

Phytoremediation as a biotechnological tool for environmental restoration

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Layout of the presentation

I – Phytoremediation: scope of application to environmental restoration

II – Case study I – tannery wastewater treatment

III - Case study II – restoration of industrial sediments

IV - Case study III – metal uptake from contaminated soil

V - Case study IV – sustainable forestry

I – Phytoremediation: scope of application to environmental restoration

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Phytoremediation

“...is an emergent technology that uses plants (and rhizosphere microorganisms) to remove, degrade or immobilize chemical contaminants from polluted soils, sediments and water”.



Phytoremediation – Why?

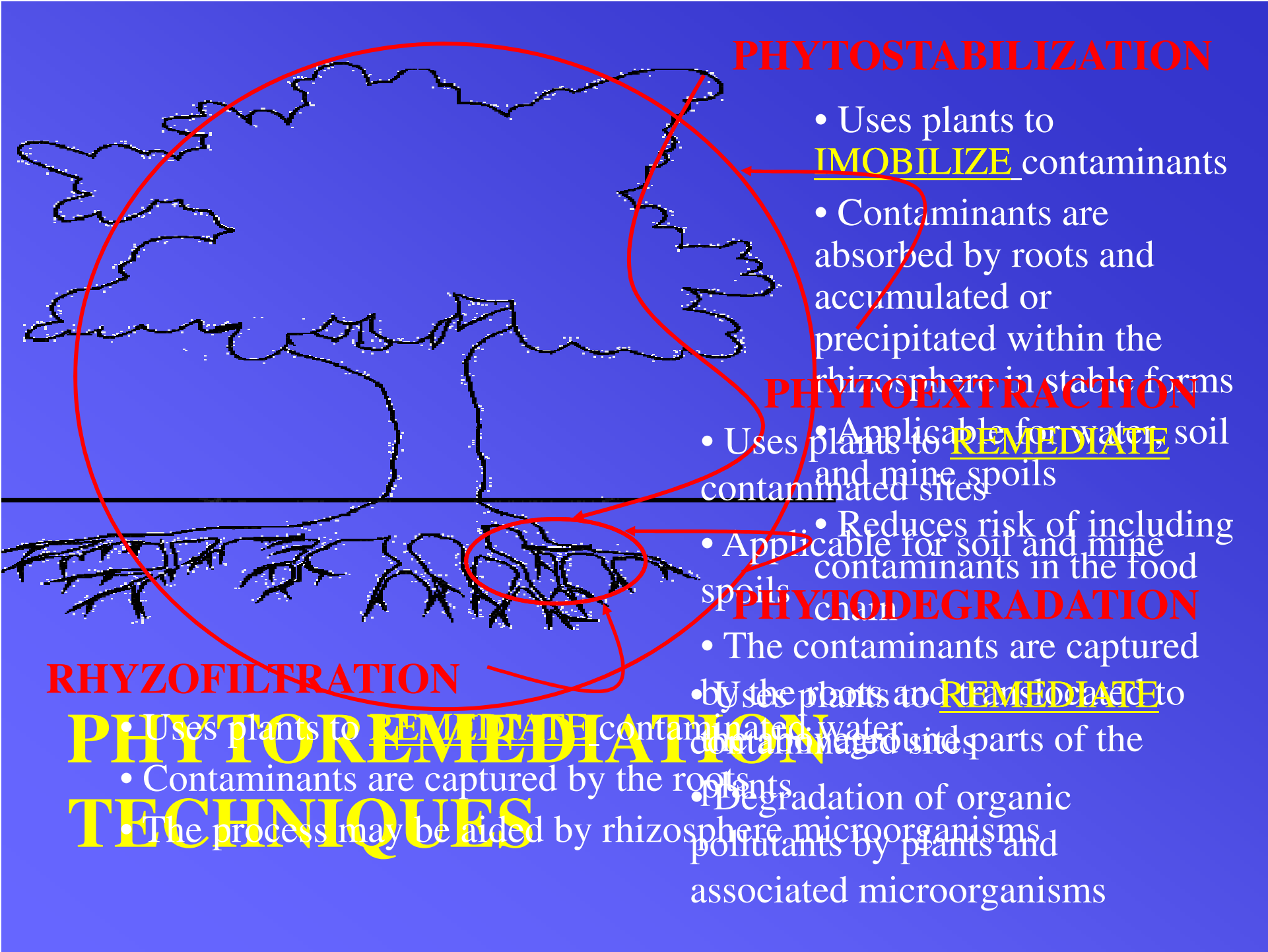
Advantages

- ✓ Performed *in situ*
- ✓ Cost reduction
- ✓ Acceptance from the public and regulatory agencies
- ✓ No disturbance to the landscape

**SUSTAINABLE
TECHNOLOGY**

Disadvantages

- ✗ High concentrations of the contaminant may become toxic to the plants
- ✗ Contamination risk of the food chain through animal consumption
- ✗ Takes long periods of time



PHYTOSTABILIZATION

- Uses plants to IMOBILIZE contaminants
- Contaminants are absorbed by roots and accumulated or precipitated within the rhizosphere in stable forms

PHYTOEXTRACTION

- Uses plants to REMEDiate contaminated sites
- Applicable for water and mine spoils
- Reduces risk of including contaminants in the food chain

PHYTODEGRADATION

- The contaminants are captured by the plants and REMEDiate to

RHYZOFILTRATION

- Uses plants to REMEDiate contaminated water
- Contaminants are captured by the roots
- The process may be aided by rhizosphere microorganisms

PHYTOREMEDIATION TECHNIQUES

- Degradation of organic pollutants by plants and associated microorganisms

Phytoremediation - water and soil

Constructed
wetlands



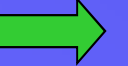
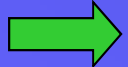
Diferent plants
and growth
substrates



Wastewater
treatment



Mycorrhizal
fungi



Isolation

Field trials



Plant growth
on stressed
and polluted
sites



Metal uptake by
local plants



Effect of mycorrhiza



I – Phytoremediation: scope of application to environmental restoration

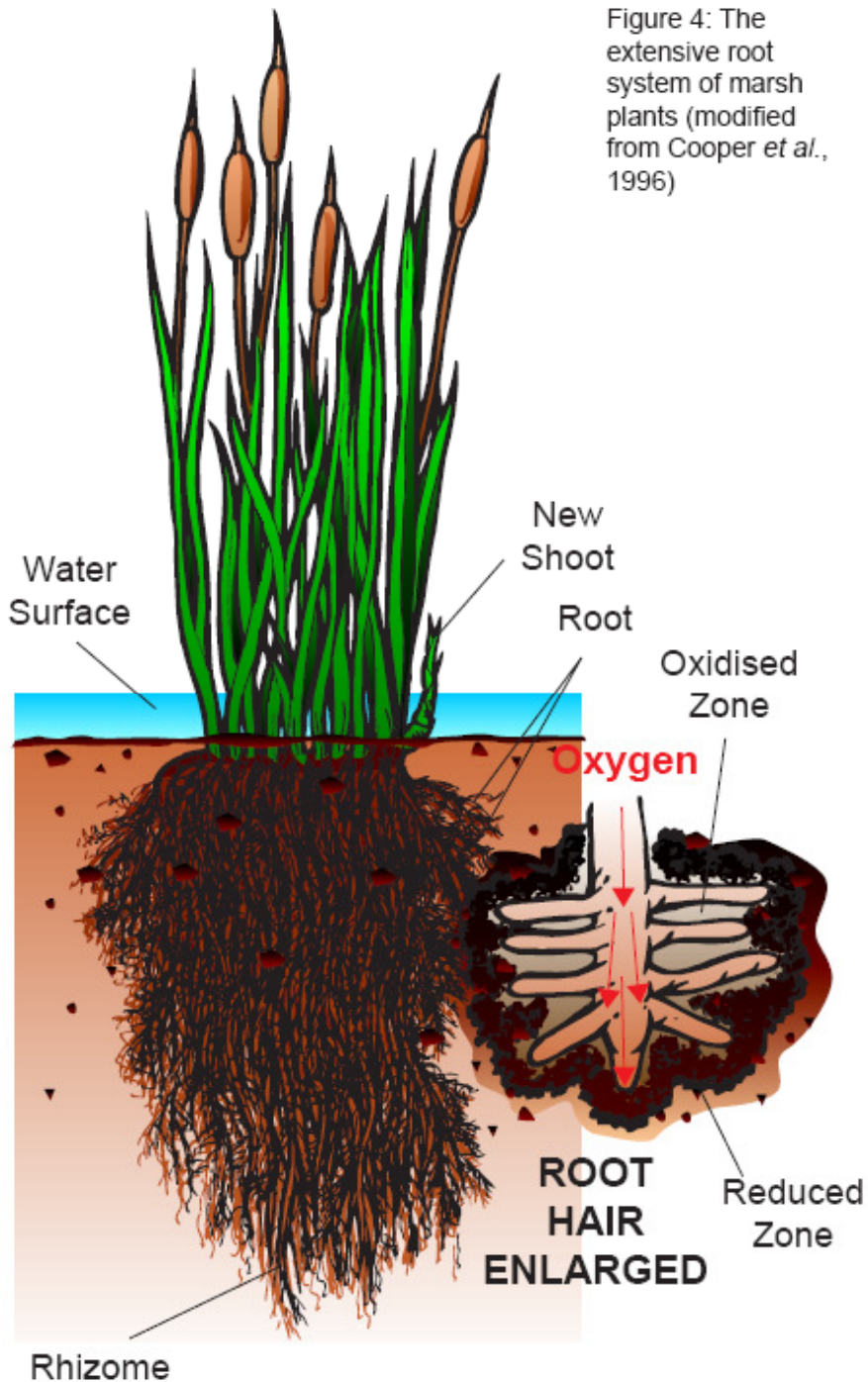
II – Case study I – tannery wastewater treatment

