Soil-plant relationships and contamination by trace elements: A review of twenty years of experimentation and monitoring after the Aznalcóllar (SW Spain) mine accident

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ABSTRACT

Soil contamination by trace elements (TE) is a major environmental problem and much research is done into its effects on ecosystems and human health, as well as into remediation techniques. The Aznalcóllar mine accident (April 1998) was a large-scale ecological and socio-economic catastrophe in the South of Spain. We present here a literature review that synthesizes the main results found during the research conducted at the affected area over the past 20 years since the mine accident, focused on the soil-plant system. We review, in depth, information about the characterization of the mine slurry and contaminated soils, and of the TE monitoring, performed until the present time. The reclamation techniques included the removal of sludge and soil surface layer and use of soil amendments; we review the effects of different types of amendments at different spatial scales and their effectiveness with time. Monitoring of TE in soil and their transfer to plants (crops, herbs, shrubs, and trees) were evaluated to assess potential toxicity effects in the food web. The utility of some plants (accumulators) with regard to the biomonitoring of TE in the environment was also evaluated. On the other hand, retention of TE by plant roots and their associated microorganisms was used as a low-cost technique for TE stabilization and soil remediation. We also evaluate the experience acquired in making the Guadiamar Green Corridor a large-scale soil reclamation and phytoremediation case study.

Keywords:
Guadiamar Green Corridor
Heavy metals
Mine spill
Phytoremediation
Soil amendments
Soil pollution

Contents

1. Introduction

Trace elements (TE) are potential environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons. Since the industrial revolution, anthropogenic impacts have caused a progressive release of TE into the environment (Nriagu and Pacyna, 1988). As a consequence, soils, being the basic and most essential component of terrestrial ecosystems, have received an important load of TE, which might impair their functions and threaten the delivery of their ecosystem services. Unlike other pollutants, TE cannot be chemically or biologically degraded. Even worse, some of them can concentrate along the food chain and eventually accumulate in the human body (Adriano, 2001; Kabata-Pendias, 2011; Wu et al., 2010). Therefore increasing attention has been paid in recent years to the remediation of contaminated soils, in which the use of plants to remove or stabilize these metal ions is particularly emphasized.

Past and present mining activities have a significant impact on the environmental quality of the surrounding area (Doumas et al., 2016). The mining industry is expected to grow in the coming decades, following the expected trends in metal demand, which has increased as a result of both a growing world population and the increasing per-capita requirement (Elshkaki et al., 2016; Patiño Douce, 2106). However, the grade of most ore reserves is declining, worldwide, from high-grade, low-bulk to low-grade, high-bulk (ICMM, 2012; Van der Ent et al., 2015). This trend is causing a growing problem related to the disposal and management of an increasing volume of waste generated by the mining industry (Van der Ent et al., 2015). Besides tailings, which are usually devoid of vegetation and highly exposed to wind and water erosion (Mendez and Maier, 2008; Santamaría, 2005), the huge volume of water-rich sludge produced during the flotation of valuable minerals (sulfides) requires a parallel and adequate disposal, which usually involves storage in constructed dams. Poorly constructed or heavily charged dams may lead to the rupture of these reservoirs (Van Niekerk and Viljoen, 2005), resulting in the spread of pollutants into vast land areas, as occurred at the Aznalcóllar mine complex (SW Spain) in late April 1998 (Ayala-Carcedo, 2004; Grimalt et al., 1999).

The Aznalcóllar accident was the second largest of the 59 major mine accidents in the world at that time (221 cases, according to Ayala-Carcedo, 2004) and the largest reported in Europe to the date of the accident (Nikolic et al., 2011). It had catastrophic ecological and socio-economic consequences, including the death of all fishes (about 73,000 kg) and shellfishes in the water courses as well as many amphibians, birds, and mammals. In addition, the mine complex was closed, with the consequent loss of jobs (CMA, 2001, 2003a,b, 2008; Grimalt and Macpherson, 1999; Grimalt et al., 1999). The importance of this accident motivated a special issue of the journal *The Science of the Total Environment*, with 22 articles documenting the main environmental impacts (Grimalt and Macpherson, 1999). The subsequent restoration project is considered one of the few large-scale examples of soil reclamation using phytoremediation approaches (Domínguez et al., 2008). The contaminated and remediated area is a natural laboratory where a large number of studies have been conducted, contributing to the advancement of our understanding of the fate of TE in different ecosystem compartments, the effectiveness of different remediation approaches, and the evolution of ecosystems after a major soil pollution episode. A search of the scientific literature yielded 435 publications related to the Aznalcóllar accident (Figure 1), with 11,356 citations and an H index of 56, Grimalt et al. (1999) being the most cited paper (244 citations). The main categories of the publications were “Environmental Science” (61%), “Water Research” (11%), “Toxicology” (8%), and “Soil Science” (7%). Thus, this pollution episode and its subsequent management program can be considered one of the key references for researchers studying TE dynamics in terrestrial ecosystems.

Here, we present a literature review that incorporates the main results yielded by the work conducted in the affected area over the past 20 years after the mine accident, focusing on the soil-plant system. Additional information on the effect of the spill on the air, water, sediment, and fauna can be seen in Table A.1, including references relevant to these ecosystem compartments.

The implementation of robust monitoring programs is crucial to the remediation of contaminated environments (Peña-Fernández et al., 2014). We discuss here the main lessons learned during 20 years of monitoring and experimenting on the plant-soil relationships in this singular case study, as well as the gaps in our knowledge that still exist.

2. The Aznalcóllar mining accident

The Aznalcóllar accident was caused by the shear failure of the dam settlement, an over-consolidated marly clay formed during the Miocene (known as Guadalquivir blue marl), through a vertical joint and a bedding plane, provoked by a progressive failure process under high pore pressure (Ayala-Carcedo, 2004). Thus, the accident was caused by the excessive volume of waste deposited in the dam, which had been enlarged several times to increase its storage capacity. At the time of the accident the dam was between 21 and 27 m high (Ayala-Carcedo, 2004; Gil-Toja et al., 2005; Grimalt et al., 1999). The spill affected 4286 ha of alluvial soils of the Agrio and Guadiamar river valleys, which were flooded by ca. 6×10^6 m^3 of slurry composed of acidic water loaded with TE, finely divided metal sulfides, and materials used in the refining/floating process. A strip of 40 km long and 300 m wide, along both rivers, was covered by a layer of highly heterogeneous thickness (from <2 to >30 cm) of black sludge (Cabrera et al., 1999; López-Pamo et al., 1999) (Figure A.1).

An emergency soil clean-up procedure started shortly after the mine spill, whose cost amounted to almost 170 million euros (Arenas et al., 2003). This large-scale restoration plan included the purchase of the land by the Regional Government (formerly devoted to crops and pastures) and the design of a public nature reserve, acting as a ‘Green Corridor’ between the lowlands (Doñana National Park) and the mountains (Sierra Norte Natural Park). The affected and remediated area was declared as the protected landscape ‘Guadiamar Green Corridor’ (GGC) in 2003 (Arenas et al., 2003; CMA, 2008).

During the clean-up operations about 8 x 10^6 m^3 of sludge together with a variable layer of top soil (10-30 cm) were removed and disposed of in a mine open-pit. In the more accessible areas (former croplands), soil remediation was carried out by adding amendments - inorganic (calcium carbonate -rich amendments such as sugar beet lime) and organic (compost or manure) - followed by the afforestation of ca. 2700 ha with autochthonous species (Burgos et al., 2013; CMA, 2003b; Domínguez et al., 2008). In some sites, arsenic immobilization was performed by application of red soil (Antón-Pacheco et al., 2001). Despite these clean-up and remediation measures, soils of the affected zone continued to have trace-element concentrations higher than background values, and occasionally higher than values before restoration (Cabrera et al., 2008), with a fairly irregular distribution. This was due to the heavy machinery used for sludge removal, and to the operations of liming, manuring, and harrowing that broke up and buried part of the sludge layer in the soil (Cabrera et al., 2008). Sixteen years after the accident soil and sediment pollution was still particularly high in the river banks and bed, where the limited accessibility made reclamation operations more difficult than in the floodplains (Domínguez et al., 2016a).

3. Contaminant source: The slurry

The characterization of the contaminant source is vital to the implementation of remediation programs (Wyke et al., 2014). In the Aznalcóllar mine accident the slurry was made up by a mixture of acidic water and sludge.

