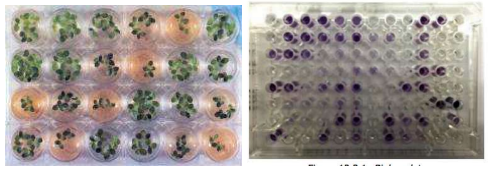


Ecotoxicity Tests

Biological impact?



How? What?



-. BIOLOGICAL INDICATORS (biomaker)

Bioindicator: Is a **quantificable characteristic** of biochemical, physiological, toxicological or ecological process or function (**biomarker**) **that correlate with 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 Methodology **Ecotoxicity Tests**

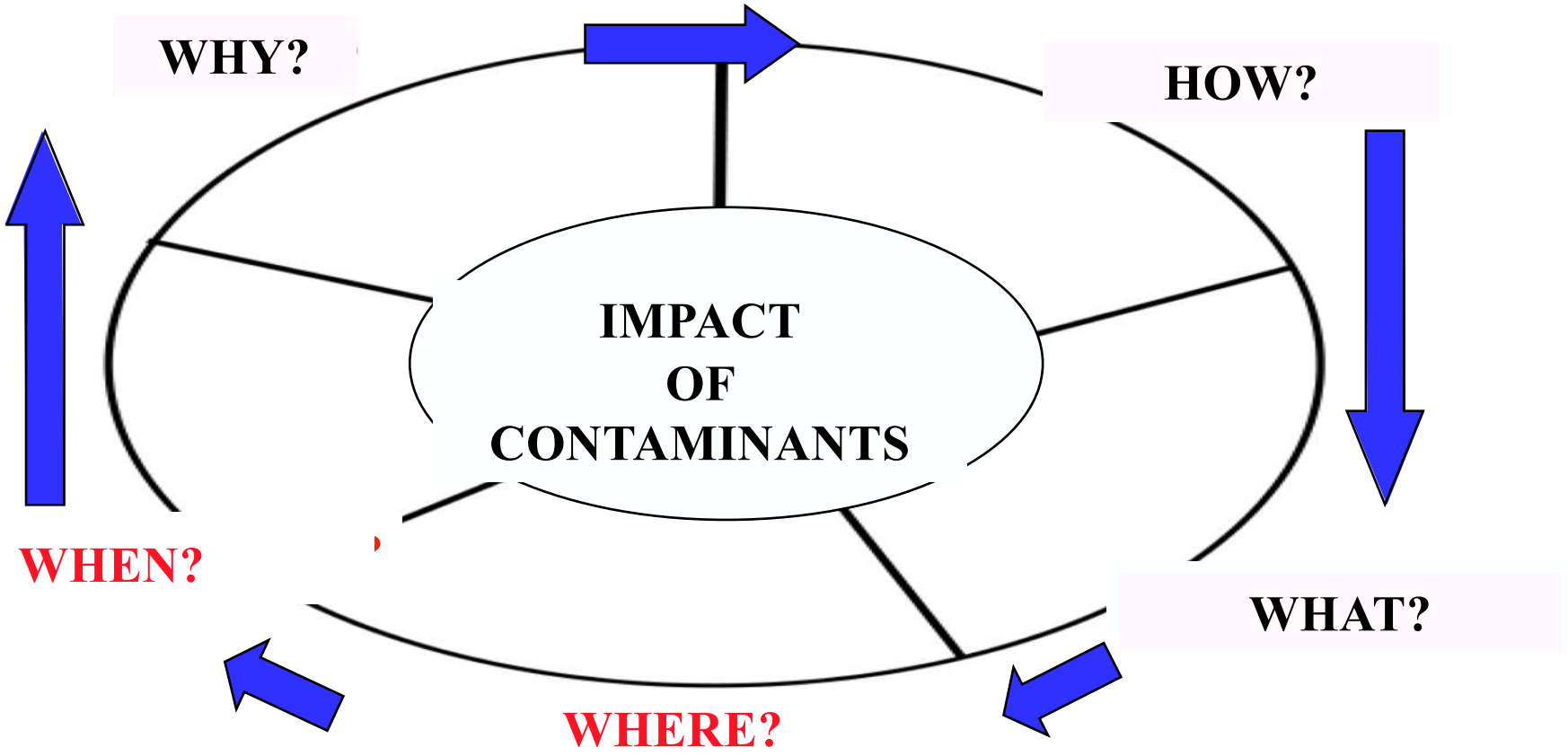
Root elongation bioassay

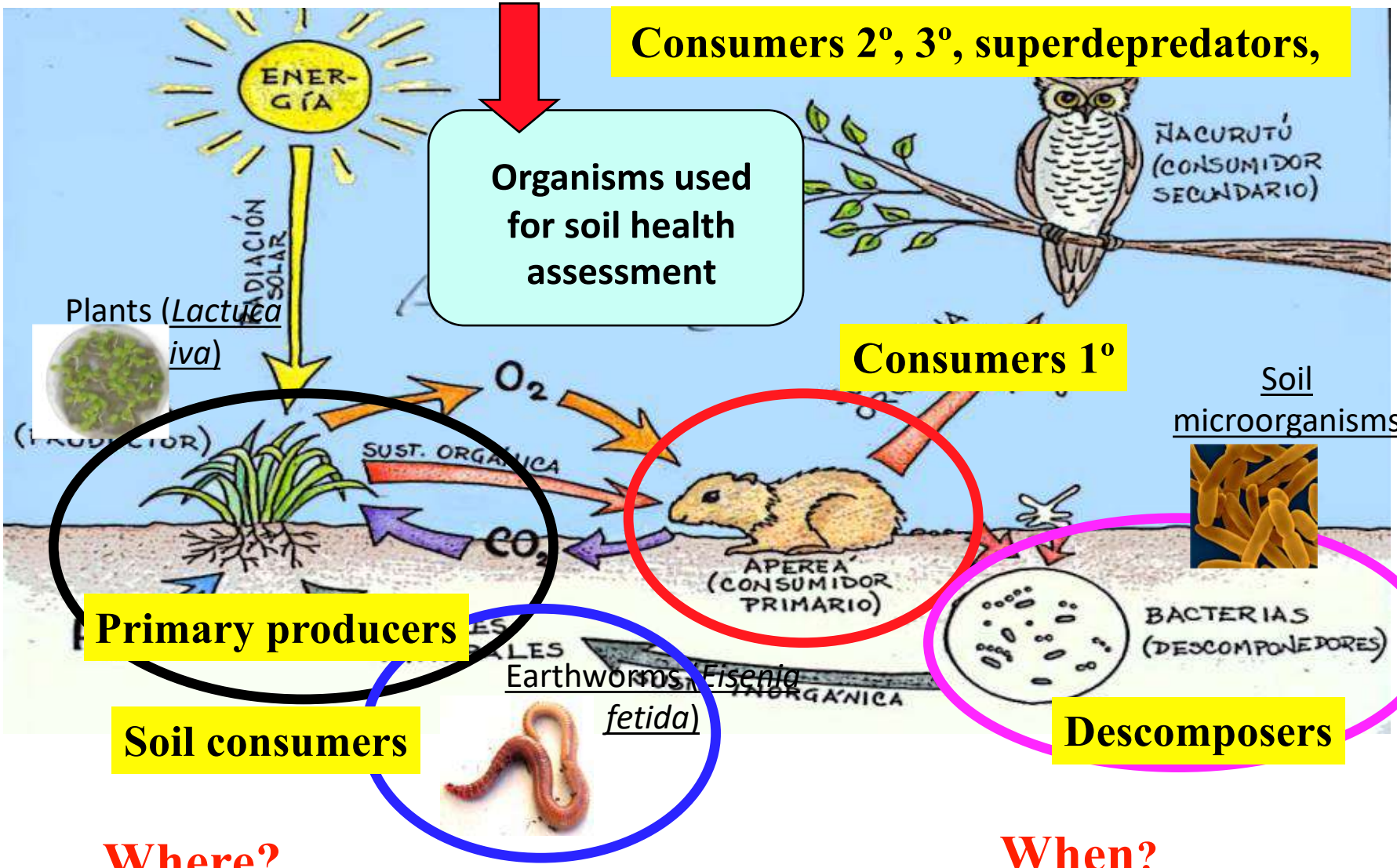
Control 0h

Control 72h

MONITORING IMPACT OF CONTAMINANTS WITH BIOINDICATORS

the essential issues





Where?

Real receptors of pollution

-. Relevant
-. Representative
-. Final

When?

The best time to monitor

-. Life cycle
-. Seassons of the year
- Events (regular, eventual)

Organisms used for soil health assessment in bioassays

Primary Producers

Cucumis sativus l.

