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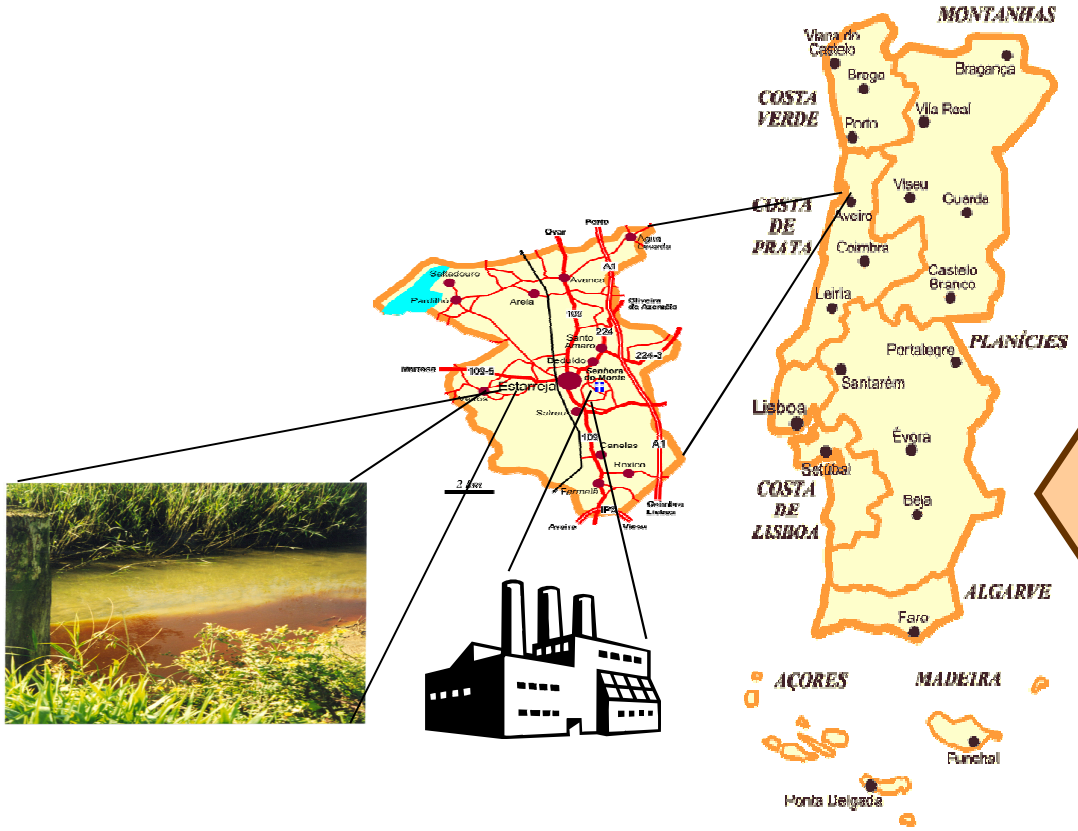
HEAVY METAL ACCUMULATION AND RELATION WITH SOIL CONTAMINATION IN *RUBUS ULMIFOLIUS* GROWING IN ESTEIRO DE ESTARREJA, PORTUGAL

Ana P. G. C. Marques, Helena Moreira, António O. S. S. Rangel, Paula M. L. Castro

Site-specific constraints can render the known- to-date species used for phytoremediation inadequate

Enlargement of the data concerning plant species valuable for phytoremediation



Investigation of the potential for phytoremediation of plants indigenous to a historically HM contaminated site: Esteiro de Estarreja

✓**Discharge of solid residues in the surrounding area, with consequent contamination of the aquiferous;**

✓**Conducting of the wastewaters of the factories into the stream nearby ("Esteiro de Estarreja").**

✓**High permeability of the soils**

➢**Levels of metals in the sediments of the stream remain above the limits established by EC Directive 86/278/EC**

➢**The contamination occurs mainly in the top 20 cm layer of the soil and near the former exit of wastewaters**

General view of the stream

Total metal (mg/ kg soil)

Zn	898.9 (125-3620)
Pb	835.4 (16- 3740)
As	1495 (4 5- 5620)
Fe	16.8
Cr	26.0
Ni	37.3
Cu	0