There was no information available on the composition of the acid water of the slurry. According to Cabrera (2000) its composition should have been very similar to that of the Guadiamar River 12 km downstream from the tailing dam a few hours after the accident: low pH (ca. 5) and high concentrations of TE (e.g., 0.27 mg L^{-1} of As, 0.85 mg L^{-1} of Cd, 3.6 mg L^{-1} of Pb and 463 mg L^{-1} of Zn). In contrast, in the same place and a month later, the pH value was 6.6 and the concentrations of TE had decreased considerably (As 0.072 mg L^{-1}, Cd 0.068 mg L^{-1}, Zn 97.1 mg L^{-1}). A year later, the water composition (e.g., pH 7.4; As 0.0016 mg L^{-1}, Pb <0.01 mg L^{-1}, Zn 0.127 mg L^{-1}, Cabrera, 2000) was within the normal range of values found before the accident (Arambarri et al., 1996). The low pH and high TE concentrations of the acid waters immediately after the flood and near the dam contrasted with the higher pH (ranging between 6.15 and 9.42) and lower levels of contaminants of the toxic waters once they were channeled in the ‘Entremuros’ (meaning ‘between-dikes’) area, 40 km downstream from the dam (Garralón et al., 1999). These changes resulted from dilution by the river water and TE precipitation. Even so, these channeled waters were chemically neutralized (Garralón et al., 1999) before flushing them into the Guadalquivir River. These high pH values in the 'Entremuros' waters were also reported by Alzaga et al. (1999) when studying the organic compounds in waters, sludge, and affected soils.

The pyritic sludge was characterized according to its sedimentological (Gallart et al., 1999), structural (Simón et al., 1999), mineralogical (López-Pamo et al., 1999; Sastre et al., 2004), and chemical properties (Alzaga et al., 1999; Cabrera et al., 1999; Doménech et al., 2002a; Galán et al., 2002; López-Pamo et al., 1999; Sastre et al., 2004; Simón et al., 1999; Vidal et al., 1999). Basically, the pyrite sludge was characterized by a fine grain composition: between 50 and 90% of the particles had a mean diameter ranging from 4.5 to 13 µm (Alastuey et al., 1999; Gallart et al., 1999). This fine grain size was a great concern because of its potential impact on air quality during the soil clean-up operations, in which tonnes of soil were removed using heavy machinery (Alastuey et al., 1999; Querol et al., 1999).

The sludge was composed mainly of pyrite (68-78%, w/w) with minor proportions of sphalerite, chalcopyrite, galena, and arsenopyrite (heavy metal-bearing sulfides), as well as other minerals such as chlinochlore, quartz, calcite, and gypsum (Alastuey et al., 1999; López-Pamo et al., 1999). According to its mineralogical composition, the sludge had high concentrations of Fe and S (up to ca. 40%) and also high levels of potentially toxic TE such as Ag, As, Bi, Cd, Co, Cu, Hg, Pb, Sb, Se, Tl, and Zn (Alastuey et al., 1999; Cabrera et al., 1999; López-Pamo et al., 1999). Table 1 shows a complete analysis of the sludge chemical composition, comparing its concentrations of Fe, S, and TE with normal ranges in soils (Cabrera et al., 1999).

The partial oxidation of sulfides resulted in the formation of a white sulfate crust on the top of the sludge, after the evaporation of the interstitial fluids during the dry periods (Alastuey et al., 1999). This complex process - that involves hydration, oxidation, and hydrolysis reactions generating acidic conditions and solubilization of elements - affected not only the sludge but also the contaminated soils (Dorronsoro et al., 2002; García et al., 2009; Simón et al., 1999, 2001). Regarding to the mobility of these elements in the sludge, calcium showed the highest mobility (water-extractable levels), followed by Cd, Co, Cu, Mn, Ni, Pb, Sr, and Zn; by contrast, S, Fe, Ag, Sb, and Tl had relatively lower mobility and As, Cr, and V had the lowest mobility (Alastuey et al., 1999).

4. Concentrations of trace elements in the affected soils

Soils were contaminated by both the TE dissolved in the acidic water and by the sludge rich in these elements. The acidic water penetrated throughout the soil profile and the TE reacted with soil components, becoming retained through different processes, while the sludge entered the soil through pores and cracks, also contributing to the increase in the total content of TE in the soils. The first studies on the soil contamination caused by the mine-spill were carried out before the clean-up operations; that is, before the sludge crust was mechanically removed (Alastuey et al., 1999; Cabrera et al., 1999; Díaz-Barrientos et al., 1999; López-Pamo et al., 1999; Simón et al., 1999; Vidal et al., 1999). During soil sampling the sludge crust covering the soil was carefully removed and collected for analysis; then, soil samples were taken at different depths (0-5, 5-10, 10-15, 15-20, and 20-50 cm). Thus, the contamination detected in these studies mainly resulted from the large amounts of TE dissolved in the acidic waters of the slurry entering the soils (especially Cd, Cu, and Zn; Simón et al., 1999) and from the particulate fraction of the slurry that could have penetrated through fissures and cracks of the soils.

At this initial stage the soils were contaminated mainly by Ag, As, Au, Bi, Cd, Cu, Hg, Pb, Sb, Tl, and Zn (Alastuey et al., 1999; Cabrera et al., 1999; Simón et al., 1999). Table 2 shows the total TE values in affected and unaffected soils in 1998, just after the mine accident (Cabrera et al., 1999). There was a significant effect of soil texture on TE mobility. The concentrations of TE below 20-cm depth decreased in soils with a clay content greater than 25% to values close to the background levels of the Guadiamar valley soils (Cabrera et al., 1999). In contrast, TE contamination penetrated below this depth in coarser soils (located mainly at the Northen section of the Guadiamar Valley), being noticeable down to at least 50-80 cm.

Paradoxically, concentration levels of TE increased in the soil after the remediation operations, which was attributed to the mixing and burying of the remains of the sludge layer (left on the soil surface) by the heavy machinery used for sludge removal, liming, and manuring (Cabrera et al., 2005, 2008; Nagel et al., 2003; Simón et al., 2008a). The amount of sludge buried in this way reached a maximum of 5% (w/w) in the first centimeter of the soil (Domènech et al., 2002b). In consequence, the soils affected by the mine spill continued to exhibit a high concentration of TE several years after the accident, when compared to the background values of the Guadiamar valley soils (see results for 4, 7, or 16 years after in Cabrera et al., 2008 and Domínguez et al., 2008, 2016a) (Table 2). One of the main consequences of the sludge deposition was the strong acidification caused by the sulfide oxidation, with weathering of primary minerals and carbonates in calcareous soils. This process enhanced the potential leaching of TE, especially in acid soils, a circumstance requiring a rapid intervention to neutralize the acidity (Martín et al., 2007; Ordóñez et al., 2007; Simón et al., 2002, 2005a,b; Vázquez et al., 2011).

After the sludge removal and soil remediation (amendments addition and afforestation), different surveys have monitored the evolution of soil contamination over the years, using a range of techniques. Although most of the TE analyses were performed by wet digestion (triacid digestion, aqua regia, etc.), some authors used alternative techniques such as by Portable X-ray Fluorescence (PXRF), near-infrared spectroscopy (NIRS), multitemporal imaging spectroscopy, and spectral mixture analysis (Romero-Freire et al., 2016; Font et al., 2005; Kemper and Sommer, 2002; Kemper et al., 2005), obtaining similar results for the TE concentrations. These studies highlighted the irregular distribution of the residual contamination along the GGC (e.g., Aguilar et al., 2004a; Burgos et al., 2006; Cabrera et al., 2008; Domínguez et al., 2016a; Galán et al., 2002; Kemper and Sommer, 2002; Kraus and Wiegand, 2006; Martín et al., 2004, 2008; Martín-Peinado et al., 2015; Simón et al., 2007, 2008b; Vanderlinden et al., 2006a, among others). In addition, the area has been monitored using Geographical Information Systems and Remote Sensing to evaluate the affected surface (López-Pamo et al., 1999; Moreira Madueño et al., 2005).

The total As concentration in the contaminated soils was frequently greater than the intervention value established for agricultural Andalusian soils (Galán et al., 2002), indicating (according to the definition of these intervention values) an impairment of the functional properties of the soil, and a level of contamination above which a serious case of soil contamination is deemed to exist, thus needing soil reclamation. In contrast, the levels of other potentially toxic elements, such as total Cd and Hg, were below the maximum acceptable concentrations (according to Aguilar et al., 1999), that is, within the range between no observable adverse effect level and the lowest observable adverse effect level. Nevertheless, as discussed below, (available) Cd is currently the most worrying element in the area, due to its high mobility (Pérez de Mora et al., 2010), while elements such as As, Cu, Pb, and Tl present a comparatively low mobility and are less taken up by plants (Domínguez et al., 2008; Martín et al., 2004).

