

# ACCUMULATION, TRANSFER AND REMEDIATION OF Cd IN SOILS AFFECTED BY THE AZNALCÓLLAR MINE SPILL (SW SPAIN): A DECADE OF EXPERIENCE (1998-2008)

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## ABSTRACT

Cadmium is a non-essential trace element with great potential to escape the soil-plant barrier, resulting in readily transmission through the food chain and high-risk for potential consumers. Although Cd was not a major component of the Aznalcóllar mine toxic spill (SW Spain, 1998) short- and mid-term studies have shown that residual Cd contamination in soil is a major problem for a safe and sustainable reutilization of the affected area ( about 4500 ha). We summarize a decade of experience dealing with soils contaminated with Cd in a dynamic and changing landscape “the Guadiamar Valley”, a formerly agricultural area redesigned as a nature reserve “Green Corridor” after the accident. The accumulation, availability and remediation of Cd in soil and its implications for the soil-plant-animal system are discussed within the context of the impact and mitigation of environmental disasters.

## CADMIUM IN SOILS: SOURCES, USE AND TOXICITY

The chemical composition of the parent rock is the main factor controlling the Cd content of the soil and thus the weathering of minerals from the parent rock is the major natural source of Cd in soils. Other natural phenomena which may contribute to the local accumulation of Cd in soils may include volcanic emissions, natural fires and dust storms

(Ross, 1994; Naidu et al. 2001). The earth's crust mean value of Cd is  $0.1 \text{ mg kg}^{-1}$  (Alloway, 1990), whilst ranges of  $0.01\text{-}2.0 \text{ mg kg}^{-1}$  (Bowen, 1979) and  $0.06$  and  $1.1 \text{ mg kg}^{-1}$  (Kabata-Pendias and Pendias, 1992) have been proposed as normal for non-contaminated soils.

In addition to natural phenomena there are various anthropogenic activities that can act as punctual and/or diffuse sources of Cd accumulation in soils and in other environmental compartments (water and air). These include: a) mining-related activities derived from the transport and processing of ores, the disposal of generated mine wastes, and the abandonment of fully-exploited mines (Gustafsson et al., 1999; Grimalt et al., 1999); b) industrial activities related to the manufacturing of iron and steel, batteries, textiles, plastics and refineries (Aguilar et al., 1999); c) atmospheric deposition of contaminated particles generated during the combustion of fossil fuels (power plants, automobiles, etc.) (Ross 1994); d) phosphate fertilizers and sewage sludge used in agriculture (Adriano, 2001) and e) landfills containing municipal wastes (Ross, 1994).

Cadmium is produced commercially as a by-product of the Zn industry, in fact, no ores are mined exclusively to provide Cd. Cadmium is mostly used in batteries (Ni-Cd and Ag-Cd) (35%), as protective agent for iron and steel against corrosion (25-30%), in electroplating (autoindustry), in pigments (cadmium sulfide and cadmium selenide), and as a stabilizer for polyvinyl plastics and as alloys (5%) (Litz et al., 2004). Other uses of Cd include photography, lithography, process engraving, rubber curing, and as a fungicide primarily for golf course greens (Adriano, 2001). As an impurity in Zn, significant amounts of Cd are present in galvanized metals. Consequently, Cd can be found in a wide variety of consumer goods, and virtually all households and industries have products that contain some Cd.

Until relatively recent, Cd was considered a non-essential trace element with unknown biological role. Lane et al. (2000, 2005) found for the first time a Cd-dependant anhydrase in marine diatoms. Cadmium has the same function as Zn in other anhydrases, but the diatoms live in environments with very low Zn concentrations. Hence they have evolved to use Cd instead of Zn under these particular conditions. Apart from this example, Cd has no other known-biological role. Nonetheless, Cd can be ubiquitously found in biological tissues. In the case of plants Cd is mainly passively absorbed via the roots or the leaves. As a result, high uptake of Cd may occur in contaminated soils. Consumption of contaminated food (e.g. vegetables) and water is a potential source of Cd intake by animals and/or humans. In the decades following World War II, Japanese mining operations contaminated the Jinzu River with Cd and traces of other toxic metals. As a consequence, Cd accumulated in the rice crops growing along the riverbanks downstream of the mines. The local agricultural communities consuming the contaminated rice developed Itai-itai disease and renal abnormalities, including proteinuria and glucosuria (Nogawa et al., 2004). Other routes of Cd exposure for humans include tobacco smoking and inhalation of Cd-containing fumes in industrial settings.

A major problem associated with Cd accumulation in soils is its relatively high mobility in relation to other metals, resulting in readily absorption by plants and subsequent translocation from roots to shoots. Here Cd may accumulate at levels that are not phytotoxic, but possibly hazardous for potential consumers (animals and humans) in the mid- and long-term through bioaccumulation and biomagnification (Litz et al., 2004). Nonetheless, biomagnification of Cd and other metals is still a matter of debate, since mercury is the only metal that has been so far unequivocally demonstrated to undergo biomagnification (Wright and Welbourn, 2002). As a rule, transmission of trace elements through the food chain is affected by the soil-plant barrier described by Chaney and Giordano (1977). There are two

main mechanisms by which this transmission is prevented. The first one relates to those elements, including Ti, Si, Zr, Au, Ag, V, and Hg, that are very insoluble and/or strongly adsorbed to soil particles or in roots. The second mechanism includes Zn, Cu, Ni, Mn, Cr, As, and F, and results in plant phytotoxicity, before these elements reach levels in the edible parts of plants often considered harmful to consumers (Basta et al., 2005). There are a few elements, however, that escape the soil-plant barrier namely Cd, Se and Mo. However, only Cd is ranked (place 7 out of 275 substances) as a priority pollutant by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA 2007).

In this work we summarize the experience gained over the last decade on Cd accumulation, transfer and remediation in the Guadiamar Valley (SE, Spain), an area affected by the greatest mine spill disaster in the history of Spain. Most of the data presented here belongs to numerous studies carried out by our group at the IRNAS-CSIC Research Centre in Sevilla (Spain). Significant results from other groups working on the affected area are also discussed. After a brief introduction to the Aznalcóllar mine accident, we discussed the evolution of Cd in the soil over time, that is: immediately after the accident, following remediation works and long after remediation works terminated. A second part is focused on the stabilization of Cd in the soil via assisted natural remediation, which is practically the only reliable technique for treating extensive areas moderately contaminated with inorganic contaminants (Adriano et al., 2004; Martin and Ruby, 2004). Finally, data related to plant biomonitoring along the Guadiamar Valley is presented and the entry of Cd in the food chain and transmission from primary producers to primary consumers is examined.

## The Aznalcóllar Mine Accident

The Aznalcóllar mine area (SE Spain) belongs to the Iberian Pyrite Belt (250 km long and 40-60 km wide), the largest deposit of massive sulfides worldwide (Leistel et al., 1998). This mining complex exploited the ore reserves of two open-pit mines: Aznalcóllar until 1992 and Los Frailes since 1997. The processing of the ore consisted of grinding, treating of the particles with  $\text{SO}_2(\text{g})$  and slaked lime ( $\text{Ca}(\text{OH})_2$ ) and finally separation by differential floating of Cu, Pb and Zn at different pH values. The residues from this process were composed mainly of pyrite with minor proportions of other sulfides (López-Pamo et al., 1999). The tailings from the floating process were dumped in two different ponds separated by a dam: one for the tailings coming from the processing of Cu-rich pyroclast and another pond for the tailings coming from the processing of complex pyrite.

In the early morning of April the 25<sup>th</sup> 1998, the tailing-dam dike enclosing pyritic mine sludge was broken. About 6  $\text{hm}^3$  of slurry composed of acidic water (3-4  $\text{hm}^3$ ) and sludge (2  $\text{hm}^3$ ) laden with heavy metals and other toxic elements, finely divided metal sulfides (mainly pyrite), and materials used in the refining floating process, flooded ca. 4500 ha of land along the Agrio and Guadiamar river valleys. A long strip, approximately 300 m wide and 40 km long, was covered by a layer (2-30 cm thick) of black sludge (Cabrera et al., 1999; Grimalt et al., 1999) (Figure 1). Dissolved and particulate trace elements in the slurry polluted the soils and severe As, Bi, Cd, Cu, Pb, Sb, Tl and Zn pollution was observed in most of the sludge-affected soils (Cabrera et al., 1999; 2008a). About 1  $\text{hm}^3$  of sludge was deposited on the river bench while the other half was deposited on the river margins, mainly agricultural land (López-Pamo et al., 1999). Approximately 25  $\text{hm}^3$  of slurry remained in the tailings dam.

