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ORIGINAL PAPER

Afforestation of a trace-element polluted area in SW Spain: woody plant performance and trace element accumulation

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Abstract Trace element soil pollution can have ecotoxic effects on plants, which could negatively affect the restoration of a degraded area. In this work, we studied the revegetation success in different sites within a trace element-polluted area (Guadiamar River Valley, SW Spain). We analysed the survival and growth patterns of afforested plants of seven Mediterranean woody species, and their relation to soil pollution, over 3 years. We also analysed the trace element accumulation in the leaves of these species. The area was polluted mainly by As, Cd, Cu, Pb and Zn (soil total concentrations up to 250, 3.6, 236, 385 and 510 mg kg^{-1}, respectively). The woody plant performance was very different between sites and between species; in the riparian sites, plant survival rates were nearly 100%, while in the upland terrace sites species such as *Quercus ilex* and *Ceratonia siliqua* showed the lowest survival rates (less than 30%) and also the lowest relative growth rates. There were no significant relationships between plant performance and soil pollution in the riparian sites, while in the upland sites mortality, but not growth, was related to soil pollution, although that could be an indirect effect of different substrate alteration between sites. The accumulation of soil pollutants in the studied plants was low, with the exception of *Salicaceae* species, which accumulated Cd and Zn in the leaves above 1 and 200 mg kg^{-1}, respectively. We discuss the results with regard to the afforestation of trace-element polluted areas.

Keywords Soil remediation · Plant survival · Relative growth rates · Mediterranean woody species · Heavy metals

Introduction

Atmospheric and soil pollution are regarded as important factors contributing to forest decline (Hüttermann et al. 1999; Brydges et al. 2000; FAO 2005). Trace elements, an important group of soil pollutants, usually occur in (by definition) very low concentrations in natural soils. However, different human activities have altered their biogeochemical cycles and have increased the trace element levels in agricultural and forest soils during the last decades (Adriano 2001; Kabata-Pendias and Pendias 2001).

In the forest systems, an increase in the soil trace element concentrations may have ecological consequences at different levels. At the soil level, the structure and biological activity of microbial communities may be altered (Jentschke and Godbold 2004; Pennanen 2001) and the degree of mycorrhizal associations may decrease (Del Val et al. 1999; Hartley-Whitaker et al. 2000). Nutrient cycles and plant nutrition could be altered due to these effects (Naidu et al. 2001). At the plant level, trace element effects are diverse at metabolic, subcellular and cellular levels, which could adversely affect plant performance (Prasad and Hagemeyer 1999). Particularly, root growth inhibition is one of the first toxicity symptoms (Schulze et al. 2005), which may affect seedling establishment.

The revegetation of trace element-polluted areas is a difficult task, because of the presence of many growth-limiting factors. Ecotoxic effects of soil pollution can be an

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important factor. In addition, other factors such as high irradiance, nutrient deficiencies and poor substrate structure, which commonly characterize these areas (Tordoff et al. 2000; Walker 2002), can be also detrimental to plants. Moreover, the accumulation of high concentrations of trace elements into the above-ground biomass of plants should be avoided, because that would facilitate their entry into the food chain (Salomons et al. 1995) and also increase the trace element concentration on the soil surface upon litter fall (Johnson et al. 2003; Watmough et al. 2005).

For most woody plant species, seedling establishment is the critical phase in their life cycle (Rey and Alcántara 2000; Pulido and Díaz 2005). In particular, under Mediterranean-type climate, summer drought is the main cause of mortality during the first years after afforestation plantings (Navarro-Cerrillo et al. 2005; Pausas et al. 2004). Root growth inhibition caused by trace elements pollution could reduce their resistance to drought and hence increase mortality. Growth of those planted saplings may also be reduced, due to the cost of tolerance to the chemical stress (Hagemeyer 1999).

The aim of this work was to monitor the revegetation success in a trace element-polluted area (Guadiamar River valley, SW Spain) and to analyse its relation to residual soil pollution. This area was affected by a huge mine spill in 1998, which polluted the soil with several trace elements. The area was afforested with native woody species after soil remediation, one of the largest cases of soil remediation in Europe in the last decades. Five years after the remediation programme, revegetation success was assessed by monitoring plant survival and growth for the main planted woody species. Trace element concentrations of plants and soils were analysed and related with survival and growth rates of afforested saplings in the same sites. Specific questions were: what was the performance of the different woody species, in terms of survival and growth? Were there any differences in the survival and growth patterns between sites, due to soil pollution? Did the studied species accumulate soil pollutants into their above-ground biomass? Based on the results, what are the most suitable species for the restoration of the Guadiamar Valley and other similar areas?

Methods

Site description and studied species

The Guadiamar River Valley in SW Spain (37°30′ to 37°13′N, 6°13′W) lies inside the Iberian Pyrite Belt, the largest massive sulphide province in Western Europe (Fig. 1). The area has a semi-arid Mediterranean climate with mild rainy winters and warm dry summers. The average annual temperature is 19°C (minimum 9°C in January and maximum 27°C in July). The average annual rainfall is 448 mm and the potential evapotranspiration is 1,139 mm (period 1971–2004). Nevertheless, the annual rainfall can be very different for different years. For example, during our study period (years 2003, 2004 and 2005), the total rainfall during the hydrologic year (from September to August) was 693, 869 and 242 mm, respectively (CSIC meteorological station at Coria, Seville). Soils of the Guadiamar floodplain are mostly neutral or slightly alkaline, with the exception of terraces on the northern right bank, which have low pH. Soil texture varies from loamy sand to silty clay (Cabrera et al. 1999).