Mercury was only occasionally measured in the contaminated soils, despite being an important component of the sludge with a potentially high toxicity. Cabrera et al. (2008) reported that the Hg content was 6.6 times greater in the affected soils (0-20 cm) than in unaffected soils (three years after the accident), although with a very irregular distribution (0.404 ± 0.420 mg kg^{-1}, mean value ± SD). In general, this level is rather similar to those reported by Galán et al. (2002) and Kemper and Sommer (2002).

Mercury and other less mobile elements, such as As and Pb, reached their highest values (greatest contamination) close to the tailing dam (in the first 5-8 km away from the mine; Cabrera et al., 2008; Vanderlinden et al., 2006b). Martín-Peinado et al. (2015) estimated that, within the first 18 km away from the mine, a irregular pattern of spots with bare soils covered about 7% of the total affected area, despite the remediation; these spots had acidic soils with remnants of sludge. In contrast, beyond 18 km from the mine, the soils were predominantly neutral or alkaline and the bare area decreased to 0.4%. The potential toxicity of these bare soils has been evaluated by using different toxicity bio-assays (Escoto Valerio et al., 2007; García-Carmona et al., 2017; Romero-Freire et al., 2016), which showed the extremely adverse conditions for plant growth and soil fauna that prevail in these spots. García-Carmona et al. (2017) recommended mixing the remediated and bare soils to reduce the pollutant burden of these spots.

The river beds and banks are hotspots of residual contamination (Domínguez et al., 2016a; Galán et al., 2002). Concentrations up to 3000 mg kg^{-1} of Pb, 650 mg kg^{-1} of As, and 9 mg kg^{-1} of Cd were detected in the river banks 16 years after remediation, in the survey by Domínguez et al. (2016a). This study highlighted that the solubility of the most mobile TE (Cd and Zn) was different across the river section, these TE being more soluble in the river channel and banks than in the floodplain. This study also found evidence of a redistribution of Cd and Zn along the basin, while As and Pb remained at shorter distances from the mine. The authors concluded that application of new remediation techniques in river banks should be a priority for land managers, to prevent a further redistribution of Cd and Zn downstream. The conservation status of riparian ecosystems in the Guadiamar basin was already bad before the accident, but the mine spill made it worse (Arribas et al., 2005).

Taking together all the recent surveys in the area, it can be concluded that, in general, the residual contamination in the Guadiamar floodplain does not necessary imply a serious risk for the native biota, given its relative immobilization within the soil matrix; however, the particular situation of the polluted bare spots and river banks should be remediated. According to Adriano (2001), “pollution” only exists when harmful effects on the ecosystem are detected (see sections below).

Clearly, this favorable evolution of soil contamination in the floodplain was possible due to the sludge removal and remediation works, as shown by the study and monitoring of a “control” plot of 2000 m^2 - where sludge was not removed, soils were not cleaned, and amendments were not applied. The sludge-covered soil in this plot was analyzed at different depths and at different times after the accident: one month (at 0-125 mm; Dorronsoro et al., 2002), three years (0-570 mm; García et al., 2009), seven years (0-60 cm; Álvarez-Ayuso et al., 2008), and 12 years (0-100 cm; Burgos et al., 2013). These studies showed that the progressive oxidation and hydrolysis of sulfides in the deposited sludge resulted in a strong acidification and weathering of carbonates, re-dissolution of the most mobile elements (Cd, Cu, and Zn), and their penetration into deeper layers (Dorronsoro et al., 2002; García et al., 2009) - similar to the situation observed in the bare soils close to the mine and in the river banks (Domínguez et al., 2016a; Martín-Peinado et al., 2015). In contrast, consistent leaching of the most mobile elements was not observed in the remediated, adjacent soils, separated from the sludge-covered plot by only a few meters. With time, an increase in the pH of the topsoil (from pH ca. 3, seven years after the accident, to pH ca. 6, 12 years later) has been observed in this non-remediated plot, possibly facilitated by its progressive colonization by natural vegetation (as discussed in Burgos et al., 2013). Despite this natural remediation by plants, these results demonstrate that sludge removal and soil remediation were necessary to avoid the leaching of the most mobile elements and to minimize the risk of contamination of groundwater sources, close to the Doñana National Park (Burgos et al., 2013).

5. Soil remediation by amendments addition

Remediation of the affected soils was carried out by assisted natural remediation (ANR), a practical approach to accelerate natural remediation (NR). The latter refers to a variety of processes that take place naturally in the soil (sorption, precipitation, complexation) and reduce the mobility and bioavailability of TE. The ANR technique consists of the enhancement of those processes that stabilize TE in the soil by the use of inorganic or organic amendments. This *in situ* immobilization technique that reduces TE mobility and availability is less expensive and less disruptive to the natural environment than conventional excavation and disposal methods (Adriano et al., 2004; Xiong et al., 2015).

There is no consensus for standardized methods to estimate the mobility of TE in soil (often referred as availability of TE for soil organisms; Kabata-Pendias, 2004); in fact, various solutions (i.e.: acids, neutral salts, chelating agents, and others) are used for TE extractions (Kabata-Pendias, 2004). In the Guadiamar case study, solutions of $CaCl_2$, NH_4NO_3, CH_3COOH, and chelating agents such as DTPA and EDTA have been used to assess bioavailable TE concentrations (Murillo et al., 1999; Nagel, 2003; Vidal et al., 1999). As mentioned before, Cd was found to be the most mobile element in the affected soils. Zinc and, to a lesser degree, Cu also showed a potentially high mobility (especially in acidic scenarios), while elements such as As, Bi, Pb, and Tl had a very low mobility (Vidal et al., 1999).

Clemente et al. (2015) reviewed the suitability of different amendments with respect to the restoration of soils contaminated by TE. However, detailed information about amendments actually applied on a large scale in the contaminated soils (after the sludge removal) is very scarce. Three types were used: calcic amendments (sugar-beet lime in particular) for acid soils (at rates of 20, 30, or 50 t ha^{-1} depending on the soil acidity), iron oxide-rich red soil where the As concentration was high (at rates of 500, 700, or 900 t ha^{-1} depending on the As level), and different organic matter-rich composts to recover soil fertility, but without any dose specification (Antón-Pacheco et al., 2001). According to Cabrera et al. (2005; 2008), the organic amendments were added at a rate of 15-25 t ha^{-1}. The availability of TE (estimated by extraction with 0.05 M EDTA) decreased in amended soils, although it was still much higher than in non-affected soils (data from December 2001) (Madejón et al., 2006b) (Figure 2).

More specific information about the efficacy of amendments was provided by a field experiment performed at the “El Vicario” study site (from 2002 to 2016), inside the GGC (Madejón et al., 2006a, 2009a, 2010a; Xiong et al., 2015). In addition, experiments with contaminated soils and amendments were carried out in containers, pots, and ‘microcosms’ (Burgos et al., 2010; Ciadamidaro et al., 2016, 2017; Montiel-

Rozas et al., 2016a; Pérez de Mora et al., 2005, 2007a). In these experiments several organic amendments were used: leonardite (LEO), litter (LIT), municipal waste compost (MWC), biosolid compost (BC), ‘alperujo’ compost (AC), and cow manure (CM). Inorganic amendments tested included lime and sugar beet lime (SL), sometimes in combination with organic materials (e.g., LEO + SL), and other types of materials, such as red soil, iron oxides, zeolites, and benthonites (Aguilar et al., 2007; Cama et al., 2005; Querol et al., 2006). The amendments were applied at different rates, with single or repeated applications and varying experimental time lengths. In general, the amendments increased the soil pH and organic carbon content, while reducing soluble TE concentrations in the soil and TE concentrations in plants (Burgos et al., 2008; Madejón et al., 2006a, 2009a; Pérez de Mora et al., 2011; Walker et al., 2004; Xiong et al., 2015).

Under field conditions, lime and SL were especially effective at increasing pH in the heavily polluted soils (from values as low as 2.5 to near neutrality), which facilitated the establishment of a noticeable plant cover (Cama et al., 2005; Clemente et al., 2003, 2006; Madejón et al., 2006a; Pérez de Mora et al., 2006 a,b, 2011; Xiong et al., 2015). Liming in combination with other amendments, such as soils rich in iron oxides and clay, was a suitable treatment for loams and sandy loam soils, with high gravel content (Aguilar et al., 2004b). Nevertheless, Simón et al. (2010) pointed out that liming should be properly controlled because “excessively high pH” could limit As immobilization and, at the same time, the effectiveness of $CaCO_3$ could be limited by the precipitation of gypsum and iron hydroxysulfates on the carbonate surface, reducing its capacity to react with the acidic solution.

Among the organic amendments, LEO was found to be an excellent material for long-term carbon sequestration in contaminated lands because of its molecular composition, rich in relatively stable aromatic and lignin-derived compounds (Montiel-Rozas et al., 2016b).