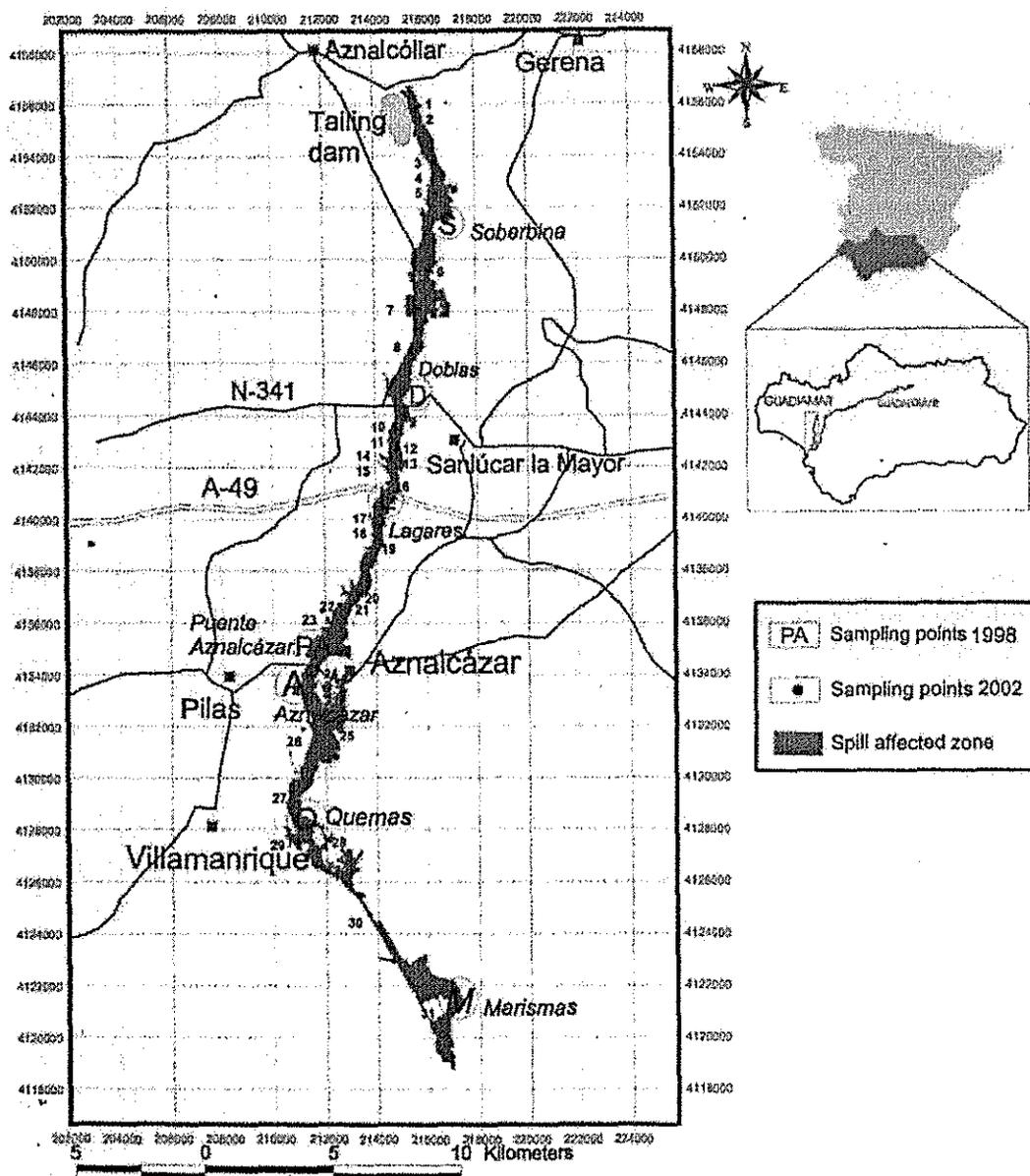


Figure 1. The Guadiamar River. Extent of the affected zone and sampling areas (adapted from Cabrera et al., 2008).

The accident was caused by the appearance of a fracture at 14 m depth in the marl layer beneath the tailings pond. The fracture was formed as a consequence of the overpressure generated by the dam and tailings on the interstitial water of the marl layer beneath the dam. In addition, leaching of acid water from the dam and subsequent weathering of the  $\text{CaCO}_3$  of the marl clays could have contributed to the accident. The mining company Boliden officially recognized in February 1999 the erroneous design of the dam.

Contamination of soils in the Guadiamar Valley was a three-stage process (Vidal et al., 1999). Firstly, the acid waste waters flooded the Agrio and Guadiamar rivers and their banks. The degree of penetration of the waste water was determined by the texture and structure of the soils, which were mostly loam sandy and clay, calcareous soils. The second phase was characterized by the arrival of the sludge, formed mainly by fine particles between 4.5 and 12  $\mu\text{m}$  of pyrite with low amounts of clay, quartz and gypsum (Querol et al., 1998). The thickness of sludge deposition was determined by the geographical structure of the basin.

Deposition decreased with distance from the source, so areas near the Guadalquivir marshlands were affected only by the acid waste waters (Cabrera et al., 1998). After deposition, the sludge oxidized partially at the surface of the deposition layers into iron sulphate species, which led to the precipitation of iron hydroxides in the wettest areas (third phase) (Querol et al., 1998). Finally, short rain events during the following month could have leached partially contaminants down the soil profile.

Remediation works started soon after the accident. An emergency program consisting of three phases was conducted: first of all the toxic sludge and a variable layer of topsoil (10-30 cm) were mechanically removed; a second phase comprised the addition of liming materials to immobilize cationic trace elements in soil; finally organic amendments and iron-rich clayey materials were incorporated into the affected soils to improve their organic matter content and stabilize anionic contaminants such as As, respectively (Aguilar et al., 2004).

After these short-term measures, the Regional Government “Junta de Andalucía” decided to establish a green corridor along the Guadiamar River connecting the lowland protected area “Doñana National Park” (the largest bird reserve in Europe) with the forested mountains of the “Sierra Norte Natural Park” (CMA, 2003). Within the frame of the “Guadiamar Green Corridor Programme” the local authorities focused on three main environmental aspects: a) the immobilization of the contaminants, b) the restoration of a natural vegetation cover using autochthonous plants, and c) additional monitoring activities to evaluate the mobility of contaminants and their potential risks to the environment and to living organisms.

## Cadmium Concentrations in the Sludge and in Affected Soils Before and After Remediation

### *Cadmium concentrations in the sludge and affected soils*

The chemical composition of the sludge was strongly related to the ore composition and the efficiency of the ore recovery process. Several authors reported similar results and showed that the main contaminants of the spill were: As, Bi, Cd, Cu, Hg, Pb, Sb, Tl and Zn (Alastuey et al., 1999; Cabrera et al., 1999; 2008; López-Pamo et al., 1999; Simón et al., 1999; Galán et al., 2002). Cabrera et al. (1999) reported a maximum concentration of Cd of 36 mg kg<sup>-1</sup> and a minimum value of 15 mg kg<sup>-1</sup>. Based on data from different authors the sludge had a mean Cd concentration of 26 mg kg<sup>-1</sup> (Table 1).

**Table 1. Maximum, minimum and mean concentrations of Cd (mg kg<sup>-1</sup>) in the sludge.**

maximum	minimum	mean	reference
25.0	31.0	27.7	Alastuey et al., 1999
36.8	22.9	28.6	Simón et al., 1999
36.4	15.1	25.1	Cabrera et al., 1999
28.8	20.4	23.5	Galán et al., 2002

**Table 2. Mean concentrations and ranges for Cd in soils non-affected and affected by the spill. Values are expressed in mg kg<sup>-1</sup>.**

non-affected		affected		normal	critical	reference
mean	range	mean	range	range*	range**	
0.33	0.12-1.06	1.69	0.12-22.0	0.01-2.0	3-8	Cabrera et al., 1999
0.30 <sup>x</sup>	0.1-0.9	2.2	0.4-5.9			Simón et al., 1999
			0.33- 6.00			Vidal et al., 1999
0.3		1.9	1.1-3.0			Galán et al., 1999

<sup>x</sup>median value. \*Bowen (1979) and\*\*Kabata-Pendias and Pendias (1992).

**Table 3. Mean values and standard deviations (in parenthesis) of Cd concentrations, pH and CaCO<sub>3</sub> content in soils of the northern sector of the Guadamar River (adapted from Cabrera et al., 2008b).**

depth (cm)		Soil 1 (n=6)	Soil 2 (n=23)	Soil 3 (n=11)	Soil 4 (n=5)
0-20	Cd (mg kg <sup>-1</sup> )	3.10 (3.00)	1.12 (0.87)	1.32 (1.41)	0.42 (0.21)
20-40		2.87 (2.74)	0.69 (1.31)	1.76 (4.09)	0.26 (0.05)
0-20	pH	7.5 (0.1)	4.7 (1.1)	4.5 (1.1)	7.5 (0.1)
20-40		7.4 (0.5)	5.4 (1.1)	5.1 (1.0)	7.6 (1.0)
0-20	CaCO <sub>3</sub> (g kg <sup>-1</sup> )	104 (82)	<DL	<DL	125 (48)
20-40		53 (65)			121 (56)
Mean values of Cd (mg kg <sup>-1</sup> ) before the sludge removal					
0-20			1.02		
20-40			0.24		
Background values (north sector)			0.18		
Background values (whole valley)			0.33		

Soil 1: Typic Xerofluvent calcareous; Soil 2; Typic Xerofluvent non-calcareous; Soil 3: Typic Xerochrept; Soil 4: Calcixerollic Xerochrept.

