

The bryophyte diaspore bank in soils affected by the Aznalcóllar (Seville, SW Spain) mine disaster

Alejandro Ordóñez, Juan Guerra & María J. Cano

Departamento de Biología Vegetal, Área de Botánica, Facultad de Biología, Universidad de Murcia, Spain.

Correspondence

J. Guerra

Tel: + 34 968 364981

Email: jguerra@um.es

Received: 20 December 2002

Accepted: 28 April 2003

Abstract

The tailings dam failure of the pyrite mine in Aznalcóllar released toxic tailings slurries and liquid that contaminated soils and water throughout the Guadiamar basin. In this study we attempt to ascertain the recovery state of the zone four years after the dam failure, based on a study of the soil diaspore bank and the method of cultivating soil plots and determining the germinated species. Four localities were selected in the middle zone of the basin. Once the plots were established (five in each locality), they were divided into four groups, depending on the culture conditions. Soil samples were also taken for the analysis of heavy metals and edaphic parameters. Data seem to show that four years after the dam failure soils have started a slow recovery and heavy metal levels are decreasing.

Keywords: Aznalcóllar, Bryophyte, Diaspore bank, Guadiamar river, Heavy metals.

Resumen

El banco de diásporas de briófitos en suelos afectados por el desastre minero de Aznalcóllar (Sevilla, SO España).

La ruptura de la presa de contención de la mina de pirita de Aznalcóllar provocó la liberación de lodos tóxicos que contaminaron el suelo y el agua de toda la cuenca del río Guadiamar. En este estudio hemos pretendido averiguar el estado de recuperación de la zona cuatro años después del vertido. Para ello nos hemos basado en la observación del banco de diásporas del suelo, más concretamente el método de cultivar muestras de suelo para después determinar las especies que germinan. Se seleccionaron cuatro localidades en la zona media de la cuenca. Una vez tomadas las muestras de suelo (cinco por localidad) necesarias, se separaron en cuatro grupos en función de las condiciones de cultivo. También se tomaron muestras de suelo para el análisis tanto de metales pesados como de los parámetros edáficos. Los datos parecen indicar que tras cuatro años los suelos comienzan una lenta recuperación y los niveles de metales pesados comienzan a descender.

Palabras clave: Aznalcóllar, Briófitos, Banco de diásporas, Río Guadiamar, Metales pesados.

Introduction

On the 25th of April, 1998, the retention dam at the pyrite mine in Aznalcóllar (Seville, Spain), burst, allowing the contents of the tailings reservoir (about 3,62 Hm³ of contaminated water and 0.88 Hm³ of toxic sludge) to flow freely into the rivers Guadiamar and Agrio, both tributaries of Guadalquivir river. The sludge flowed about 40 km, reaching the vicinity of Puente de Don Simón (Seville). In all, approximately 5,500 ha were affected, 293 of which corresponded to the river course itself.

The waste, composed of sludges and water, had a great ecological and socio-economic impact, leading the Provincial and National government to draw up a scientific and technical plan of action to restore the lost ecological functionality.

The mine at Aznalcóllar is located in the Iberian pyrite belt, in the SW of the Iberian Peninsula. The mineralogical composition is 83,1% pyrite (FeS₂), 5,4% blende (ZnS), 2,1% galena (PbS), 1,4% chalcocopyrite (CuFeS₂), 0,9% arsenopyrite (FeAsS) and 7,1% gangue (Almodóvar et al. 1998). After extraction, the tailings contain Pb, Zn, As and Cu (Simón et al. 1998).

The upper basin of the Guadiamar river presents two clearly differentiated zones. The metamorphic and igneous rocks of the headwaters imparting a markedly impermeable nature, while towards Aznalcóllar (the zone most affected by the accident) detritic materials from the Andaluciense-Tortonense Miocene (progressively more recent and permeable towards the south) predominate; lastly, there are sands and silts from the mud flats of the lower Guadalquivir.

The climate is Mediterranean, becoming more continental towards the higher parts of the basin, in Sierra Morena. There are two bioclimatic belts in the zone: thermomediterranean in the Guadalquivir plain, below 400 m a.s.l. and mesomediterranean in the open upper plains and shaded valleys. Three ombrotypes are evident: humid in the higher zones of the Aznalcóllar and Escacena mountains, subhumid as we drop in height and latitude, and lastly, in the mud flats, a dry ombroclimate appears (Guerra et al. 2002).

The soils most affected by the toxic waste materials were typic and vertic fluvisols, and typic regosols, most presenting characteristics that appear favourable for neutralising the negative impact of the accident. These are neutral or slightly alkaline soils (pH 7-8) with small variations in their organic matter (0,8 - 2,9%) and iron content and a carbonate content of 0-20%. Other characteristics are more variable; for example, the texture varies from very

clayey to sandy loam, the structure from massive to angular blocks, and the gravel content from 0 to 43% (Simón et al. 1998).