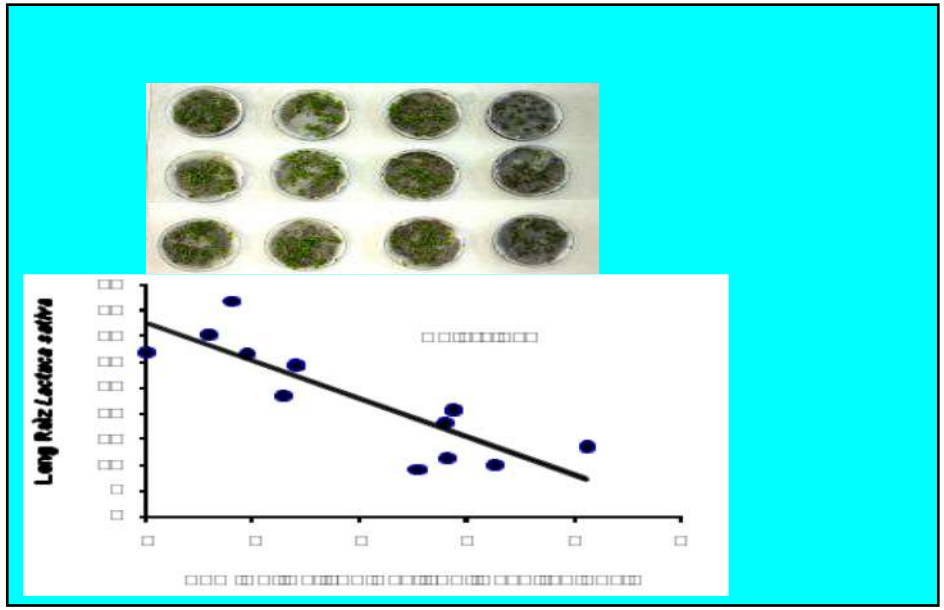
- Bio-metric parameters
- Physiological and biochemical parameters

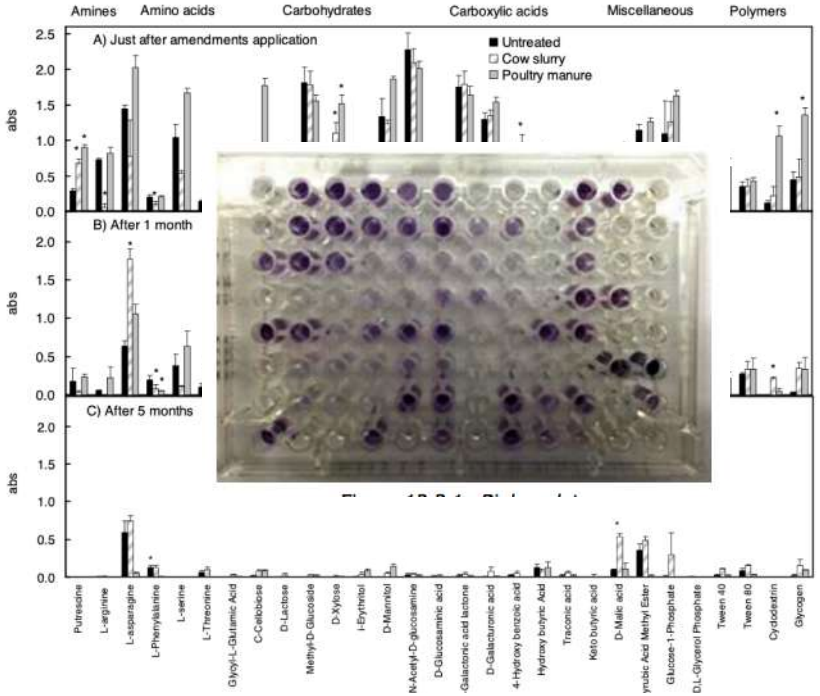
Descomposers

Soil microorganisms

- Basal respiration
- Induced Respiration
- Diversity

BIOASSAYS OF ECOTOXICITY ON SOILS





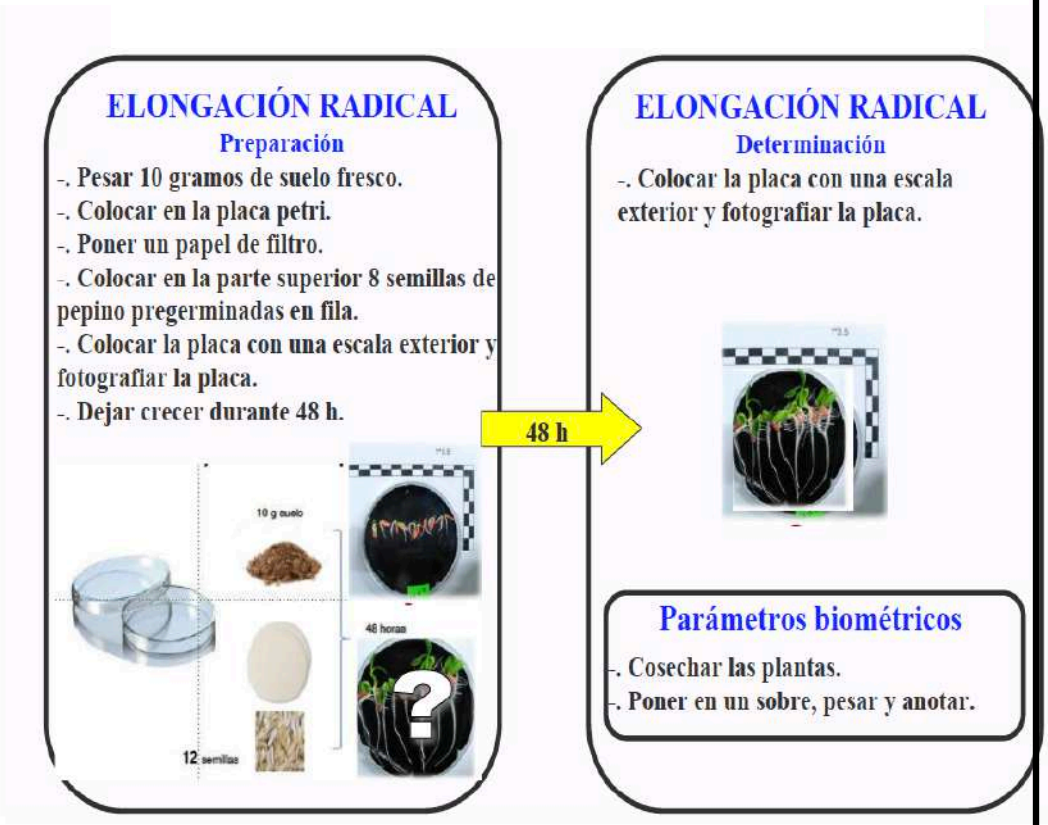
Where? When?

Organisms used for soil health assessment: bioassay

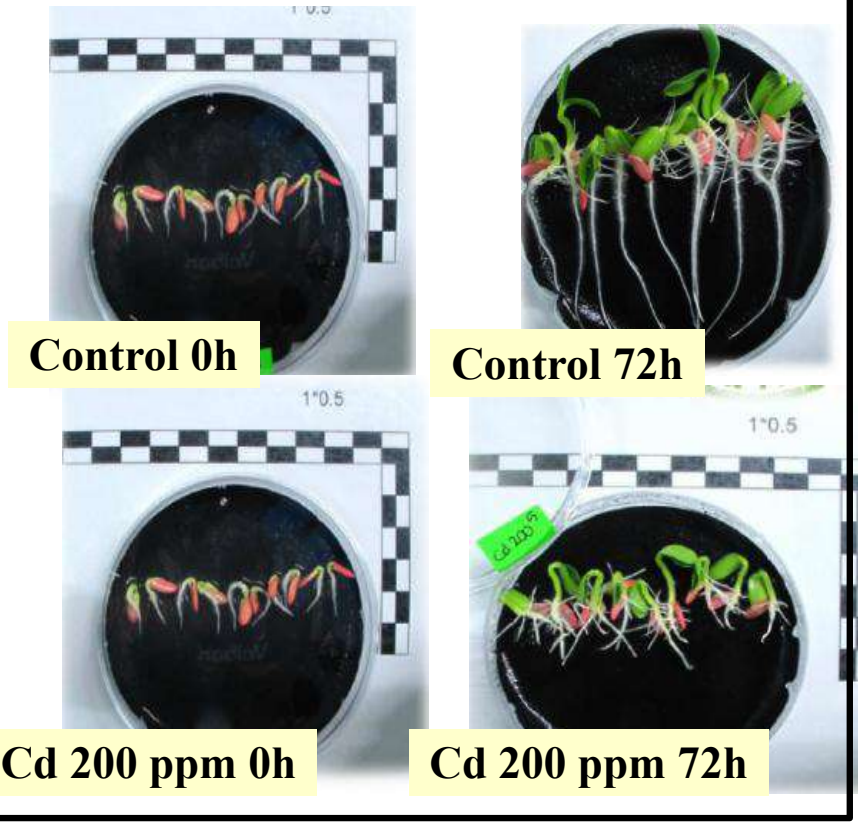
Cucumis sativus l.