Source: Environmental Impact Studies in the area (1994)

➢**Survey of the main colonising species of Esteiro de Estarreja - *Phragmites australis*, *Rubus ulmifolius* and *Solanum nigrum* - and determination of their Zn accumulation**

AMF colonisation of the roots of the plants collected near the former wastewaters exit

Accumulation of Zn in the whole plant samples collected near the former wastewaters exit

	Pb	Zn	As
S. nigrum	2,6	1130	5,4
R. ulmifolius	6,0	714	31,2
P. australis	2,7	374	2,9

Survey of metal accumulation in plant species indigenous to Esteiro de Estarreja

Solanum nigrum **grown in Zn contaminated matrices : effect of AMF on Zn accumulation**

Application of manure and compost to contaminated soils and its effect on Zn accumulation by *Solanum nigrum* inoculated with AMF

EDDS and EDTA-enhanced Zn accumulation by *Solanum nigrum* inoculated with AMF grown in contaminated soil

Zn, As, Pb and Ni accumulation in Rubus. ulmifolius growing in Esteiro de Estarreja

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Main results

✓**Accumulation studies showed that increasing concentrations of available zinc in the matrix induced increased metal accumulation in the tissues of this species** → **the plant was able to grow with levels of available Zn of 100 mg/kg dry soil**

1450 mg Zn kg^{-1} (L)

3240 mg Zn kg^{-1} (L)

3810 mg Zn kg^{-1} (L)

✓**Enhanced accumulation levels were observed in all the sections of *S. nigrum* plants inoculated with *G. claroideum* (83%) and *G. intraradices* (49%)**

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Main results

✓**When EDTA or EDDS were applied to the soil, AMF colonisation decreased and there was no effect of the selected AMF on Zn accumulation by the plant**

✓**The Zn concentrations in water-extracts of the soils collected at the time of harvest were increased by the addition of EDTA or EDDS by up to 4.0- and 3.1-folds, respectively.** → Higher metal mobility !!

The accumulation of Zn in all plant tissues generally increased with the addition of both chelating agents → application of EDTA would reduce the number of crops to achieve soil clean-up from 23 to 13

A chelate-enhanced phytoextraction strategy using *S. nigrum* and EDDS or EDTA still appears as time consuming and risky concerning contamination dispersion

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✓**The establishment of *S. nigrum* in combination with OM amendments provided a reduction in the quantity of Zn leached of ca. 70 to 80% → reduction of metal dissemination**

✓**The addition of manure resulted in a reduction in Zn accumulation in *S. nigrum* of up to 80%** (reduction promoted by compost addition→ 48%)

Phytostabilisation employing *S. nigrum* and manure appears thus as a realistic scenario for the remediation of Zn contaminated soils

Survey of metal accumulation in plant species indigenous to Esteiro de Estarreja

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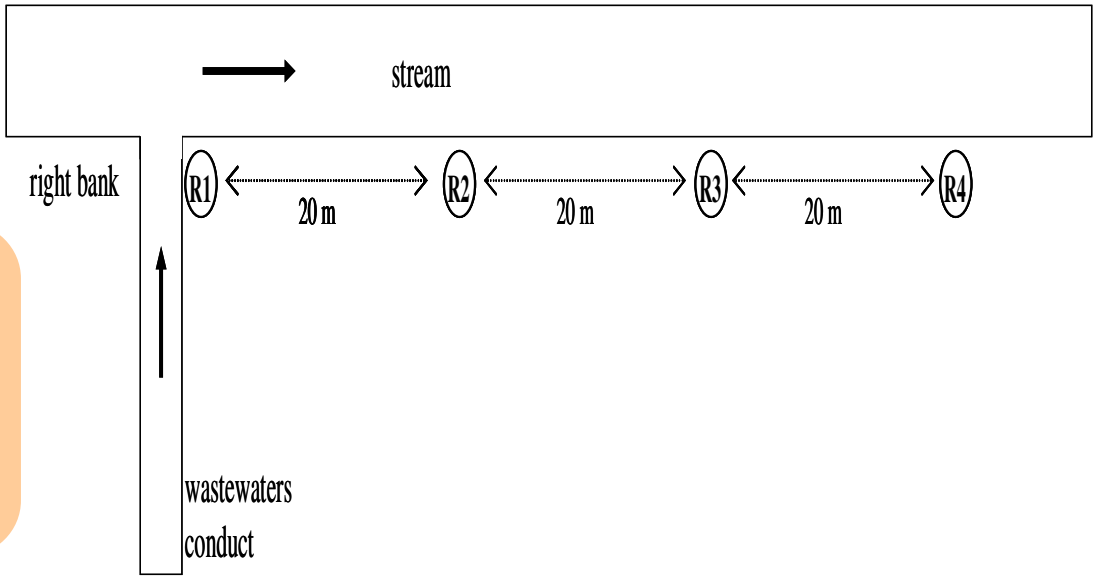
AMF colonisation of the roots of the plant samples collected along the banks of the stream