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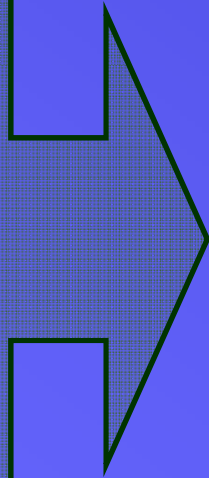
Figure 4: The extensive root system of marsh plants (modified from Cooper *et al.*, 1996)



Removal of organic and inorganic contamination from wastewater

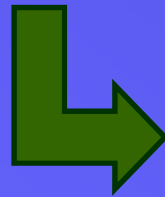
Microbial degradation in bulk soil or rhizosphere

Case study: Tannery industry



Wastewater:

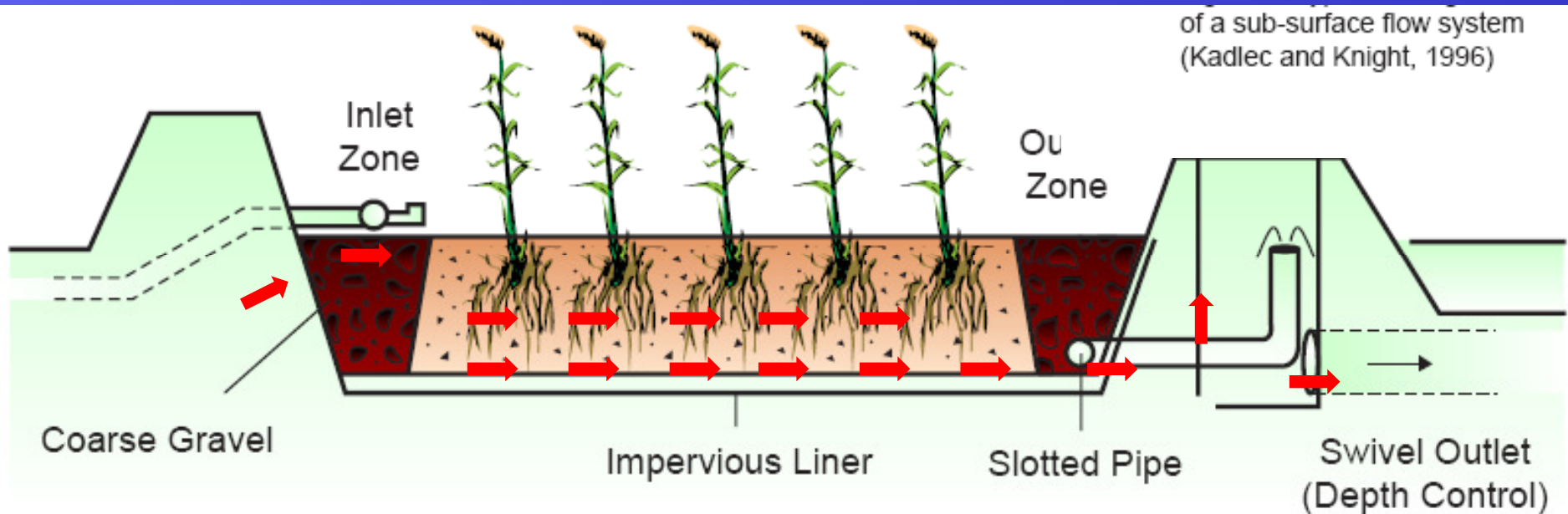
- High organic loading
- Suspended solids
- Chromium



Phytotoxic effluent

Constructed wetlands

Sub-surface Horizontal Flow



Typical configuration of a sub-surface flow system
(Kadlec and Knight, 1996)

Plant species



Typha latifolia
(cattail)



Typha latifolia (cattail)