- Root elongation bioassay

Implementation on Standardized Methodology



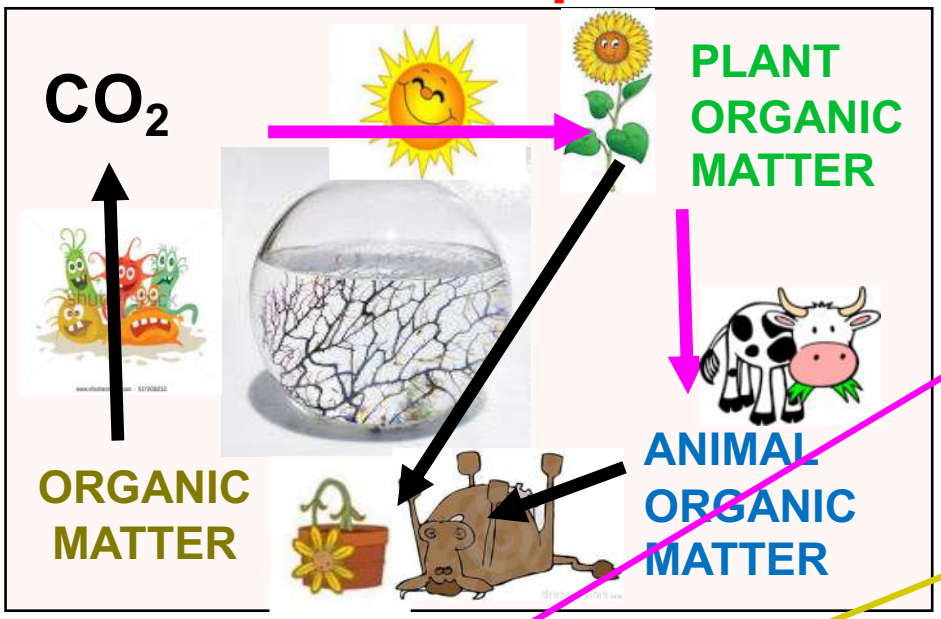
Root Elongation Bioassay

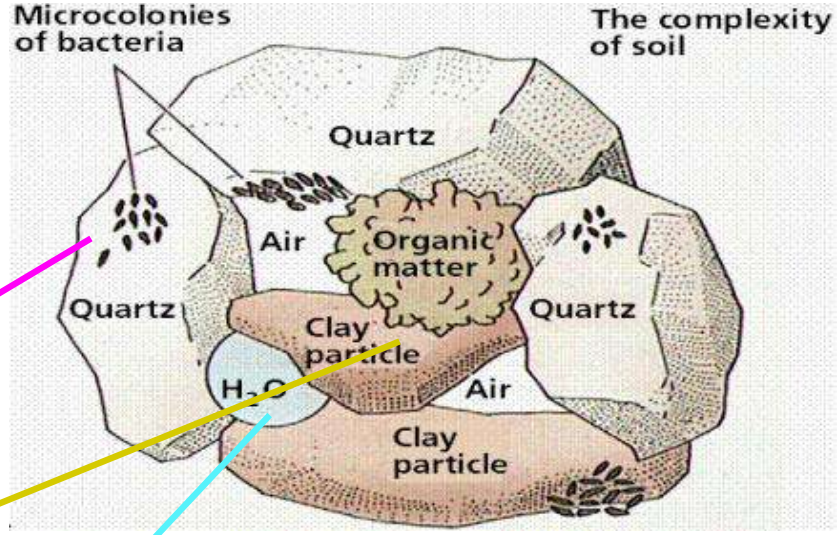


Soil microorganisms

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

Essential processes in soils: Cicle of C





ORGANIC MATTER

Microbial respiration

$CO_2 + H_2O \rightarrow H_2CO_3$

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

$2NaOH + H_2CO_3 \rightarrow Na_2CO_3 + 2H_2O$

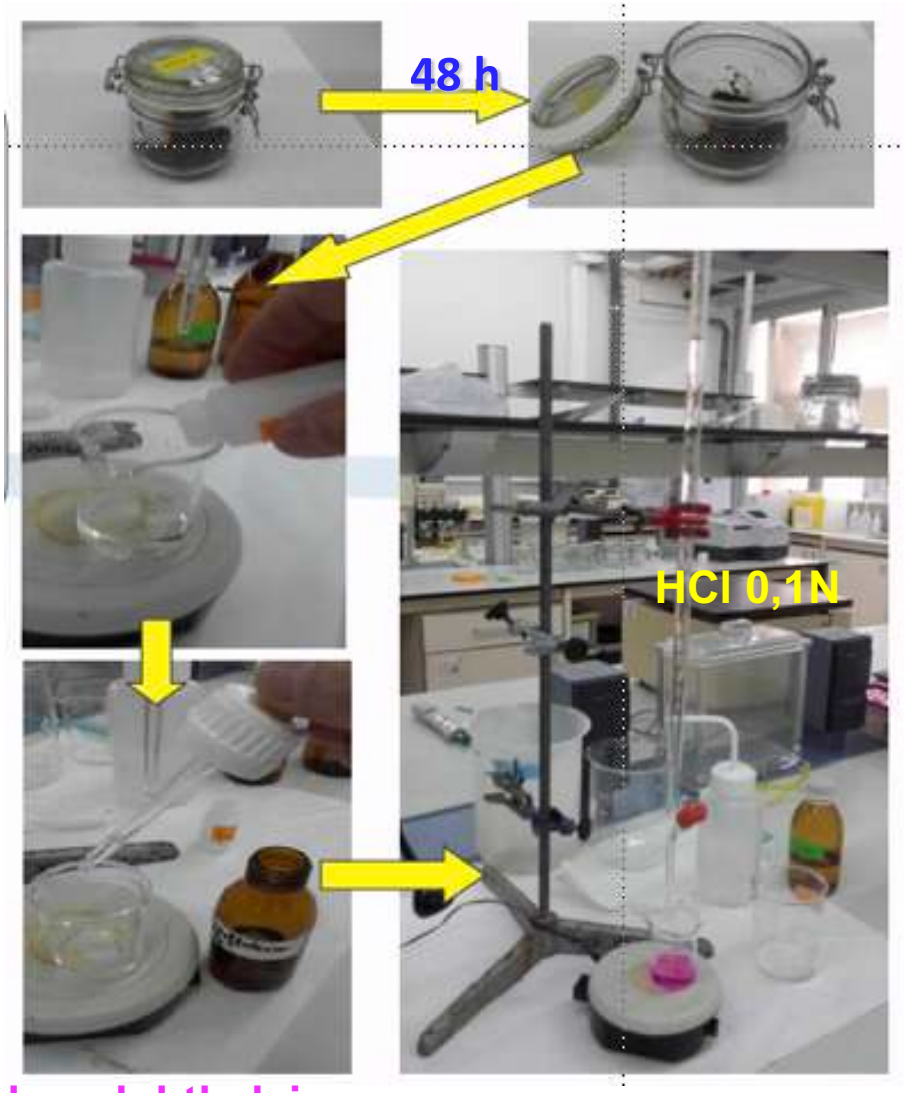
Titration of non neutralized NaOH

$NaOH + HCl \rightarrow NaCl + H_2O$

Soil microorganisms

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

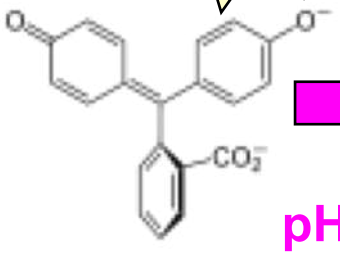
Basal Respiration (microbial activity)



phenolphthalein

¿WHY DOES PHENOLPHTHALEIN CHANGE COLOR?