Accumulation of metals in the roots, stems and leaves of the plant samples collected along the banks of the stream (4 spots separated 20 m from each other)

Levels of metals in soils collected along the banks of the stream (4 spots separated 20 m from each other)



Results: Soil caractheristics

Collection site	pH	Water content (%)	Organic content (%)	N (mg kg^{-1})	P (mg kg^{-1})
R1	7.13 ± 0.4	1.46 ± 0.09	10.1 ± 0.2	3513 ± 4	201 ± 8
R2	6.49 ± 0.1	1.4 ± 0.1	8.4 ± 0.3	1596 ± 10	282 ± 4
R3	6.33± 0.2	1.6 ± 0.1	7.9 ± 0.3	3359 ± 5	255 ± 8
R4	7.14 ± 0.1	1.60 ± 0.09	9.5 ± 0.2	1118 ± 13	44 ± 4

Results: Soil total and available metal levels

Collection site	Metal (mg kg^{-1} dry soil)							
	As		Pb		Ni		Zn	
	Total	EDTA extract	Total	EDTA extract	Total	EDTA extract	Total	EDTA extract
R1	3078 ± 117	43 ± 2	1400 ± 9	204 ± 5	96 ± 5	2.23 ± 0.05	957 ± 74	87 ± 2
R2	952 ± 13	26 ± 3	371 ± 1	67 ± 10	135 ± 10	9 ± 1	715 ± 47	71 ± 2
R3	1180 ± 24	8.7 ± 02	611 ± 7	77 ± 3	63 ± 2	2.01 ± 0.05	853 ± 13	42 ± 2
R4	1126 ± 27	20 ± 3	540 ± 9	83 ± 9	105 ± 3	3.7 ± 0.4	713 ± 55	35 ± 2