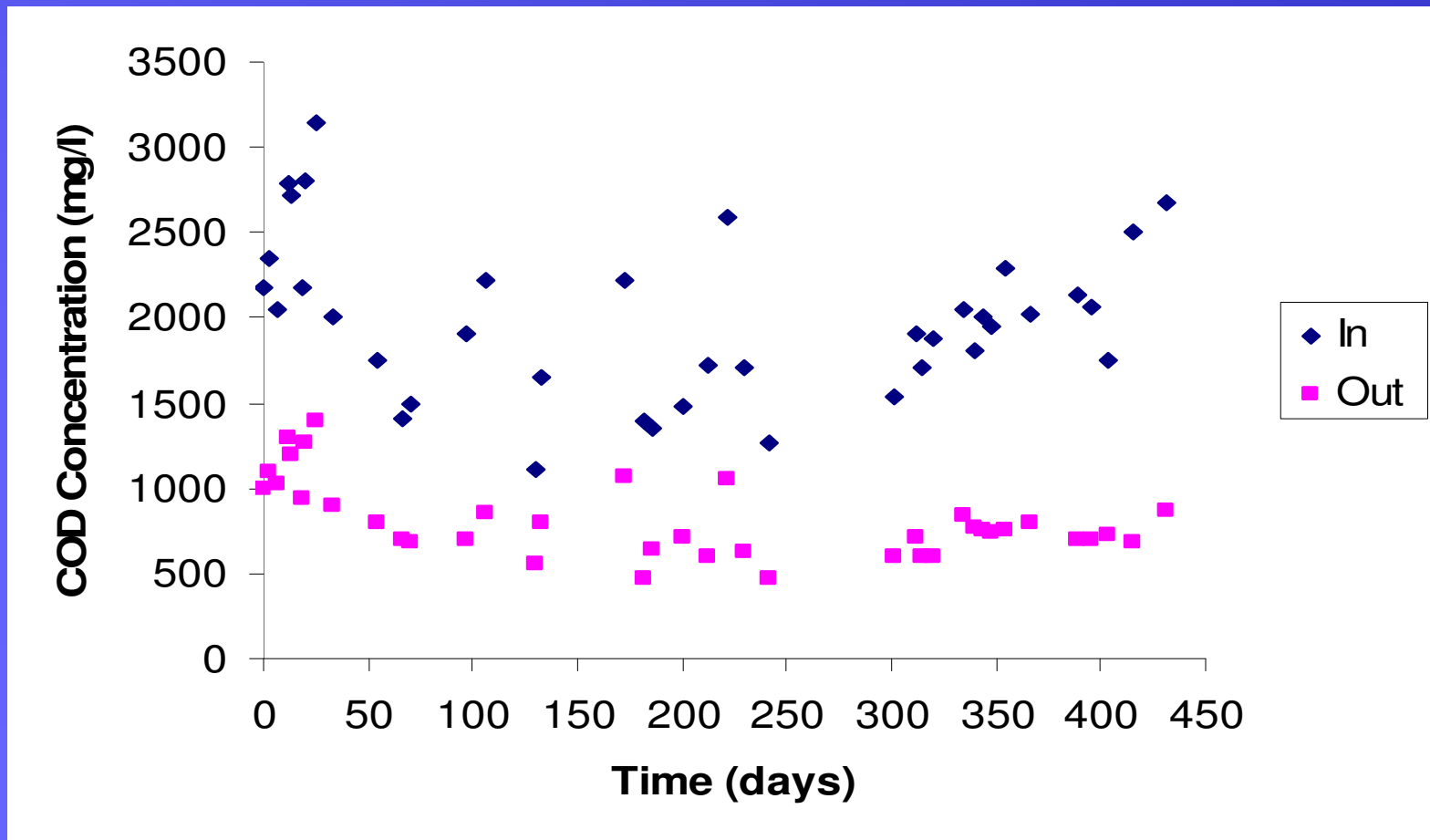


Iris pseudacorus

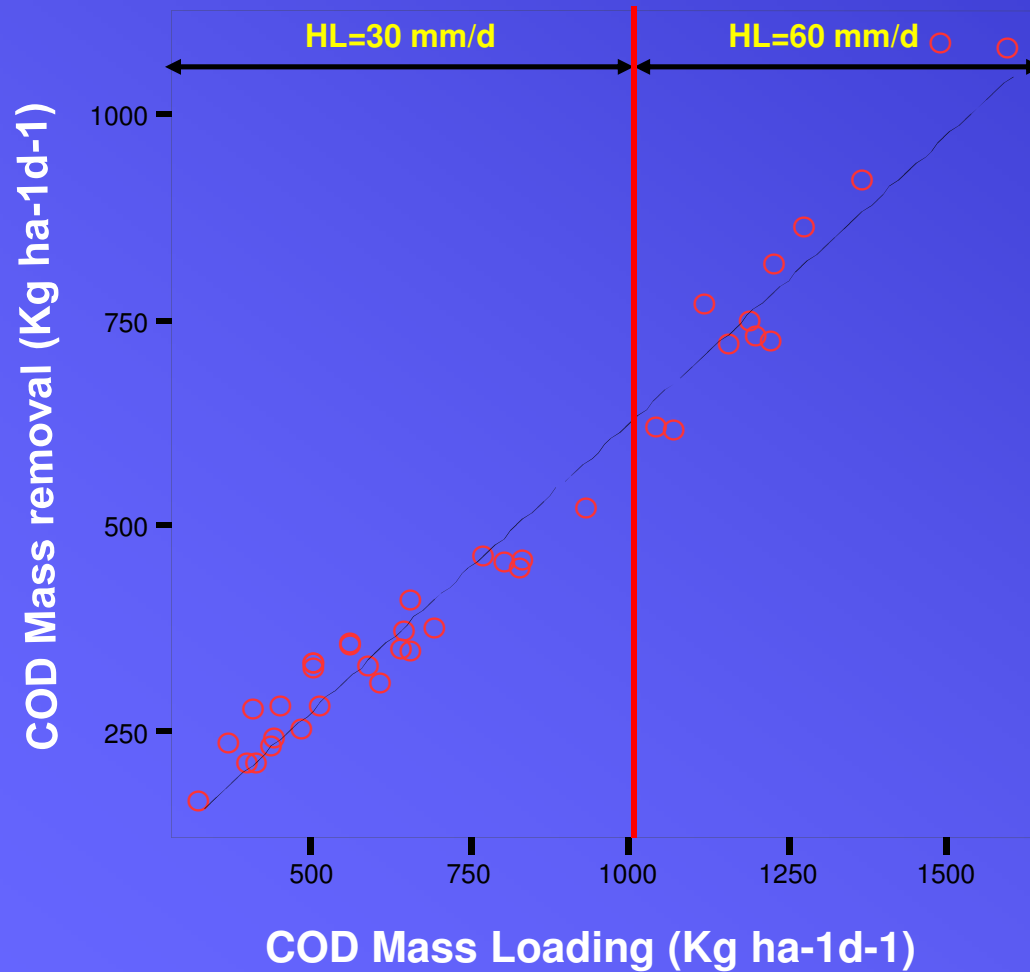


Phragmites australis
(common reed)

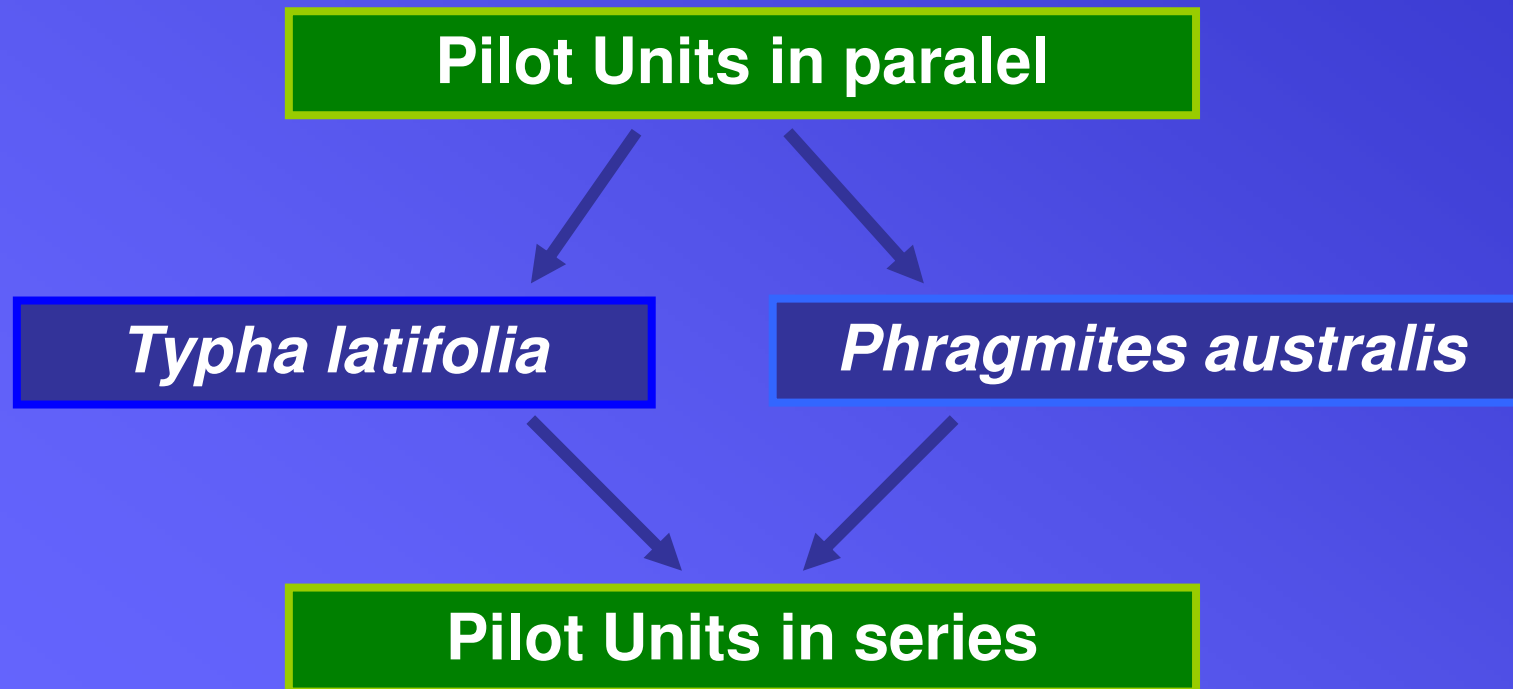
Efficiency of the pilot units - example



Removal efficiency for organic loading



Pilot Units



Pilot Units – *Typha latifolia*



Water characterization Series I

4 - 7
July
2005


3rd EUROPEAN
BIOREMEDIATION
CONFERENCE

Hidraulic loading	60mm/d → 480 Kg BOD ₅ ha ⁻¹ d ⁻¹				
Parameters	In	Out 1		Out 2	
pH	6.57(8.46-5.25)	8.15(8.38-7.95)		8.11(8.25-7.90)	
COD, mgO ₂ l ⁻¹	1297(1455-1091)	523(600-480)	59%	240(268-225)	54%
BOD ₅ , mgO ₂ l ⁻¹	720 (800-660)	355 (390-320)	51%	175 (195-160)	51%
TSS, mg l ⁻¹	35(43-26)	10(12-8)	72%	5 (6-4)	52%
Total P, mgP l ⁻¹	0.66(0.95-0.26)	0.64(0.96-0.26)		0.62(0.82-0.27)	

Water characterization Series I

4 - 7
July
2005

3rd EUROPEAN
BIOREMEDIATION
CONFERENCE

Hidraulic loading	180mm/d  1700 kgBOD ₅ ha ⁻¹ d ⁻¹				
Parameters	In	Out 1		Out 2	
pH	7.83(8.10-7.66)	8.23(8.65-8.09)		8.21(8.55-8.10)	
COD, mgO ₂ l ⁻¹	1579(2138-1082)	868(1200-570)	45%	595(840-380)	32%
BOD ₅ , mgO ₂ l ⁻¹	797(960-720)	570(670-520)	22%	446(523-410)	21%
TSS, mg l ⁻¹	54(63-42)	12(14-9)	78%	4(4-3)	66%
Total P, mgP l ⁻¹	0.24(0.30-0.15)	0.24(0.32-0.17)		0.27(0.40-0.16)	

4 - 7

July

2005

Pilot Units - *Phragmites australis*


3rd EUROPEAN
BIOREMEDIATION
CONFERENCE



Water characterization Series II

4 - 7
July
2005


3rd EUROPEAN
BIOREMEDIATION
CONFERENCE

Hidraulic loading	60mm/d  480 Kg BOD ₅ ha ⁻¹ d ⁻¹				
Parameters	In	Out 1		Out 2	
pH	6.57(8.46-5.25)	7.94(8.33-5.84)		8.17(8.38-7.99)	
COD, mgO ₂ l ⁻¹	1297(1455-1091)	530(590-500)	59%	249(265-235)	53%
BOD ₅ , mgO ₂ l ⁻¹	720 (800-660)	363(400-333)	50%	181(200-160)	50%
TSS, mg l ⁻¹	35(43-26)	10(13-7)	71%	6(6-5)	52%
Total P, mgP l ⁻¹	0.66(0.95-0.26)	0.62(0.86-0.25)		0.58(0.72-0.27)	

