ZINC ACCUMULATION AND HISTOLOCALISATION IN *SOLANUM NIGRUM* GROWN IN CONTAMINATED SOIL: EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI

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PHYTOREMEDIATION OF TOXIC METAL CONTAMINATED SOILS

The experience in phytoremediation suggests that collecting plant species existing in contaminated soils may be effective for selecting potential plants to use in this remediation technique. The region of Estarreja appears as a strong candidate for this kind of research as for many years several chemical facilities of the region have discharged its solid residues in an improvised park in the surrounding area, and conducted its wastewaters into a stream nearby ("Esteiro de Estarreja"). In spite of the levels of zinc (amongst other metals as Pb, Hg and As) in the sediments remaining above the limits established by the European legislation, the vegetation on the banks remains proliferous. One of the main indigenous species, *Solanum nigrum* L. (black nightshade), has shown to accumulate up to 1130 mg Zn/kg tissues when collected at the site.

The role of arbuscular mycorrhizal fungi (AMF) in phytoremediation of heavy metals is not clear and the analysis of these undergoing rhizosphere interactions seems to be a promising patch for the optimization of the metal uptake by the plant.

The aim of this study was to assess the influence of AMF on plant biomass production and Zn accumulation in *S. nigrum* plants growing in the naturally contaminated soil and to determine the location of the accumulated Zn in plant tissues.

Fig.1: General view of the stream

Fig.2: General view of the growth experiment

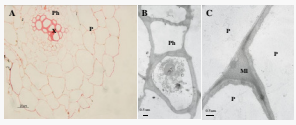
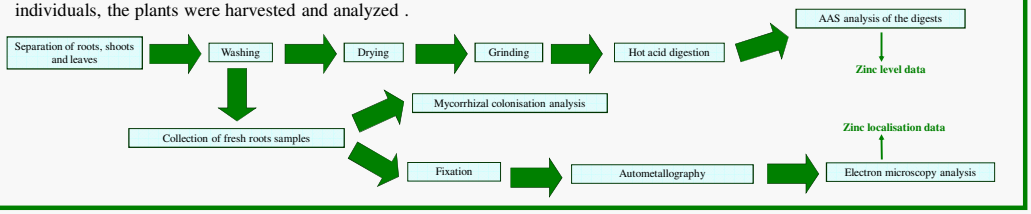


Fig. 3. Root section of *Solanum nigrum* inoculated with *Glomus* sp. BEG140 grown in control sand. After autometallography was performed on the root pieces, no labeling was detected in control plants. A) General structure of the root as seen in cross section under the light microscope; cortical parenchyma cells (P), phloem (Ph), xylem (X). B) Electron microscopy image of a detail of the phloem cells (Ph). C) Region of contact of three cortical parenchyma cells (P), middle lamellae (Ml).

MATERIAL AND METHODS

The experiment was a factorial design with two matrix Zn levels [acid washed sand, considered as the control (no detectable Zn), and contaminated soil, with 426 mg Zn/kg dry soil] and six mycorrhizal fungi treatments (no AMF, *Glomus* sp. BEG140, *Glomus claroideum*, *Glomus mosseae*, *Glomus intraradices* and a mixture of all the AMF isolates). After reaching the maturity of the individuals, the plants were harvested and analyzed .



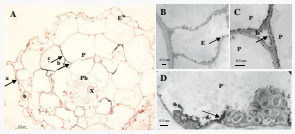


Fig.4. Root sections of *Solanum nigrum* inoculated with *Glomus* sp. BEG140 grown in contaminated soil. A) Light microscopy image of a root cross section; xylem (X), phloem (Ph), cortical parenchyma cells (P) and the epidermal cells (E). The arrows indicate Zn deposits, as revealed by the dark staining in the epidermis (a), the parenchyma cells of the cortex (c) and intercellular spaces (b). B, C and D) Electron microscopy analysis reveals Zn deposition (arrows) in the epidermal cells (E), in intercellular spaces (Is) and in the cytoplasm of cortical parenchyma cells (P).

