Heavy Metal Uptake by *Brassica* Species Growing in the Polluted Soils of Aznalcóllar (Southern Spain)

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INTRODUCTION

On 25 April 1998 a pyrite slurry spill occurred into the Agrio and the Guadiamar rivers (Aznalcóllar, Seville, Southern Spain) which contaminated a wide area (40 km length, 0.5 km wide) in the proximity of the Doñana National Park, the largest reserve of bird species in Europe. Immediately after the spill, the Ministry of the Environment in Spain and the Autonomous Council of Andalucia began soil-reclamation activies in order to reduce to a minimum the impact caused by leaching of the toxic heavy metals in the affected area. After physically removing the sediments the soils remained polluted by trace metals such as Pb, Cu, Zn, Cd, Tl, Bi, Sb and As (Simon et al. 1999).

The remediation of large volumes of contaminated soil by conventional technologies results very expensive. Phytoremediation has emerged recently as an alternative to the engineering-based methods. Species of the genus *Brassica* have been shown to accumulate moderate levels of heavy metals (Nanda-Kumar et al. 1995; Ebbs and Kochian 1997).

The objective of this work was to determine the potential of *Brassica carinata* and *Brassica juncea* accessions to clean-up contaminated soils, based on their growth responses and heavy metal uptake, growing in field trials at Aznalcóllar for two years.

MATERIALS AND METHODS

Brassica carinata (accession BC850) and *Brassica juncea* (accession Z-1) were seeded directly into the field during 1999 and 2000. Field trials had a size of 50 m x 20 m and both experimental sites were located in the area named "El Vicario" close to the pyrite mine of Aznalcóllar (Fig. 1). The sowings were made on March 15, 1999 and February 25, 2000. In addition, *B. carinata* and *B. juncea* were also cultivated on an unpolluted soil as control.

During the field trial 1999, plants were harvested when reached the top of the vegetative period on June. The plants had low biomass due to the late sowing and a hydric stress along the time of plant growing.