$CO_2 + H_2O \longrightarrow H_2CO_3$

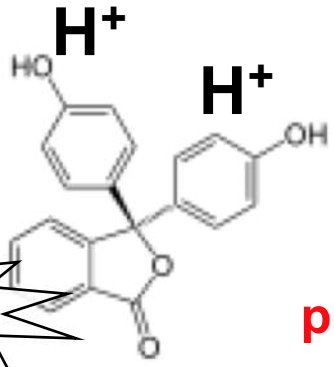


Basic

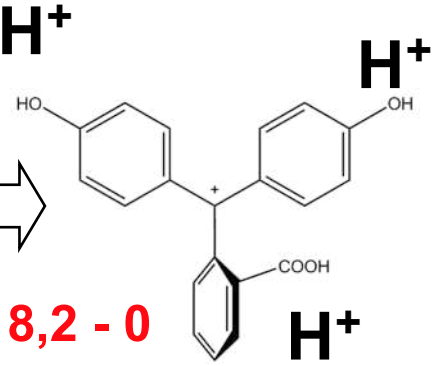
Fuchsia

pH= 12 - 8,2

pH= 8,2



pH= 8,2 - 0

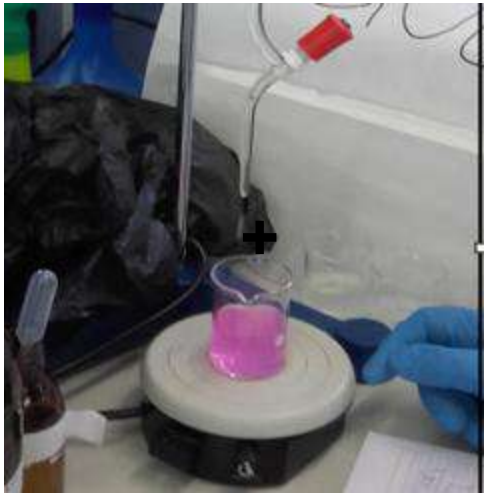


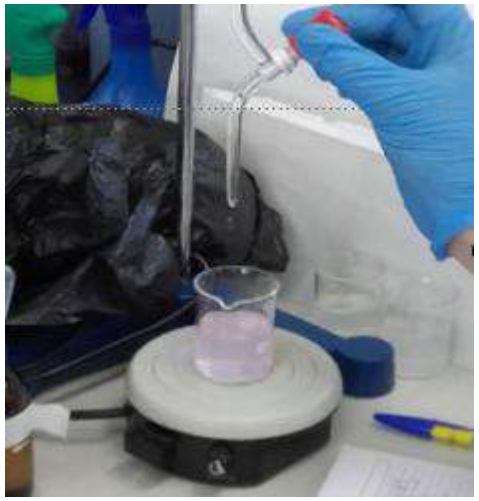
acid

clear

H^+ Cl^-

$NaOH + HCl \longrightarrow NaCl + H_2O$



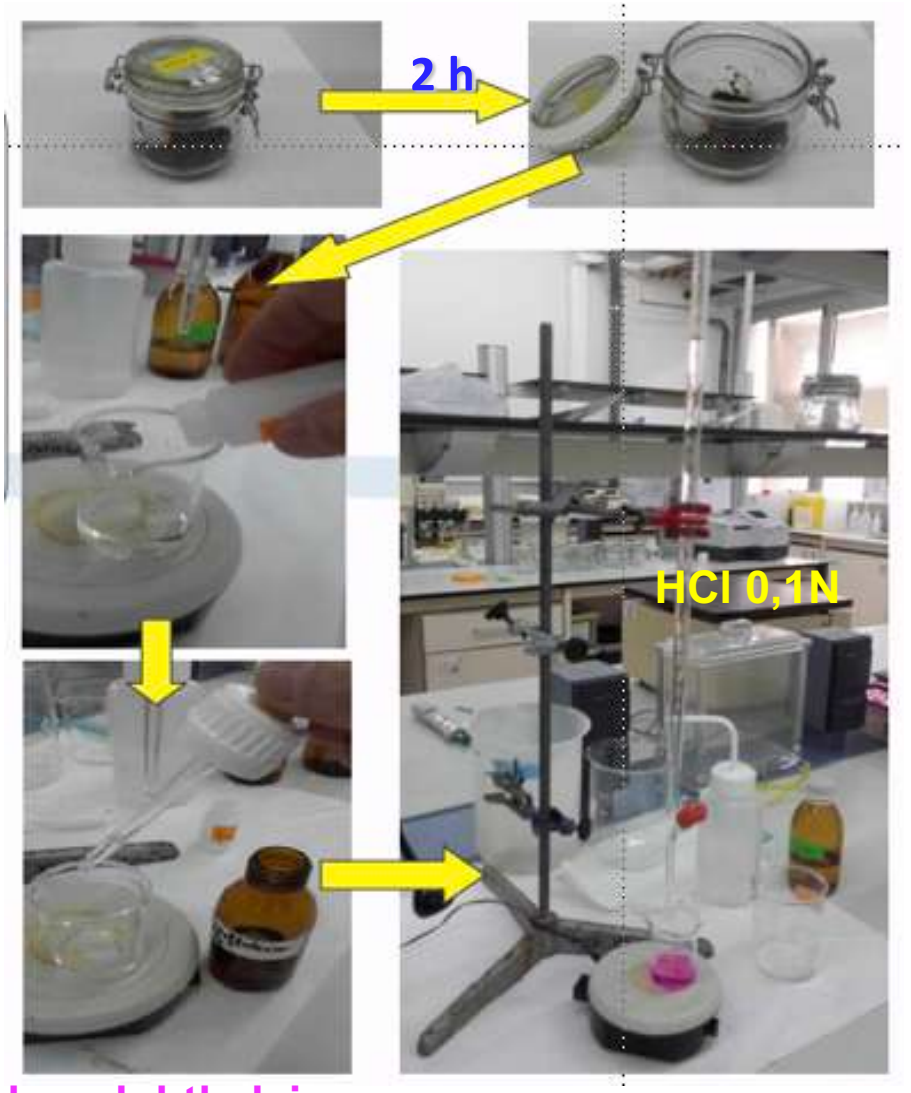


Soil microorganisms

- Induced Respiration= Microbial Biomass (glucose added to soils)

Induced Respiration (microbial biomass)

glucose



phenolphthalein

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

Objetives

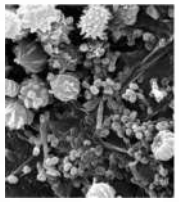
- Use a **ecofriendly technology** for soil remediation

PHYTOREMEDIATION

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

BIOLOGICAL INDICATORS

Soil microorganisms

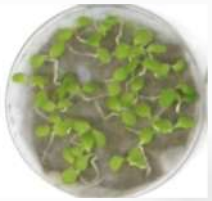


- Soil enzyme activities
- CLPPs

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

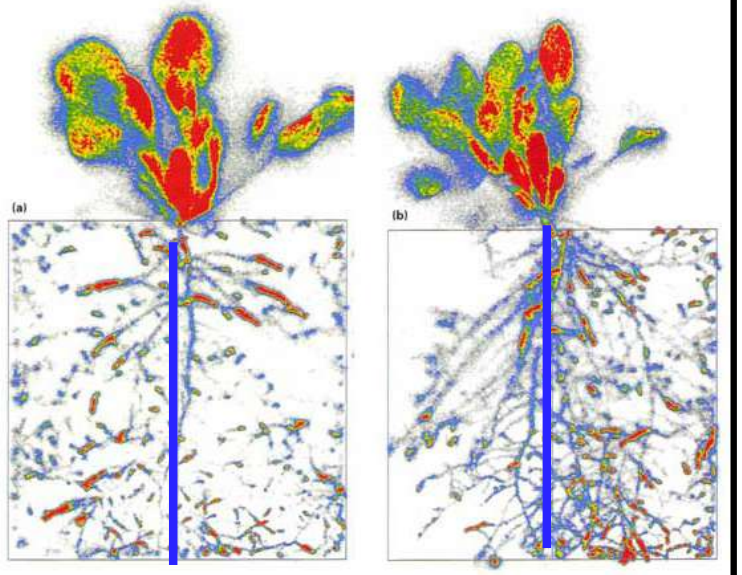
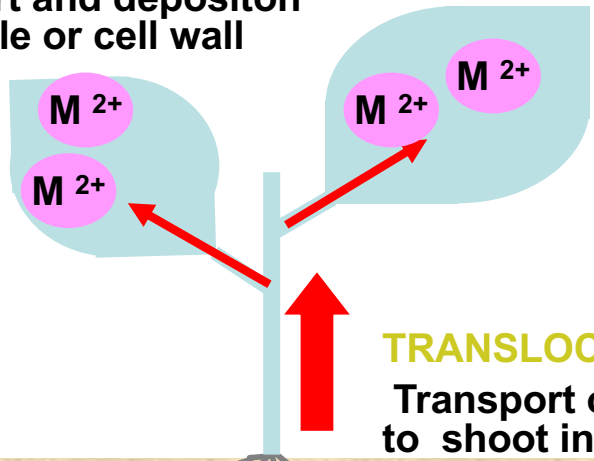


Fig. 6. The effect of localized Zn-enrichment on the allocation of ^{11}C within the roots of *Thlaspi caerulescens* in treatment 1000/1000 (a) and treatment C/1000 (b). Radiographic images were taken 48 h after labelling. The red threshold (points with highest ^{11}C activity) was set at 50 counts.