Water characterization Series II

4 - 7
July
2005

3rd EUROPEAN
BIOREMEDIATION
CONFERENCE

Hidraulic loading	180mm/d  1700 kg BOD ₅ ha ⁻¹ d ⁻¹			
Parameters	In	Out 1		Out 2
pH	7.83(8.10-7.66)	8.19(8.54-8.06)		8.25(8.61-8.07)
COD, mgO ₂ l ⁻¹	1579(2138-1082)	905(1189-650)	42%	628(820-450) 31%
BOD ₅ , mgO ₂ l ⁻¹	797(960-720)	578(700-530)	27%	456(550-412) 21%
TSS, mg l ⁻¹	54(63-42)	11(15-8)	80%	4(5-3) 65%
Total P, mgP l ⁻¹	0.24(0.30-0.15)	0.30(0.46-0.18)		0.25(0.32-0.20)

Instalação dos leitos

unidades piloto: *Typha latifolia*

Carga hidraulica

Entrada (Kg/had)

saida U1

saida U2

180mm/d

3800-1950

48-41%

33-30%

60mm/d

870-650

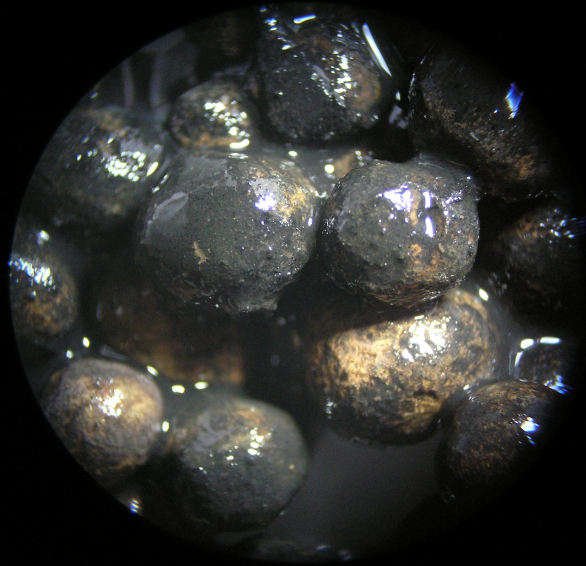
66-52%

60-50%

Instalação dos leitos

unidades piloto: *Phragmites australis*

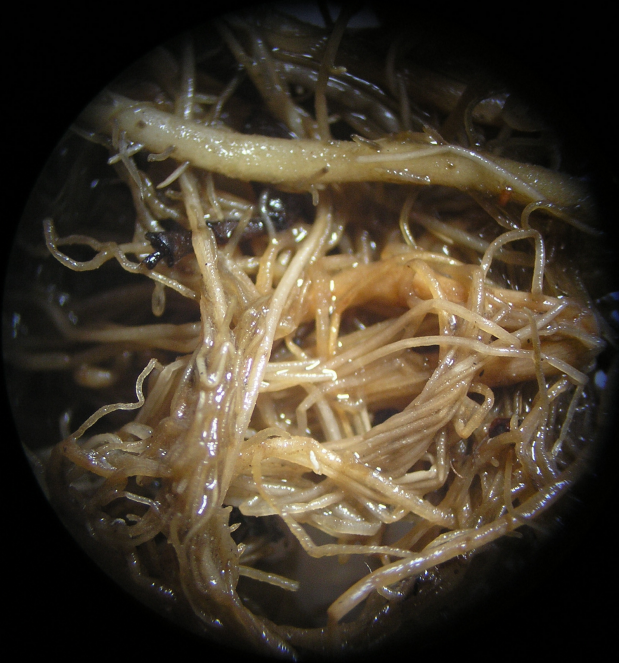
Carga hidraulica	Entrada (Kg/had) Conc	Conc saida U1	Conc saida U2
180mm/d	3800-1950	(47-39%)	(32-29%)
60mm/d	870-650	(64-52%)	(56-50%)



Filtralite MR



Typha



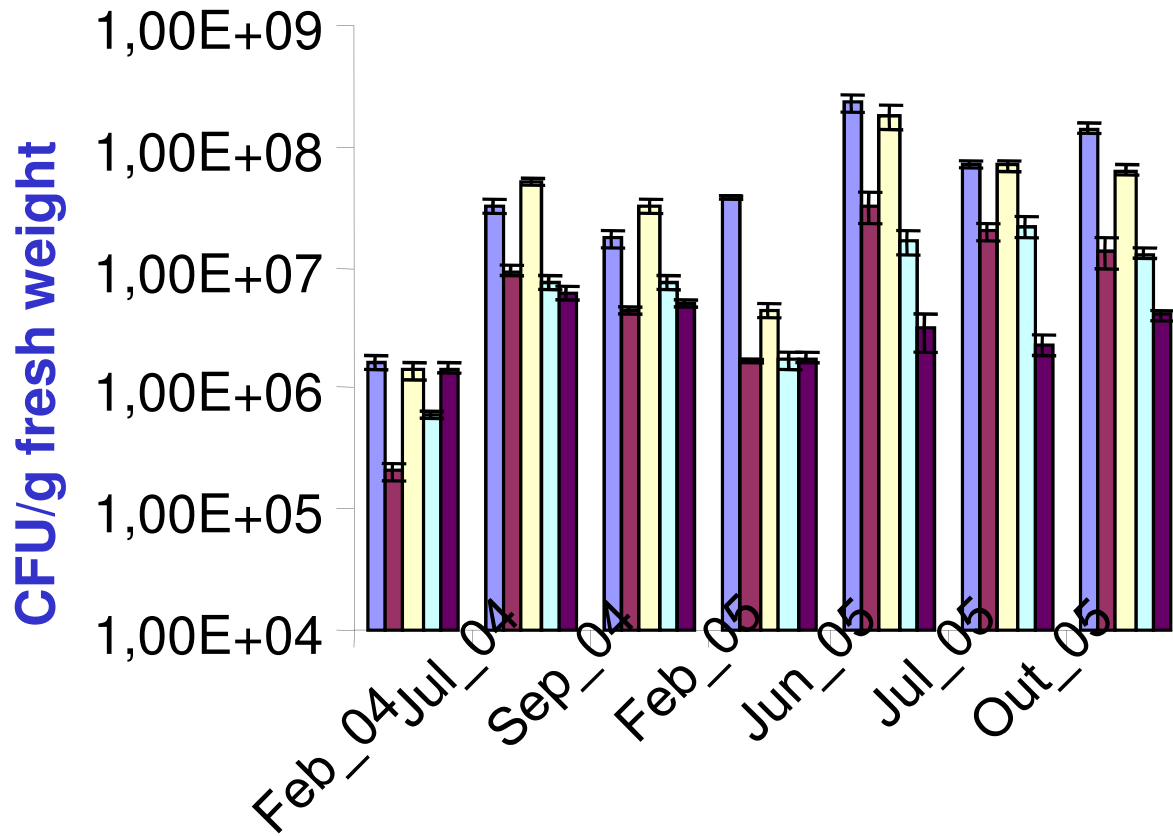
Typha



Phragmites

Culturable bacteria in the pilot units

- Typha root
- Typha substrate
- Phragmites root
- Phragmites substrate
- control substrate