The present contribution is a study of the initial recovery process of soils altered by the spill, where the appearance of bryophytes, lichens and some algae (mainly cyanophytes that intervene in nitrogen fixation) is an excellent indicator of the soil's recovery potential (Brasell et al. 1986), since they retain water in the upper levels of the soil and they prepare the edaphic environment for the appearance of vascular plants. Such phenomena have been widely studied and they are perfectly demonstrated in ecological succession processes, for example, in burnt soils (De Las Heras et al. 1990, De Las Heras 1992, De Las Heras et al. 1994, During et al. 1987, Gloaguen 1990, Herranz et al. 1991, Puche & Gimeno 2000). The diaspore bank is a good starting point for studying the state of a damaged ecosystem, especially if we compare it with the vegetation developed on unaffected soils (Bisang 1996, During & Lloret 2001, Hébrard 2001, Milberg & Persson 1994, Sundberg & Rydin 2000, Trabaud et al. 1997).

In the area studied, the diaspore bank was in an unusual condition, since a large part of the topmost soil layer had been removed mechanically along with the sludge, and with it a good part of the diaspore bank. Moreover, since the river course is tidal, many of the seeds and propagules come from the upper stretches of the basin, which, to a certain extent, alleviated any losses in the diaspore bank.

Material and methods

Several procedures have been described for studying the diaspore bank of a soil. One direct method, described by Malone (1967), consists of extracting and isolating diaspores for identification and counting under a binocular microscope. An alternative, indirect method, consists of taking soil samples and counting the seedlings corresponding to viable diaspores that grow in a given cultivation or incubation conditions (During 1997, Martínez & Maun 1999), while yet another method observes the appearance of seedling *in situ* (Thompson et al. 1977, Roberts 1981).

The first method provides an approximation of the total quantity of propagules in the soil, both viable (those that regenerate the vegetation) and nonviable. The second method only includes the viable diaspores but shows exactly which species regenerate the plant cover. The last method does not allow the control in the culture conditions. In the present study the second method was chosen because the small size of the bryophyte diaspores rendered their isolation im-

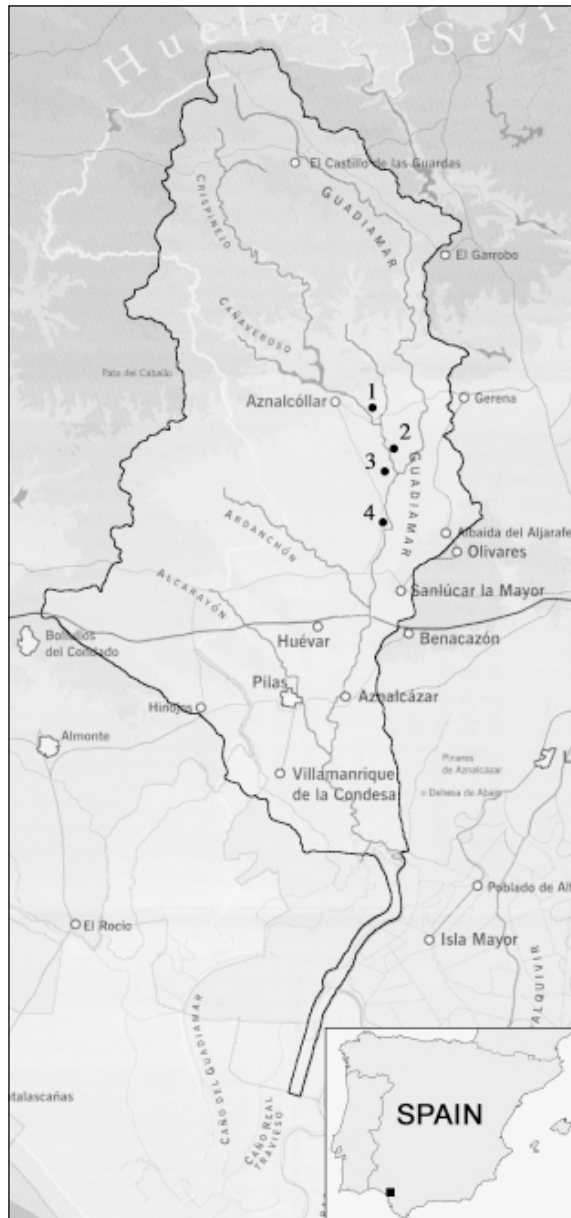


Figure 1. Guadiamar river basin with localities studied (1-4).
 Figura 1. Cuenca del río Guadiamar con las localidades estudiadas (1-4).

possible; furthermore, the distance between the locality studied and the place where the samples were processed did not allow for the suitable study.