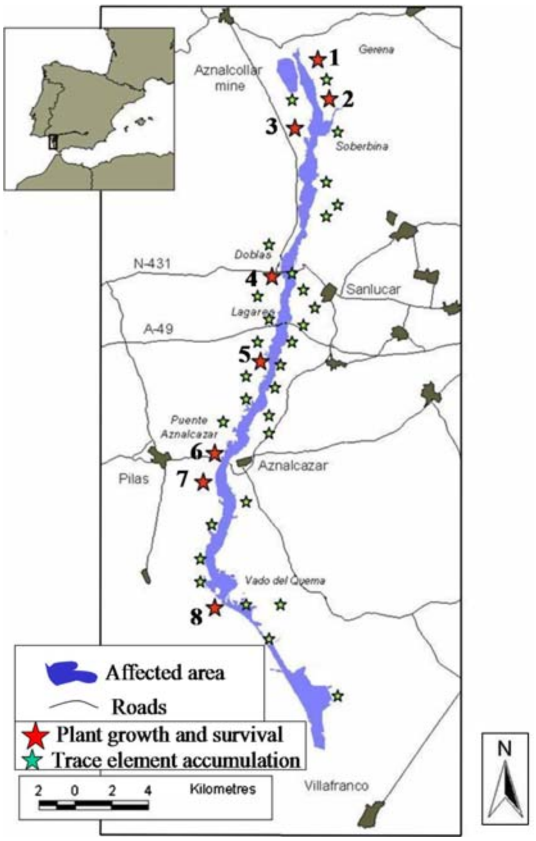


Fig. 1 Situation of the Guadiamar River Valley in the Iberian Peninsula and location of the sampling sites. Adapted from ITGE (1998)

In 1998, the failure of a large mine tailing dam at Aznalcóllar (Seville) released about 2 M m^3 of trace element-contaminated sludge and 4 M m^3 of acid waters into the Guadiamar River. The resulting flood inundated 4,286 ha of the basin southward towards the Doñana National Park (Grimalt et al. 1999). The affected soils, mostly under agricultural production, were burdened with high concentrations of As, Cd, Cu, Pb, Tl and Zn (Cabrera et al. 1999).

After the accident, an emergency cleanup removed the sludge and the polluted topsoils, which were transported and deposited in the nearby opencast mine. Organic matter and calcium-rich amendments were added with the aim of immobilizing trace elements and improving soil fertility (CMA 2003). Afforestation started in 1999, after the purchase of affected lands by the regional administration. Depending on the local habitat conditions, the target tree and shrub species to afforest were those typical of riparian forests, such as *Populus alba*, *Fraxinus angustifolia* and *Salix atrocinerea* or those typical of drier upland forests, such as *Quercus ilex* subsp. *ballota, Olea europaea* var. *sylvestris, Ceratonia siliqua, Phillyrea angustifolia, Pistacia lentiscus, Rosmarinus officinalis* and *Retama sphaerocarpa*. Seedlings were grown in a local nursery, and then planted when they were 1-year old. Planting density ranged from 480 to 980 plants per hectare.

We focussed our study on seven woody species that were the most used in the restoration project of the Guadiamar Valley: *Quercus ilex* subsp. *Ballota* (Desf.) Samp. (holm oak), *Olea europaea* var. *sylvestris* Brot. (wild olive tree) *Ceratonia siliqua* L. (carob tree) *Populus alba* L. (white poplar), *Fraxinus angustifolia* Vahl (ash), *Salix atrocinerea* Brot. (willow) and *Tamarix africana* Poir. (tamarisk).

Plant survival and growth

Eight sites were selected within the study area, from the mine tailing southward the Doñana National Park (40 km length, Table 1 and Fig. 1); two of them were not affected by the spill. *Quercus ilex*, *Olea europaea* and *Ceratonia siliqua* were present at six of the eight sampled sites (in the upland terraces of the floodplain, including those unaffected by the spill). *Populus alba, Fraxinus angustifolia* and *Salix atrocinerea* were sampled from two spill-affected sites, within the riparian bands. *Tamarix africana* was collected from three spill-affected sites (two within the riparian bands and one in the upland terraces).

In each site, three 50 m lines were randomly established; on these lines all individuals of the studied species were marked. The total number of marked plants was 195 (23.3 ± 3.4 plants per site, mean ± standard error). For each plant, different morphometric parameters were measured: shoot height (stretched distance from ground level to the highest living bud), main stem basal diameter (average of three measurements at 4 cm from the ground with digital callipers) and two opposite diameters of the crown. These two diameters were used to calculate the crown projected area (CPA), as the elliptical crown surface projected on the ground (Rey-Benayas and Camacho-Cruz 2004). The measurements were first made in June 2003, and repeated in July 2004 and July 2005. At each time, the survival status was also assessed. Since the sites were afforested between 1999 and 2000, the measured plants were 4–5 years old at the start of the study.

Soil and plant chemical analyses

Soil and plant (leaves) samples were taken for chemical analyses in autumn 2003. At each of the eight sites, between four and six soil samples were taken at 0–25 cm, using a spiral auger of 2.5 cm of diameter. They were mixed to obtain a composite soil sample for each site. The samples were dried at 40°C and crushed to pass through a 2 mm sieve, for the determination of general properties: pH (1:2.5 soil–water suspension), Kjeldahl N, available P (Olsen et al. 1954) and K (Bower et al. 1952) and texture (Gee and Bauder 1979). A fraction of the soil sample was ground to <60 µm for trace element analysis. Total trace element concentrations were determined by ICP-OES (inductively coupled plasma spectrophotometry), after digestion of the samples with HNO_3 and HCl (“aqua regia”).

Available soil trace element concentrations (Cd, Cu, Pb and Zn) were estimated from the total concentrations and soil pH by a regression model (Table 2). This model was obtained for soils from the Guadiamar River from a later study on the soil factors affecting the trace element bioavailability in the area (Domínguez et al. 2008a). In that

Table 1 Soil and vegetation types in each of the sampled sites

Site	Soil type (FAO)	Vegetation type	Plant species (planting density, plants ha^{-1})
1 (UNAFF)	Fluvisol	Upland terrace	*C. siliqua* (60), *O. europaea* (60), *Q. ilex* (80)
2 (AFF)	Fluvisol	Upland terrace	*C. siliqua* (60), *Q. ilex* (80)
3 (AFF)	Fluvisol	Upland terrace	*C. siliqua* (60), *Q. ilex* (80)
4 (AFF)	Fluvisol	Upland terrace	*C. siliqua* (60), *O. europaea* (60), *Q. ilex* (80)
5 (AFF)	Fluvisol	Riparian site	*F. angustifolia* (100), *P. alba* (280), *S. atrocinera* (200), *T. africana* (200)
6 (UNAFF)	Calcic luvisol	Upland terrace	*C. siliqua* (60), *O. europaea* (60), *Q. ilex* (80)
7 (AFF)	Fluvisol	Riparian site	*F. angustifolia* (100), *P. alba* (280), *S. atrocinera* (200)
8 (AFF)	Fluvisol	Upland terrace	*F. angustifolia* (50), *O. europaea* (100), *T. africana* (230)