The incorporation of organic amendments was also effective at reducing the leaching of more mobile TE, such as Cd, Cu, and Zn (by 40–70% in comparison to untreated soils) (Pérez de Mora et al., 2007a). Organic amendment application reduced the exudation of low-molecular-mass organic acids from the roots of some herbs (Montiel-Rozas et al., 2016a). However, application of organic amendments should be made with caution, given that the increase in soil organic matter could enhance the availability of certain elements, such as Sb, to plants (Nakamaru and Martín-Peinado, 2017).

The Cu-binding capacity of BC and LEO was studied by Soler-Rovira et al. (2010). Despite the fact that the pH of the soil-amendment system is the most important chemical property governing Cu availability in amended soils, soil organic matter and the humic acid (HA) fraction may also be important factors. In particular, binding sites formed by N-, S-, and O-containing acidic functional moieties in HAs may play an important role in Cu (II) behavior. Binding sites involving N may exert a relatively more marked influence than those involving S and O in soils amended with BC, while sulfurated and oxygenated acidic sites may be relatively more important in soils amended with LEO.

Other studies have evaluated the response of afforested wild olive saplings (*Olea europaea* var. *sylvestris*) to a fulvic acid-rich amendment five years after the accident. Although fulvic acids are known to increase the availability of TE in soil, this organic amendment did not lead to the accumulation of a phytotoxic concentration of any TE in any plant tissue (Murillo et al., 2005)

The long-term monitoring of the experimental site, where treatments with different application periodicity were tested, revealed that the necessity for re-treatment of the contaminated soil was amendment- and element-dependent (Madejón et al., 2010a; Pérez de Mora et al., 2011). In the long-term, SL-amended soil showed the highest pH after one or successive applications (at a rate of 30 Mg ha^{-1} $year^{-1}$), the TE availability diminishing over time. Subsequent applications of amendments only caused consistent beneficial effects in the soils treated with organic amendments (Xiong et al., 2015). The results of the long-term experiment suggested that the durability and sustainability of amendment incorporations were high, the effects of the amendments being noticeable more than 12 years after application. At the same time, TE solubility decreased in the unamended plots, a clear sign of NR (Xiong et al., 2015) (Figure 3).

Soil remediation in the GGC led also to a consistent recovery of biochemical functionality, measured as soil enzyme activities and general microbiological rates (Carreira et al., 2008; Hinojosa et al., 2004 a,b; Pérez de Mora et al., 2005, 2006c). Enzyme activities were strongly inhibited in the most polluted areas (Carreira et al., 2008; Hinojosa et al., 2004 a,b). By contrast, remediated soils reached higher enzyme activities than non remediated areas, with urease and β-glucosidase showing the greatest discrimination between degrees of contamination. In a laboratory study, Hinojosa et al. (2008) reported that arylsulfatase (that hydrolyzes aryl sulfates) was the soil enzyme with the highest sensitivity to the presence of sludge. After amendment with lime, acid and alkaline phosphatases exhibited the highest percentage of recovery and β-glucosidase the lowest (Hinojosa et al., 2008). In general, the use of amendments and/or a plant cover improved the biological status of the soils, increasing microbial biomass carbon, enzyme activities, and the maximum rate of glucose mineralization, especially in organically amended soils (Pérez de Mora et al., 2005). However, the differences in enzyme activities among treatments (type -MWC, BC, LIT, LEO and SL-) and dose of amendment) varied, depending on the activity studied. For most treatments, the dehydrogenase and aryl-sulfatase activities (sensitive indicators of heavy metal toxicity) showed the greatest responses to amendment application. At the same time, the structural diversity (measured by ARDRA; amplified ribosomal DNA restriction analysis) of bacterial and fungal communities changed under the different treatments (Pérez de Mora et al., 2006c), linked to the distinct nature of the amendments employed and the development of a plant root system.

In the case of fungi, Montiel-Rozas et al. (2016c) studied the effects of both contamination and amendments application on mycorrhization processes. The arbuscular mycorrhizal (AM) community associated with the studied plants (*Lamarckia aurea* and *Chrysantemum coronarium*) under field conditions responded to different levels of contamination as a result of the amendments application (Montiel-Rozas et al., 2016c). Somewhat contrasting results were reported by the same authors for other plants (*Poa annua*, *Medicago polymorpha*, and *Malva sylvestris*) and other experimental conditions (a medium-term experiment in pots). In this case, soil contamination had a secondary role with regard to structuring the fungal communities, while the initial content of soil total organic matter was the main factor affecting the AM community (Montiel-Rozas et al., 2017).

In the long-term experiment, vegetative colonization was clearly enhanced by amendments, although some plants also colonized the non-amended (control) plots at slower rates (Xiong et al., 2015). All these results showed that ANR using amendments had potential for success on a field scale, reducing TE availability in soils and therefore their entry into the food web. Nonetheless, it is important to remark that high quality by-products should be employed as amendments to minimize TE input into soil (Madejón et al., 2006a; Pérez de Mora et al., 2007b).

Other studies have evaluated the response of *Paulownia fortunei* to amendment application, using contaminated soils from the GGC. The plantation of these fast-growing trees with industrial use (for cellulose or biofuel production) could be a profitable alternative to the ANR approach. A general improvement in soil biochemical quality was detected when organic amendments were applied to the containers containing soil from the GGC, in which saplings were grown, in comparison to non-amended soils. This tree species was considered adequate to improve the economic balance of the revegetation of TE-contaminated soils (Madejón et al., 2014, 2016; see also section 7 on afforestation).

6. Trace element contamination effects on crops and wild herbs

Plants in the Guadiamar Valley have been exposed to TE since the Roman times (about 2,000 years ago), when exploitation of polymetallic sulfides in the area started. A few years before the accident, Cabrera et al. (1987) reported high concentrations (mean values in mg kg^{-1}) of Fe (1950), Cu (45), Pb (51), and Zn (272) in *Mentha rotundifolia* and of Pb (8.5) and Zn (202) in *Typha latifolia* plants collected close to the Aznalcóllar mine; all these values were greater than those in plants of the same species growing in adjacent areas, not influenced by the mining activities. After the mine accident, there was a priority interest in assessing the concentrations of potentially toxic elements in plant leaves, fruits, and seeds, and in evaluating the consequences for the food web. All presented plant concentrations are based on dry weight basis.

6.1. Contamination effects on crops

The accident affected the riparian and dry forests, and also crops and orchards along the flooded area. The first results obtained for spill-affected plants were reported by

Murillo et al. (1999), who analyzed sunflower and sorghum crops that had been directly affected by the spill; samples were taken two months after the accident - thus, before the remediation operations. Although most plants near the river channel were washed away or killed by the spill, many others, more distant from the river, had their stem bases submerged into the sludge and continued to grow and to produce seeds. Unexpectedly, these crops grew better than those not affected by the spill, and had higher nutrient concentrations in aerial tissues (K, Ca, and Mg in the case of sunflower, and N and K in the case of sorghum) - indicating a certain 'fertilizing' effect of the flood(by mobilization of nutrients from the soil), despite its potential toxicity. In general, none of the TE studied, in both crops, reached levels that were phytotoxic or toxic for humans or livestock.

The use of plants to remove contaminants from the soil is termed phytoextraction. After the remediation operations, different crops were tested for phytoextraction purposes, in particular those that hyper-accumulate TE, like species belonging to the Brassicaceae. The species assayed - such as *Brassica napus*, *B. carinata*, and *B. juncea* - showed certain capacity for extracting metals, especially Zn and Tl (Clemente et al., 2005; Del Río et al., 2000, 2005; Soriano and Fereres, 2003); however, the total crop uptake was moderate, casting doubts on the feasibility of using these crops for the decontamination of the area (Bernal et al., 2005).

Trace element accumulation in the leaves and seeds of other crops, such as barley and triticale (Soriano and Fereres, 2003) and sunflower (Madejón et al., 2003), was even lower than in *Brassica* species. Sunflower was considered as an adequate crop for phytoremediation when using EDTA to enhance TE extraction (Alcántara et al., 2005); however, this practice is questionable because this chelator is both toxic and non-biodegradable (Wu et al., 2010).

An alternative technique is to use plants to immobilize contaminants in the soil, an approach termed phytostabilization. White lupin (*Lupinus albus*) showed high retention of heavy metals in its root nodules and a capacity to increase soil pH, thus it was considered adequate for phytostabilization; on the other hand, it was disesteemed for phytoextraction purposes (Vázquez et al., 2006). Trace element concentrations in white lupin tissues were increased by using different chelators, NTA (nitrilotriacetic acid) being particularly effective; however, a parallel increase in TE in soil leachates, above the maximum permissible levels (USEPA, 1997), was also recorded (Peñalosa et al., 2007), questioning the environmental feasibility of this technique.