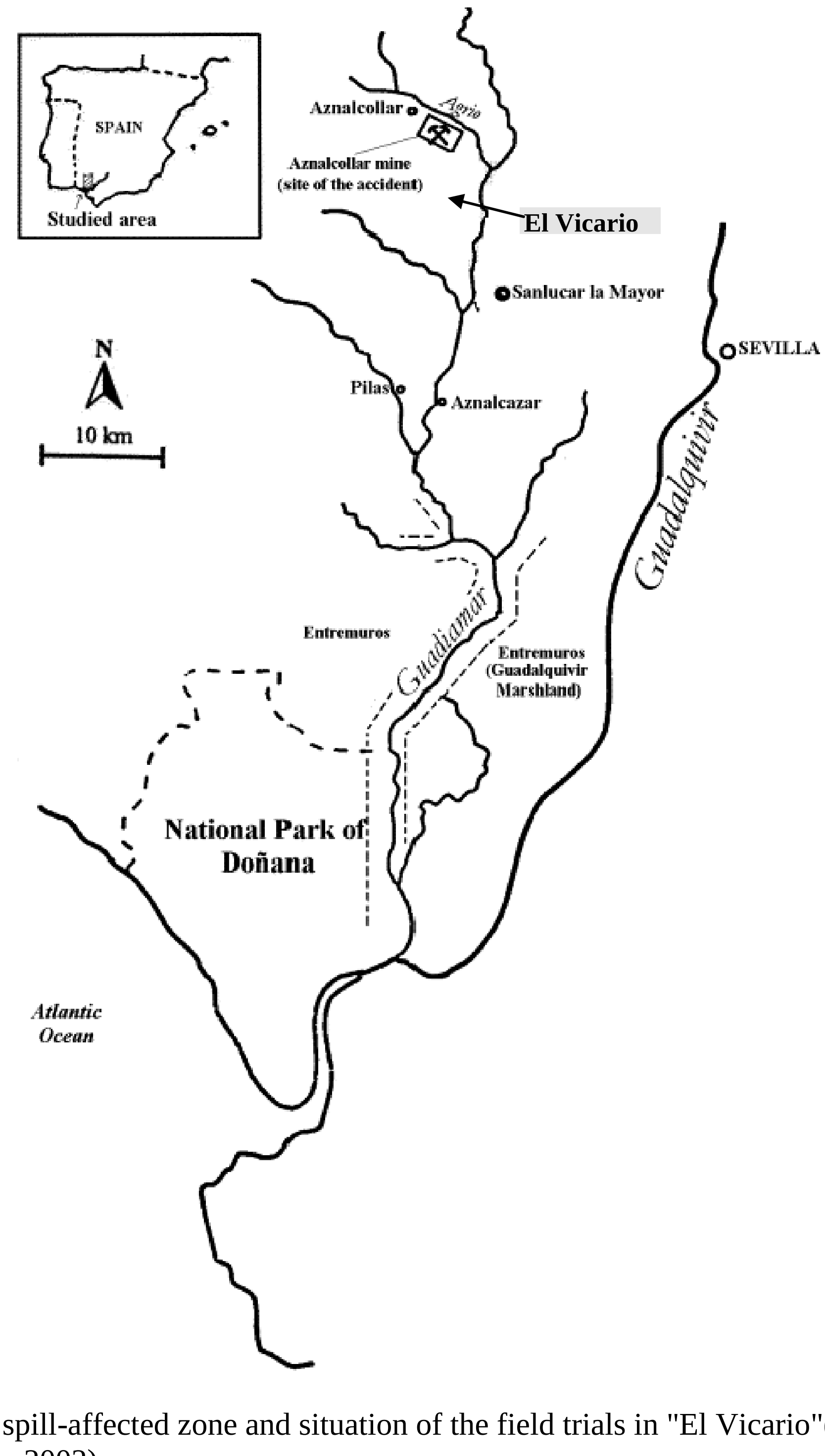

Fig. 1. Area of the spill-affected zone and situation of the field trials in "El Vicario"(adapted from Santos et al., 2002).

In the second year trial, plants were harvested on June. In this season, the plants had higher biomass than in 1999 due to the greater duration of the growing period and higher rainfall.

From each plot, 5 soil samples were taken (1-30 cm deep) in both years. The preparation of the soil samples and analysis of heavy metals were made by the method described by Simon et al. (1999).

Plant sampling. At each plot, ten rows were selected at random and three plants in each row were collected for laboratory analysis. All plants were divided into leaves, stems, roots, pods and seeds. The preparation of the samples and determination of heavy metals was performed as described Del Río et al. (2002a). Heavy metal concentrations for soils and plants were calculated on a dry weight basis.

Student *t*-test was used to assess differences between paired means from the same plant species cultivated both at polluted and unpolluted soils.

RESULTS AND DISCUSSION

Total mean concentrations of heavy metals (Pb, Zn, Cu and Cd) in the soil before sowing are shown in Table 1. The results obtained showed that the soil contained low concentration of Cd and high concentrations of Pb, Zn and Cu.

Table 2 compares the ability of *B. carinata* and *B. juncea* plants to accumulate heavy metal in shoots during two years. Concentrations of Cd were below detection limit of the spectrophotometer and were not showed.

In both years, *B. juncea* plants accumulated higher amounts of Pb and Zn than *B. carinata* plants. The Cu concentrations of *B. juncea* and *B. carinata* plants in the polluted soils did not differ significantly from plants harvested in unpolluted soils.during two years. This could be explained by multiple interactions among metals in the soil, which can affect the capacity of the plant for uptaking as has been observed in *B. juncea* (Ebbs and Kochian, 1997) and in barley (Luo and Rimmer, 1995). The concentrations of Zn in plants of *B. juncea* and *B. carinata* were higher in comparison with those reported by Ebbs et al. (1997), taking into account that *Brassica* spp (*Brassica juncea*, *Brassica rapa* and *Brassica napus*) in their study were grown in contaminated soils with 11700 $mgkg^{-1}$ of Zn.