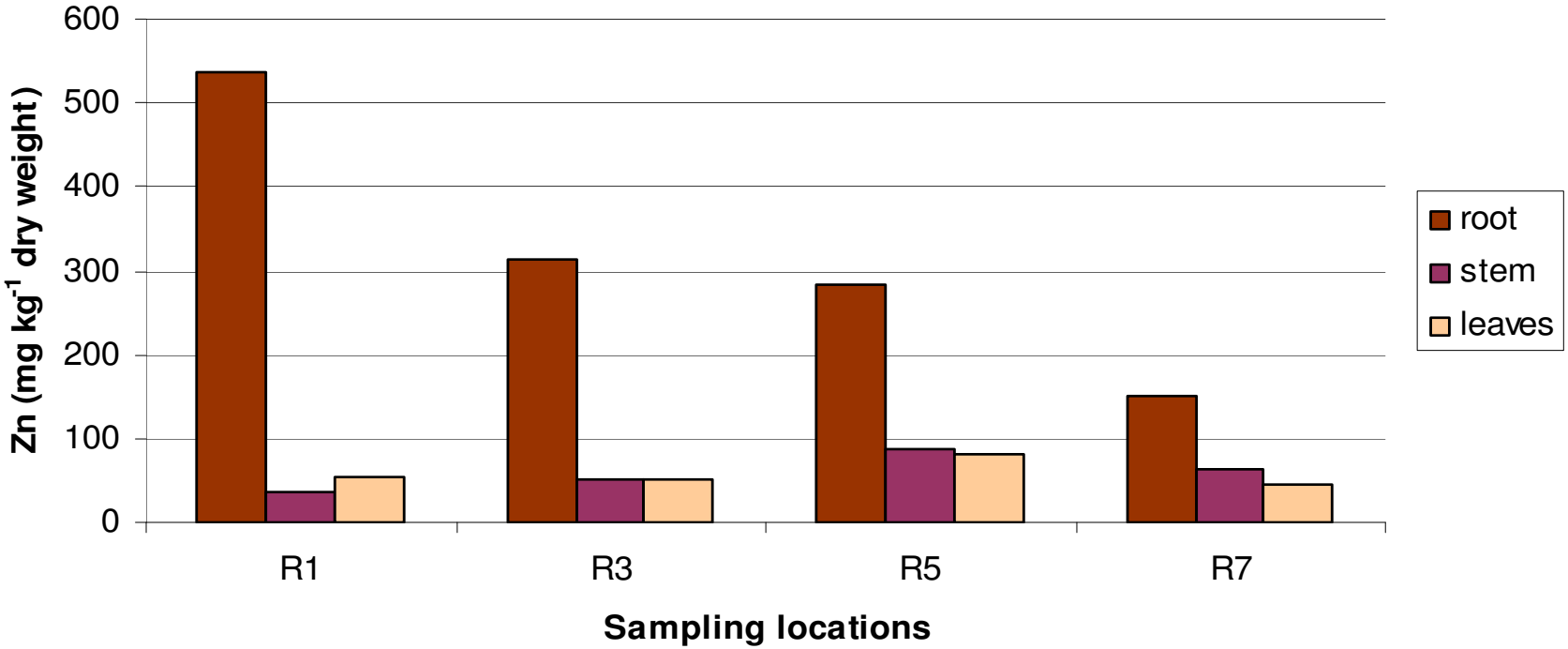
✓Total metal levels higher than those proposed as normal in soils

→

2 to 100 mg Ni kg^{-1}
2 to 300 mg Pb kg^{-1}
0.1 to 40 mg As kg^{-1}
70 a 400 mg Zn kg^{-1}