Key Processes in Metal Hyperaccumulation

SHOOT ACCUMULATION

Transport and depositon in vacuole or cell wall

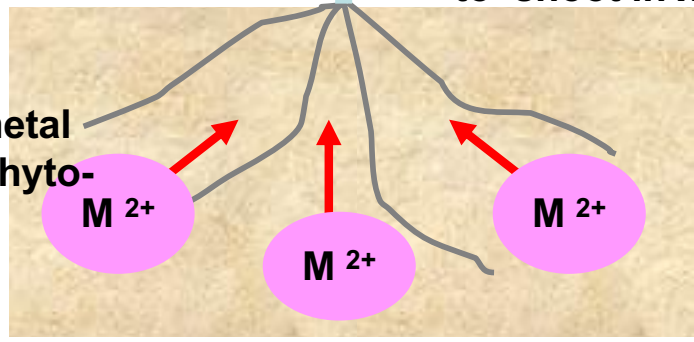


TRANSLOCATION

Transport of metal to shoot in xylem

CHELATION

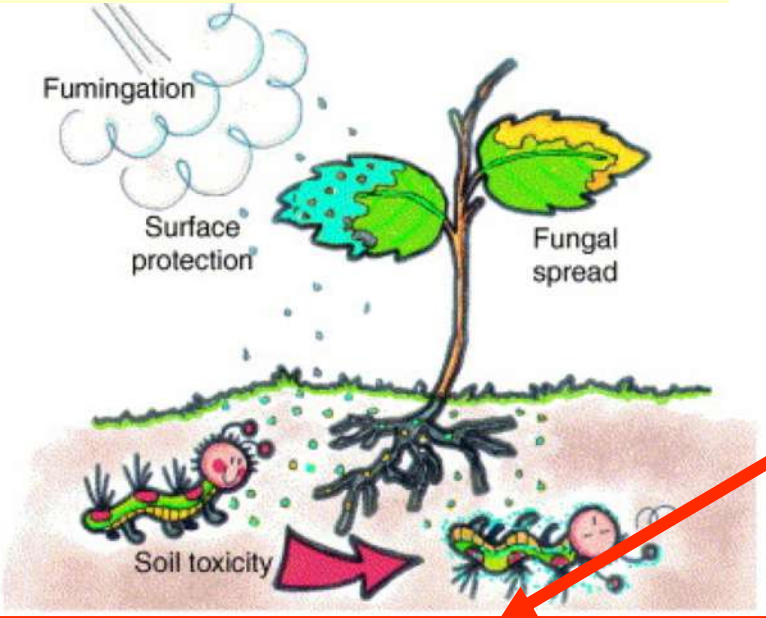
Ligands to chelate metal in root to minimize phyto-toxicity



METAL ACQUISITION

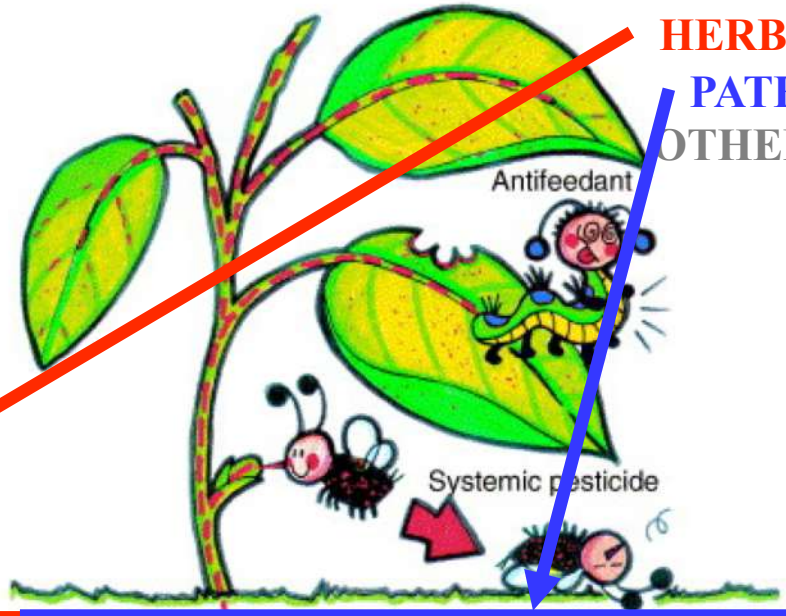
Effective dissolution and uptake of specific metals