AFF affected by the spill, *UNAFF* unaffected

Table 2 Regression models for the estimation of the soil available trace element concentrations (log-transformed, in mg kg^{-1}), with the soil pH and total concentrations as predictors

Element	Model equation	Model r^2
Cd	-1.80 pH + 0.55 Cd_{total} + 0.38	0.70
Cu	-3.77 pH + 0.84 Cu_{total} + 1.1.0	0.80
Pb	-0.69 pH + 0.67 Pb_{total} − 1.14	0.47
Zn	-9.11 pH + 1.99 Zn_{total} + 1.87	0.57

Adapted from Domínguez et al. 2008a

study (comprising 36 soil samples), the available trace element concentrations were analysed by extraction with ammonium nitrate and determination by atomic absorption spectrophotometry. It was concluded that Cd, Cu and Zn had the highest available concentrations and that soil pH was the most influential factor for trace element availability. In consequence, the available concentrations of Cd, Cu and Zn can be estimated from the soil pH and the total concentrations. The regression coefficient for Pb was lower than for the rest of the elements, so the estimated values for Pb presented here are only tentative.

For the analysis of plant trace element accumulation, an extensive plant sampling was carried out along the Guadiamar River Valley (Fig. 1). A total of 38 sites, including the 8 sites where we measured plant growth and survival, were selected; 4 of these sites were not affected by the spill. At least 5 individuals of the studied species were selected at each site, making a total of 75 sampled individuals for leaf analysis. Fully expanded leaves were taken from the outer canopy of each plant and a composite leaf sample per species and site was obtained. Samples were dried at 70ºC and digested with HNO_3 (Jones and Case 1990). As, Cd, Cu, Pb and Zn concentrations were determined by ICP-MS (inductively coupled plasma-mass spectroscopy). The quality of the plant analyses was assessed by analyzing reference material BCR 62 (olive tree leaves, Community Bureau of Reference; Colinet et al. 1982). For all elements, our experimental values were between 80 and 109% of the certified values.

Data analysis

Plant relative growth rates (RGR; Hunt 1982) were calculated with the morphometric measurements, as RGR = $(\ln X_1 - \ln X_0)/t$, where X_1 and X_2 are the different measurements at different time intervals (t, in years).

In order to assess the residual soil pollution levels, pollution load index (PLI; Tomlinson et al. 1980) was calculated for each site. This is an integrative index of the concentrations of several soil pollutants and compares the concentration factor (CF) for each pollutant (total concentrations) with respect to background levels (CF = $C_{\text{polluted soil}}:C_{\text{background}}$). The PLI was calculated as the *n*th root of the *n* CF (PLI = $(CF_1 \times CF_2 \times \ldots CF_n)^{1/n}$). In our case, PLI was calculated considering As, Cd, Cu, Pb and Zn (main trace elements in the spill) concentrations, and the background levels were those reported by Cabrera et al. (1999) for unpolluted soils from the Guadiamar River Valley.

Relative growth rates for the whole period (2003–2005) were used for the statistical comparison between sites. Since growth data did not fit normality, even after different transformations, nonparametric tests were used. For the three species that were present both in the affected and unaffected sites (*Q. ilex*, *O. europaea* and *C. siliqua*), Mann–Whitney *U* tests were performed to compare the growth rates in both types of sites. For all the species, Kruskal–Wallis tests were used for the comparison of RGRs between sites.

Plant chemical data fitted normal distributions after log-transformation. Therefore, one-way ANOVAs were used for the comparison of the leaf trace element concentrations between affected and unaffected sites. The level of significance used was 0.05. All statistical analyses were performed with STATISTICA v. 6.0 (StatSoft Inc., Tulsa, USA).

Results

Soil characteristics

The soils of the Guadiamar Valley (0–25 cm depth) had a loamy texture and were mostly basic (pH above 6.7, with two acidic exceptions; see Table 3). Soil fertility was very variable; for example, K availability ranged from 4 to 370 mg kg^{-1} and P availability from 7.2 to 29.2 mg k^{-1} (Table 3). They were polluted by several trace elements, namely As, Cd, Cu, Pb and Zn (Table 4). In comparison to the unaffected soils (sites 1 and 6), in the most polluted sites the As concentrations were up to 12 times higher, 10 times higher for Pb, 5 times for Cu, 4.5 times for Zn, and 2 times higher for Cd. Furthermore, in all the polluted sample sites, the As levels were above the range of normal values for agricultural soils (40 mg kg^{-1}; Bowen 1979), and exceeded the intervention values suggested in different countries (55 mg kg^{-1} in Holland; 50 mg kg^{-1} in Andalusia, South Spain). For other elements, namely Cd, Cu and Pb, some sites exceeded the regional permitted values: <3, 100 and 200 mg kg^{-1}, for Cd, Cu and Pb, respectively (Aguilar et al. 1999).

The Pollution Load Index (PLI) describes the total pollution burden for each site. There was a broad heterogeneity in the degree of soil pollution within the study area

Table 3 Soil general properties in each sampling site, within the Guadiamar River Valley

Site	pH (H_2O)	N (%)	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Silt (%)	Clay (%)	Sand (%)	Texture (USDA)
1	4.2	0.10	15.1	3.9	34.6	9.7	55.7	Sandy loam
2	6.7	0.08	19.8	59	39.1	10.1	50.8	Loam
3	6.8	0.11	26.3	22	36.1	19.7	44.2	Loam
4	3.2	0.07	7.7	156	38.8	18.8	42.4	Loam
5	7.3	0.07	7.7	120	43.4	23.3	33.3	Loam
6	7.3	0.08	9.1	249	51.0	19.7	29.3	Silt loam
7	7.4	0.07	7.7	156	38.8	18.8	42.4	Loam
8	7.2	0.12	29.2	370	41.3	16.9	41.8	Loam