✓Levels of EDTA extractable Pb, As, Ni and Zn representing up to 13, 0.7, 2.4 and 10 % of the total metal contents

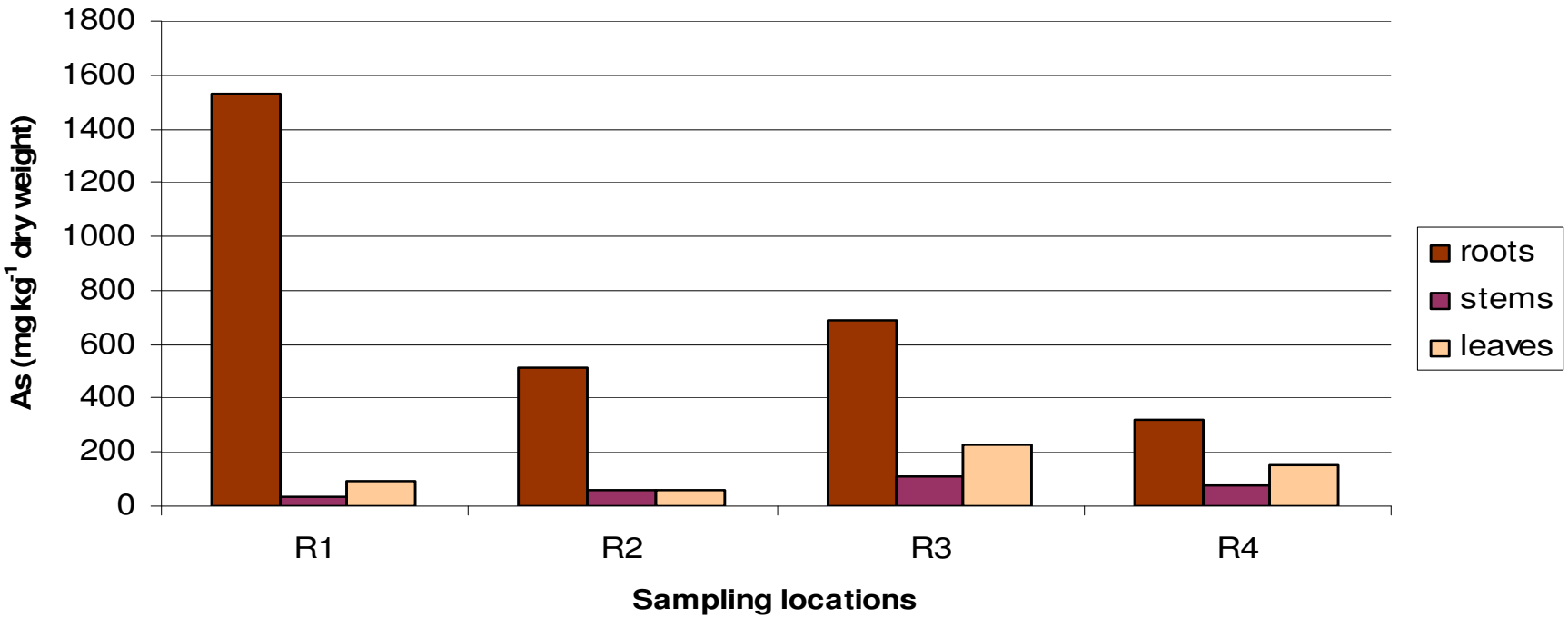
Results: Zn accumulation by *Rubus ulmifolius*