Soils affected by the spill showed concentrations of As, Bi, Cd, Cu, Hg, Pb, Sb, Tl and Zn significantly higher than the geochemical background values of the area within the first 50 cm of the soil profile (Cabrera et al., 1999; López-Pamo et al., 1999; Simón et al., 1999). Table 2 summarizes Cd concentrations in unaffected soils and in soils affected by the mine-spill before remediation works started. Ranges for Cd concentrations in affected soils varied greatly between authors, although a mean value of approx. 2 mg kg<sup>-1</sup> was reported in most cases (Table 2). This could be due to the fact that these data do not necessarily correspond to the same zone. Table 2 shows that Cd concentrations were significantly higher in affected soils than in control soils. In general, mean values of Cd reported by different authors were within the normal range for soils, but in many cases, concentrations within and above the critical range for soils were also observed (Table 2).

#### *Cadmium concentrations in soil after sludge removal*

The first remediation measure carried out was the removal of the sludge and a variable layer of topsoil (10-30 cm) depending on the area. Despite the clean-up operations, results

from various studies showed that affected soils still presented higher concentrations of Cd and trace elements than unaffected soils (Nagel 2000; Madejón et al., 2002; 2004).

Nagel (2000) studied Cd and trace element concentrations in four soil units in an area closed to the dam (37° 24' - 37°28' N, 06°12' – 06°14' W) six months after the accident. Results from that sampling showed that the degree of contamination was independent of the soil type (Table 3). Total Cd and trace element concentrations were generally higher near the soil surface than at greater depths. In some cases, higher concentrations of Cd were recorded after the removal of the sludge than before.

Madejón et al. (2002) investigated available Cd and trace element concentrations in soil in two former agricultural areas upstream from the mine (not affected by the spill) and three former agricultural areas downstream from the mine (affected by the spill and cleaned). Despite sludge and topsoil removal Cd availability (estimated using a 0.05M EDTA extracting solution) was significantly higher in all downstream areas (0.85-2.15 mg kg<sup>-1</sup>) than in non-affected areas located upstream from the mine (0.01-0.033 mg kg<sup>-1</sup>).

In a similar study focused on soils located on the floodplain of the Guadiamar River sustaining riparian forests, total and available Cd (0.05M EDTA-extractable) concentrations were determined at two depths (0-25 cm and 25-40 cm) in two control areas upstream from the mine and five spill-affected areas downstream from the mine (Madejón et al., 2004). Results showed that total and available Cd concentrations at both depths were higher in spill-affected soils than in controls despite the clean-up operations. At the same time, Cd concentrations decreased down the soil profile (Table 4) as reported in other studies in different areas of the Guadiamar Valley (Cabrera, 2000; Burgos et al., 2008). In general, penetration of contaminants within the sludge and acidic waters in the soil profile was highly influenced by soil texture, being significantly lower in clay soils compared to those with a loam or sandy texture (Cabrera, 2000).

#### *Cadmium concentrations in soil after termination of remediation works*

Cabrera et al. (2008a, b) and Domínguez et al., (2008) recently reported results from two extensive soil samplings carried out along the Guadiamar valley in 2002 and 2005, three and six years after the remediation works terminated. Results from this study showed that Cd and trace element concentrations (As, Cu, Hg, Pb and Zn) were generally higher in 2002 than in 2005, although differences were not always significant. Except As, Cd and trace element concentrations in both samplings were below the intervention levels proposed for andalusian soils by Aguilar et al. (1999), but above the geochemical background levels described by Cabrera et al., (1999) indicating residual soil contamination.

**Table 4. Mean values (mg kg<sup>-1</sup>) and standard deviations of total and available (0.05M EDTA-extractable) Cd at two depths in soils affected (n = 25) and not affected by the spill (n = 10) (adapted from Madejón et al., 2004).**

	Surface soil (0-25cm)		Deep soil (25-40cm)		background
	affected	non-affected	affected	non-affected	
Total	4.29±0.53	1.56±0.15	3.64±0.41	1.57±0.16	0.01-2
Available	1.25±0.19	0.03±0.01	1.17±0.24	0.02±0.01	

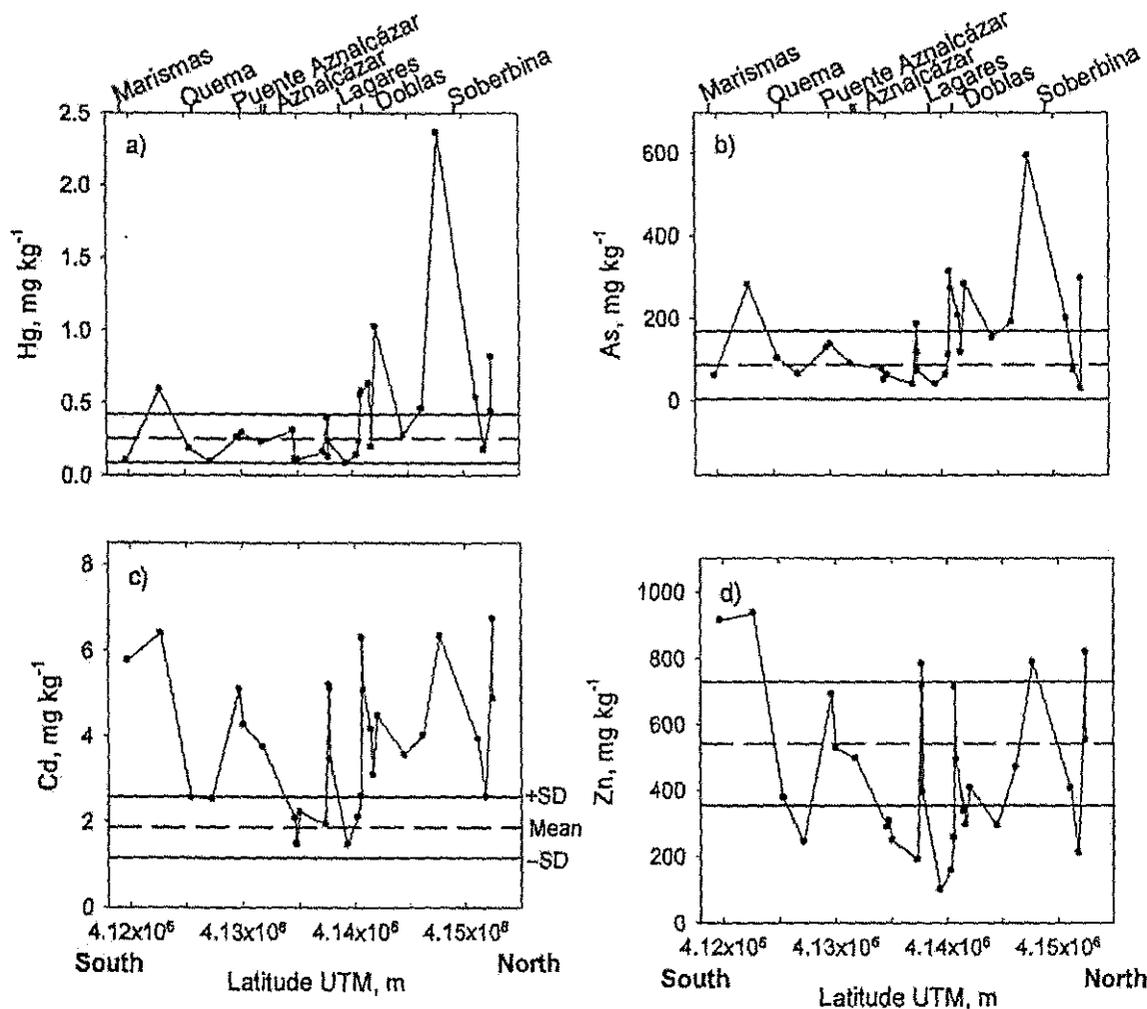


Figure 2. Mean concentrations values of: a) mercury; b) arsenic; c) cadmium and d) zinc in soils along the Guadiamar River in 2002. Mean values (dashed lines) and standard deviations (straight lines) in 1998 are also indicated (adapted from Cabrera et al., 2008).

Figure 2 shows concentrations of As, Hg, Cd and Zn in soils along the Guadiamar River starting in Soberbina (closest point to the mine) and heading south to Marismas (furthest point from the mine). Figures 2a and 2b show that Hg and As concentrations were clearly highest near the mine and decreased as distance from the mine increased. A similar pattern was found for Pb (data not shown), the most severe contaminant together with As. In contrast, Cd and Zn concentrations followed a very similar pattern with marked differences between sampling areas and no distance-related influence of the sampling location to the mine (Figures 2c and d). Using Principal Component Analysis different associations of trace elements (Cd-Zn and As-Hg-Pb) were found (Cabrera et al., 2008). Similar results were reported by Galán et al. (2002) after a systematic sampling around the Quema ford, approximately 30 km downstream of the mine tailings. The study was carried out after the sludge was removed and the preliminary soil treatments had been concluded.