Soils were collected during autumn 2003

Table 4 Trace element total concentrations ($mg\ kg^{-1}$) in the soils from the studied sites

Site	As	Cd	Cu	Pb	Zn	PLI
1	19.6	1.67	45.3	51.5	152	1.71
2	61.3	1.93	79.3	124	196	3.10
3	249	3.66	151	328	510	7.79
4	174	3.13	236	385	312	7.19
5	62.5	3.00	216	126	378	4.74
6	15.6	0.88	14.3	16.0	61.5	0.75
7	75.6	2.81	132	141	460	4.69
8	51.6	2.7	110	129	466	4.09
Background levels	18.9	0.33	30.9	38.2	109	1
Normal range	0.1–40	0.01–2	2–250	2–300	1–900	
MAV	20	3	100	200	300	

Pollution Load Index (Tomlinson et al. 1980), background values for the Guadiamar Valley (Cabrera et al. 1999), normal ranges in agricultural soils (Bowen 1979) and maximum allowable values (MAV) for Andalusia (South Spain, Aguilar et al. 1999) are indicated. Soils were collected during autumn 2003

(Table 4). The northern and central areas (sites 1–4), closest to the mine dam, were the most polluted sites. Sludge was stored in these sites during the cleanup operations, before its transport to the opencast mine. In these areas, irregularly distributed sludge patches could frequently be observed. Although site 1 was not affected by the spill (it is near the mine, but upwards from the dam), soils there had slightly higher trace element content than the background values (PLI > 1) from the Guadiamar Basin.

The estimated available trace element concentrations were relatively low. Maximum concentrations were recorded in site 4, which showed high total concentrations and low pH. In this site, estimated Cd, Cu, Pb and Zn availability were 0.31, 15.4, 1.75 and 170 $mg\ kg^{-1}$, respectively. On average, the available fraction of Cd, Cu, Pb and Zn represented 5.3, 1.5, 0.5 and 7%, respectively, of the corresponding total concentrations. Therefore, Cd and Zn were the most labile elements in the soils from our study area. The maximum availability percentage was observed in site 4, where a 10 and a 55% of the total soil Cd and Zn, respectively, was available for plants.

Plant survival

The survival patterns were very different between species (Table 5). For the riparian species (*P. alba, F. angustifolia, S. atrocinerea,* and *T. africana*) and for *O. europaea*, the survival rates were 100%. In contrast, for *C. siliqua* and *Q. ilex*, the survival rates were low during the study period, decreasing to 30 and 20%, respectively (Table 5). For some species, sprouting ability was very important for survival, especially between 2004 and 2005, when rainfall was especially low (see the section "Site description and studies species"). For example, 50% of *S. atrocinerea* individuals had a die-back of above-ground parts during the summer 2004, but they all resprouted afterwards in the autumn. Likewise, around 40% of *O. europaea* and *F. angustifolia* showed a sprouting behaviour between summer 2004 and summer 2005. In the case of *C. siliqua*, all the surviving plants had resprouted at least once during the whole study period. For *Q. ilex*, the sprouters were just a 22% of the surviving plants.

Among the sites, the lowest survival rates were recorded in those sites located in the northern areas, closest to the mine. The highest mortality rate was recorded at site 3, where 90% of *C. siliqua* and 97% of *Q. ilex* were dead by

Table 5 Survival rates (%) of seven woody species in the afforested sites of the Guadiamar Valley

Species	Site							
	1	2	3	4	5	6	7	8
Ceratonia siliqua	50	40	10	25	–	20	–	–
Fraxinus angustifolia	–	–	–	–	100	–	100	100
Olea europaea	100	–	–	100	–	100	–	–
Populus alba	–	–	–	–	100	–	100	100
Quercus ilex	50	22	3	25	–	75	–	–
Salix atrocinerea	–	–	–	–	100	–	100	–
Tamarix africana	–	–	–	–	100	–	–	100

Values correspond to the whole study period (2003–2005), with the exception of site 6 (2003–2004), burnt in late 2004 the end of the monitoring period. At site 1, which was not affected by the spill, the mortality rate was 36.3%. As mentioned above, the survival rates at the riparian sites (sites 5 and 7) were 100% (Table 5).

Plant growth

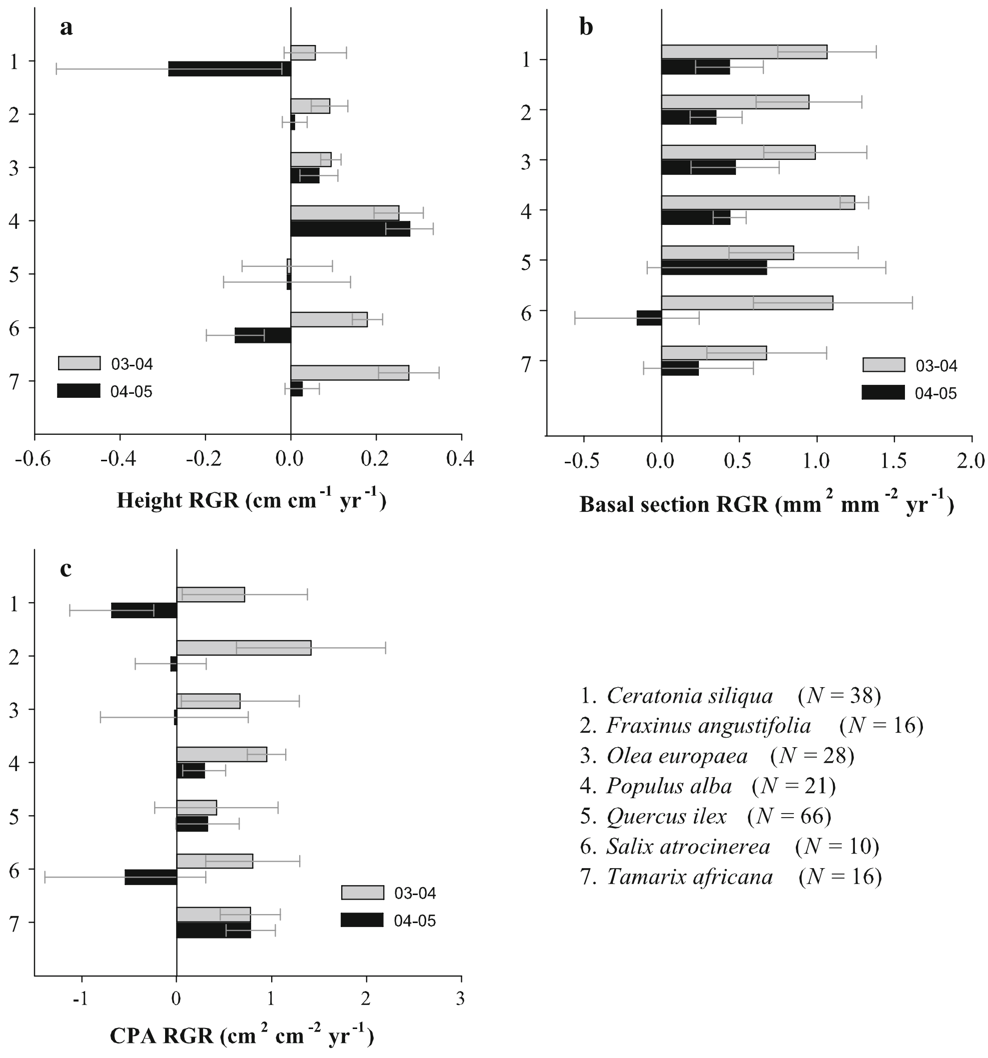
The relative growths rates (RGR), in terms of shoot height (Fig. 2a), stem basal section (Fig. 2b) and crown projected area (Fig. 2c), varied among species and years. For the second period (2004–2005), the growth rates were comparatively lower, and in some cases had negative values due to shoot dieback during summer drought and later resprouting, e.g. *C. siliqua* and *S. atrocinerea*.