DGGE analysis is underway

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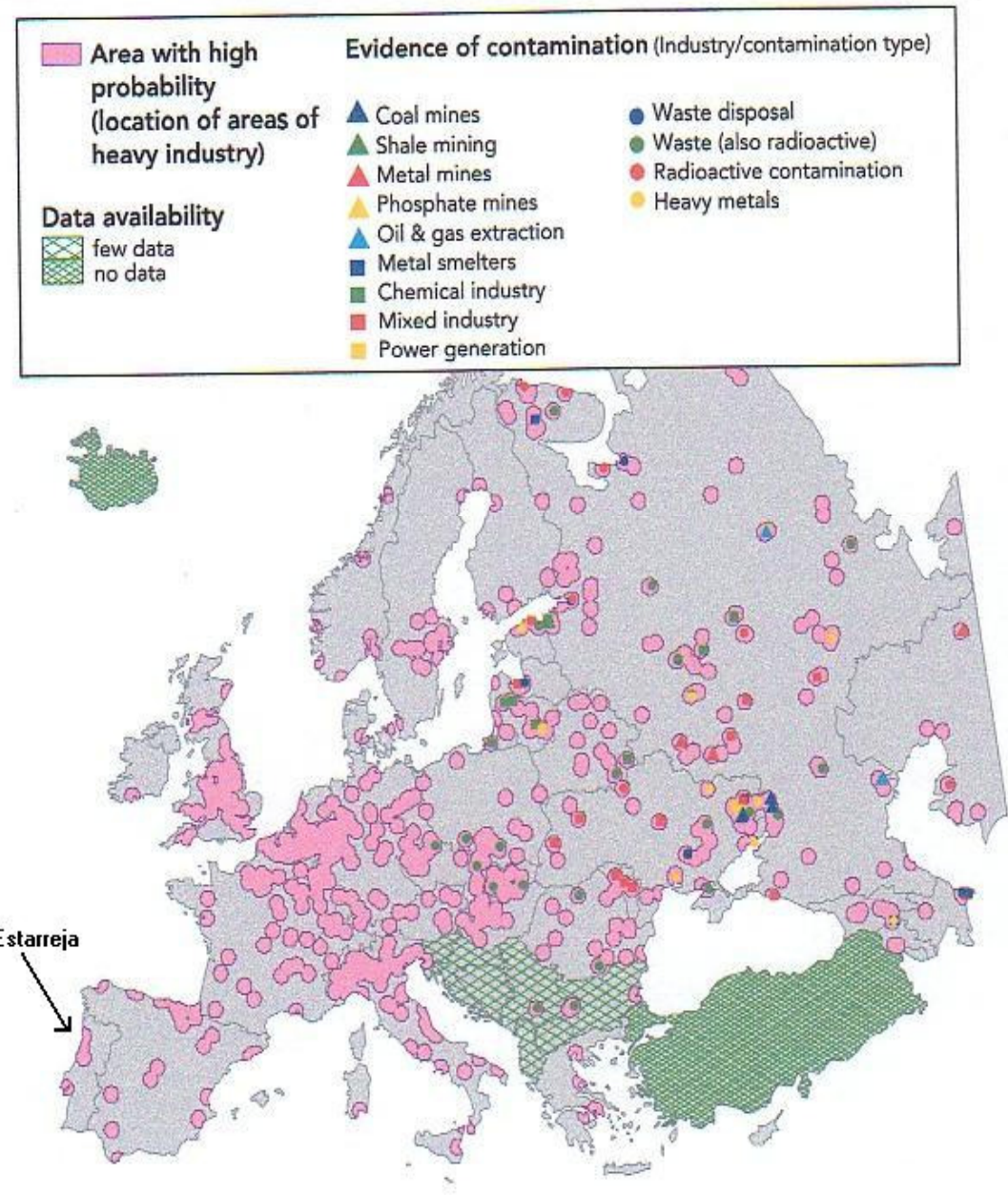
V - Case study IV – sustainable forestry

Estarreja – Industrial site

Cerca de 2 mil milhões
ha de solo sofrem de
degradação
antropogénica na
Europa e a recuperação
de solos degradados
pode tornar-se a
“prioridade do século
para a sustentabilidade”

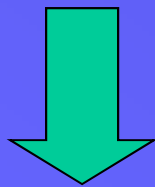
Figure 1

Probable problem areas of local
contamination in Europe

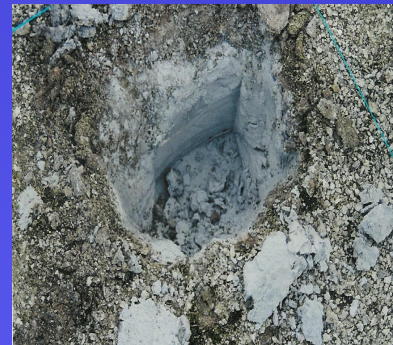


Industrial sediment

- Acetylene and PVC production
- $\text{Ca}(\text{OH})_2$ residues
- 300 000 ton, 10 ha
- High pH (11.8-12.6) and salinity
- Low nutrient levels (ex. N e P)



Scarce vegetation and limited plant diversity



Phytorestoration
Phyto-microbial complexes

Phytotechniques

Plants have to grow
under stress....

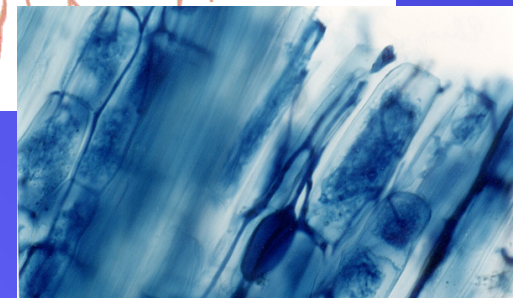
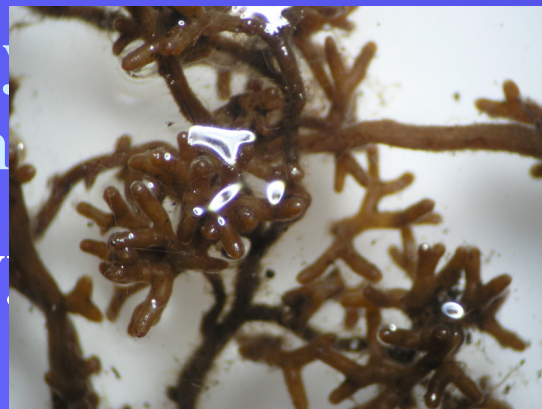
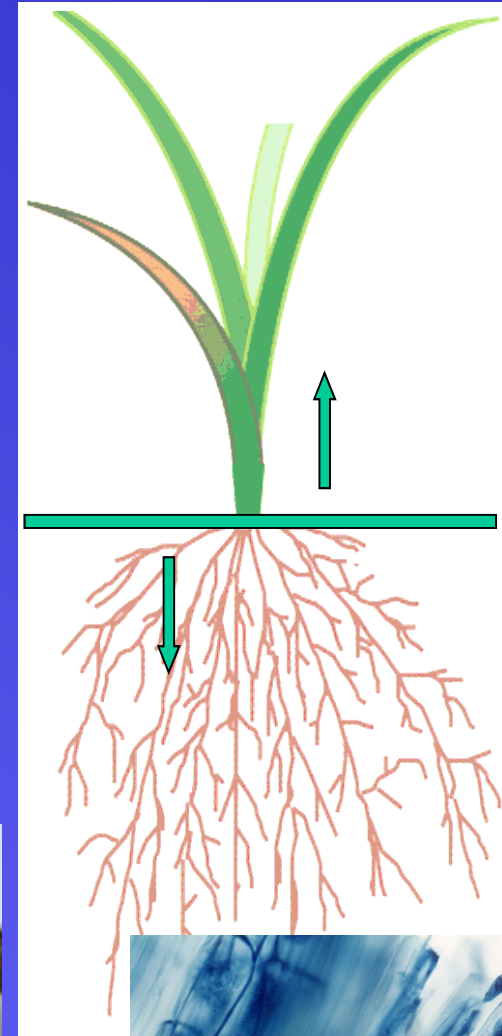


Symbiosis between
adapted plants, bacteria
and fungi...

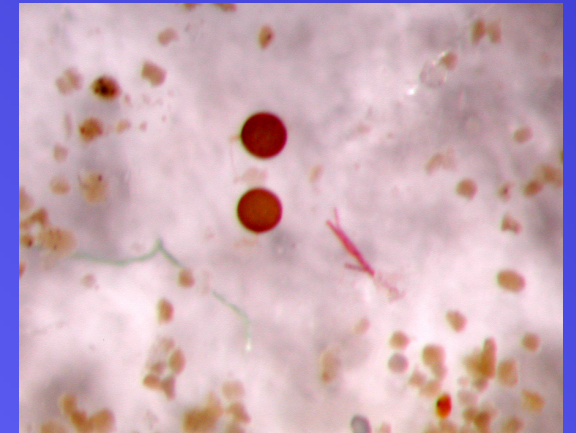
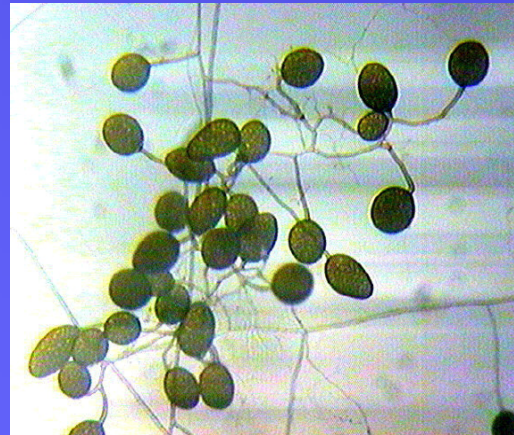


Mycorrhizal fungi are crucial components

- Group of soil microorganisms
- Form symbiotic associations with plants roots
- Improve plant growth and reproduction
- Capture mineral nutrients from soil
- Receive carbohydrates from associated plants
- Influence heavy metal uptake