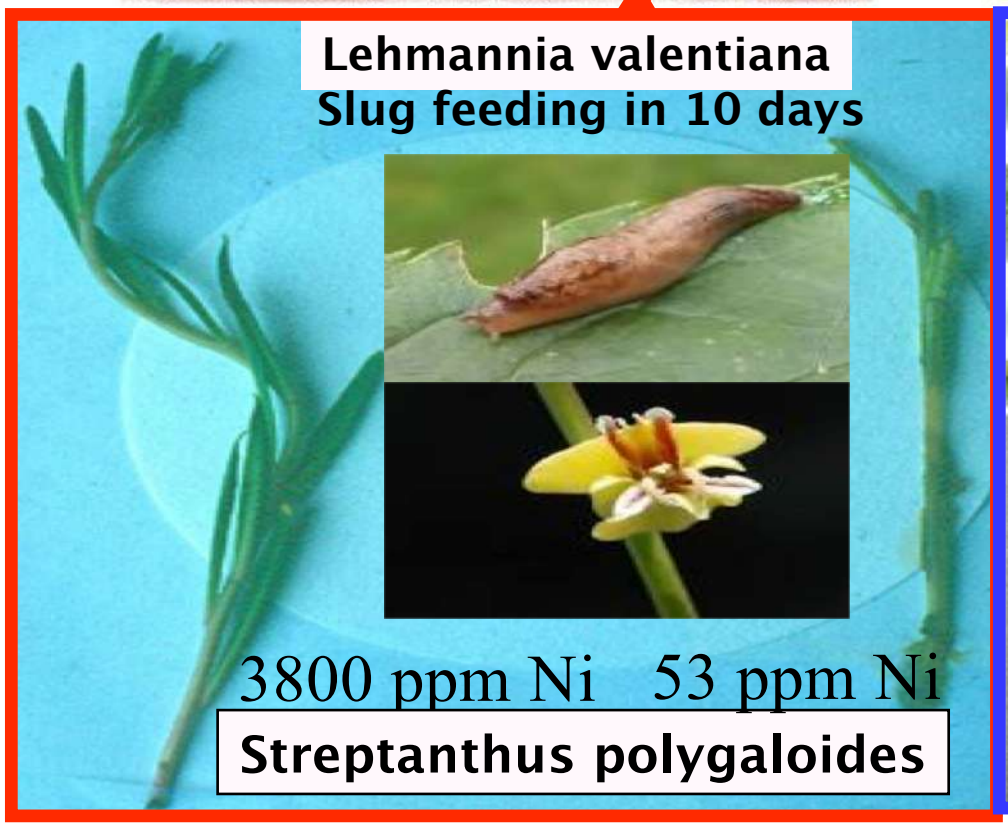
HUMAN ARTIFICAL DEFENSE

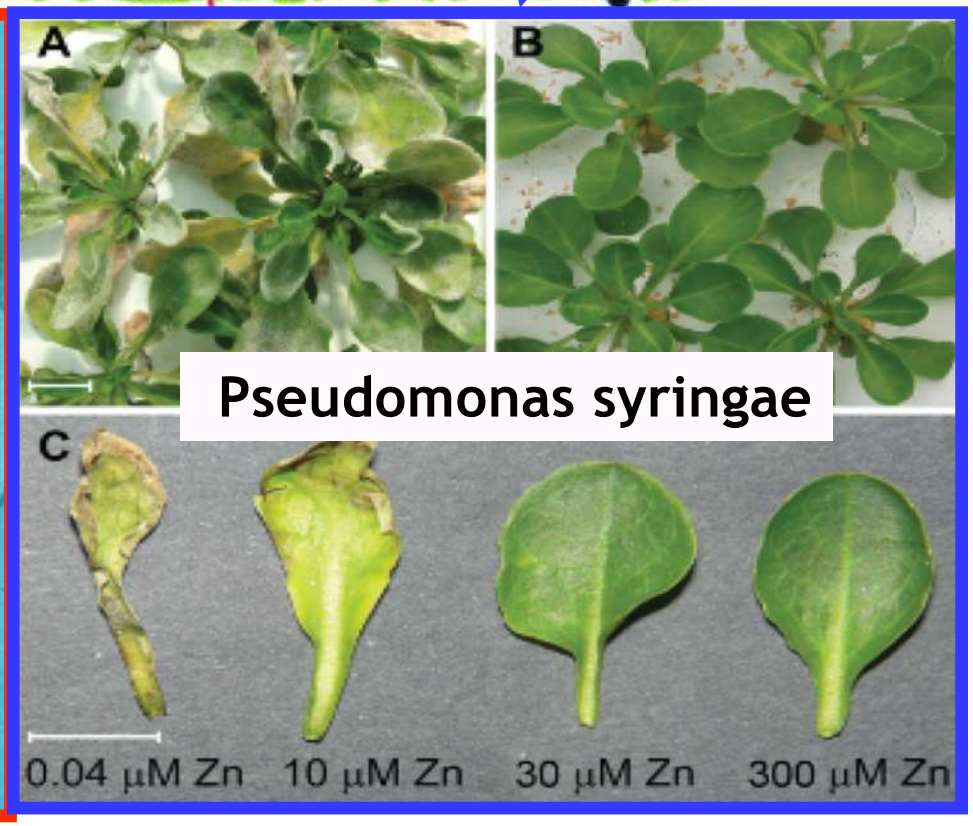


NATURAL METAL DEFENSE

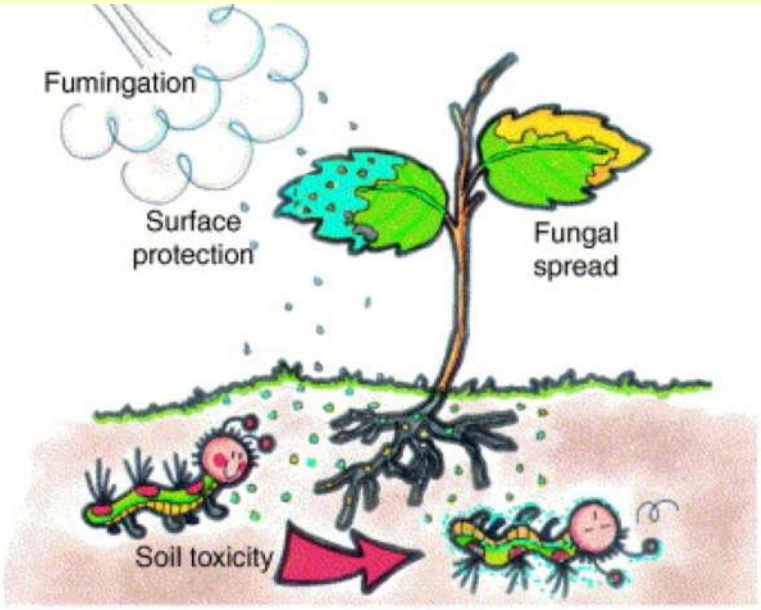
HERBIVOROUS
PATHOGENS
OTHER PLANTS



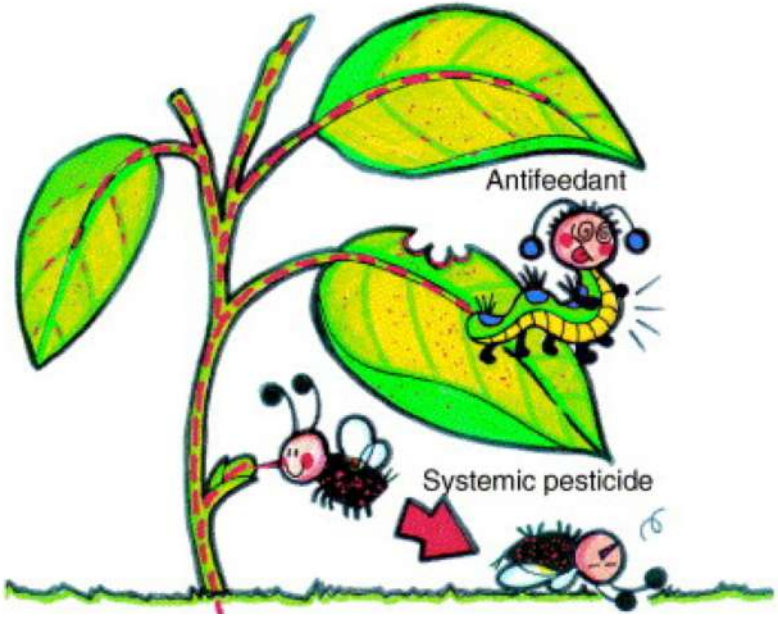


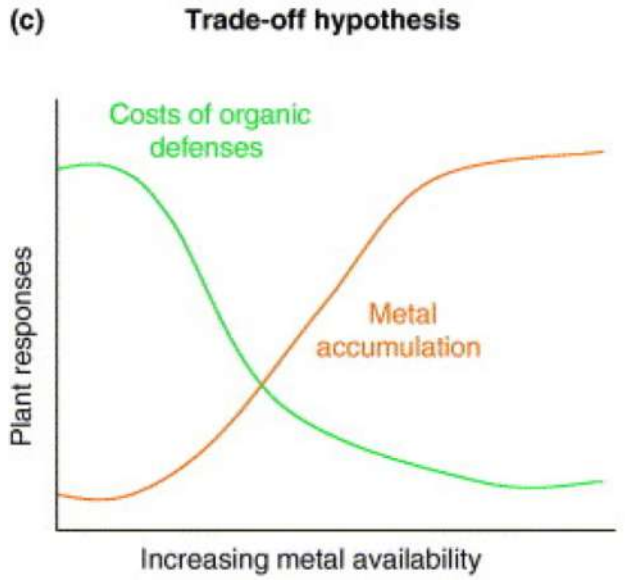


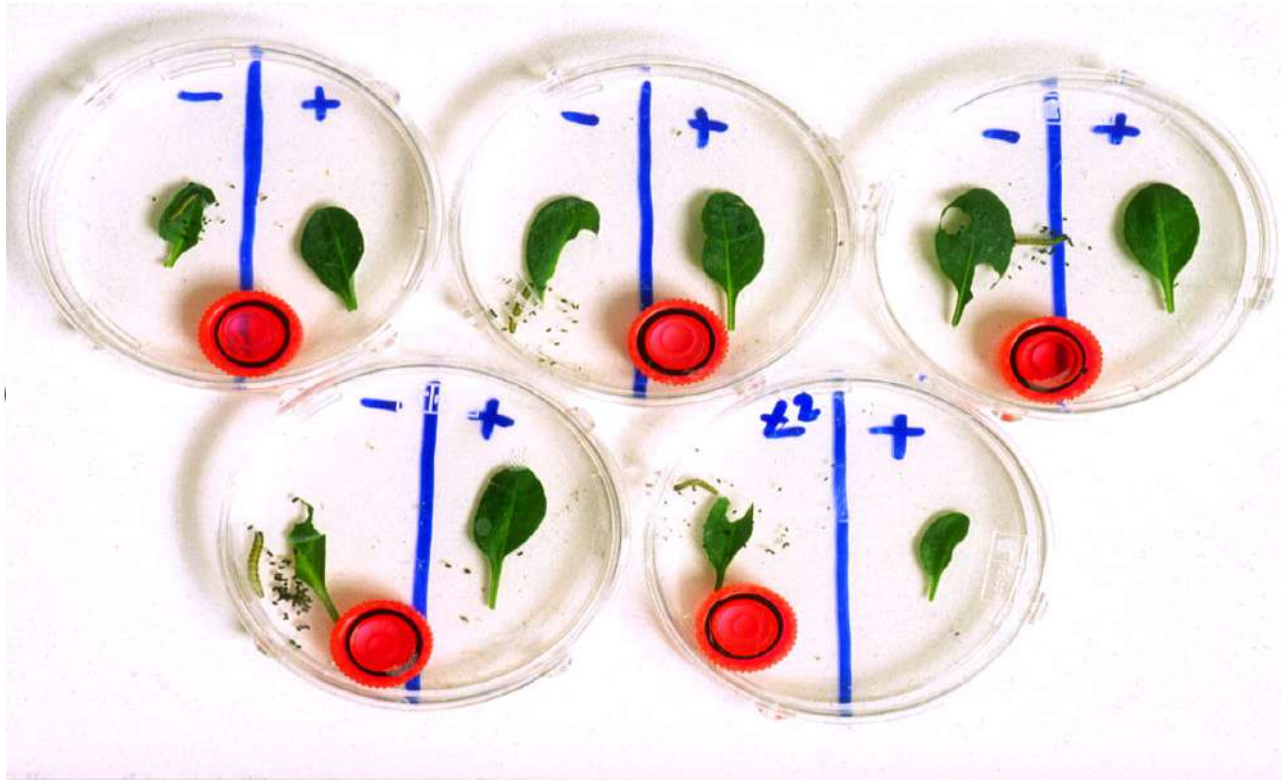
HUMAN ARTIFICAL DEFENSE



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

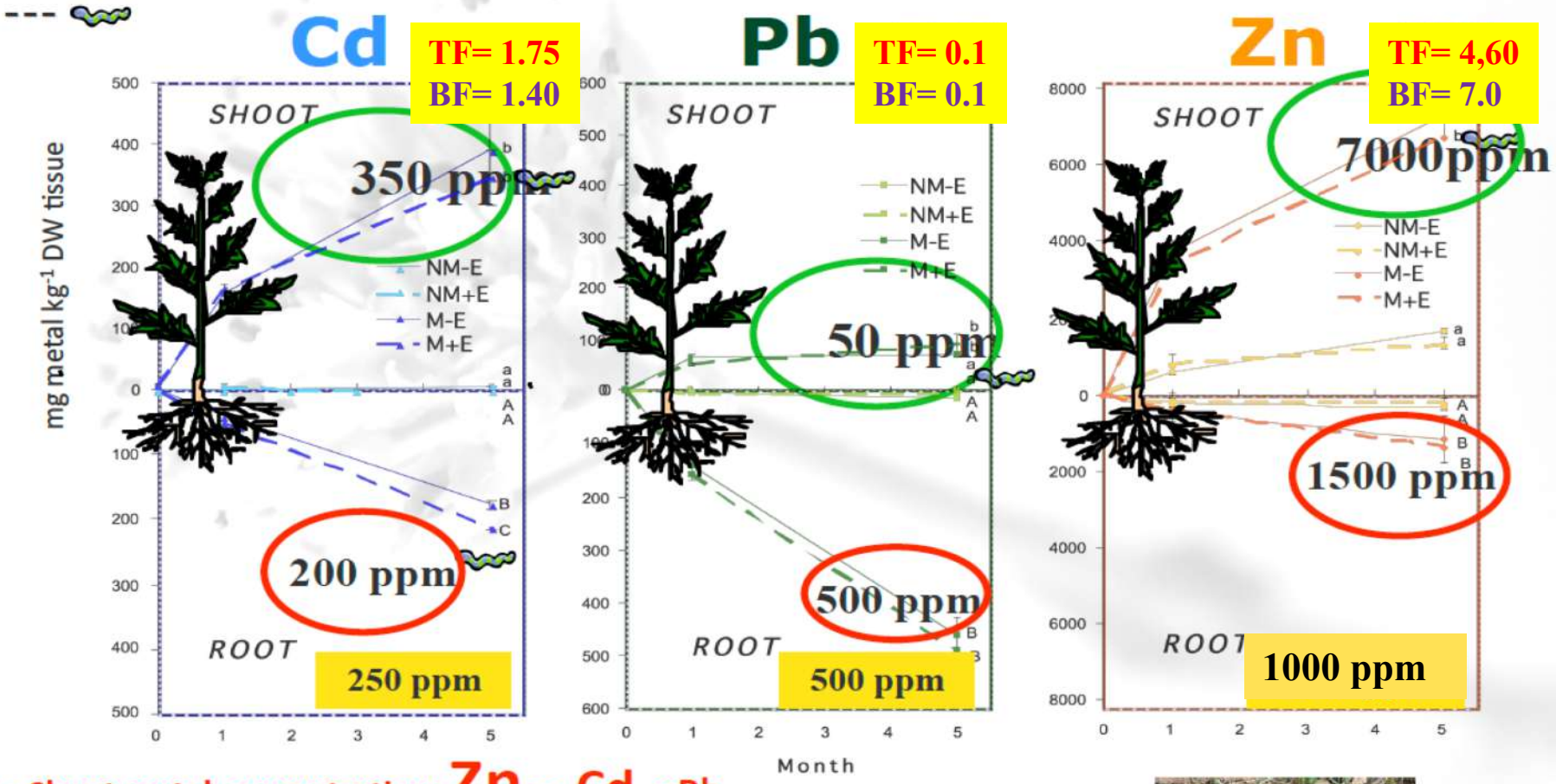
Texture	**Clay loam**
pH	**5.2**
OM (%)	**4.12**
Total N (%)	**0.23**
C/N	**10.4**
EC (dS m^{-1})	**0.08**
P (mg kg^{-1})	**26.4**

- 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}** (***N. caerulescens*** Lanestosa ecotype)