For the first period (2003–2004), the highest growth rates were observed for the riparian species; growth in height was remarkable for *T. africana* (up to 0.2 cm cm^{-1} year^{-1}) and *P. alba*, and crown growth for *F. angustifolia*. Stem width growth was rather similar among species, although the highest mean value (1.2 mm^2 mm^{-2} year^{-1}) was also shown by *P. alba*. In contrast, the lowest growth rates for height and crown were shown by *Q. ilex*.

During the second measured period (2004–2005), growth rates were lower for most of the species. The exceptions were the consistent growth in the height of *P. alba*, in the crown of *T. africana* and in the stem width of *Q. ilex*. Partial or total shoot dieback was stronger during the 2004 summer, affecting many species, which in consequence showed negative growth values of height and crown width. These were more remarkable for *C. siliqua* and *S. atrocinerea*. In the case of *S. atrocinera*, the loss of aerial biomass in the sprouting individuals was total, and the saplings resprouted from the stem base, producing new branches. Therefore, the mean growth of stem width for this species was also negative for this period.

Relationships with soil pollution

Survival rates decreased at higher levels of residual soil pollution for *Q. ilex* and *C. siliqua* (Fig. 3a), while the other



1. *Ceratonia siliqua* ($N = 38$)
2. *Fraxinus angustifolia* ($N = 16$)
3. *Olea europaea* ($N = 28$)
4. *Populus alba* ($N = 21$)
5. *Quercus ilex* ($N = 66$)
6. *Salix atrocinerea* ($N = 10$)
7. *Tamarix africana* ($N = 16$)

Fig. 2 Mean values (*bars*) ± standard errors (*lines*) of relative growth rates (RGR) for shoot height (**a**), main stem basal section (**b**), and crown protected area (**c**), for the seven studied species

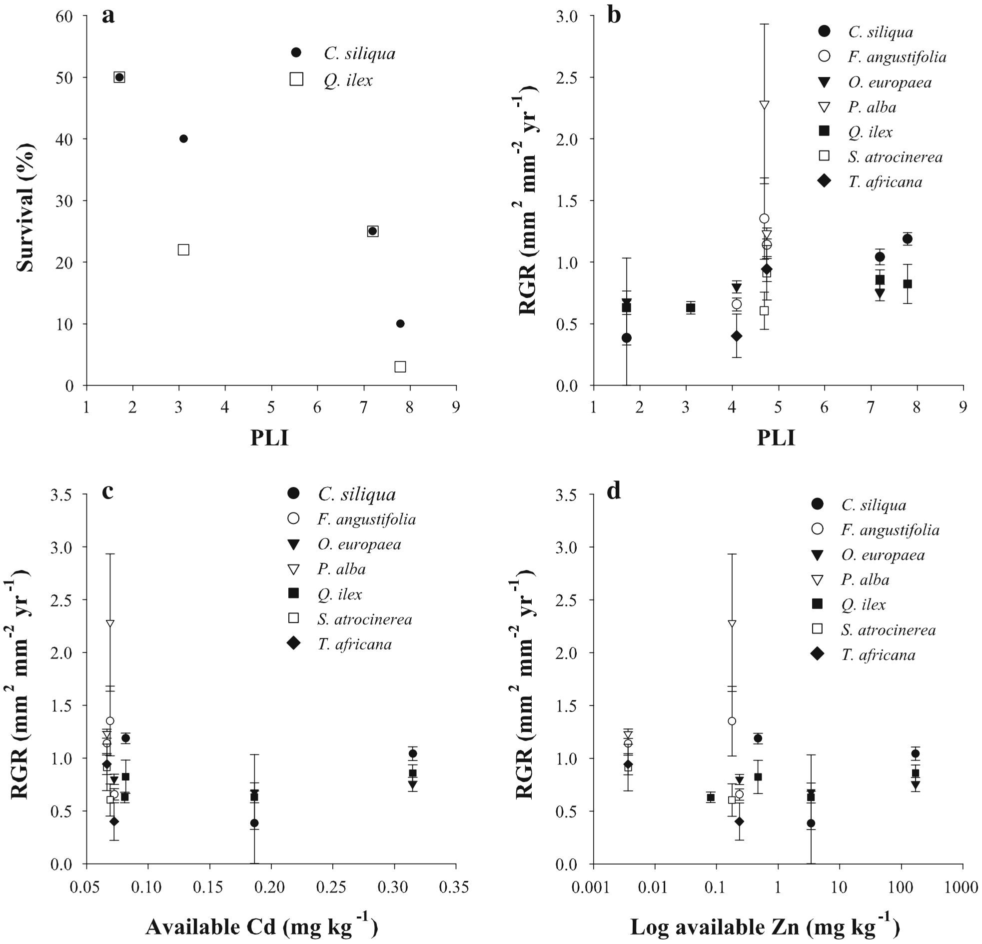


Fig. 3 Relationships between soil pollution (indicated by a Pollution Load Index, PLI) and plant performance: plant survival (**a**), and stem basal section growth (**b**). Relationships between availability of Cd (**c**) and Zn (**d**) in polluted soils and stem basal section growth of the different studied species