✓**Zn in the roots generally above the normal levels (10 to 100 mg kg^{-1})**

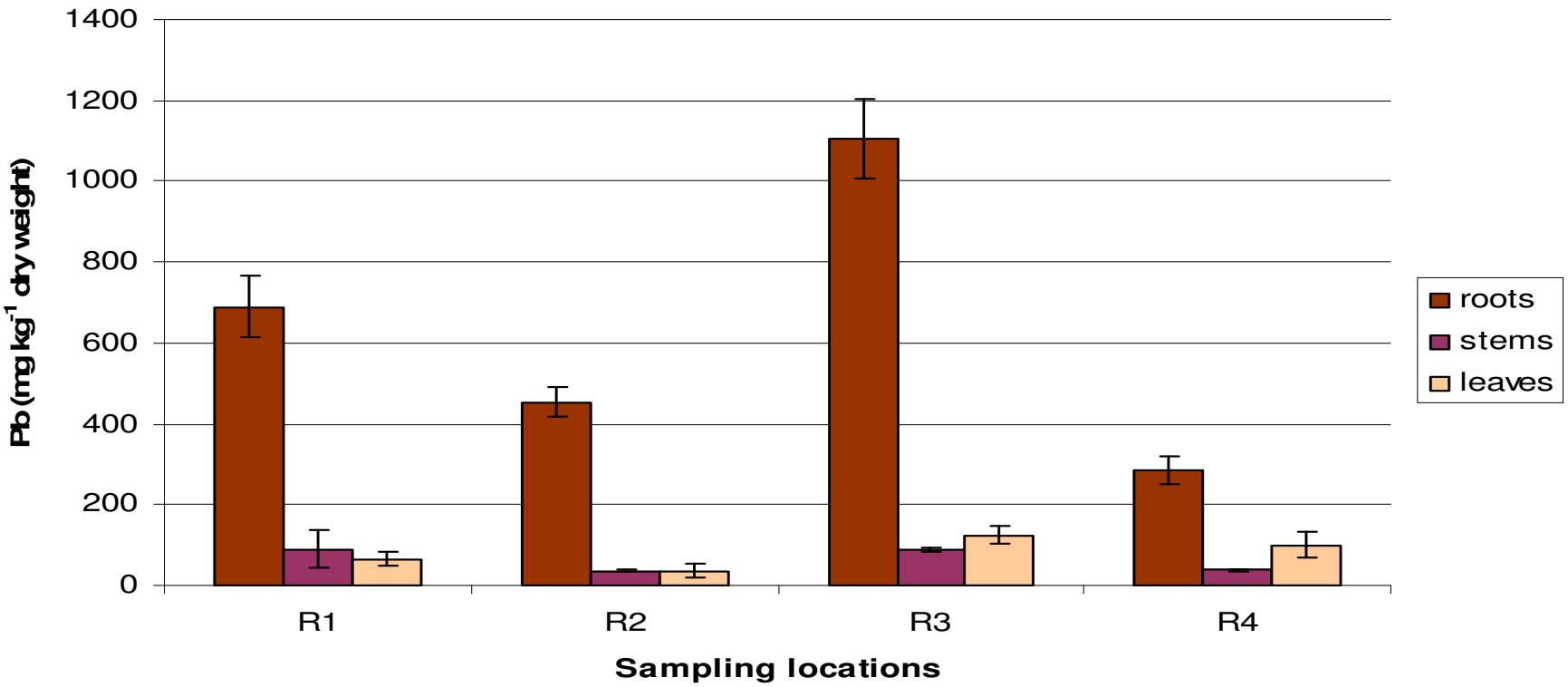
✓**Exclusion from aboveground tissues**

Results: As accumulation by *Rubus ulmifolius*



- ✓ As in the roots generally above the normal levels (5 mg kg^{-1})
- ✓ Accumulation mainly in the root tissues

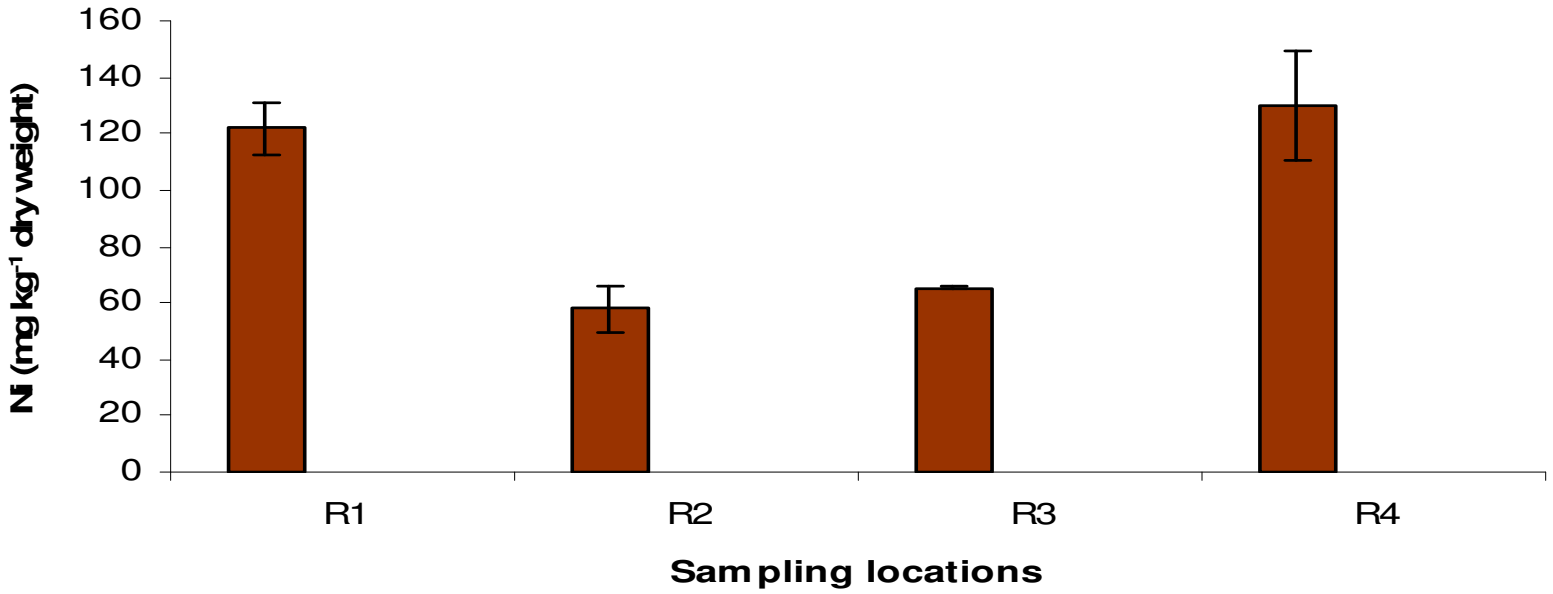
Results: Pb accumulation by *Rubus ulmifolius*