- Earthworm treatment: 15 earthworms pot^{-1} (***Eisenia fetida***)

Translocation Factor (TF): metal in plant root/metal in plant shoot
Bioconcentration Factor (BF): metal in plant shoot/metal in soil

Chemical indicators

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

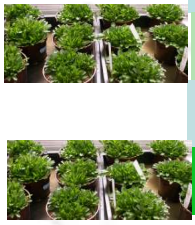
A case study: Monitoring soil health with biomarkers during a phytoremediation process

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} 33%	344^{b} 42%	672^{b} 37%	79.9^{b}	34.2^{b} 27%	495^{b} 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*)

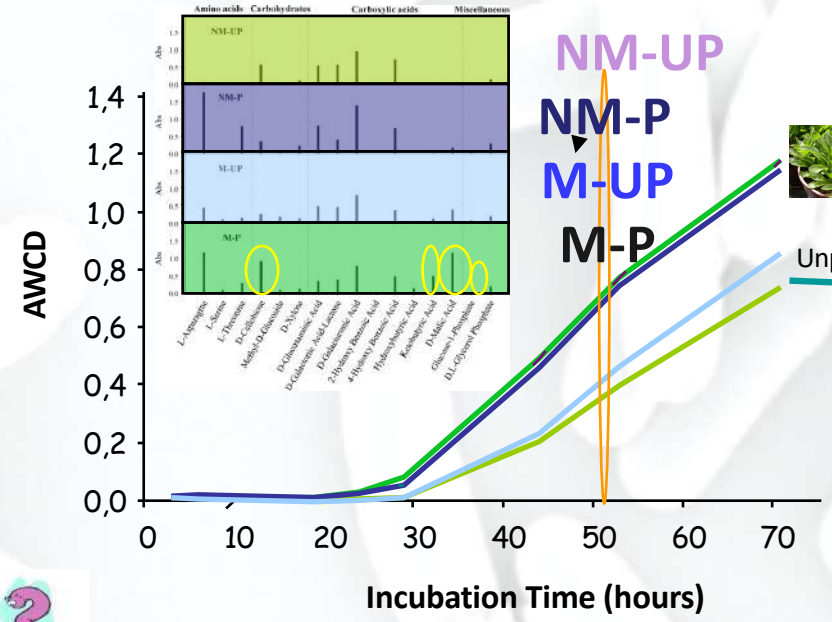
- Survival
- Biomass
- NRU

Plants (*Lactuca sativa*)

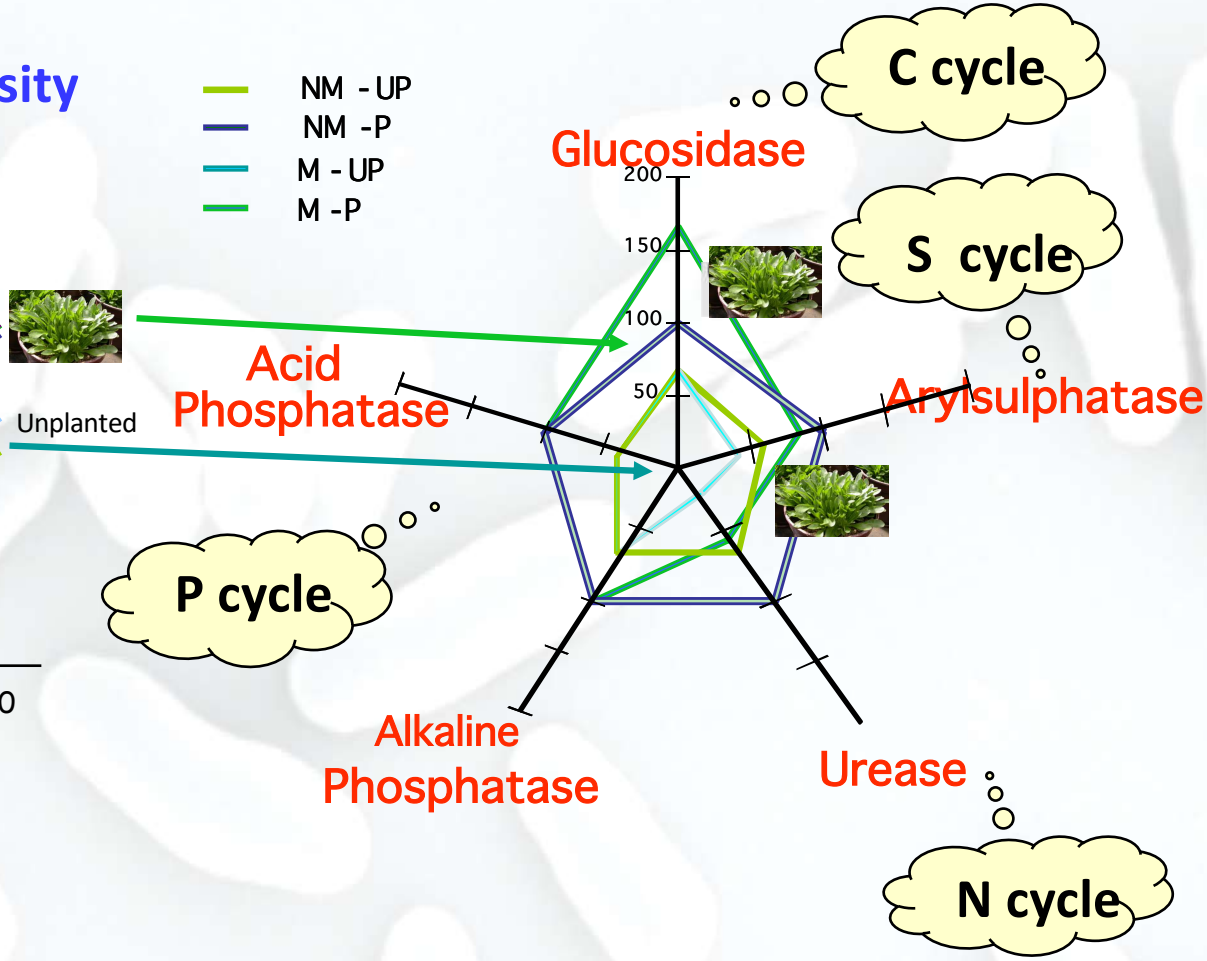
- Bio-metric parameters
- Antioxidant response