five species had about 100% survival, independent of the pollution level of the site. The relative growth rates (for the whole 2003–2005 period) were not related to the level of soil pollution, considering the total concentrations in the PLI (see example for stem basal diameter in Fig. 3b), and neither considering the estimated availability (see examples for Cd and Zn in Fig. 3c, d).

Moreover, there were no significant differences in growth rates between spill-affected and unaffected sites for the three common species: *C. siliqua*, *O. europaea and Q. ilex* (Table 6). When comparing the RGRs for each species between sites, we only found significant differences for the basal diameter growth in *F. angustifolia* and *T. africana* (Kruskal–Wallis tests: $H = 8.29$, $P = 0.015$ and $H = 5.10$, $P = 0.024$, respectively). In both cases, the basal diameter RGR was significantly higher in the most polluted sites.

Trace elements in plants

When comparing the trace element accumulation between the affected and unaffected sites (*C. siliqua*, *O. europaea* and *Q. ilex*), we detected some significant differences. For *C. siliqua*, As and Zn concentrations in the unaffected sites were 0.048 and 27.6 mg kg^{-1} respectively, which were significantly lower than those in the affected sites ($F = 5.58$, $P = 0.034$ and $F = 9.73$ and $P = 0.008$, respectively). Cadmium and Zn levels in the leaves of *O. europaea* in the affected sites were significantly higher ($F = 22.9$, $P = 0.034$ and $F = 5.14$ and $P < 0.001$, respectively) than the concentrations in the unaffected sites (0.012 and 40.7 mg kg^{-1},

Table 6 Results of the Mann–Whitney tests (*U* and *P*-values) for the comparison of relative growth rates (basal section and shoot height) between spill-affected and nonaffected sites

Species	Basal section		Shoot height	
	U	*P*	*U*	*P*
C. siliqua	3	0.133	1	0.266
O. europaea	18	0.141	24	0.494
Q. ilex	4	0.327	2	0.071

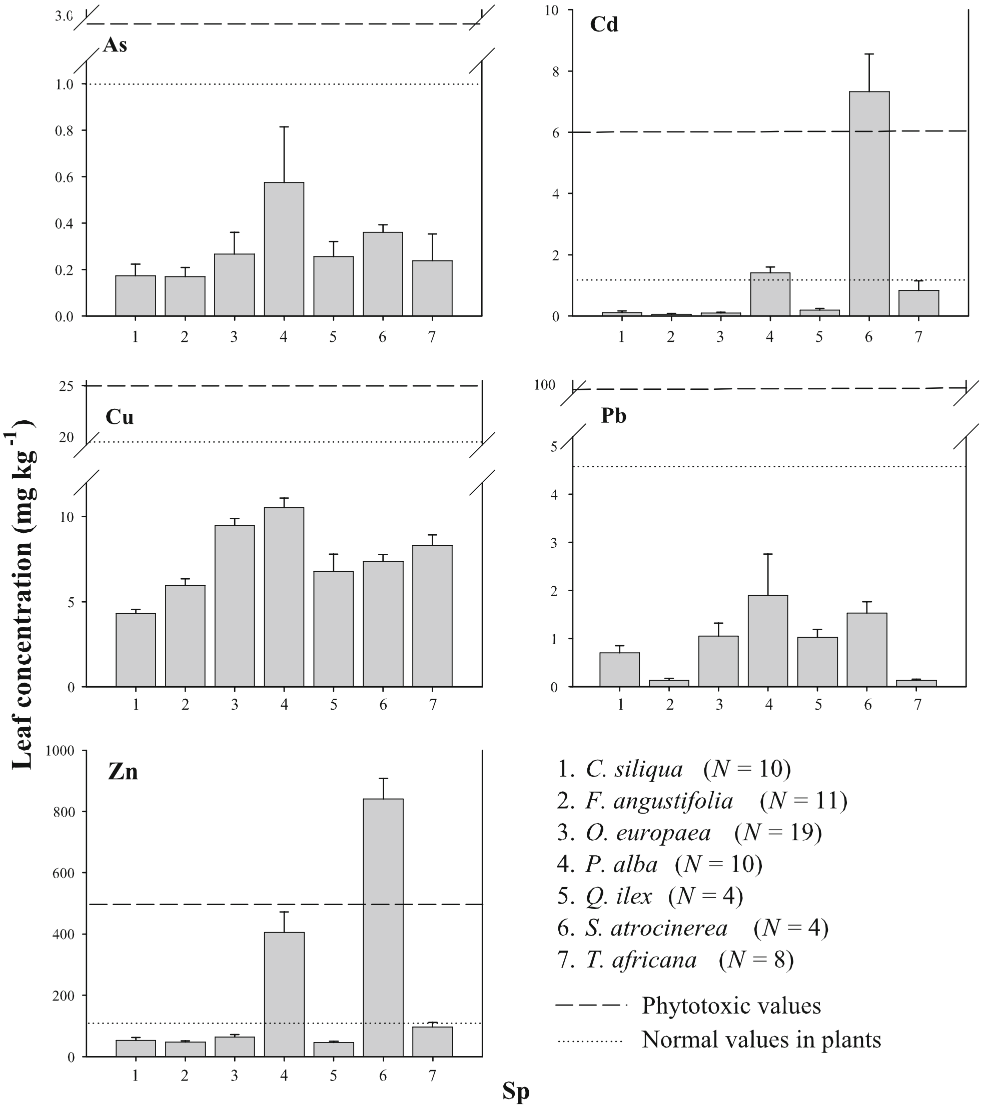


Fig. 4 Mean values (*bars*) ± standard errors (*lines*) of trace element concentrations in the leaves of the studied woody plants, collected from polluted sites. Normal levels for higher plants and phytotoxic levels (reported by Chaney 1989) are indicated