Considering the low TE extraction achieved by most crops and its questionable viability for large areas (Van der Lelie et al., 2001), phytoextraction was finally discarded as a management approach. However, Robinson et al. (2003) suggested that afforestation, with a 30-year rotation and wood harvesting, could contribute to long-term phytoextraction of TE and be profitable. Eventually, the regional administration established the aforementioned Green Corridor, where agricultural activity was forbidden.

6.2. Contamination effects on wild herbs

The establishment of an herbaceous vegetation cover, based on autochthonous species well adapted to the local conditions, contributes to the sustainable phytostabilization of polluted lands. However, continuous monitoring of TE uptake by the herbaceous plants is needed in order to regulate and avoid (as much as possible) the transfer of potentially toxic elements along the terrestrial food web.

In one of the first surveys after the accident and clean-up operations, del Río et al. (2002) analyzed 99 species of 29 families for As and heavy metals. Among these species, they found remarkably high concentrations of TE in *Amaranthus blitoides* (Amaranthaceae), with up to 120 mg kg^{-1} of As, 152 mg kg^{-1} of Cu, 450 mg $kg^{-1}$of Pb, and 1138 mg kg^{-1} of Zn, as well as high Cd concentrations (up to 9.7 mg kg^{-1}) in *Chamaemelum fuscatum* (Asteraceae). Alcántara et al. (2005) also highlighted comparatively high TE accumulation in some wild plant species collected in 1999. The most representative families were Asteraceae (22.4%), Poaceae (11.3%), Polygonaceae (7.0%), Brassicaceae (6.0%), Fabaceae (5.6%), Chenopodiaceae (4.5%), and Cyperaceae (2.3%). In these surveys the TE concentrations in many plant species were above the critical levels of toxicity for livestock and other herbivores, which justified the maintenance of the monitoring of TE in wild plants in the polluted area.

Madejón et al. (2002) monitored two common wild grasses, *Cynodon dactylon* and *Sorghum halepense*, 18 months after the accident, comparing plants growing on sludge-covered soil, on remediated soil, and on adjacent, non-affected soils. The results showed that on the sludge-covered plot some TE reached levels that could be toxic in the food web, while on the remediated soils only Cd reached levels in the grass tissues that could be considered of concern. For this reason, the TE in *C. dactylon* were determined two years later (2000 and 2001; Madejón et al., 2006b): with the exception of Cd, the TE concentrations in plant tissues decreased with time, being almost similar in sludge-covered plots and in non-affected soils. The aging of most TE in soils (Kirby et al., 2012; Ma et al., 2006) was further corroborated by the plant surveys carried out during 2007-2009 along the GGC, which showed that the residual contamination of the affected area did not have a negative effect on pasture TE accumulation or growth. The addition of amendments also affected the herbaceous plant cover and composition by, especially, changing soil pH (Burgos et al., 2008; Madejón et al., 2006a; Pérez de Mora et al., 2006a,b, 2007a,b, 2011). In a later study, carried out 11 years after the accident, in the sludge-covered plot, species such as *Poa annua, Medicago truncatula*, and *Plantago lagopus* exhibited TE concentrations that were normal for higher plants and tolerable for livestock (Burgos et al., 2013), confirming the process of long-term metal aging, even in the more polluted sites of the GGC. However, other plant species - especially those of the Brassicaceae, Asteraceae, and Plantaginaceae families - still had comparatively high TE concentrations. Madejón et al. (2005a, 2007) reported that the concentrations of Tl (and other TE) in *Diplotaxis catholica*, *Hirschfeldia incana*, *Plantago lanceolata*, and *Raphanus raphanistrum* were above the normal levels in plants (according to Kabata-Pendias, 2011).

The management of the afforested area included control of the herbaceous cover, to reduce competition and the fire risk. Mechanical control is expensive, may affect biodiversity, and generates greenhouse gas emissions. An alternative means of control was implemented, namely eco-grazing using horses, with the condition that their meat was not consumed (Madejón et al., 2009b). The concentrations of TE in the pastures (based on grasses and legume species) were tolerable for the grazing horses (NRC, 2005), whose nutrient requirements were covered (Madejón et al., 2010b). Only the relatively high Cd concentration could be somewhat excessive for more sensitive animals consuming a diet based on Cd-rich members of the Asteraceae and other plant families (Madejón et al., 2012a). In general, it stood out that, regardless of the pasture composition, grazing should be avoided on regenerating pastures in autumn, due to the comparatively greater concentrations of TE in the herbage and the soil adhering to it. Horses usually feed closer to the ground, using shorter grasses than other animals (Menard et al., 2002).

Wild herbaceous plants are also grazed by terrestrial snails, which are harvested locally for human consumption and thus represent a potential health risk. Madejón et al.

(2013a) found TE concentrations in the snail bodies that were of concern for human consumption; therefore, it was advisable to avoid collecting them for human consumption in the GGC.

Biofuel production is another alternative use for contaminated soils. Different thistle species, growing spontaneously in the GGC, have been evaluated for biomass production for energy purposes (Domínguez et al., 2017). Among them, *Silybum marianum* showed by far the highest production of biomass, being able to colonize highly contaminated sites. In general, the TE accumulation in thistle species was lower than the upper limit of normal levels in plants (according to Chaney, 1989). As an exception, *S. marianum* accumulated high levels of Cd, but this did not affect its calorific value.

Besides the terrestrial vegetation, the wetland vegetation was also severely impacted by the spill that contaminated wetland ecosystems with the acid waters. As in the terrestrial plants, the analysis of the aquatic macrophyte *Scirpus maritimus* also showed a progressive decrease in the uptake of TE by vegetation over time. Two months after the accident (samples of 1998) Meharg et al. (1999) reported a mean Zn concentration of 1390 mg kg^{-1} in *S. maritimus* tubers, with an extreme value of 3145 mg kg^{-1}, while two years later (samples of 2000) Madejón et al. (2006c) reported a maximum of 180 mg kg^{-1}. The Cd accumulation in the tubers also decreased, from maximum values of 18.2 mg kg^{-1} in 1998 (Meharg et al., 1999) to 0.9 mg kg^{-1} two years later (Madejón et al., 2006c). The accumulation of potentially toxic elements in *S. maritimus* tubers was of great concern because they are massively consumed by Greylag geese (*Anser anser*) wintering at Doñana National Park. A consistent temporal reduction in the Cd and Zn concentrations in *Typha dominguensis* and *S. maritimus* was also reported by Pain et al. (2003). In contrast to Cd and Zn, the As concentrations in the tubers were consistently low, even in the first studies. The high concentration of As in the mine sludge (up to 4000 mg kg^{-1}; Cabrera et al., 1999) caused some social alarm due to its known toxicity. However, the acid waters entering the channeled 'Entremuros' site contained relatively little As in solution (Pain et al., 1998), although some As could have reached this area in As-rich suspended particulates (Taggart et al., 2005, 2009). The As level reported by Madejón et al. (2006c) in the *Scirpus* tubers, about 1.0 mg kg^{-1} in 2000, was very similar to the levels recorded two years earlier (1.1 mg kg^{-1} in 1998; Taggart et al., 2005).

Taking all these studies in wild herbaceous species into account (Table A.2), it could be concluded that a general trend of a decrease in TE accumulation over the years has been observed. However, different plant species have different TE accumulation patterns and different responses to TE availability in soils (Baker, 1981); thus, continuous monitoring of TE in plants along the GGC is advisable, to detect any potential risk for the terrestrial food web.

7. Afforestation of remediated soils

After the cleaning-up of the sludge and soil remediation, the subsequent afforestation program included the planting of more than 20 species of native shrubs and trees in the affected area (Domínguez et al., 2008). For the tree-soil relationships, different research stages consisted of the evaluation of: the growth and survival of the planted saplings, TE accumulation in plants and their use as biomonitors of soil contamination, and the effects of trees on soils and their potential for phytoremediation.

7.1. Contamination effects on tree growth and survival

The performance of the plantations (growth and survival rates, plant nutrient status) was monitored for 3-5 years after afforestation. In addition to soil contamination, planted individuals had to resist summer drought, high irradiance, the alteration of soil structure, and competition from herbaceous plants, which resulted in very low survival rates of tree saplings in some highly altered sites (Domínguez et al., 2010a). The survival rate differed among the seven woody species monitored. Holm oak (*Quercus ilex* subsp. *ballota*) saplings were more sensitive to drought than those of other sclerophyllous tree species, such as wild olive (*Olea europaea* var. *sylvestris*); the holm oak saplings planted in dry terraces suffered high mortality during the first years after planting. Greenhouse experiments showed a relative tolerance of holm oak (in terms of survival) to increasing concentrations of Cd by a nutritive solution with up to 200 mg L^{-1}, although this exposure affected root mass, length, and diameter (Domínguez et al., 2009). Therefore, the combination of impaired root growth and high sensitivity to drought probably provoked the observed mortality of oak seedlings in the field.