Soil microbial biomarkers

Microbial Functional diversity



Key Soil Enzyme Activities

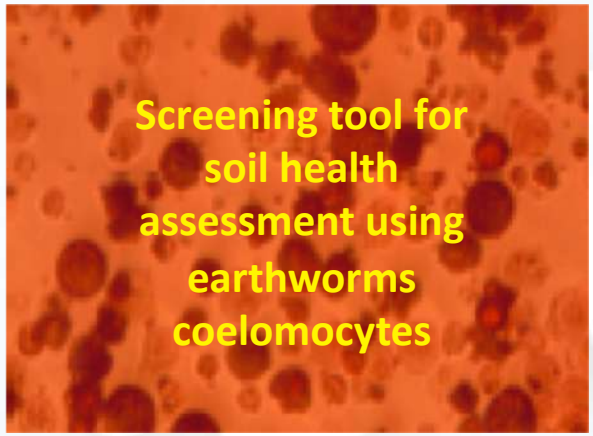


Decrease of **extractable and total metal content, and presence of *T. caerulescens* plants had the major effect on soil microbial parameters**

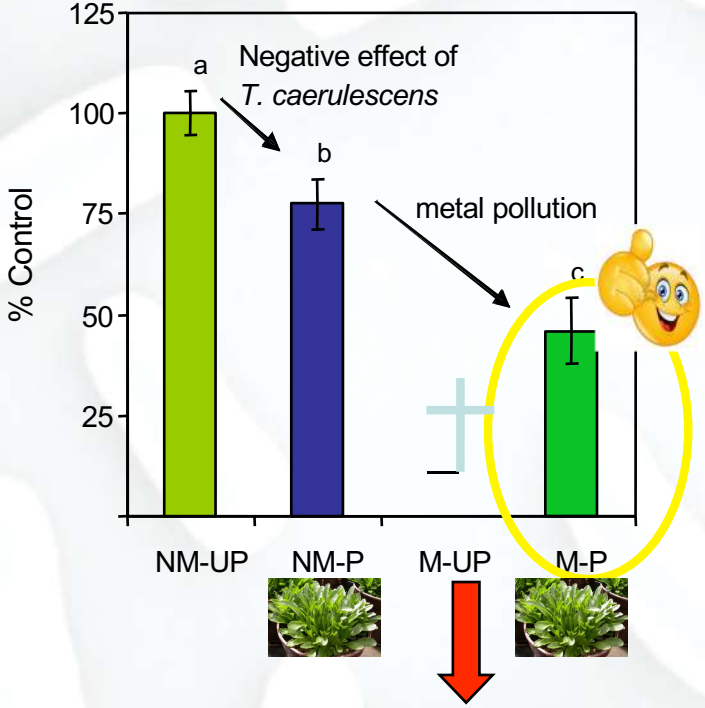
Soil health recovery assessment at the end of the experiment

Animal (earthworm) biomarkers

Neutral Red Uptake

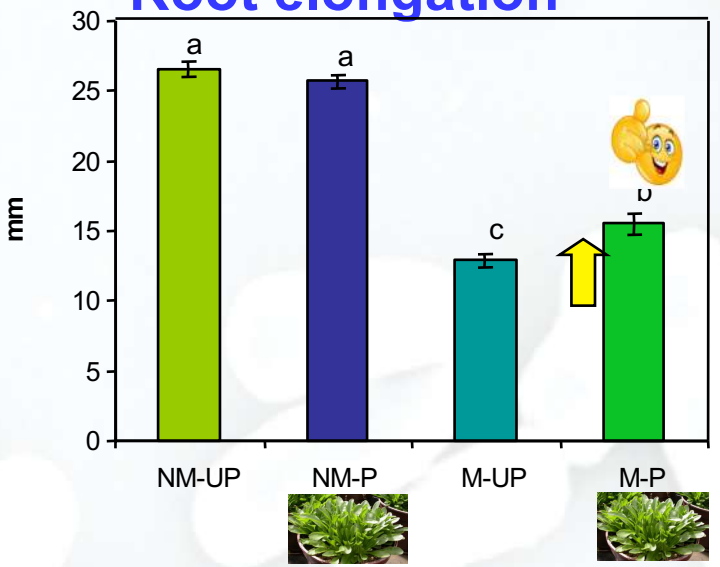


	Survival (%)	Weight (mg)
NM-UP	100	238
NM-P	100	252
M-UP	16.6	220
M-P	83.3	238

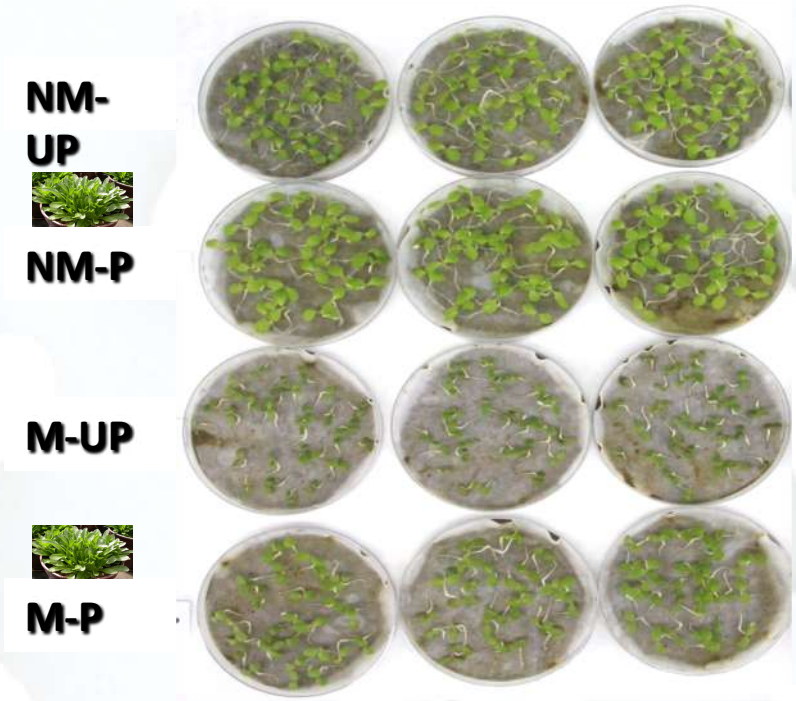


Plant (L. sativa) biomarkers

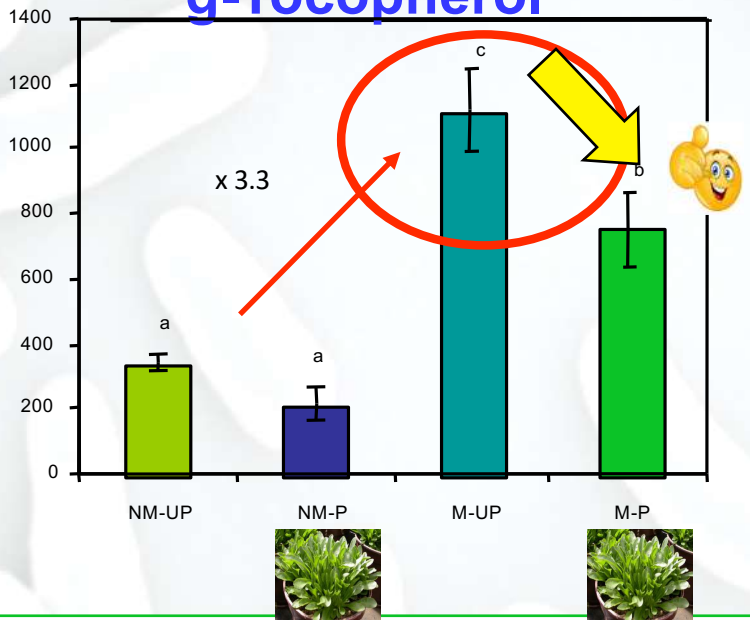
Root elongation



3 days exposure



g-Tocopherol



Phytoremediation reduced soil phytotoxicity
Phytoremediation reduced oxidative stress

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

INNOVATIVE ASPECTS

- Innovative **technologies for soil remediation:**

PHYTOREMEDIATION /vermiremediation

- Innovative **techniques for soil health and toxicity assessment: BIOLOGICAL INDICATORS**

Soil microorganisms

- Soil enzyme activities
- CLPPs

Earthworms (*Eisenia fetida*)

- Survival
- Weight
- NRU

Plants (*Lactuca sativa*)

- Bio-metric parameters
- Antioxidant response

***Ultimate goal*: "reduction or *elimination of pollutants* and *recovery of soil health*"**