✓**Pb in the roots generally above the normal levels (0.1 to 5 mg kg^{-1})**

✓**Exclusion from aboveground tissues**

Results: Ni accumulation by *Rubus ulmifolius*



✓**Ni in the roots generally above the normal levels (1 to 10 mg kg^{-1})**

✓**Ni was not detected in the leaves and stems of the plants**

Results: metal accumulation

✓***R. ulmifolius*** **tolerant to Zn, As, Pb and Ni**

✓**Metal exclusion strategy from stems and reproductive tissue retaining the metal in the roots**

✓***R. ulmifolius*** **never accumulated more than 1000 mg kg^{-1} of any of the metals in the aboveground tissues, a criteria indicated for As, Pb or Ni hyperaccumulators or 10000 mg kg^{-1} in the case of Zn**

Resistance of *R. ulmifolius* to the metals can be achieved by an avoidance mechanism such as the precipitation and association with cell walls or detoxification in vacuoles

Results: Soil total-available metal relations

✓**Soil: Generally, strong positive correlations between total Zn, As, Pb and Ni concentrations and the metal levels in the available fraction**

Total As vs. EDTA extractable As → 0.77

Total Pb vs. EDTA extractable Pb → 0.96

Total Nis vs. EDTA extractable Ni → 0.85

Total Zn vs. EDTA extractable Zn → 0.85

Levels of available Zn, As, Pb and Ni are positively related with total metal in the soil

Results: Soil – plant relations

✓**Soil-Plant: Positive correlations between the level of all metals in the soil and Zn, Pb and As accumulation in the roots of *R. ulmifolius***

Zn, As, Pb and Ni in the soil are positively related with the metal uptake at the root level

Total As vs. EDTA extractable As → 0.71

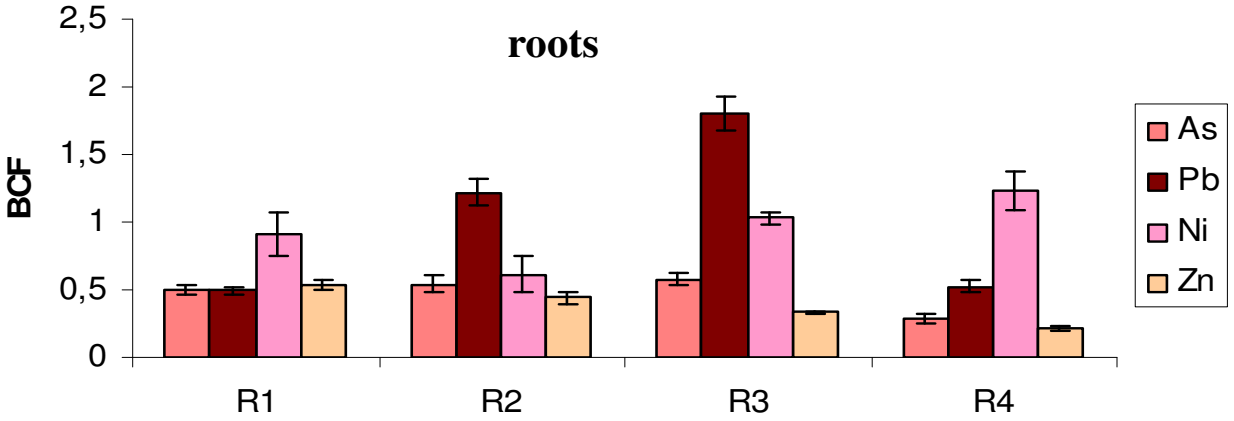
Total Pb vs. EDTA extractable Pb → 0.61

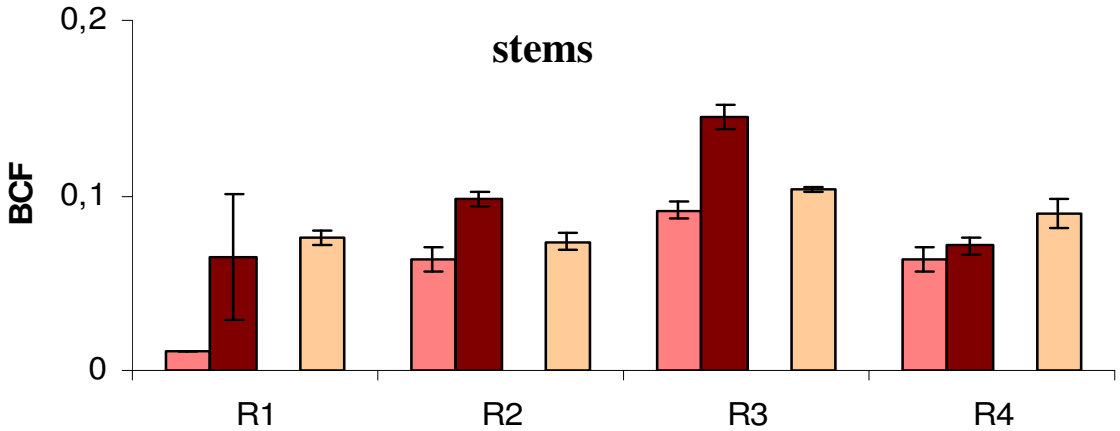
Total Nis vs. EDTA extractable Ni → 0.67