Survey and isolation of arbuscular mycorrhizal fungi



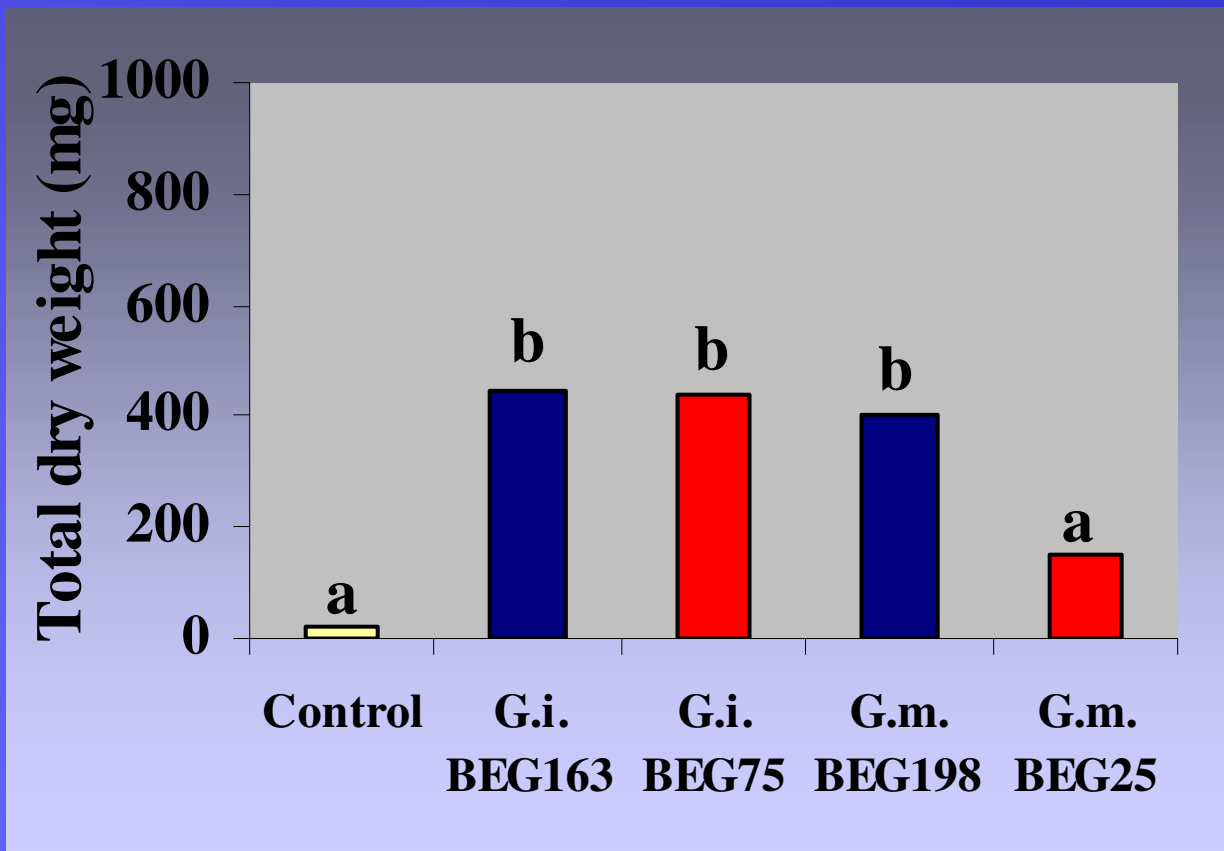
Glomus intraradices *Glomus geosporum*

Study of the effect of native and non-native AMF on plant establishment in the industrial sediment



Inoculated with native and non-native *G. intraradices* and *G. geosporum*





AMF play a critical role in the growth and establishment of native plant species (ex. *Conyza bilbaoana*)

The use of adapted AMF as inoculants is recommended for the phytorestoration of the highly alkaline anthropogenic sediment

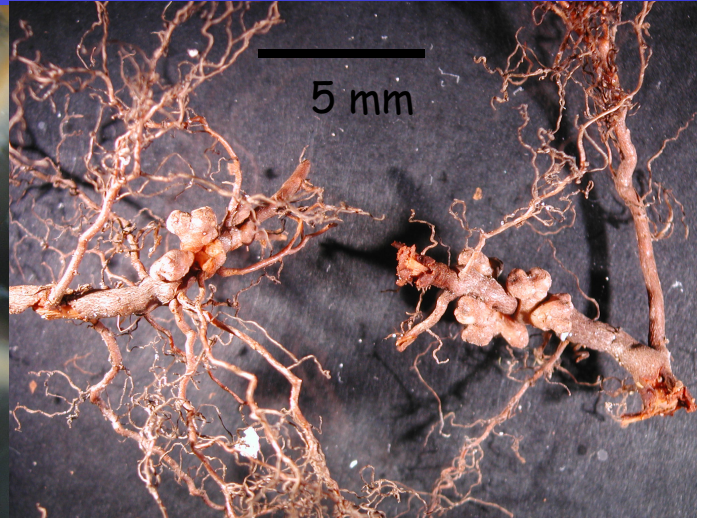
Study of the effect of AMF and bacteria on plant establishment in the industrial sediment