Such associations seem to be closely related to the origin of these elements in the spill. Arsenic, Hg and Pb entered the soil mainly in the solid phase (pyrite sludge, particulate elements), whereas Cd and Zn were also found strongly associated with the liquid phase (acid waters, dissolved elements). Based on this, the relative mobility of Cd and Zn should be much higher than that of the other elements and hence larger horizontal transfer (downstream)

would be expected for Cd and Zn. The distinct nature of the above-mentioned elements within the spill not only influences horizontal transport of contaminants, but also their vertical mobility. In this way, dissolved elements such as Cd penetrated into the soil to a depth that largely depends on their interactions with soil constituents. On the contrary, penetration of particulate elements such as As and Pb should be much lower as the potential entry route for these was via surface run-off entering soil cracks and pores.

Remarkably Cd and trace element concentrations in most locations from the 2002 and 2005 samplings were higher than mean values in 1998, immediately after the accident. Similar results were reported by other authors (Nagel 2000; Burgos et al., 2008). This is due to the sampling strategy used in 1998, where soil samples were carefully taken avoiding the sludge layer. In this manner no mixing between soil and sludge material occurred. During remediation operations and the restoration process, remains of sludge were left on the soil surface and subsequently buried; this could be the main contact between soil and sludge. As a result, total soil trace element concentrations increased and patchy or irregular distributions for Cd and other elements were observed in many restored areas (Antón-Pacheco et al., 2001; Cabrera et al., 2007; Burgos et al., 2006; 2008).

Results from all these studies showed that soils of the affected area are still contaminated despite the clean-up operations and the remediation measures carried out by the local authorities. The question remains how to deal with this residual contamination and to what extent this is hazardous for the environment and the soil-plant-system.

### ***Remediation of soils contaminated with Cd and other trace elements***

Remediation of trace element-contaminated soils is still a challenge, since trace elements cannot be decomposed in the environment unlike degradable organic contaminants or short-lived radionuclides (Knox et al., 2001). Remediation options for trace element-contaminated soils are based on physical, chemical or biological techniques (Vangronsveld and Cunningham, 1998) and can be classified as *ex situ*, if the soil is excavated and treated at the affected site or transported and treated somewhere else, or *in situ* if the soil is not excavated and is treated at the affected site. Excavation of trace element-contaminated soils (*ex situ* techniques) may be impractical due to the excessive cost involved, the magnitude (area, depth, volume) of the soil contamination, and the degree of disruption incurred at the affected site. Within the *in situ* techniques (isolation, removal/extraction and stabilization) few of them are cost-effective and reliable for land treating of extensive contaminated areas.

Assisted natural remediation is based on the use of amendments and/or plants to accelerate processes (sorption, precipitation and complexation reactions) that occur naturally in soils to reduce the mobility and bioavailability of toxic elements (Bolan and Duraisamy, 2003). Natural attenuation processes on their own may not be sufficient to mitigate risks from trace elements. This process may also enhance microbial activity, plant colonisation and development and nutrient cycling in the affected soils (Pérez-de-Mora et al., 2005; 2006a). Plant cover also prevents re-movement of contaminant particles and may reduce migration of contaminants to groundwater. Numerous amendments have been used to immobilize Cd and other trace elements in contaminated soils (Knox et al., 2001). These include lime, zeolites, apatite, Fe and Mn oxides, alkaline composted biosolids, clay minerals and industrial by-products such as beringite. Although many studies have shown positive effects of amendment application in reducing trace element solubility and availability (Vangronsveld et al., 2000),

there is still concern regarding the longevity of remediation. Those amendments that promote sequestration of trace elements in non-labile pools, such as the inorganic fraction of biosolids or surface complexation by covalent bonding have greater potential longevity (Li et al., 2001). Reacidification of soil may reverse the action of amendments that make soils alkaline. Mineralization of organic matter present in biosolids may also release trace elements in potentially bioavailable forms. Traditionally, repeated applications of amendments have been recommended to maintain trace element immobilization, but more work is required to refine these procedures.

Despite some limitations, assisted natural remediation might be more suitable for land treating extensive areas than other *in situ* techniques such as phytoextraction. At present, phytoextraction (which uses hyperaccumulator plants to extract and accumulate trace elements in shoots) has not been fully developed. It presents some constraints for effective *in situ* reclamation of soils contaminated with various trace elements such as adaptation to local conditions and low biomass; in addition, the extraction efficiency diminishes with subsequent yields and plants tend to accumulate only one or two elements (Kamnev and van der Lelie, 2000). Herbivory must also be controlled to avoid contaminant entry in the food chain (Khan et al., 2000).

In the case of the Aznalcóllar mine accident, the local authorities decided to follow a stabilization strategy for the control of the residual contamination once the sludge was removed. This included the utilization of amendments and local vegetation well adapted to the harsh and arid conditions of the area (low nutrient status, drought and extremely high temperatures). In order to assess the potential of incorporating amendments into the soil to stabilize Cd and other trace elements and the longevity of assisted natural remediation, we initiated two long-term experiments back in 2002, one under semi-field conditions and the second under field conditions.

Pérez-de-Mora et al. (2006b, c; 2007a, b) carried out a 3-year semi-field study in which various amendments and/or a plant cover (*Agrostis stolonifera* L.) were tested for their potential to stabilize Cd and various trace elements (As, Cu, Pb and Zn) in a moderately contaminated soil affected by the Aznalcóllar mine spill. Results from these studies showed that the incorporation of sugar beet lime and composts (biosolid and municipal waste) into the soil significantly reduced Cd and trace element availability (0.01M CaCl<sub>2</sub>-extractable) compared with other amendments (litter and Leonardite) and controls (Figure 3). In addition, the soils treated with these amendments showed better plant performance and lower accumulation of Cd and trace elements in aboveground parts (Pérez-de-Mora et al., 2006c). Regular monitoring of leachates from the different treatments also showed reduced leaching of Cd (40-70% reduction at the end of the experiment) under the influence of these amendments (Figure 4) (Pérez-de-Mora et al., 2007a). The authors attributed the success of these amendments mainly to the readily alkalization of the soil following their initial application. Using a sequential extraction procedure Pérez-de-Mora et al. (2007b) observed that trace elements from these by-products were mainly related to the residual fraction of these materials and hence they were unlikely to be released following chemical changes in the soil (Pérez-de-Mora et al., 2007b). Furthermore, the authors found that residual Cd in soil was mainly related to the presence of metal sulfides (recovered in the residual fraction) and the formation of iron oxides, hydroxides and oxyhydroxides which co-precipitate metals after oxidation of sulfides (Fracción 2, Figure 5). Only 10% of total Cd was found as available, whilst no association with the organic matter pool was found in contrast to other relatively

mobile elements such as Cu and Zn (Figure 5). This suggests that mobility of residual Cd depends largely on the redox state of the soil and its pH. Mineralization of organic matter may thus play a secondary role in these soils.

Burgos et al. (2006; 2008a, b) and Madejón et al. (2006; 2009a) carried out a series of studies in an experimental field named “El Vicario” (37° 26'21''N, 06°12'59''W), affected by the toxic Aznalcóllar mine spill. In the study of Burgos et al. (2006) total, available (0.05M EDTA-extractable) and soluble (0.01M CaCl<sub>2</sub> extractable) Cd and trace element concentrations in soil before and after incorporation of amendments (only one amendment application) were investigated. While no significant differences between control and amended subplots were reported for total and available concentrations, solubility of Cd, Cu and Zn was found to decrease in amended soils. This effect was most pronounced in those subplots amended with sugar beet lime (Table 5). Based on statistical and geostatistical evidence the authors suggested that the alkalinizing effect of the amendments on the soil pH, and particularly of sugar beet lime, was the main cause for this phenomenon.

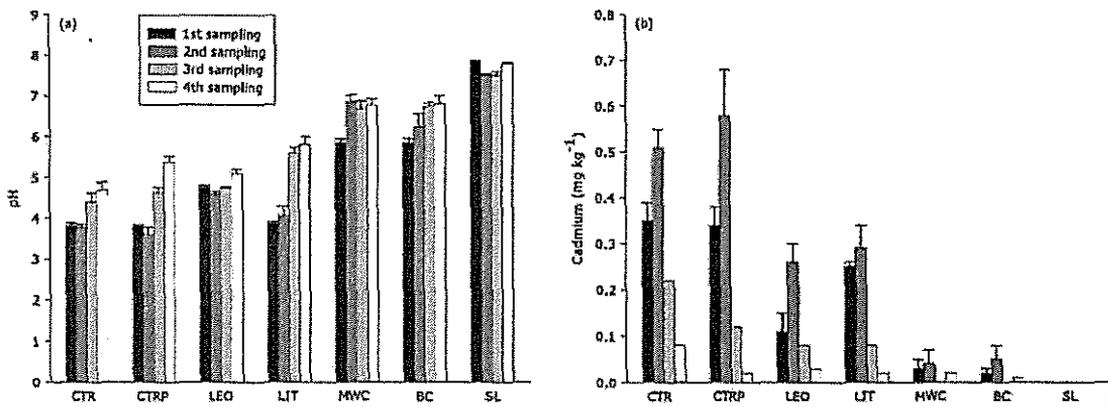


Figure 3. Mean values and standard errors of soil a) pH and b) 0.01M CaCl<sub>2</sub> extractable Cd in the different treatments. BC = biosolid compost; CTR = control without plant; CTRP = control with plant; LEO = leonardite; LIT = litter; MWC = municipal waste compost and; SL = sugar beet lime (adapted from Pérez-de-Mora et al., 2006b).