The metal accumulation by plant was low caused probably by the low bioavailability of metals in soils. Before sowing, the Autonomous Council of Andalucia performed chemical treatments (soil amendments with calcium carbonate and ferric oxides) to fix metals in the soil, which decreased the uptake of heavy metals by plants. Although others studies have shown the genetical capacity of *Brassica spp.* to accumulate heavy metals (Nanda-Kumar et al. 1995; Ebbs and Kochian 1997; Del Río et al., 2002b) in hydroponic and pot cultures, it is common to find cases of low bioavailability, thus preventing the remediation process, under many different circunstances (pH, solid soil constituents, metal, etc) in field (Raskin et al., 1997).

The distribution of Pb, Zn and Cu in the different parts of *B. carinata* and *B. juncea* plants was different between years. In the first year the levels of Pb and Cu present in *B. juncea* were in the order leaves>stem>pods>seeds>roots. Instead, Zn was present in the different parts of plant in the following order: stem>leaves>pods>seeds>roots. *B. carinata* showed the same pattern for all the metals but pods and seeds had the lowest levels. In the second year, the levels of all the elements were in the order stem>leaves>roots in both species. This is attributed to the fact that the agronomic conditions were more favourable in 2000 than the previous year thus plants producing higher biomass, mainly located in stems.

CONCLUSIONS

Data obtained in this work indicate these species are more effective in removing Zn and Pb than Cu. The uptake of heavy metals in *Brassica* plants could be increased by enhancing metal availability in these soils. For instance, maintaining a moderately acid pH in the soil through the use of ammonium containing fertilizers or soil acidifiers or applying certain chelates (Blaylock et al., 1997).

Since the potential of a species for phytoextraction depends not only on the tissue metal concentration but also on shoot biomass production, and taking into account the differences observed respect to plant growth in both years, it is important to establish more favourable agronomic conditions. Agronomical practices as irrigation, fertilization, planting and harvest time and the timing of amendment application need to be developed to maximize the effectiveness of phytoremediation of the *Brassica* species.

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Table 1. Concentrations (mean ± standard deviation) of total heavy metals in experimental area "El Vicario" (0-30 cm. in depth) during 1999 and 2000.

	Element (mgkg^{-1} dry weight)			
year	**Pb**	**Zn**	**Cu**	**Cd**
1999	309±96.2	461±48.3	127±14.6	1.5±0.2
2000	263±129	748±60.5	138±32.3	3.0±1.2

Table 2. Concentrations of Pb, Zn and Cu (mgkg^{-1} dry weight) in *Brassica carinata* and *Brassica juncea* plants growing at polluted (p) and unpolluted soils (up) during 1999 and 2000. Data expressed as mean ± standard deviation.

		B. carinata		***B. juncea***	
		mgkg^{-1}		**mgkg^{-1}**	
year		**(up)**	**(p)**	**(up)**	**(p)**
1999	**Pb**	1.6*[1]±0.1	4.4±0.7	0.07**[1]±0.2	6.2*[2]±1.3
	Zn	35.9*[1]±5.1	81.1±21.3	37.5**[1]±4.7	94.1*[2]±17.3
	Cu	14.8ns[1]±0.3	6.7±1.0	14.9ns[1]±0.4	5.8ns[2]±3.3
2000	**Pb**	3.4**[1]±0.1	23.5±4.8	4.4*[1]±0.2	28.6*[2]±4.2
	Zn	15.1*[1]±2.6	67.8±23.0	18.9**[1]±1.9	132*[2]±79.8
	Cu	4.9ns[1]±0.2	7.5±1.1	7.0ns[1] ±0.6	7.5ns[2]±0.2

[1] Comparisons between heavy metal concentrations in plants on polluted (p) and unpolluted (up) soils. ns=no significant, *; ** y ***= Significantly different at p<0.05, p<0.01 and p<0.001, respectively.
[2] Comparisons between heavy metal concentrations in plants of *B. carinata* and *B. juncea* on polluted soils. ns=no significant, *; ** y ***= Significantly different at p<0.05, p<0.01 and p<0.001, respectively.

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