In order to propose alternative planting schemes to improve holm oak establishment, some strategies designed to mitigate abiotic stress during the summer were tested in the area. Facilitation using nurse shrubs, such as *Retama sphaerocarpa*, was proven to be very effective at decreasing oak seedling mortality over the dry season (Domínguez et al., 2015). The shade provided by the nurse shrub protected plants from the damaging effects of high irradiance, extreme temperatures, and water loss during the summertime. Also, seedling survival rates were slightly higher under a pioneer shrub (*R. sphaerocarpa*) than under a late successional shrub (*Phillyrea angustifolia*) or tree (*Olea europaea*). Possibly, seedlings growing under *R. sphaerocarpa* might benefit from the higher light availability, in comparison to the conditions under the canopy of the late successional shrubs/trees. The fact that the nurse effect of *Retama* shrubs was due mainly to the amelioration of extreme microclimatic conditions, and not because of lower TE mobility or higher microbial activity underneath the shrub cover, was confirmed in a further study by Domínguez et al. (2016b).

Although other abiotic stresses (high irradiance and drought) might be more critical to the survival of the plantations than TE contamination, the soil chemical conditions imposed by the mining spill had an important role in the nutritional status of the plantations. In some sites, intensive acidification of the soil took place, due to the oxidation of the sulfides deposited onto the soil (Domínguez et al., 2016a). Low soil pH resulted in nutrient deficiencies in afforested oak and wild olive saplings, in particular of P - as indicated by a high N:P ratio (Domínguez et al., 2010b). The previously mentioned facilitation experiment also showed that the oak seedlings that emerged in more acidic soils were less likely to survive the dry season than those that emerged in neutral soils (Domínguez et al., 2015).

7.2. Trace elements accumulation and biomonitoring of soil contamination

A main objective of the first studies after the mine accident was to evaluate the toxicity risks for the food web derived from the accumulation of TE in the leaves and fruits of the surviving adult trees. Monitoring of two tree species typical of the Mediterranean forests in the area, wild olive and holm oak, showed comparatively low values of the most toxic elements (As, Cd, Pb, and Tl) in their leaves and fruits (Madejón et al., 2006e). Only during the first year after the accident did the leaves of holm oak (spiny and tomentose underneath) and the fleshy fruits of olive trees (non-washed samples) reach worrying levels of contamination; these exceptionally high levels were derived from the frequent clouds of dust produced during sludge removal and soil clean-up operations in 1999 (Madejón et al., 2005b; Querol et al., 1999). Fortunately, even in non-washed samples, the TE concentrations decreased with time and, in consequence, the toxicity risk to the food web diminished to a normal level.

In contrast to holm oak and olive tree, white poplar (*Populus alba*), a tree species abundant in the riparian forest, accumulated high levels of TE in leaves. In particular, the maximum values recorded for Cd (15.4 mg kg^{-1}) and Zn (1312 mg kg^{-1}) in trees surviving after the accident were in the phytotoxic range (Madejón et al., 2004). The ability of members of the Salicaceae (poplars and willows) to accumulate Cd and Zn in their leaves is well-known; in fact, they have been proposed for the phytoextraction (removal of metals by plant harvesting) of both elements in contaminated sites (Robinson et al., 2000). The concentrations of TE in poplar leaves were significantly correlated with the soil availability (EDTA-extractability) for Cd, Zn, As, Mn, and Cu, but not for Fe, Ni, or Pb (Madejón et al., 2004). Given this ability of poplar leaves to provide quantitative information on the availability of certain TE in soils, it was proposed that they could be used to biomonitor soil contamination, in particular by Cd and Zn (Madejón et al., 2004, 2006d).

White poplar leaves were assessed again, 12 years after the accident (Madejón et al., 2013b). While the As and Pb concentrations had significantly decreased in comparison to the previous analysis, Cd and Zn had not changed significantly over time (Figure 4). The fruiting catkins also showed significant accumulation of Cd and Zn, suggesting that the barriers impeding metal translocation to seeds were weak. Despite this accumulation of TE in seeds at contaminated sites, germination and plantlet growth were not affected (Madejón et al., 2015).

In addition to the direct potential effect of TE-rich leaves on herbivores, it is important to consider the effects of poplar litter, loaded with Cd and Zn, on the general soil health. Experiments with white poplar litter were carried out under both field and controlled conditions (greenhouse containers and microcosm incubations; Ciadamidaro et al., 2013, 2014a,b,c, Madejón et al., 2012b). Poplar litter addition did not increase the soil Cd and Zn concentrations significantly, nor did it reduce plant growth (Ciadamidaro et al., 2013, 2014a). On the contrary, the organic matter added through the litter had positive effects on some aspects related to the cycles of carbon (microbial biomass carbon, water soluble carbon, β-glucosidase activity) (Madejón et al., 2012b) and nitrogen (Ciadamidaro et al., 2014b). The litter input, like the exudates from the poplar roots, increased the pH in acidic soils and improved other variables related to soil quality - such as total and soluble organic carbon, microbial biomass carbon and nitrogen, enzyme activities, and, especially in neutral soils, the potential nitrification rate (Ciadamidaro et al., 2014b, c; Madejón et al., 2012b).

The long-term monitoring of white poplars in the area suggests that leaves of this tree can be used as long-term biomonitors of soil TE bioavailability (Figure 4). More recently, the potential of other tree species as biomonitors of long-term soil contamination has been also explored. *Eucalyptus camaldulensis* (red gum) is a tree species from Australia widely planted in poor-soil conditions in the Mediterranean region. It was abundant in the Guadiamar basin before the accident, planted for pulp production. Monitoring of the regrown trees in contaminated sites from the GGC rendered low values of TE in leaves, below phytotoxicity levels. However, the correlations with soil availability ($CaCl_2$-extracted) were significant for Cd, Mn, and Zn, but not for Cu or Pb. Therefore, *E. camaldulensis* was proposed for the biomonitoring of soil Cd, Mn, and Zn in contaminated sites (Madejón et al., 2017).

7.3. Potential of trees for phytostabilization of trace elements

Phytostabilization is the use of plants and associated microorganisms to immobilize contaminants in the soil. Trees and shrubs could have a greater potential for phytostabilization than herbaceous plants, because of their extensive root systems that contribute to physical soil stabilization, reducing the spread of contaminants through soil erosion (Mendez and Maier, 2008). Those tree and shrub species with reduced transfer of TE from the soil to aboveground organs will be best suited to this soil remediation measure (Bolan et al., 2011; Madejón et al., 2017).

Most of the afforested trees in the GGC (with the exception of Salicaceae species) showed comparatively low TE concentrations in their aboveground tissues, usually within the normal ranges for higher plants (Domínguez et al., 2008). In general, the TE concentrations in aboveground tissues of trees and shrubs were far lower than those in herbaceous plants; bearing in mind also their larger root systems, these woody species could be considered as suitable phytostabilizers of TE-contaminated soils (Domínguez et al., 2008, 2009, 2010a). In particular, holm oak was very efficient at retaining Cd in its roots. Although soil acidification at some sites increased the availability of Cd and other TE, the leaves of holm oak trees growing on acidic soils (with high TE availability) did not accumulate more Cd than leaves of trees growing on neutral or basic soils (Domínguez et al., 2009). Greenhouse experiments, in which holm oak seedlings were exposed to soil solutions highly contaminated by Cd, showed an extreme ability of these seedlings to accumulate Cd in their fine roots (up to 7 g kg^{-1} dry root), thus supporting the suitability of holm oak for phytostabilization (Domínguez et al., 2011). Mastic shrub (*Pistacia lentiscus*) seedlings also retained Cd in their roots, but were more sensitive to Cd than oak seedlings in terms of growth and survival. In the same experiment, Tl was retained much less than Cd by the roots of both species;

fortunately, the Tl availability in soil was comparatively low in the affected soils of the study area (Martín et al., 2004; Vidal et al., 1999) and, therefore, its concentration in the leaves of woody plants was low under field conditions (Domínguez et al., 2008; Madejón et al., 2006e). The mycorrhizal fungi associated with holm oak roots could also contribute to TE retention in roots and reduced transfer to aboveground organs. Ongoing studies in the GGC are assessing the diversity of ectomycorrhizal fungal species and the role of the fungal community in plant health and TE accumulation (Alvárez-López et al., unpublished).