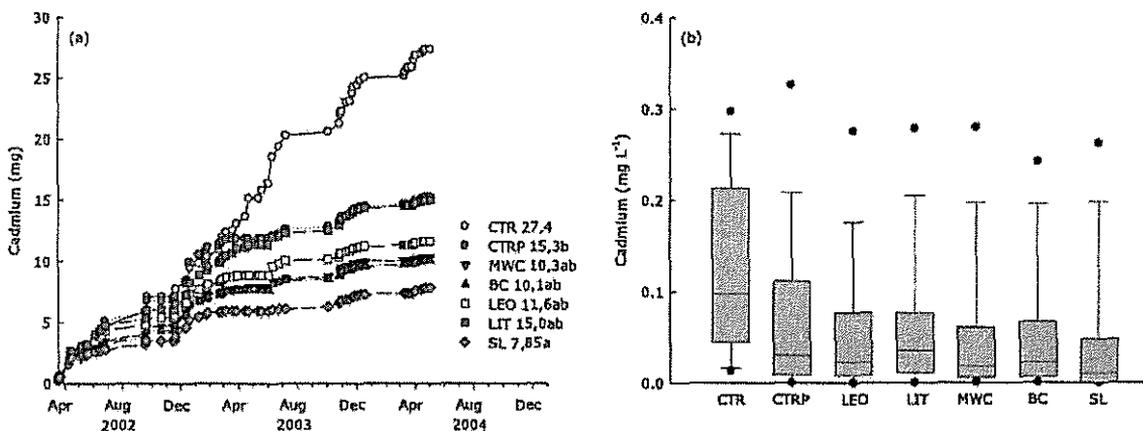


Figure 4. (a) Cumulative Cd leached (mg container<sup>-1</sup>) for each treatment during the experimental period. Different letters indicate significant differences between mean values ( $p < 0.05$ ). b) Box and whiskers diagram of Cd concentrations in drainage water for each treatment. Straight lines inside the boxes indicate median values (adapted from Pérez-de-Mora et al., 2007a).

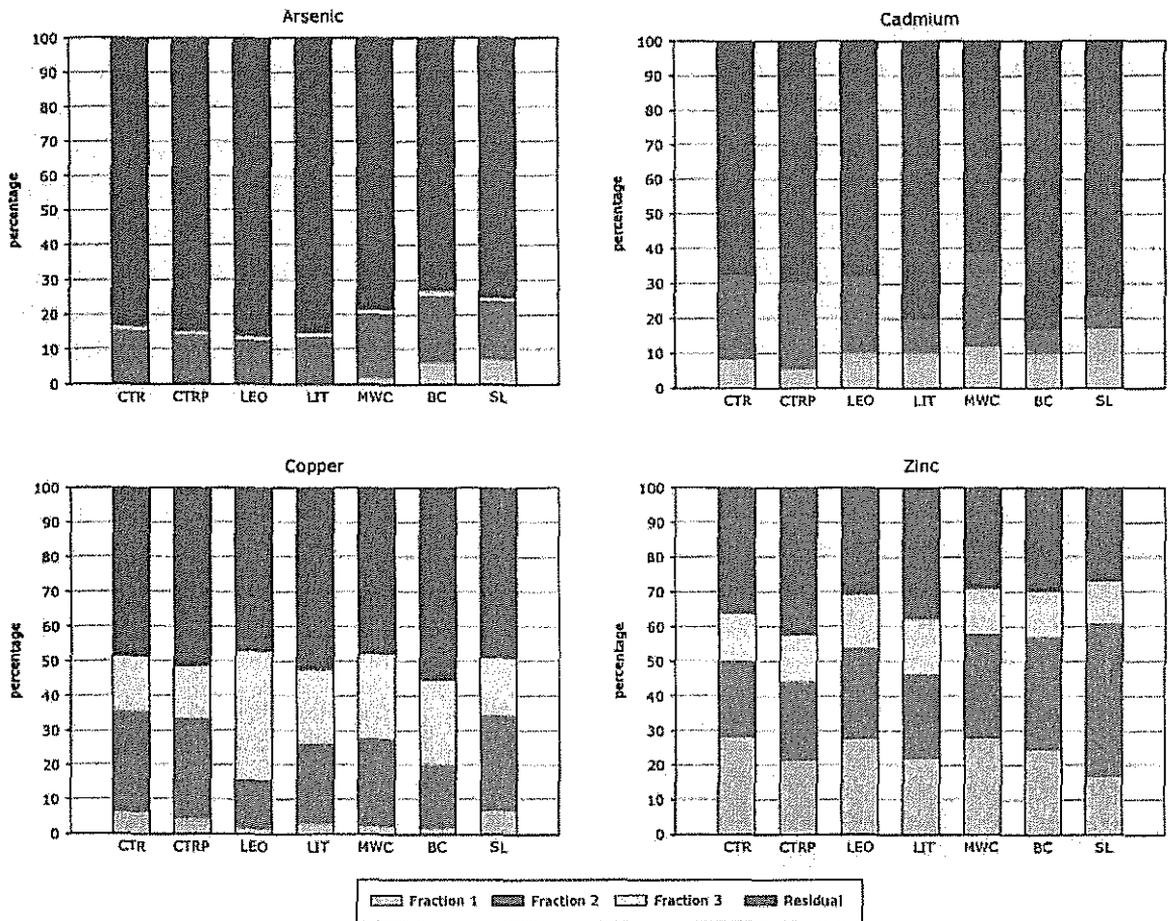


Figure 5. Sequential fractionation of a) arsenic, b) cadmium, c) copper and d) zinc. Each fraction is supposed to represent: available Cd (Fraction 1); Cd associated with iron oxides/oxyhydroxydes (Fraction 2); Cd associated with organic matter (Fraction 3); residual/recalcitrant Cd (adapted from Pérez-de-Mora et al., 2007b).

**Table 5. Mean values of pH, TOC, total S and soluble (0.01M CaCl<sub>2</sub>) Cd, Cu and Zn in soils treated with different amendments after one year of application (adapted from Burgos et al., 2006).**

Variable	Treatment			
	NA	BC	LEOSL	SL
pH	3.19a	4.67bc	4.17ab	6.01c
TOC (%)	0.81a	1.38b	1.50b	1.18ab
S (mg kg <sup>-1</sup> )	6434a	5506a	8059a	5395a
Soluble (0.01M CaCl <sub>2</sub> )				
Cd (mg kg <sup>-1</sup> )	0.62c	0.31a	0.47bc	0.19a
Cu (mg kg <sup>-1</sup> )	27.3b	6.10a	21.7a	11.7a
Zn (mg kg <sup>-1</sup> )	152b	76.2a	93.9a	65.9a

Values followed by the same letter in the same row do not differ significantly ( $p < 0.05$ ). BC = biosolid compost; LEOSL = leonardite + sugarbeet lime; NA = not amended; SL = sugarbeet lime; TOC = total organic matter.

**Table 6. Cadmium concentrations (mg kg<sup>-1</sup>) in above-ground parts of various species growing on an experimental field plot (adapted from Burgos et al., 2008b).**

Species	Treatment			
	NA	BC	LEOSL	SL
<i>Poa annua</i> (2004)	1.4±0.4	0.9±0.2	0.6±0.2	0.7±0.2
<i>Lamarckia aurea</i> (2004)	0.8±0.4	0.5±0.1	0.5±0.1	0.4±0.2
<i>Bromus rubens</i> (2005)	0.2±0.1	0.1±0.1	0.1±0.1	<0.1
<i>Lamarckia aurea</i> (2005)	0.6±0.1	0.2±0.1	0.2±0.1	0.1±0.1

BC = biosolid compost; LEOSL = leonardite+sugarbeet lime; NA = not amended; SL = sugarbeet lime.

Madejón et al. (2006a) studied the effect of the same treatments on natural vegetation growing on the experimental plot following a second amendment application. All treatments improved considerably plant performance in comparison with non-amended soil as shown by higher biomass yields, percentage of plant cover and number of species. Amendment treatments notably reduced Cd and trace element accumulation in above-ground parts of various species analyzed: concentrations of Cd in shoots of *Oxalis pes-caprae* L., *Poa annua* L. and *Lamarckia aurea* (L.) Moench growing on amended soil were reduced by 35-60% compared to non-amended controls. Burgos et al. (2008) confirmed the durability of this trend after analyzing Cd and trace elements concentration in shoots of representative species growing on the same experimental field one and two years after the second amendment application. Lower Cd concentrations in shoots of *Poa annua* L. and *Lamarckia aurea* (L.) Moench in 2004, and *Bromus rubens* L. and *Lamarckia aurea* (L.) Moench in 2005 were reported for the three treatments with amendments with respect to the non-amended control (Table 6). These studies also confirmed previous results by Pérez-de-Mora et al. (2006b, c) at semi-field scale, which showed that estimation of phytoavailability of Cd and other trace elements was generally better using 0.01M CaCl<sub>2</sub> than 0.05M EDTA. The latter seemed to overestimate concentrations of some trace elements such as Cu and Pb in shoots. It is therefore necessary to handle data from extraction procedures with caution and preferentially test at least two of them. A complementary plant survey should be carried out at least in those areas in which plant material is available and the same species grows.