respectively). Likewise, the As concentrations in the leaves of *Q. ilex* trees growing in the affected sites were significantly higher ($F = 32.1$, $P = 0.011$). However, despite these significant increases, in the affected areas the trace element concentrations were always within the normal ranges for higher plants in these three species and also very much lower than the phytotoxic levels (Fig. 4). The same occurred for *T. africana* and *F. angustifolia.* In contrast, *P. alba* and *S. atrocinerea* (both of the Salicaceae family) in the affected areas showed relatively high concentrations of Cd and Zn in their leaves. In the case of *P. alba*, the mean concentration of Cd (1.4 mg kg^{-1}) was only slightly higher than the normal values for plants (1 mg kg^{-1}, Chaney 1989), while Zn concentrations (mean of 405 mg kg^{-1}) were well in excess of these values (150 mg kg^{-1}; Chaney 1989). The highest Cd and Zn levels were detected for *S. atrocinera*, which showed mean Cd and Zn concentrations up to 7 and 850 mg kg^{-1}, respectively. These concentrations were higher than those considered toxic for some higher plants (5 and 500 mg kg^{-1}, respectively; Chaney 1989). For both *P. alba* and *S. atrocinerea*, the Cd concentrations were higher than those levels that may adversely affect livestock (0.5 mg kg^{-1}; Chaney 1989). As mentioned above, for the rest of the elements the mean concentrations were relatively low, with *P. alba* having the maximum As, Cu and Pb concentrations (0.6, 10.9 and 1.8 mg kg^{-1}, respectively; Fig. 4). Interestingly, these average values of trace elements accumulated in the leaves of planted saplings were not significantly related to the growth recorded for the species in the same site.

Discussion

Residual soil pollution of the restored area

Polluted areas require an active management in order to reduce the risk derived from pollution to the health of humans and the ecosystem. In the case of trace elements, soil remediation should be achieved, with the aim of decreasing the bioavailability of such pollutants. Revegetation of polluted soils minimize wind and water erosion, thus reducing the risk of migration of trace elements (Robinson et al. 2003). Monitoring of the remediated sites plays a central role in their management; based on the monitoring results, useful information can be obtained about the most suitable soil amendments to apply and the plant species to afforest.

In this study, we have monitored different variables of soils and plants from the Guadiamar Valley (SW Spain). Five years after the restoration of this mine spill affected area, the soils were still polluted by several trace elements. Despite the removal of the superficial polluted soil layer, the sludge containing these elements could penetrate into the surface soils, and total concentrations in the top 25 cm of soils were still relatively high. The heterogeneity in the pollution levels between sites was caused by several factors, such as the irregular sludge deposition (Grimalt et al. 1999), the heterogeneity in soil texture along the study area, which conditioned the degree of penetration and leaching of sludge through the soil profile (Cabrera et al. 1999), and the irregular cleanup operations. In many sites, the pollution levels were higher than the intervention values, especially in the case of As. These high trace element concentrations justified the intervention of the regional administration after the spill. Despite reducing the mobility of such pollutants by the remediation activities, the area requires a long-term monitoring, due to the high persistence of trace elements in soils. The dynamics of these elements in the soil is complex, and despite retention being possibly the main process, the bioavailable levels could increase under certain conditions, such as soil oxidation, acidification or loss of organic matter (Adriano 2001).

The estimated availability of trace elements in the afforested soils was relatively low. In comparison, it was lower than the availability analysed (using EDTA extractant) during the first years after the accident in similar sites of riparian forests and upland terraces (Madejón et al. 2004, 2006). Subsequent studies have shown that soil pH is the most important factor that determines the bioavailability of trace elements, rather than other soil factors such as organic matter content, soil texture or cation exchange capacity (Domínguez et al. 2008a and unpublished). Therefore, the monitoring of the area should consider not only the total pollution burden of the sites, but also the concurrence of acidic soil conditions, which could increase the risk of toxicity by trace elements.

Afforested woody plant survival

The afforested plants in the Guadiamar Valley had to resist the particular soil conditions, in addition to the summer drought, high irradiance and the alteration of soil structure that characterized the area after the cleanup operations. The performance of the studied plants was very different between species and between sites.

Riparian species (including *T. africana*) showed the highest survival rates. *Populus* and *Salix* species present physiological mechanisms that allow them to survive in highly disturbed riparian environments, such as a high anchorage ability and vegetative reproduction (Karrenberg et al. 2002, 2003). The fast growth of the riparian species, which can reach a height of several metres in 1–2 years after being planted promote the establishment of favourable micro-environmental conditions underneath and have a buffering effect on temperature and soil moisture (Conner et al. 2000; Dulohery et al. 2000). Thus, fast-growing trees can enhance the middle-term plant survival in restored sites (Gardiner et al. 2004; Twedt 2006). In this study, plants were firstly marked 3–4 years after being planted, so the initial mortality of the saplings was not registered. At the time of sampling, the forest structure in the riparian sites was much more developed than in the upland terraces, favouring the high survival rates of the saplings during the study period, despite especially dry periods as in 2004–2005. *P. alba* and *T. africana* showed the best performance, maintaining high growth rates even during the driest growth period. In general, *Populus sp.* has lower water requirements than *Salix* sp. and a lower tolerance to inundation (Francis et al. 2005). Some studies have reported good results in the afforestation of Mediterranean riverbanks with *P. alba* (Martínez and Martín 2001). Plants of *Tamarix* sp. are physiologically better adapted to tolerate high water stress than *Populus* or *Salix* sp. (Horton et al. 2001; Lite and Stromberg 2005), and the restoration of semi-arid riverbeds with *T. africana* is usually successful (Salinas and Guirado 2002). Under the conditions of the Guadiamar River Valley, these two species, *Populus alba* and *Tamarix africana*, gave good results for the revegetation of the riparian edges.