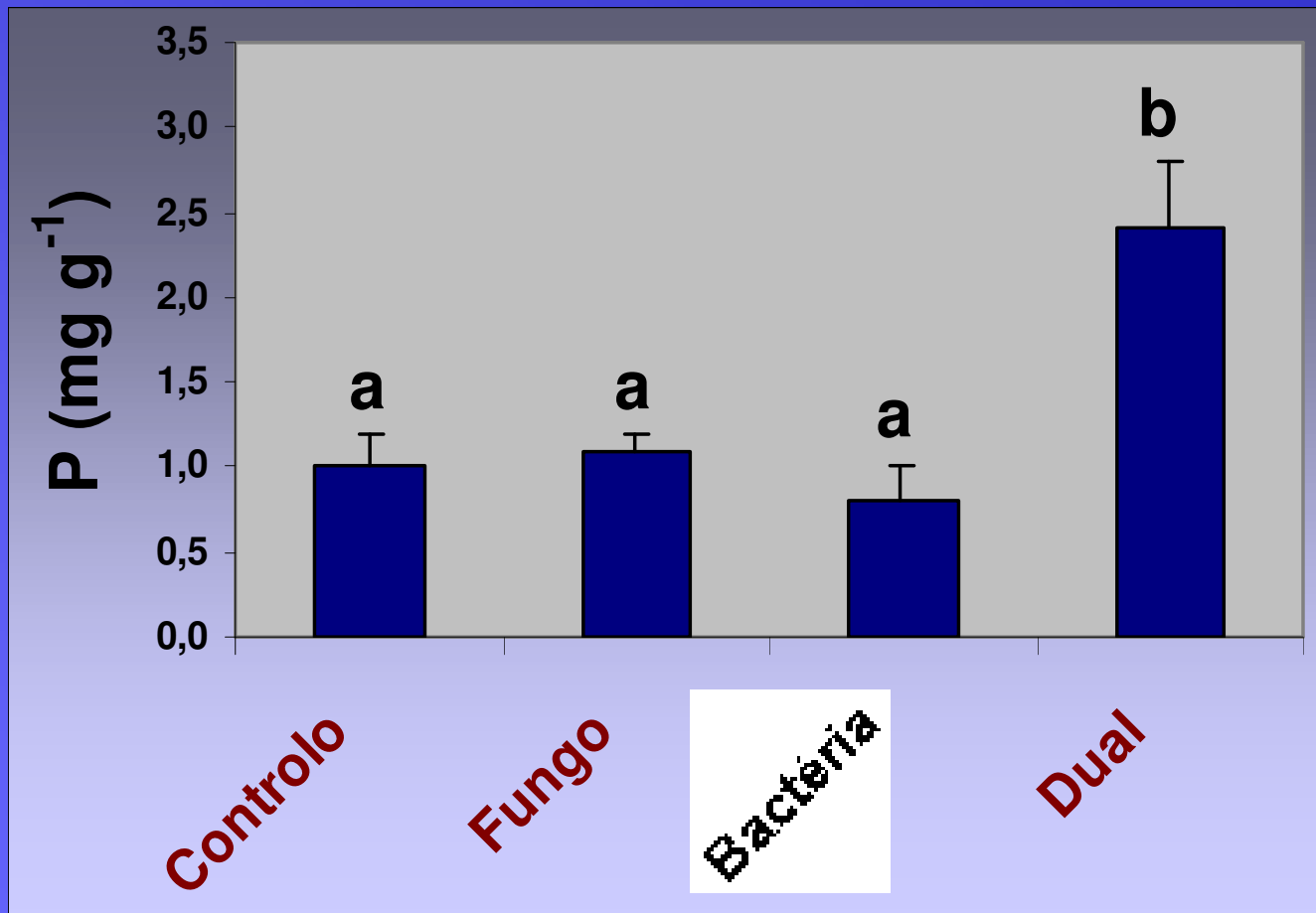
Alnus glutinosa



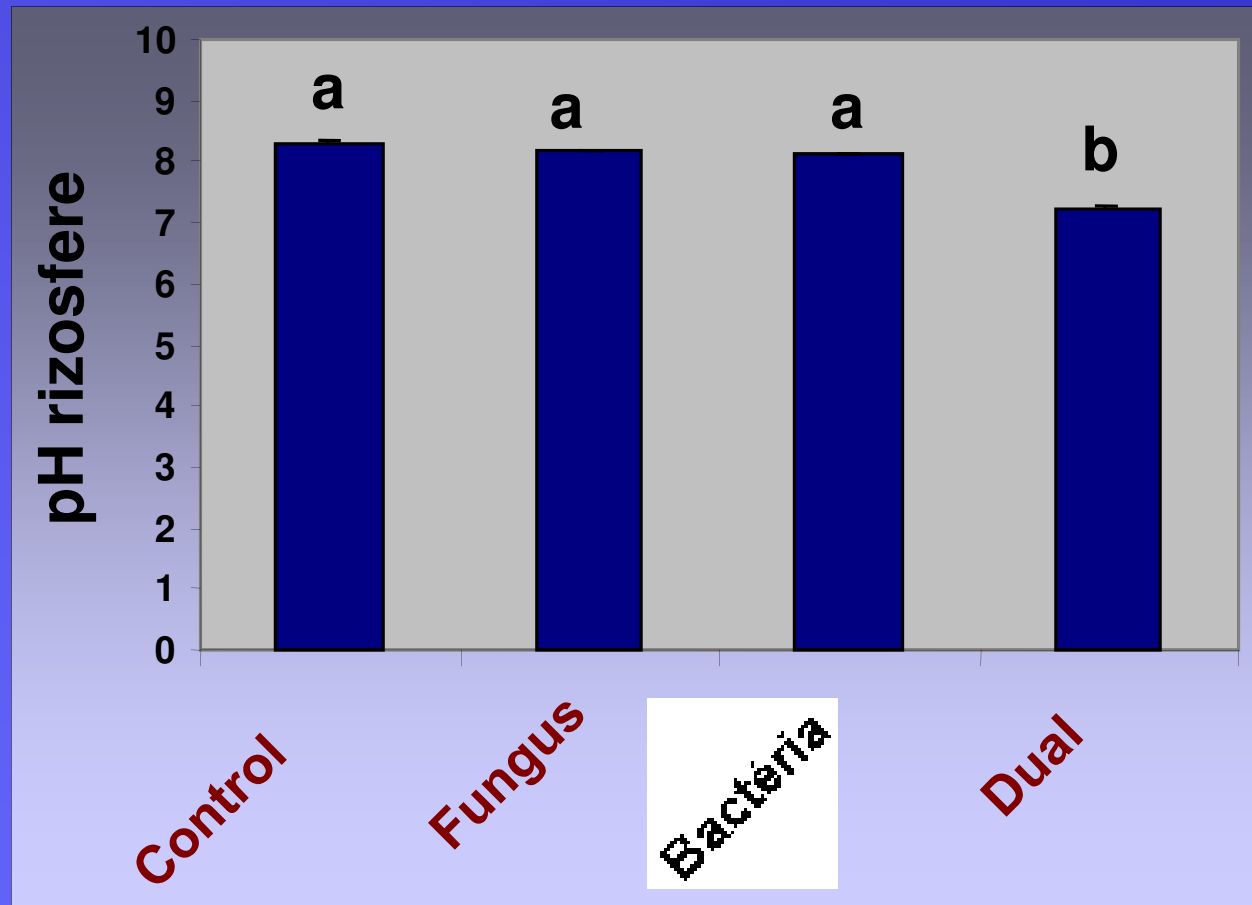
Glomus intraradices



Frankia sp.



Synergistic effect between AMF and nitrogen fixing bacteria (*Frankia*) led to improved plant benefit in phosphorus nutrition



Synergistic effect between AMF and nitrogen fixing bacteria (*Frankia*) led to an alleviation of high pH stress by reducing rhizosphere pH

Long-term field trial on-going





Plants still growing on the site

I – Phytoremediation: scope of application to environmental restoration

II – Case study I – tannery wastewater treatment

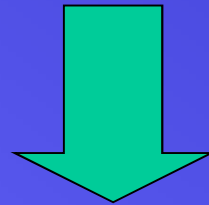
III - Case study II – restoration of industrial sediments

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Heavy metal contaminated soil

- ↪ Discharge of solid residues in the surrounding area
- ↪ Conducting of industrial wastewaters into a stream nearby (“Esteiro de Estarreja”)



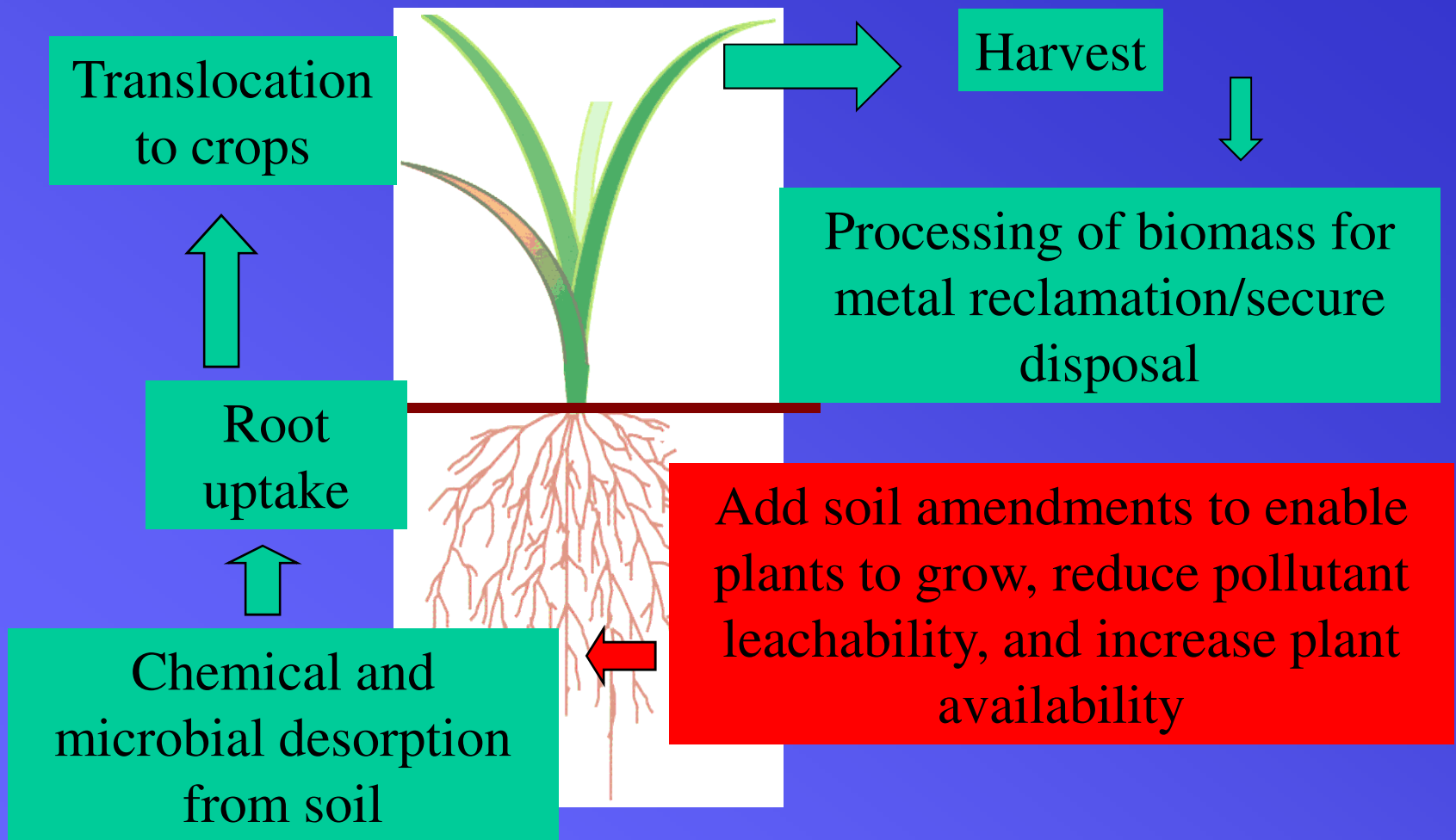
Total metal in the banks (mg/ Kg soil)

Zn	898.9 (125-3620)
Pb	835.4 (16-3740)
Hg	66.6 (0.3-275)
As	1495 (45-5620)



Levels higher than European legislation

Plant based decontamination of Metal-contaminated soils



Factors to take in account

Plant

- Rapid growth
- High biomass rate
- Capacity to tolerate and accumulate the contaminant
- Adequate root length
- Adequate to the nature of the contamination

Contaminant

- Bioavailability of the contaminant
- Existence of multiple contamination

Survey of local plants



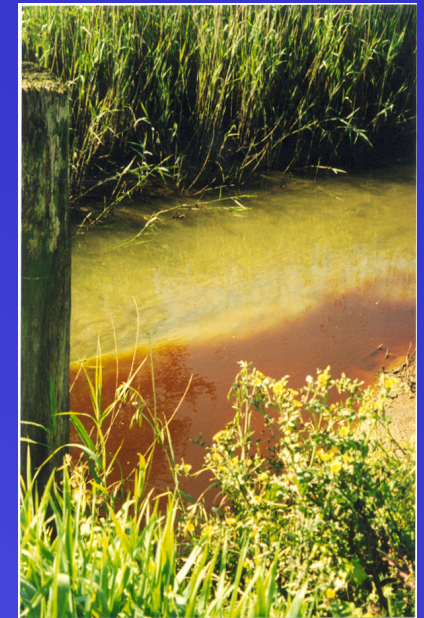
Rubus ulmifolius



Convolvulus sp.