### *Cd transfer within the soil-plant-animal system*

Biomonitoring is essential to assess the risks associated with the accumulation and mobility of trace elements in the environment. This is particularly important for Cd, since plants can tolerate and accumulate relatively high amounts of this element (up to 700 mg kg<sup>-1</sup>) (Chaney, 1989). Such concentrations may be, however, toxic for animal or human consumption. Additionally plants may accumulate Cd on aboveground parts via contaminated dust deposition. Cadmium originating from dust/soil particles may be equally taken up by herbivores feeding on contaminated plants. Since the Aznalcóllar mine accident several studies have addressed Cd and trace element accumulation on plants that survived the spill. The aim was to evaluate the potential entry of trace elements in the food chain and the associated risks for consumers. One of the first studies (Madejón et al., 2002) investigated bioaccumulation of Cd and other trace elements in wild grasses (*Cynodon dactylon* and *Sorghum halepense*) growing in three different areas: a) unaffected areas (control); b)

remediated or cleaned-up areas and c) non-remediated. The authors found that while various elements (As, Cd, Cu and Pb) reached toxic concentrations for livestock in above-ground biomass of both species in sludge-covered, non-remediated areas, Cd was the only element that reached toxic levels in cleaned-up and remediated soils. In order to evaluate the effect of dust deposition on the aboveground biomass of these grasses, washed and unwashed samples were analyzed. In all cases unwashed samples showed higher concentrations of Cd and other trace elements than washed samples.

In another study focused on white poplar (*Populus alba*) growing in riparian forest affected by the spill, Madejón et al. (2004) compared trees from contaminated areas with trees growing on non-affected soils. The authors reported that among the elements studied, Cd showed the highest concentration factor ( $[Cd]_{\text{plant}}/[Cd]_{\text{soil}}$ ) for both leaves (18 times) and stems (10 times). In the most extreme cases, concentrations as high as  $15 \text{ mg kg}^{-1}$  were reported for leaves. This value is within the phytotoxic range for plants ( $5 - 700 \text{ mg kg}^{-1}$ ; Chaney, 1989), but still far from the extreme concentrations ( $200 \text{ mg kg}^{-1}$ ) reported by Robinson et al. (2000) for white poplar growing on soils severely polluted by Cd. Toxic Cd concentrations were also found in fruits of wild olive (*Olea europaea*) growing on spill-affected soils (Madejón et al., 2006b). These fruits are the main source of food for wintering birds in Mediterranean forests such as *Turdus* spp. and *Sturnus* spp and hence they could suffer some Cd intoxication. Previous work (Benito et al., 1999) had reported high levels of Cd in blood from birds feeding in the area. In this case, the relatively high concentration of Cd in the fruits was attributed to the deposition of contaminated dust on the fruit surface rather than to translocation mechanisms within the plant. Nonetheless, Madejón et al. (2006b) recorded a time-dependent decrease of Cd concentrations in wild olive tree fruits from 1999 to 2001, which may be related to the cessation of the remediation works with heavy machinery.

More recently, Domínguez et al. (2008, 2009a) investigated the distribution of trace elements in soils and woody plants from the Guadiamar Valley 7 years after the accident. The authors studied the factors determining the availability of Cd and other trace elements in the soils, and their transfer to the aboveground biomass of three tree and five shrub species in a total of 19 locations along the Guadiamar River, three of them unaffected by the spill. They compared the adult trees surviving the spill with the afforested saplings, used in the remediation project. Despite high total concentrations of trace element in remediated soils, available trace elements (extracted with  $\text{NH}_4\text{NO}_3$ ), were relatively low, with the exception of some extremely acidic sites ( $\text{pH} < 4$ ) where the availability of cationic trace elements significantly increased. Cadmium was the most available metal, in relative terms; 15 % of the total Cd in the soil was extracted with  $\text{NH}_4\text{NO}_3$ . The concentrations of Cd and other trace elements in the aboveground parts of the woody species were, on average, within the normal ranges for higher plants ( $0.1 - 1 \text{ mg kg}^{-1}$ ). The exception was the high Cd and Zn accumulation by poplar leaves in the spill affected area. Cadmium accumulated up to  $3 \text{ mg kg}^{-1}$  in leaves from poplar trees growing on affected soils. For the other woody species, the low leaf concentrations of Cd may be due to a high capacity of Cd retention in fine roots, as described for Holm oak (*Quercus ilex*) under controlled conditions (Domínguez et al, 2009b). In general, results from biomonitoring and mobility studies on Cd based on (sequential) extraction procedures agree underpinning the importance of Cd as a major risk in the area despite a relatively low concentration in the sludge and low uptake by most woody species (with exception of poplar and willows).

**Table 7. Daily predicted intake of trace elements (Mean values  $\pm$  SD) by consumption of pasture growing on affected (n=90) and control (n=10) soils. Estimated food intake for horses in mg element kg<sup>-1</sup> body weight day<sup>-1</sup> (data based on a daily food intake of 21 g of plant dry weight per kg of body weight). A/C is the ratio between values at affected and control sites (adapted from Madejón et al., 2009b).**

Elements	Season	Soil	Cu	Fe	Mn	Zn
Essential	Spring	Affected (A)	0.15 $\pm$ 0.06*	3.60 $\pm$ 2.96*	1.27 $\pm$ 1.31	0.94 $\pm$ 0.70
		Control (C)	0.12 $\pm$ 0.05	1.75 $\pm$ 0.92	0.97 $\pm$ 0.15	0.34 $\pm$ 0.18
		A/C	1.25	2.06	1.31	2.76
	Autumn	Affected (A)	0.23 $\pm$ 0.12*	20.9 $\pm$ 31.3*	2.32 $\pm$ 2.41*	1.44 $\pm$ 1.48
		Control (C)	0.18 $\pm$ 0.02	5.90 $\pm$ 3.53	1.40 $\pm$ 0.31	0.86 $\pm$ 0.45
		A/C	1.28	3.54	1.66	1.67
			As	Cd	Pb	Tl
Non-essential	Spring	Affected (A)	0.006 $\pm$ 0.006*	0.006 $\pm$ 0.01	0.02 $\pm$ 0.02*	0.001 $\pm$ 0.004*
		Control (C)	0.0004 $\pm$ 0.0005	0.0005 $\pm$ 0.0005	0.007 $\pm$ 0.002	0.0001 $\pm$ 0.00005
		A/C	15.0	12.0	2.86	10.0
	Autumn	Affected (A)	0.04 $\pm$ 0.06*	0.010 $\pm$ 0.02*	0.10 $\pm$ 0.14*	0.001 $\pm$ 0.001*
		Control (C)	0.002 $\pm$ 0.002	0.0006 $\pm$ 0.0006	0.02 $\pm$ 0.01	0.0001 $\pm$ 0.00004
		A/C	20.0	16.6	5.0	10.0

\*significant differences between soils.

**Table 8. Trace element concentrations (mg kg<sup>-1</sup>, mean and SD) in horses' faeces from affected and control sites (adapted from Madejón et al., 2009b).**

Elements	Soil	Cu	Fe	Mn	Zn
Essential	Affected (A)	34.9 $\pm$ 23.9*	1914 $\pm$ 774*	187 $\pm$ 64.5*	142 $\pm$ 60.3
	Control (C)	19.4 $\pm$ 8.81	914 $\pm$ 78.8	90.6 $\pm$ 6.75	68.7 $\pm$ 19.0
	A/C	1.8	2.1	2.1	2.1
		As	Cd	Pb	Tl
Non-essential	Affected (A)	4.93 $\pm$ 3.69*	0.78 $\pm$ 0.57	13.8 $\pm$ 12.6	0.13 $\pm$ 0.11
	Control (C)	0.58 $\pm$ 0.08	0.15 $\pm$ 0.13	2.02 $\pm$ 0.37	0.02 $\pm$ 0.001
	A/C	8.5	5.2	6.8	6.5

\*significant differences between soils.

After the spill, and subsequent cleaning-up, remediation and afforestation tasks, the local government "Junta de Andalucía" banned agricultural and grazing activities in the affected area. As a result, a vigorous herbaceous cover competes with planted woody species for water and nutrients. In addition, the desiccated remains of the herbaceous vegetation (mostly annuals) present a fire hazard during the summer droughts. Mechanical control of these species is expensive, may affect biodiversity and generates greenhouse gas emissions. The fact that previous studies had shown that trace element accumulation in most plant species decreased with time and the difficulties in controlling illegal grazing by horses in the remediated area, made the local authorities reconsidered the possibility of permitting grazing

for non-edible animals in the Guadiamar Valley. Recent work by Madejón et al. (2009b) evaluated the potential ingestion of Cd and other trace elements (As, Cu, Pb, Tl and Zn) from pasture by horses and the possibility of using grazing as a management tool for herbaceous vegetation in the Green Corridor. The floristic composition of pastures, trace elements concentrations in the dominant herbs, and trace element concentrations in horse faeces and hair were analyzed. In general, Cd concentrations were enhanced in pastures where *Asterecea* dominated over *Poacea*. As horses select mainly *Poaceae* for feeding, other diets based on plants different from *Poaceae* are currently in study attending their potential risk for other herbivores. Data derived from predicted intake of trace elements by consumption of pasture growing on affected and control soils showed that the intake ratio affected/control was highest for Cd together with As and Tl (Table 7). However, analysis of faeces showed regulated absorption of essential elements, while non-essential elements such as Cd were preferentially excreted (Table 8). Furthermore, elemental content of horse hair did not differ in animals feeding on contaminated areas from those grazing on pastures from control sites and trace element concentrations in herbage were found to be below the maximum tolerable limit for horses. These results provide no evidence of Cd toxicity to horses and hence grazing may be considered a phytomanagement alternative for the area. Systematic monitoring of both forage and animals in the long-term is, however, necessary.