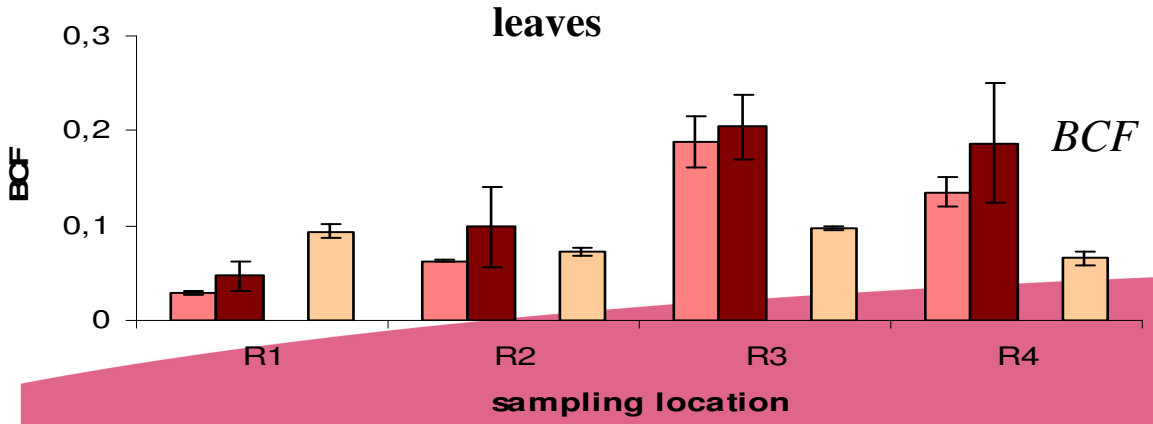
Total Zn vs. EDTA extractable Zn → 0.85

✓**No AMF were detected in the roots of *R. ulmifolius* for any collection site → sampling at different seasons would be necessary to confirm**

Results: Soil – plant relations → Bioconcentration factors







Higher BCF values were registered for the roots of *R. ulmifolius* - in some of these cases values higher than 1 were observed

$$BCF \equiv \frac{metal\ concentration\ in\ a\ plant\ section}{metal\ concentration\ in\ the\ soil}$$

- *R. ulmifolius* established successfully on this metal polluted soil
- Metals were retained in the belowground sections, as shown by the metal accumulation patterns and BCF values
- This species presents significant biomass production in the field
- *R. ulmifolius* is well disseminated throughout the region and easy to propagate

***Rubus ulmifolius* has desirable properties to be used in phytostabilisation approaches.**

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Future work:

- ✓Investigation of the effects of bacteria isolated from HM contaminated sites on *S. nigrum* growth and Zn and Cd accumulation
- ✓Evaluation of the potential of the combination of AMF and bacteria with *S. nigrum* for the phytoremediation of HM contaminated soils
- ✓Assessment of the efficacy of the tested associations under field conditions

Future work:

To survey other HM contaminated sites for indigenous flora, bacteria and AMF

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Aknowledgments

The authors wish to thank Câmara Municipal de Estarreja for the provision of access to the site.
This work was supported by Fundação para a Ciência e a Tecnologia and Fundo Social Europeu (III Quadro Comunitário de apoio), research grant of Ana Marques (SFRH/BPD/34585/2007).
This work was funded by Project MICOMETA - POCI/AMB/60131/2004 (Fundação para a Ciência e Tecnologia)

Thank you for your attention!