Besides the tree species studied in more detail (holm oak and olive tree), some shrub species were also evaluated for phytostabilization of the contamination in the GGC. We have already mentioned the function of shrubs as nurse plants, facilitating the establishment of other late-successional species underneath their canopy (Domínguez et al., 2015); they also have added value in relation to protection and facilitation of connectivity for wild fauna in the restored area (Rodríguez et al., 2009). In experimental plantations with different shrub species, the legume *Retama sphaerocarpa* showed greater survival rates than other species - such as *Myrtus communis*, *Rosmarinus officinalis*, or *Tamarix gallica* - and consistently high TE retention in its roots (de la Fuente et al., 2014; Domínguez et al., 2008; Moreno-Jiménez et al., 2008, 2011), making it a species suited to phytostabilization. Other shrubs, such as *M. communis* (de la Fuente et al., 2014; Moreno-Jiménez et al., 2008) and *R. officinalis* (Madejón et al., 2009c), have been also proposed for soil phytostabilization in the study area. However, the potential increase in soil TE availability during root decomposition has scarcely been studied. Moreno-Jiménez et al. (2009) reported that, at least for As, decomposition of roots did not lead to increased its availability for plant uptake, given that the resulting soluble forms are rapidly retained in soils.

Inputs of organic matter to soil through litter deposition, besides increasing soil C stocks, could also influence the long-term stabilization of TE in soils. Depending on the quality of the litter inputs, the effects of tree plantations on the soils underneath could lead to greater stabilization of TE in soils or, in contrast, to their solubilization - if, for instance, the accumulation of litter promotes soil acidification. An ongoing research line in the GGC is focused on the long-term footprint of the planted tree species on soils. A comparative study of seven tree species planted in a mixed design showed high variability in the chemical composition of the leaves, roots, and forest floor (coefficients of variation between 62 and 79%) 16 years after planting. The chemical footprint of the tree species on the topsoil was weak, probably due to the young age of the plantations. However, there were some differences between tree species; for example, soils under *Pinus pinea* had lower concentrations of Cd, Mn, Ni, and Zn than those under other species (Marañón et al., 2015).

8. Conclusions

The increasing release of trace elements (TE) into the environment, due to industrial and mining activities, represents a major concern for ecological systems and human health. Currently, there is a huge research effort on techniques for remediating TE-contaminated land. The Aznalcóllar mine spill and subsequent large-scale contamination were a challenge for researchers and managers. We can learn much from the experience acquired during 20 years of experimentation and monitoring in this contaminated area.

The large number of studies reviewed here represents a remarkable contribution to the understanding of TE dynamics in terrestrial ecosystems. The experience accumulated during 20 years of monitoring of soils and plants in a TE-contaminated area suggests that, despite the fact that the mobility and toxicity of contaminants could be reduced by appropriate soil management, long-term, potential exposure to TE persists, together with the consequent transfer of these TE to the food web. Thus, long-term periodical monitoring of any TE-contaminated area is required.

In the initial remediation stage, the removal of sludge was crucial. It contributed firstly to the reduction of aesthetic disaster and secondly to preventing both the penetration of the released TE into the deeper soil layers and groundwater and the contamination of adjacent soils and surface waters by runoff or wind. So, extending this to other scenarios, sludge must be removed as soon as possible.

The different approaches to soil remediation tested in this area have highlighted the use of soil amendments, with regard to their effects, types, and duration. Understanding the relationships between soils and plants was vital to evaluate the retention of TE by roots and mycorrhizas, TE exclusion from aboveground organs, and thus the potential for the phytostabilization of soil contaminants.

The contaminated and remediated area reviewed here, the Guadiamar Green Corridor, has proven to be an excellent testing ground to understand - in a realistic context - the problems associated with TE contamination, their transfer from soil to plants, the toxicity induced in the food web, the utility of bioindicators, and the application of different remediation measures. The combination of soil amendments and native non-accumulator plant species is a feasible and cost-efficient option for the management of large TE-contaminated areas.

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Table 1. Mean concentration and range (mg kg^{-1}) of trace elements and S which concentrations were higher than the upper limit of the normal ranges in soils (Bowen, 1979) in samples of sludge compared with normal soils. Adapted from Cabrera et al. (1999). Ranges for Ag, Hg and S adapted from Alastuey al. (1999*).Cabrera et al 2008** and Lopez-Pamo, 1999.

Element	Sludge		Normal soil
	Mean	Range	Range
As	2878	1028-4022	0.1-40
Au	0.55	0.25-0.90	0.01-0.02
Bi	61.8	25.2-78.8	0.1-13
Cd	25.1	15.1-36.4	0.01-2
Cu	1552	715-2035	2-250
Pb	7888	3664-9692	2-300
Sb	669	269-797	0.2-10
Tl	51.6	28.3-61.8	0.1-0.8
Zn	7096	4424-10950	1-900
Ag***	34	25-41	0.01-8
Hg**	15.1	8.14-22.1	0.01-0.5
S (%)*	35	34.1-41.8	0.003-0.16

** Cabrera et al 2008

***LópezPamo et al 1999

Table 2. Total concentration (mg kg-1) of trace elements in soils (0-20 cm) of the Guadiamar area at different years.

		As	Cd	Cu	Pb	Zn	
				mg kg^{-1}			
				Affected soils			
1998	Mean	80.4	1.69	104	234	487	Cabrera et al., 1999
	Range	9.38-1648	0.12-22.0	12.5-958	25.3-4969	56.8-5283	
1999	Mean	61.3	1.26	120	202	380	Kemper and Sommer, 2002
	Range	7.0-442	0.05-14.8	17.5-521	17.5-3332	94-3887	
2002	Mean	153	4.44	155	321	462	Cabrera et al., 2008
	Range	11.7-595	1.23-7.67	12.7-443	8-1556	40-936	
2005	Mean[1]	155	1.44	115	218	475	Domínguez et al., 2008
	Range[1]	59-408	0.45-3.11	65.8-198	75-630	190-798	
2014	Mean[1]	127	1.79	135	387	525	Domínguez et al., 2016
	Range[1]	15.3-793	0.28-9.35	28.5-428	22.8-3032	117-3413	
				Un-affected soils			
1998	Mean	18.9	0.33	30.9	38.2	109	Cabrera et al., 1999
	Range	8.37-38.5	0.12-1.06	12.3-85.0	19.5-86.3	53.9-271	

[1]Total values were calculated using pseudototal values of the papers and the corresponding recoveries factors.

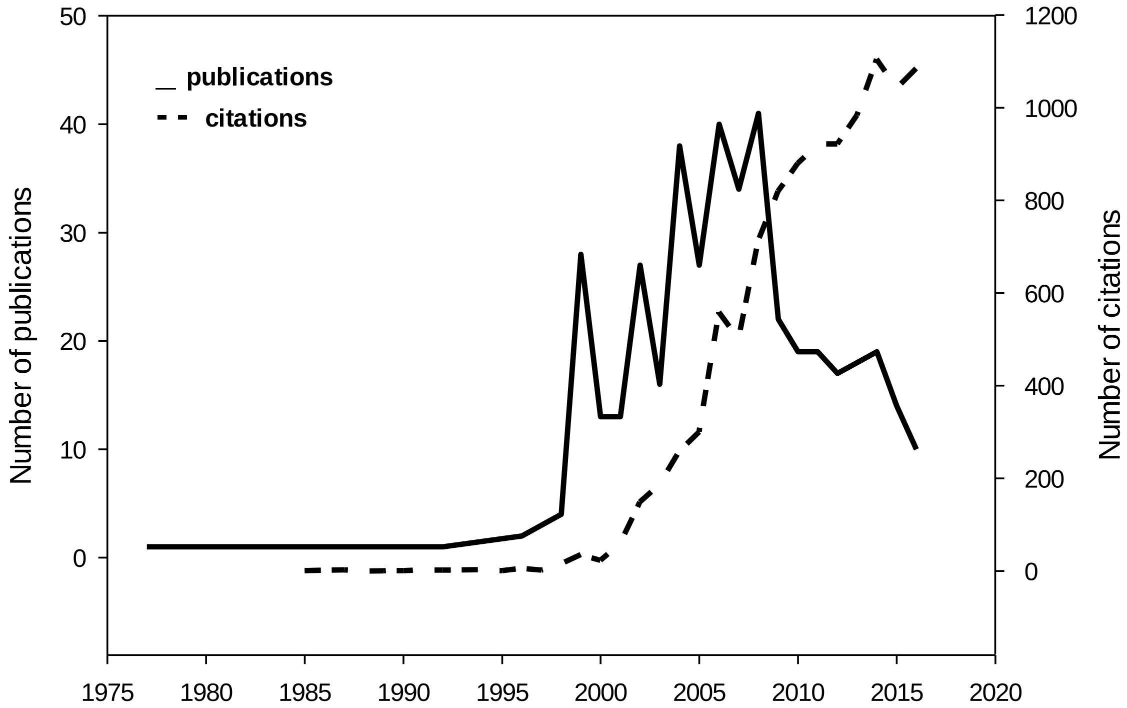
Figure captions

Figure 1. Results of a search for publications and citations with the key words "Aznalcóllar" or "Guadiamar" in the Web of Science (October 2nd 2017).