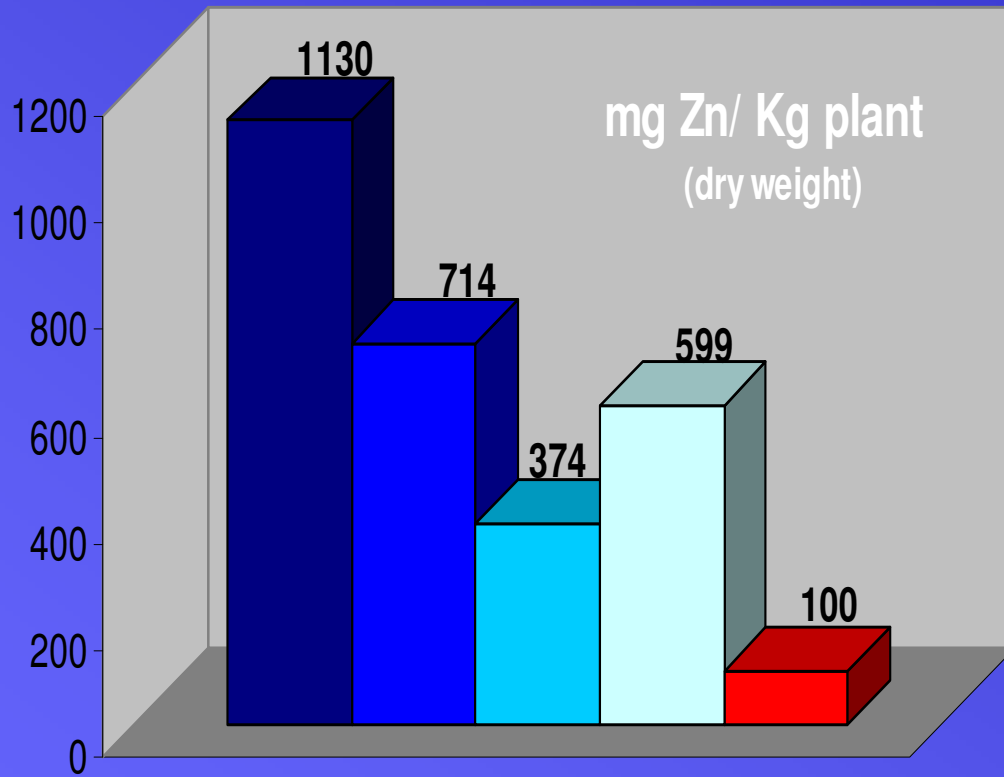
Phragmites australis



Solanum nigrum

Levels of elements in the plants

Plant \ Metal	Lead (mg/Kg dry weight)	Zinc (mg/Kg dry weight)	Arsenic (mg/Kg dry weight)	Mercury (mg/Kg dry weight)
<i>Solanum nigrum</i>	2.6	1130	5.4	9.1
<i>Rubus ulmifolius</i>	6.0	714	31.2	0.5
<i>Phragmites australis</i>	2.7	374	2.9	12.7
<i>Convolvulus sp.</i>	2.8	599	2.3	1.6



- Zn -

➤ High levels of **Zn** were detected in all the plants, especially on *Solanum nigrum*,

	Zn
<i>Solanum nigrum</i>	1130
<i>Rubus</i> sp.	714
<i>Phragmites australis</i>	374
<i>Convolvulus</i> sp.	599
Average maximum levels in plants not submitted to contamination	100

Growth experiments with *Solanum nigrum* exposed to several Zn levels and different mycorrhizal fungi

Zn= 0 ppm		matrix=sand			
fungus A	fungus B	fungus C	fungus D	fungus E	No fungi

Zn= 100 ppm		matrix=sand			
fungus A	fungus B	fungus C	fungus D	fungus E	No fungi

Zn= 500 ppm		matrix=sand			
fungus A	fungus B	fungus C	fungus D	fungus E	No fungi

Zn= 1000 ppm		matrix=sand			
fungus A	fungus B	fungus C	fungus D	fungus E	No fungi

Soil from the banks of "Esteiro de Estarreja" (426± 2 ppm)					
fungus A	fungus B	fungus C	fungus D	fungus E	No fungi



fungus A - *Glomus* sp. BEG140
isolated from a soil with high levels of Mn

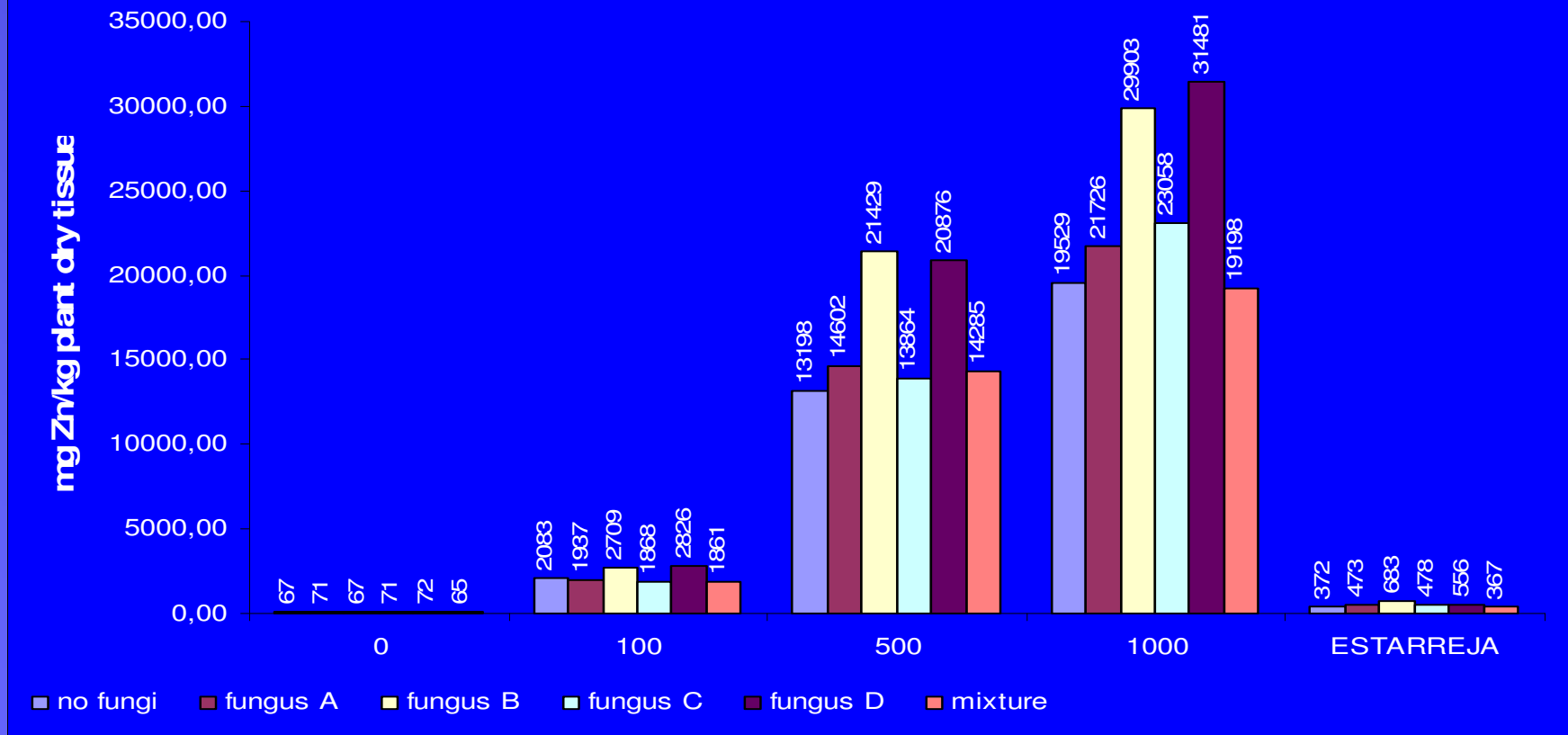
fungus B - *Glomus claroideum*
isolated from a soil with high levels of Cd and Zn

fungus C - *Glomus mosseae*
isolated from a soil with high levels of Cd and Zn

fungus D - *Glomus intraradices*
isolated from a soil with high levels of Pb

fungus E= mixture of all the isolates

Zn accumulation in the tissues of the total plant



Inoculation with *G. claroideum* and *G. intraradices*, enhanced zinc accumulation in *S. nigrum*.



The stem tissues had the higher Zn content, followed by the roots, with the leaves registering the lowest values.

Zinc level in the matrix	Percent increase in the Zn accumulation levels in the tissues (%)	
	<i>Glomus claroideum</i>	<i>Glomus intraradices</i>
100	30	36
500	62	58
1000	53	61
Estarreja	83	49

The application of *S. nigrum* with the assistance of the AMF *G. claroideum* and *G. intraradices* in the phytoremediation of contaminated soil from “Esteiro de Estarreja” is currently being tested in different conditions:

- amendment with quelating agents (EDTA and EDDS)
- amendment with organic matter sources (manure and sludges from wastewater treatment)



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Sustainable plant production in forestry