In the case of upland terrace sites, the survival rates were much lower due to the high mortality rates of saplings of *C. siliqua* and *Q. ilex*. Summer drought is the main cause of seedling and sapling mortality in Mediterranean habitats (Navarro-Cerrillo et al. 2001; Pausas et al. 2004). Weed competition and herbivory also contribute to the low survival rates in the afforestation of these types of sites (Rey-Benayas et al. 2003, 2005; Navarro-Cerrillo et al.

2005). In contrast, saplings of *O. europaea* showed a high survival. Several studies have shown that wild olive can resist difficult environmental conditions (salt marshes, mine soils) better than other tree species, such as *Q. ilex* or *C. siliqua* (Rubio et al. 2001; Clemente et al. 2004). The sprouting ability of *O. europaea* and the other species planted in upland terraces seems to be an essential strategy to recover after dieback during summer drought and survive under the conditions of the Guadiamar Valley.

Plant growth

The relative growth rates were also very different between species. As expected, riparian species showed, in general, the highest growth rates: partly because the habitat conditions were more favourable in the riparian sites; partly also because the biological characteristics of the riparian plants allowed them a faster growth. The specific leaf area (SLA) is a morphological trait contributing strongly to the growth rate of Mediterranean woody plants (Antúnez et al. 2001; Poorter and Garnier 1999). Deciduous species, such as the riparian *P. alba*, *F. angustifolia* and *S. atrocinera*, had a higher proportion of leaf area per mass unit (higher SLA) than sclerophyllous species (those in the upland terrace species), and therefore are more efficient in capturing light energy and gaining carbon (Reich et al. 1992; Ackerly et al. 2002).

A generalized decrease in growth rates was observed with time. On one hand, this is a general trend with woody plant age and higher proportion of structural tissues (Antúnez et al. 2001; Poorter and Garnier 1999). On the other hand, growth rates were particularly affected by the adverse dry conditions in the second study year, especially in the upland terraces. Sprouting ability allowed plants to survive after that dry period. However, the total growth of the sprouted plants was negative, due to the loss of above-ground biomass during the dieback.

Despite the survival of saplings by their sprouting strategies, the establishment of the above-ground woody plant cover was very slow in the upland terraces. In consequence, changes in spatial heterogeneity of the afforested area, especially in relation to light and soil moisture by the established vegetation, were also slow. Instead, a rather homogeneous matrix of weed herbaceous cover, with high radiation incidence and evaporation rates persisted. Shrub pioneer species may better resist the abiotic stress in the upland terraces. Moreover, these shrub species can promote the establishment of late successional woody plant seedlings such as *Q. Ilex* and *C. siliqua*, by a nurse effect (Callaway 1992; Castro et al. 2004; Gómez-Aparicio et al. 2004). *Olea europaea* could also be an appropriate species for the afforestation of the studied area and other similar sites. Besides its high survival rates, the shrub-like shape of the crown at the juvenile stage may facilitate the establishment of other woody plants.

Soil pollution, plant growth and trace element accumulation

There was a higher mortality of saplings of *Q. ilex* and *C. siliqua* (but not of *O. europaea*) in the highest polluted upland terraces. However, the surviving saplings there did not show a reduced growth. In the riparian sites, there were no clear relationships between the plant survival and growth rates and the level of soil pollution.

The highest polluted upland terraces were located in the northern areas, where sludge was stored during the cleanup operations. The substrate structure was more altered in these sites, since they were exposed to an intensive heavy machinery transit, and then to an intense removal of the topsoil. The sapling establishment could have been hampered by this management factor. Once the plants were established, the trace element content of soils had relatively lower importance for the sapling growth; thus there were no significant differences between the growth rates in the spill-affected and unaffected sites for the three common species. Other environmental factors (soil water availability, structure, acidity, etc.), rather than soil pollution, could be more important for the plant performance in the studied area.

In general, the trace element concentrations in the plants growing in the spill-affected sites were within the normal ranges for higher plants, and much lower than those levels considered phytotoxic, with the exception of Cd and Zn for *P. alba* and *S. atrocinerea*. These low leaf levels may indicate the low bioavailability of soil trace elements for the studied woody plants. Thus, it is possible that the application of amendments to restore the soil after the accident, together with the natural dynamics of trace elements in soils, could contribute to the apparent rather low mobilization of these elements, as reflected by plant uptake and accumulation in leaves. On the other hand, plants of these five woody species, but not those of *Salicaceae* (*Populus* and *Salix*), could actively exclude trace element uptake at the root system. Under the environmental conditions in the area, there is a limited transfer of trace elements from soils to the above-ground biomass of most woody species, which is scarcely affected by soil pH or organic matter content (Domínguez et al. 2008b). The exception is the accumulation of Cd and Zn in *Salicaceae* species. It is well known that these species can accumulate and tolerate high levels of Cd and Zn in their leaves (Madejón et al. 2004; Robinson et al. 2005). In the Guadiamar River Valley, the accumulation of Cd and Zn in *P. alba* and *S. atrocinerea* leaves may represent some environmental risk with regard to the entry of trace elements into the food chain (see discussion of this aspect in Domínguez et al. 2008b).

Conclusions

In polluted soils, pollution may be an aditional factor of abiotic stress affecting the establishment of a woody plant cover. In the study case of the Guadiamar Valley, despite the remediation activities carried out after a huge mine spill in 1998, the soils in the area were still polluted by several trace elements. A long-term monitoring of the area is needed, due to the high persistence of such pollutants in soils. However, according to the results shown here, soil pollution is not likely to be the most important factor affecting the growth of the afforested woody plants. Other factors, such as drought, high irradiance or substrate alteration may be equally or more important for the establishment of a woody plant cover in the upland terraces, where the highest mortality rates were observed.

The design of future afforestations in the upland terraces should consider the use of species that better tolerate the adverse conditions, rather than late successional species such as *Q. ilex* and *C. siliqua*. For example, the use of shrub species and *O. europaea* should be enhanced. In the riparian sites, the establishment of plant saplings was more successful, with *P. alba* and *T. africana* showing the best performance. The patterns of trace elements accumulation in leaves should also be taken into account in the management of the area. While most of the studied woody species showed low trace element concentrations in leaves, the accumulation of Cd and Zn by *P. alba* and *S. atrocinerea* may represent a risk to the food chain and may increase the trace element concentration on the soil surface upon litter fall.

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