Table 1: Influence of different AMF species on *Solanum nigrum* biomass grown in control and contaminated matrixes

AMF	Control sand			Contaminated soil		
	root	stem	leaves	root	stem	leaves
No fungi	2.2430 ± 1.0612 (6) [a]	3.4945 ± 1.0194 (6) [ab]	1.5797 ± 0.3195 (6) [a]	1.8328 ± 0.1934 (6) [a]	8.4576 ± 0.6972 (4) [a]	1.7949 ± 0.4762 (6) [a]
G. sp. BEG140	2.0750 ± 0.4300 (6) [a]	3.7018 ± 1.4697 (6) [ab]	1.8966 ± 0.6186 (6) [a]	1.8542 ± 0.4604 (6) [a]	5.6559 ± 1.3067 (5) [b]	1.5519 ± 0.5587 (6) [a]
G. claroideum	2.4744 ± 0.6489 (6) [a]	4.3592 ± 0.5679 (6) [b]	1.9261 ± 0.4561 (6) [a]	1.9748 ± 0.2625 (4) [a]	4.4544 ± 0.6065 (4) [b]	1.4866 ± 0.3446 (6) [a]
G. mosseae	2.3532 ± 0.9068 (6) [a]	2.2600 ± 0.8803 (5) [a]	1.6061 ± 0.4784 (5) [a]	1.8954 ± 0.4786 (6) [a]	3.8577 ± 0.7515 (4) [b]	1.8759 ± 0.4797 (6) [a]
G. intraradices	2.4075 ± 0.7231 (6) [a]	4.7235 ± 0.6276 (6) [b]	2.0368 ± 0.3072 (6) [a]	1.8197 ± 0.2458 (4) [a]	8.5712 ± 0.5386 (4) [a]	1.6893 ± 0.3904 (6) [a]
Mixture	1.7915 ± 0.5219 (5) [a]	3.7041 ± 0.3130 (5) [ab]	1.6946 ± 0.3476 (5) [a]	1.7942 ± 0.1879 (6) [a]	8.2986 ± 1.3144 (4) [a]	1.8044 ± 0.3219 (6) [a]

Results are expressed as means ± SD (n). Means in the same column with different letters are significantly different from each other (P<0.05) according to the Tukey test.

Table 2: Influence of different AMF species on *Solanum nigrum* Zn tissue concentration grown in control and contaminated matrixes

AMF	Control sand			Contaminated soil		
	root	stem	leaves	root	stem	leaves
No fungi	117 ± 26 (6) [a]	53 ± 6 (6) [a]	26 ± 2 (6) [ab]	1029 ± 64 (6) [a]	285 ± 16 (4) [a]	114 ± 16 (6) [a]
G. sp. BEG140	139 ± 22 (6) [a]	57 ± 10 (6) [a]	26 ± 8 (6) [ab]	1095 ± 96 (6) [a]	363 ± 11 (5) [bc]	131 ± 20 (6) [a]
G. claroideum	110 ± 8 (6) [a]	60 ± 14 (6) [a]	24 ± 3 (6) [a]	1622 ± 106 (4) [b]	411 ± 30 (4) [d]	250 ± 30 (6) [b]
G. mosseae	118 ± 10 (6) [a]	53 ± 11 (5) [a]	27 ± 10 (5) [ab]	1027 ± 102 (6) [c]	349 ± 6 (4) [b]	188 ± 11 (6) [a]
G. intraradices	144 ± 14 (6) [a]	54 ± 15 (6) [a]	28 ± 2 (6) [b]	1586 ± 59 (4) [d]	397 ± 14 (4) [cd]	253 ± 19 (6) [b]
Mixture	126 ± 7 (5) [a]	54 ± 4 (5) [a]	25 ± 5 (5) [ab]	1139 ± 109 (6) [a]	251 ± 11 (4) [c]	136 ± 11 (6) [a]

Results are expressed as means ± SD (n). Means in the same column with different letters are significantly different from each other (P<0.05) according to the Tukey test.

RESULTS AND CONCLUSIONS

- Soil contamination influenced ($P < 0.05$) positively mycorrhizal colonisation in *S. nigrum*;
- Plants grown in contaminated soil had higher shoot and lower root masses than the ones grown in control sand;
- AMF colonisation did not influence the biomass yield of *S. nigrum* when comparing with non-inoculated plants;
- *S. nigrum* plants grown in the contaminated soil accumulated up to 1622 mg kg-1 of Zn in the roots, 411 mg kg-1 in the stems and 253 mg kg-1 in the leaves;
- The inoculation with *G. claroideum* induced an increase of 58, 44 and 120% in the Zn accumulation levels for the roots, stems and leaves, respectively; when inoculated with *G. intraradices,* the increase in the accumulation levels was of 54, 39 and 122% for roots, stems and leaves, respectively;
- Dark staining and electron-dense grains resulting from the metal presence are visible through autometallography in the intercellular spaces and in the cellular walls of root sections of *S. nigrum* plants grown on contaminated media, showing the apoplast as a Zn reservoir;
- Inoculation with different AMF species caused no differences in the localisation of Zn in the intercellular spaces and cell walls of *S. nigrum* roots.

The use of *S. nigrum* inoculated with *G. claroideum* or *G. intraradices* appears to be a promising option for decontamination of Zn contaminated soil due to the enhanced extracting and accumulating capacities presented by these associations.

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