Four localities in the lower Guadiamar river basin were chosen (Figure 1), taking a total of twenty soils samples (five per site) corresponding to the arable or superficial layer (down to 5-10 cm, approximately). These samples were put in plastic trays and were covered with plastic film to prevent contamination. Some of the samples showed a slight covering of bryophytes, which is to be expected in field conditions.

In the same localities, and near zones where the samples were taken (no more than 1-2 m away) soil samples were removed for a study of their main edaphic parameters including pH, N total, C/N ratio, conductivity, etc., and their heavy metal content.

Soil samples for culture were distributed into four groups (Table 1), each of them under different culture conditions. Five trays were kept in a culture chamber at a mean temperature of 20°C, a relative humidity of 70% and a photoperiod of 16:8 (Light: Darkness). Another five trays were cultivated in a culture chamber at 20°C with a relative humidity of 70% and a photoperiod of 12:12 (Light: Darkness). The remaining ten trays were taken to a greenhouse, where five of them were left uncovered and the other five covered with plastic film (Table 5). These samples were kept in the same conditions for three months and the total and specific cover of each tray were estimated visually every fifteen days.

The following methods were used to study the edaphic parameters: pH by Peech's method (1965) in a 1:1 water extract of soil in water and in 1M KCl.

Tray (locality/condition)	Place
1.1	Greenhouse uncovered
1.2	Culture chamber 16:8
1.3	Culture chamber 16:8
1.4	Culture chamber 12:12
1.5	Greenhouse covered
2.1	Culture chamber 16:8
2.2	Culture chamber 12:12
2.3	Culture chamber 12:12
2.4	Greenhouse uncovered
2.5	Greenhouse covered
3.1	Culture chamber 12:12
3.2	Culture chamber 16:8
3.3	Greenhouse covered
3.4	Greenhouse uncovered
3.5	Greenhouse covered
4.1	Culture chamber 16:8
4.2	Greenhouse uncovered
4.3	Greenhouse uncovered
4.4	Greenhouse covered
4.5	Culture chamber 12:12

Table 1. Culture conditions of the different samples.
 Tabla 1. Condiciones de cultivo de las distintas muestras.

	pH		C.E.	C.I.C.	N	C	M.O.	C/N	CaCO ₃
	H ₂ O	KCl	dS/m	cmol ₍₊₎ /kg	g/kg	g/kg	g/kg		%
1	6,0	5,7	3,99	15,77	1,13	8,13	14,02	7,2	6,1
2	4,7	4,1	3,35	10,52	0,80	4,35	7,50	5,44	0
3	6,8	6,5	2,14	10,70	0,76	1,59	2,74	2,09	6,8
4	7,9	7,4	1,41	9,27	0,84	3,31	5,71	3,94	3,1

Table 2. General analysis of the soil samples.

Tabla 2. Análisis generales de las muestras de suelo.

Electrical conductivity (EC) by Bower and Wilcox's method (1965), expressing the results in dS/m. Cationic Exchange Capacity (C.E.C.) in ammonium acetate at pH 7, according to Chapman (1965) and expressing the results in cmol₍₊₎/kg. Total Nitrogen (N) using Kjeldahl's method, as described by Duchaufour (1970), expressing the results in g/kg. Total Organic Carbon (C) using Anne's method (1945), modified by Duchaufour (1970) and expressing the results as g/kg. Calcium Carbonate Equivalent (CaCO₃) using the Bernald calcimeter volumetric method, after calibrating with Na₂CO₃ R. A. and starting with a suitable weight of fine sand, depending on its carbonate content. Results were expressed as CaCO₃ equivalent percentages. The Textural Analysis was carried out using the Robinson pipette method as described by Kilmer and Alexander (1949). For heavy metal analysis, pulverised samples were sent to the ICP-Masses Unit of the Scientific Instrumentation Centre of the University of Granada, where heavy metals were measured with a mass spectrometer equipped with a plasma torch ionisation source and quadrupole ion filter (PERKIN ELMER Sciex-Elan 5.000 for 25 elements): Mn, Zn, Pb, Cu, As, Sb, Ba, V, Tl, Cr, Co, Bi, Cd, Ni, Sn, Y, Be, U, Th, Sc, Hg, Mo, In, Se and Au. Results are expressed in ppm.

Results

Soil samples

General analysis

Table 2 shows the results of the analysis made of the samples from the four localities.

The pH measured in the soil from the second locality indicates that the base content is very low, which may produce severe toxicity towards other elements, such as Mn, Zn and Ni (López-Ritas & López Melida 1990). The pH of the fourth locality soil could be considered normal if there were larger quantities of carbonates in the soil, but this is not the case; rather, it is the presence of large amounts of Na and Mg that is responsible for the pH of 7,9 recorded (López-Ritas & López-Melida 1990).