## CONCLUSIONS

Contamination of soils affected by the Aznalcóllar mine spill with cadmium was highly influenced by the fact that most of the Cd was mainly found dissolved within the liquid phase of the spill, rather than within the particulate or solid phase. As a consequence, the extent of Cd contamination along the Guadiamar River floodplain was relatively independent of the proximity to the mine, in contrast to other elements such as As or Pb which were mainly found within the solid phase. Despite the clean-up and remediation works carried out after the Aznalcóllar mine spill, soils are still contaminated with Cd and other trace elements. This residual contamination seems to be mainly related to remains of sludge buried in the soil during clean-up and remediation operations with heavy machinery. Nonetheless, in most cases concentrations of Cd and other trace elements are below the intervention levels proposed for soils in Andalusia.

The incorporation of amendments into the soil following sludge removal to assist natural attenuation processes shows great potential for long-term stabilization of Cd and other trace elements. It is, however, important to monitor changes in soil conditions in the long-term and evaluate the need for additional amendment doses. The use of stabilized by-products such as composts containing trace elements in recalcitrant forms will likely improve the success of this approach.

Despite the low concentration of Cd in the spill, higher soil-plant transfer of Cd occurred in remediated areas affected by the mine spill compared to the other contaminants. Accumulation of Cd and other trace elements on aboveground parts of plants via deposition of contaminated dust particles is another important source of biomass contamination. Accumulation of Cd by plants growing on contaminated soils is species-dependent and affected by seasonality. Recent data support low Cd and trace element accumulation in the

most representative woody species (both newly planted and old-survivors) of the Guadiamar Valley. However, accumulation of Cd on leaves of white poplar reaching toxic levels is still an issue of concern for phytomanagement of the Green Corridor. Fortunately, the first approaches showed that soil-plant-animal transfer of Cd and other contaminants seems to be low enough to allow grazing by horses in the Guadiamar Valley. For less selective animals it is still necessary the study of other kinds of diets.

The experience gained during the last decade following the Aznalcóllar mine accident has highlighted the role and hazards of Cd as a secondary contaminant associated with mine spills in multi-element contaminated soils and the difficulties in controlling Cd mobility efficiently even at low concentrations. A combined approach including physical, chemical and biological means has proven to be a very successful alternative for the reclamation and restoration of an extensively degraded and contaminated area. Supporting scientific monitoring activities and setting coherent political measures into motion will ensure the sustainability of the Green Corridor and its use by future generations.

## REFERENCES

- Adriano, D. C. (2001). Trace Elements in Terrestrial Environments: Biogeochemistry, *Bioavailability and Risks of Metals*, 2<sup>nd</sup> ed. Springer, New York, NY, 867.
- Adriano, D. C., Wenzel, W. W., Vangronsveld, J. & Bolan, N. S. (2004). Role of assisted natural remediation in environmental cleanup. *Geoderma*, 122(2-4), 121-142.
- Aguilar, J., Dorronsoro, C., Gómez-Ariza, J. L. & Galán, E. (1999). Los criterios y estándares para declarar un suelo contaminado en Andalucía y la metodología y la técnica para la toma de muestra y análisis para su investigación. *Junta de Andalucía*, 257.
- Aguilar, J., Dorronsoro, C., Fernández, E., Fernández, J., García, I., Martín, F. & Simón, M. (2004). Soil pollution by a pyrite mine spill in Spain: evolution in time. *Environ Pollut.*, 132, 395-401.
- Alastuey, A., García-Sánchez, A., Lóez, F. & Querol, X. (1999). Evolution of the pyrite mud weathering and mobility of heavy metals in the Guadiamar valley after the Aznalcóllar spill, South-West Spain. *Sci Total Environ.*, 242, 41-55.
- Alloway, B. J. (1990). Soil processes and the behaviour of metals. In: B. J. Alloway (ed.), *Heavy Metals in soils*. Blackie, John Wiley and Sons, Inc. New York, NY, 7-28.
- Antón Pacheco, C., Arranz, J. C., Berettino, D., et al. (2001). Actuaciones para el reconocimiento y retirada de los lodos depositados sobre el terreno, y su restauración edáfica y morfológica. *Boletín Geológica y Minero*, 112, 93-121.
- Benito, V., Devesa, V., Muñoz, O., Suñer, M. A., Montoro, R., Baos, R., et al. (1999). Trace elements in blood collected from birds feeding in the area around Doñana National Park affected by the toxic spill from the Aznalcóllar mine. *Sci Total Environ.*, 242, 309-23.
- Bolan, N. S. & Duraisamy, V. P. (2003). Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. *Aust J Soil Res.*, 41, 533-555.
- Bowen, H. J. M. (1979). *Environmental Chemistry of the Elements*. Academia Press, London, 228.

- Burgos, P., Madejón, E., Pérez-de-Mora, A. & Cabrera, F. (2006). Spatial variability of the chemical characteristics of a trace-element-contaminated soil before and after remediation. *Geoderma*, 130, 157-170.
- Burgos, P., Madejón, E., Pérez-de-Mora, A. & Cabrera, F. (2008a). Horizontal and vertical variability of soil properties in a trace element contaminated area. *Int J Appl Earth Observ Geoinform*, 10, 11-25.
- Burgos, P., Pérez-de-Mora, A., Madejón, P., Cabrera, F. & Madejón, E. (2008b). Trace elements in wild grasses: a phytoavailability study on a remediated field. *Environ Geochem Health.*, 30, 109-114.
- Cabrera, F. (2000). La contaminación por metales pesados en el valle del Guadiamar tras el vertido de Aznalcóllar. *Retema*, 74, 37-48.
- Cabrera, F., Clemente, L., López, R., et al. (1998). Contaminación por metales pesados de suelos característicos de la cuenca del Guadiamar afectados por el vertido tóxico. Proceedings from Jornadas Científicas para analizar los resultados obtenidos durante el seguimiento del efecto del vertido tóxico en el entorno de Doñana, 29 September- 2 October, El Rocío Huelva, Spain.
- Cabrera, F., Clemente, L., Díaz-Barrientos, E., López, R. & Murillo, J. M. (1999). Heavy metal pollution of soils affected by the Guadiamar toxic flood. *Sci Total Environ.*, 242, 117-129.
- Cabrera, F., Girón, I. & Moreno, F. (2007). Evolución de la contaminación con elementos traza en los suelos afectados por el vertido de Aznalcóllar. In: Bellinfante, N., Jordán, A. (eds.). Tendencias actuales de la Ciencia del Suelo. Vol 1: 220-227. Sociedad española de la Ciencia del Suelo. Huelva (Spain).
- Cabrera, F., Ariza, J., Madejón, E., Madejón, P. & Murillo, J. M. (2008a). Mercury and other trace elements in soils affected by the mine mailing spill in Aznalcóllar (SW Spain). *Sci Tot Environ.*, 390, 311-322.
- Cabrera F *et al.*, (2008b). The Soils of the Guadimar: Trace element contamination following the Aznalcóllar mine accident. The Ecological Restoration of the Guadiamar River and the Project of the Green Corridor: (in spanish). Consejería de Medioambiente. Junta de Andalucía, pp 145-164. ISBN: 978-84-96776-05-0.
- CERCLA (The Comprehensive Environmental Response, Compensation, and Liability Act) (2007). Priority List of Hazardous Substances. Agency for Toxic Substances and Disease Registry. [www.atsdr.cdc.gov/cercla/07list.html](http://www.atsdr.cdc.gov/cercla/07list.html)
- Chaney, R.L. (1989). Toxic element accumulation in soils and crops: protecting soil fertility and agricultural food-chains. In: Bar-Yosef, B., Barrow, N.J., Goldshmid, J. (Eds.). Inorganic contaminants in the Vadose Zone. Springer-Verlag, Berlin, pp. 140-158.
- Chaney, R. L. & Giordano, P. M. (1977). Microelements as related to plant deficiencies and toxicities. 234-279. In: L. F. Elliott, & F. J. Stevenson (Eds.), Soils for management of organic wastes and waste soils on metal binding. ASA, Madison, WI.
- CMA (CONSEJERÍA DE MEDIO AMBIENTE) (2003). Ciencia y restauración del Río Guadiamar. PICOVER 1998-2002. Consejería de Medio Ambiente de la Junta de Andalucía.
- Domínguez, M. T., Marañón, T., Murillo, J. M., Schulin, R. & Robinson, B. H. (2008). Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain: A large-scale phytomanagement case study. *Environ Pollut.*, 152, 50-59.