Figure 2**.** Comparison of mean values (± SE) of trace elements concentration extracted by EDTA from soils of the Guadiamar river basin: non affected by the mine spill (in white), affected before amendment addition (in black) and affected after the amendment addition (in grey) in 2001.

Figure 3. Temporal changes in soil pH and $CaCl_2$-extractable Cd concentrations in the 0–15 cm for each amendment treatment from 2003 to 2016 (similar behaviour for Cu and Zn). NA = non-amended control; SL = sugar beet lime; BC = biosolid compost; LEO = leonardite. Mean values ± standard errors are expressed as mg kg^{-1} of dry matter

Figure 4. Temporal variation in Cd (similar behaviour for Zn) and Pb (similar behaviour for As) concentrations in leaves of *Populus alba* L. at two sites from the GGC, neutral pH, white columns and 'acidic pH (grey columns), during the period 1999–2010. For each element and site bars with the same letter do not differ significantly ($p < 0.05$). Mean values ± standard errors are expressed as mg kg^{-1} of dry matter


publications
citations
Number of publications
Number of citations
50
40
30
20
10
0
1200
1000
800
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1975
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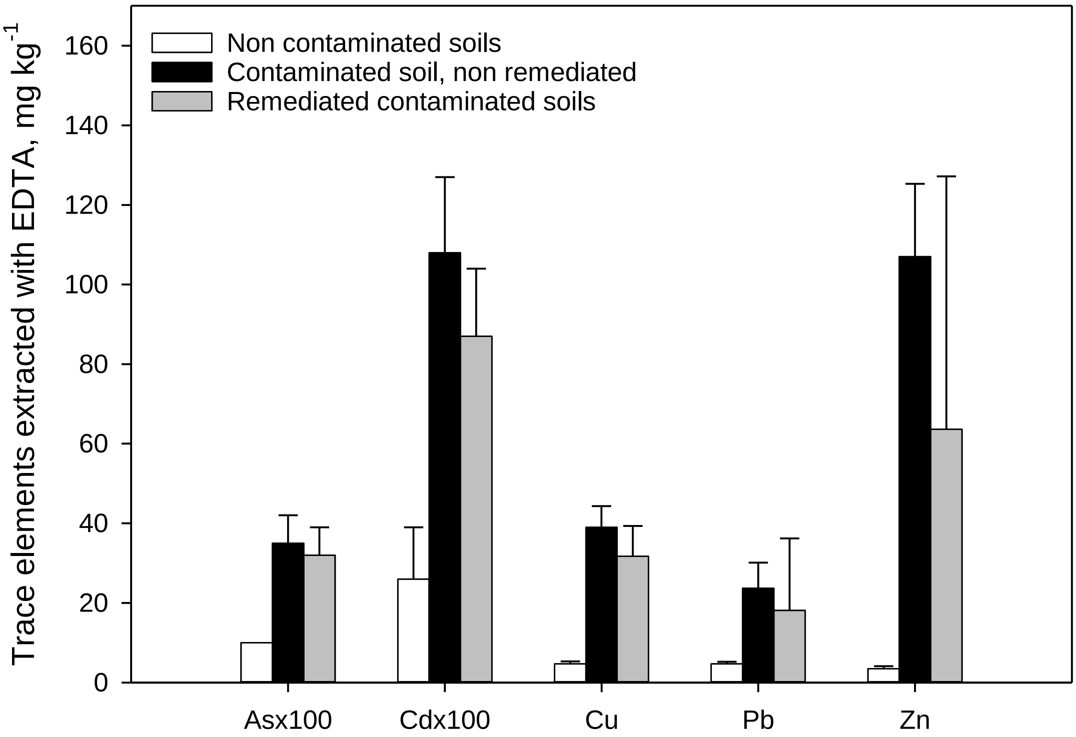
Figure 1



Non contaminated soils
Contaminated soil, non remediated
Remediated contaminated soils
Trace elements extracted with EDTA, mg kg-1
0
20
40
60
80
100
120
140
160
Asx100
Cdx100
Cu
Pb
Zn

Figure 2

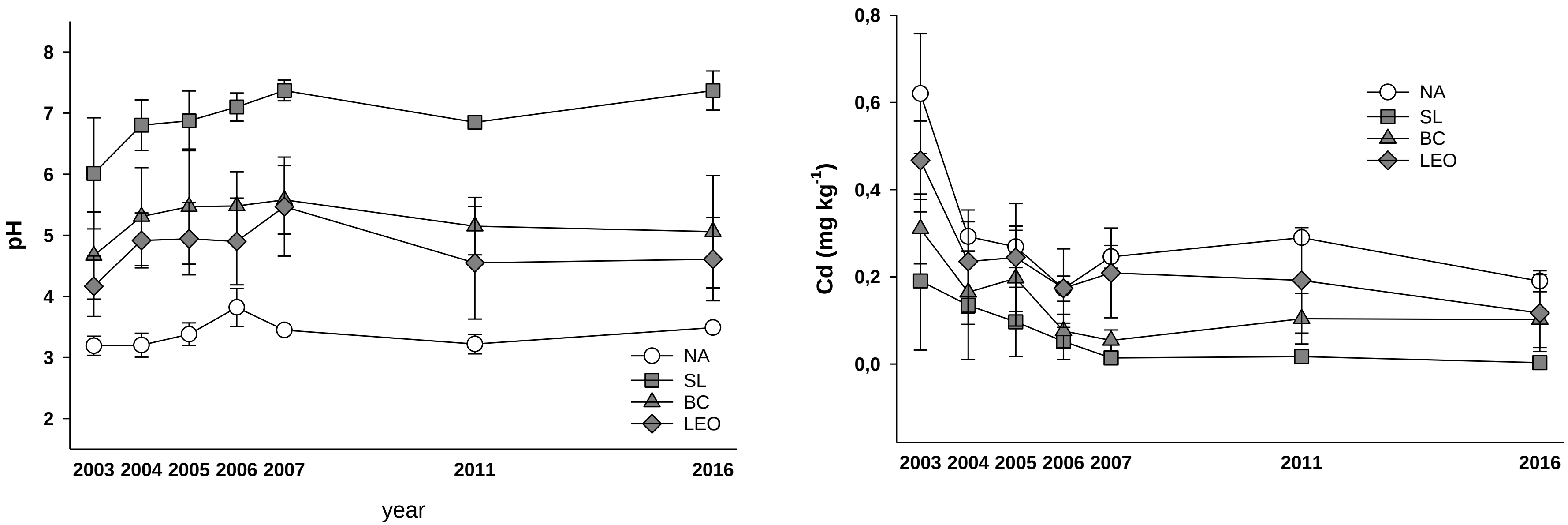

pH
8
7
6
5
4
3
2
2003 2004 2005 2006 2007 2011 2016
year
NA
SL
BC
LEO
Cd (mg kg-1)
0,8
0,6
0,4
0,2
0,0
2003 2004 2005 2006 2007 2011 2016
NA
SL
BC
LEO

Figure 3

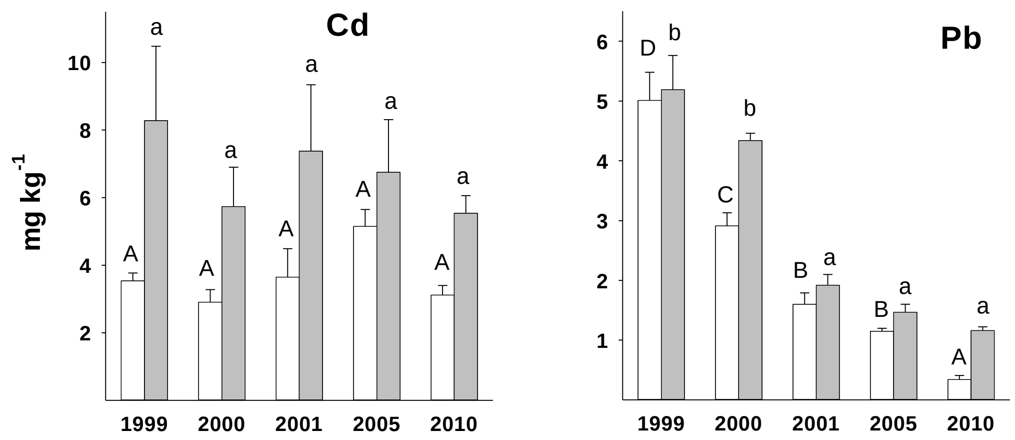


Figure 4