- Domínguez, M., Madejón, P., Marañón, T. & Murillo, J. M. (2009a). Afforestation of a trace-element polluted area in SW Spain: woody plant performance and trace element accumulation. *European Journal of Forest Research* (in press).
- Domínguez, M. T., Madrid, F., Marañón, T. & Murillo, J. M. (2009b). Cadmium availability in soil and retention in oak roots: potential for phytostabilization. *Chemosphere* 76, 480-486.
- Galán, E., Gonzalez, I. & Fernández-Caliani, J. C. (2002). Residual pollution load of soils impacted by the Aznalcóllar (Spain) mining spill after clean-up operations. *Sci Tot Environ.*, 286, 167-179.
- Grimalt, J. O., Ferrer, M. & McPherson, E. (1999). The mine tailing accident in Aznalcóllar. *Sci Tot Environ.*, 242, 3-11.
- Gustafsson, H. E., Lundgren, T., Lindvall, M., Lindahl, L. A., Eriksson, N., Jönaon, H., Broman, P. G. & Göransson, T. (1999). The Swedish acid mine drainage experience: research, development and practice. In: J. M. Azcue (ed.). *Environmental Impacts of Mining activities*. Springer-Verlag, Berlin Heidelberg, 203-228.
- Kabata-Pendias, A. & Pendias, H. (1992). *Trace Elements in Soils and Plants*, 2nd ed. CRC Press, Boca Raton, FL, 364.
- Kamnev, A. A. & van der Lelie, D. (2000). Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Biosci Rep.*, 20(4), 239-58.
- Khan, A. G., Kuek, C., Chaudry, T. M., Khoo, C. S. & Hayes, W. J. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 41, 197-207.
- Knox, A., Seaman, J. C., Mench, M. J. & Vangronsveld, J. (2001). Remediation of metal- and radionuclides-contaminated soils by *in situ* stabilization techniques. In: I. K. Iskandar, editor, *Environmental restoration of metal-contaminated soils*, Lewis Publishers, Boca Raton, FL, 21-60.
- Lane, T. W. & Morel, F. M. M. (2000). A biological function for cadmium. *Proc. Natl. Acad. Sci.*, 97(9), 4627-4631.
- Lane, T. W., Saito, M. A., George, G. N., Pickering, I. J., Prince, R. C. & Morel, F. M. M. (2005). A cadmium. *Nature*, 435(42).
- Leistel, J. M., Marcoux, E. & Thieblement, D. (1998). The volcanic hosted massive sulphide deposits of the Iberian Pyrite Belt. *Mineralia Deposita*, 33, 22-30.
- Li, Z., Ryan, J. A., Chen, J. L. & Al-Abed, S. R. (2001). Adsorption of cadmium on biosolids-amended soils. *J Environ Qual.*, 30, 903-911.
- Litz, N., Wilcke, W. & Wilcke, B. (2004). Bodengefährdende Stoffe. *Ecomed Biowissenschaften*, Landsberg am Lech, Germany.
- López-Pamo, E., Baretino, D., Pacheco, A. & Alonso, C. (1999). Contribución del ITGE a la evaluación de los efectos del accidente minero de Aznalcóllar sobre los suelos de la comarca del Guadiamar. *Tecnoambiente*, 86, 21-26.
- Madejón, E., Pérez-de-Mora, A., Felipe, E., Burgos, P. & Cabrera, F. (2006a). Soil amendments reduce trace element solubility in a contaminated soil and allow regrowth of natural vegetation. *Environ Pollut.*, 139, 40-52.
- Madejón, E., Madejón, P., Burgos, P., Pérez-de-Mora, A. & Cabrera, F. (2009a). Trace elements, pH and organic matter evolution in contaminated soils under assisted natural remediation: a 4-year study. *J Hazard. Mat.*, 162, 931-938.

- Madejón, P., Murillo, J. M., Marañón, T., Cabrera, F. & López, R. (2002). Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain). *Sci Tot Environ.*, 290, 105-120.
- Madejón, P., Marañón, T., Murillo, J. M. & Robinson, B. (2004). White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests. *Environ Pollut.*, 132, 145-155.
- Madejón, P., Marañón, T., Murillo, J. M. & Cabrera, F. (2006b). Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees. *Sci Total Environ.*, 355, 187-203.
- Madejón, P., Domínguez, M. T. & Murillo, J. M. (2009b). Evaluation of pastures for horses grazing on soils polluted by trace elements. *Ecotoxicology*, 18, 417-428.
- Martin, T. A. & Ruby, M. V. (2004). *Review of In situ technologies for remediation of lead, zinc and cadmium in soil*. Remediation, Wiley Periodicals Inc.
- Nagel, I. (2000). Schwermetallbelastung der Böden im Guadiamartal (Südspanien) nach dem Dambruch des Rückhaltebeckens der Mine "Los Frailes". *Diplomarbeit am Geographischen Institut der Eberhard-Karlsuniversität*. Tübingen.
- Naidu, R., Krishnamurti, G. S. R., Bolan, N. S., Wenzel, W. & Megharaj, M. (2001). Heavy metal interactions in soils and implications for soil microbial biodiversity. In: M. N. V. Prasad (ed.), *Metals in the Environment. Analysis by Biodiversity*. MarcelDekker Inc., New-York, 401-431.
- Nogawa, K., Kobayashi, E., Okubo, Y. & Suwazono, Y. (2004). Environmental cadmium exposure, adverse effects, and preventative measures in Japan. *Biometals*, 17(5), 581-7.
- Pérez-de-Mora, A., Ortega-Calvo, J. J., Cabrera, F. & Madejón, E. (2005). Changes in enzyme activities and microbial biomass after "in situ" remediation of a heavy metal-contaminated soil. *Appl Soil Ecol.*, 28, 125-137.
- Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P. & Schlöter, M. (2006a). Microbial community structure and function in a soil contaminated by heavy metals: effect of plant growth and different amendments. *Soil Biol Biochem.*, 38, 327-341.
- Pérez-de-Mora, A., Burgos, P., Cabrera, F. & Madejón, E. (2006b). Trace elements availability and plant growth in a mine-spill-contaminated soil under assisted natural remediation. Part I. Soils. *Sci Tot Environ.*, 363, 28-37.
- Pérez-de-Mora, A., Burgos, P., Cabrera, F. & Madejón, E. (2006c). Trace elements availability and plant growth in a mine-spill-contaminated soil under assisted natural remediation. Part II. Plants. *Sci Tot Environ.*, 363, 38-45.
- Pérez-de-Mora, A., Burgos, P., Cabrera, F. & Madejón, E. (2007a). "In Situ" Amendments and Revegetation Reduce Trace Element Leaching in a Contaminated. *Soil Water Air Pollut.*, 185, 209-222.
- Pérez-de-Mora, A., Madrid, F., Cabrera, F. & Madejón, E. (2007b) Amendments and plant cover influence on trace element pools in a contaminated soil. *Geoderma*, 139, 1-10.
- Querol, X., Alastuey, A., García Sánchez, A. & López, F. A. (1998). Seguimiento de la meteorización y toxicidad de los lodos. Proceedings from Jornadas Científicas para analizar los resultados obtenidos durante el seguimiento del efecto del vertido tóxico en el entorno de Doñana, 29 September- 2October, El Rocío Huelva, Spain.
- Robinson, B., Mills, T. M., Petit, D., Fung, L. E., Green, F. S. & Clothier, B. E. (2000). Natural and induced cadmium-accumulation in poplar and willow: implications for phytoremediation. *Plant Soil*, 227, 301-306.

- Ross, S. M. (1994). Sources and forms of potentially toxic metals in soil-plant systems. In: S. M. Ross (ed.), *Toxic Metals in Soil-Plant Systems*. Wiley & Sons, Chichester, 3-25.
- Simón, M., Ortiz, I., García, I., Fernández, E., Fernández, J., Dorronsoro, C. & Aguilar, J. (1999). Pollution of soils by the toxic spill of a pyrite mine (Aznalcóllar, Spain). *The Sci Total Environ.*, 242, 105-115.
- Vangronsveld, J. & Cunningham, S. D. (1998). Introduction to the concepts. In: J. Vangronsveld (Ed.), *Metal-contaminated soils: in-situ inactivation and phytoremediation*. Berlin, Springer, 1-15.
- Vangronsveld, J., Mench, M., Lepp, N. W., Boisson, J., Ruttens, A., Edwards, R. R., Penny, C. & van der Lelie, D. (2000). In situ inactivation and phytoremediation of metal- and metalloid -contaminated soil: field experiments. In: J. Wise, D. Trantolo, E. Cichon, H. Inyang, & U. Stotsmeister (Eds.), *Bioremediation of contaminated soils*. Dekker, New York, NY, 859-884.
- Vidal, M., López-Sánchez, J. F., Sastre, J., Jiménez, G., Dagnac, T., Rubio, R. & Rauret, G. (1999). Prediction of the impact of the Aznalcóllar toxic spill on the trace element contamination of agricultural soils. *Sci Total Environ.*, 242, 131-148.
- Wright, D. A. & Welborn, P. (2002). *Environmental Toxicology: a textbook for universities*. Cambridge University Press, Cambridge UK, 630.