As regards the Electrical Conductivity of the soils, all but one shows values higher than 2 dS/m, which indicates that the most sensitive species to the presence of salts would be affected, although the values are not so high as to consider these soils as saline. However, the electrical conductivity is nearer 4 dS/m in the soil from the first locality, which can be consi-

Samples	< 2	2-20	20-50	50-100	100-250	250-500	500-1000	1000-2000	Texture
1	9,8	19,3	18,4	15,3	23,4	9,1	4,2	0,3	sandy loam
2	6,5	20,6	20,9	17,2	18,7	9,8	6,0	0,3	sandy loam
3	10,6	16,4	20,8	16,6	24,8	8,4	2,1	0,2	sandy loam
4	11,2	13,6	8,9	10,7	18,9	18,8	17,0	0,9	sandy loam

Table 3. Textural analysis of the soil samples.

Tabla 3. Análisis textural de las muestras de suelo.

	1	2	3	4		1	2	3	4
Mn	723,2	732,9	859,6	635	Cd	1,155	1,506	1,756	0,765
Zn	417,9	417,6	558,8	225	Ni	24,85	25,17	27,89	20,9
Pb	252	270,4	60,08	76,06	Sn	5,16	3,695	2,904	2,258
Cu	99,21	101,2	97,25	54,18	Y	12,93	13,9	17,12	12,33
As	107,4	127,2	29,82	33,48	Be	1,584	1,452	1,679	1,169
Sb	19,11	19,69	6,2	6,06	U	1,575	1,76	1,532	1,057
Ba	313,9	291,6	310,1	253,6	Th	7,09	7,609	6,495	4,506
V	80,13	85,83	92,8	76,38	Sc	7,488	8,17	10,32	6,938
Tl	2,053	1,76	0,762	0,644	Hg	0,079	0,056	0,091	0,044
Cr	56,47	54,76	59,45	46,06	Mo	0,586	0,56	0,587	0,283
Co	13,02	13,61	15,7	10,83	In	0,109	0,12	0,084	0,053
Bi	1,367	1,445	0,456	0,488	Se	0	0	0	0
					Au	0,149	0,131	0,096	0,093

Table 4. Heavy metal analysis in the soil samples (values expressed in ppm).

Tabla4. Análisis de metales pesados en las muestras de suelo (valores en ppm).

dered as saline and where most of the species not adapted to the presence of salts would be affected. The first locality also has the highest cation exchange capacity in all the soils.

The C/N ratio is quite low as a result of of the topmost layer of these soils having been removed. On the other hand the soils with the highest amounts of organic matter (the first and second locality) also had the highest C/N ratios, indicating that soils still preserve some of their potential for productive capacity. The opposite may be said of the soils from sites three and four, where the ratio is quite low. As in the previous case, this is related to the small amount of organic matter, indicating that these soils have lost some of this capacity.

As regards soil texture, Table 3 shows that all the soils were of the sandy loam type, indicating that they are medium soils, warm and well drained, but with a low water retention capability. These soils would be difficult to flood, but neither would they preserve a good vegetal cover since all the water would be lost by percolation.

Heavy metals

Table 4 shows the heavy metal concentrations found in the four soil samples (one per site) and measured in ppm compared with the mean values recorded by Simón et al. (1998) a few months after the mine accident in soils, water and sludges (Table 5).

In most cases the heavy metal concentration has clearly fallen, but is still important due to the increased concentration of As, Cu, Zn, Pb, Sb, Bi, Tl and Cd. Due to their toxicity we will study As, Zn, Cd, Cu and Pb in greater detail.

After the accident As showed mean values of 127 ppm, with maximum values of up to 603 ppm, while in our soil samples we found mean values of 74,5 ppm, and maximum values of 127,2 ppm. This means a fall of up to 41,4 % in the concentration of this metal. However, the level is still above the intervention level of 20 ppm in German (Kolke 1980) and Canadian (Sheppard et al. 1992) agricultural soils. It is also above the intervention level in Belgium (45 ppm; Stringer 1990) and in Holland (50 ppm; NMHP-PE 1991).

Mean Zn levels reached values of about 747,9 ppm immediately the accident, with maximum values up to 2235,2 ppm. The present study shows mean concentrations of around 404,8 ppm and maximum values of 558,8 ppm, reflecting a fall in concentration of about 45,8 %. These values are below the intervention limits for agricultural soils in Belgium (Prost 1997) and Canada (Sheppard et al. 1992), but are still above the values found in non contaminated soils (230,8 ppm).

After the accident, Cu showed a mean concentration of 132,8 ppm, with a maximum value of 400,8 ppm, while the present study points to a mean concentration of about 87,97 ppm and a maximum value of 101,2 ppm (a fall of 33,75 %). Although these

	sludges	SC	SNC	Mu		sludges	SC	SNC	Mu
Mn	787,7	681,9	845,5	737,7	Cd	29,4	2,2	0,5	1,2955
Zn	7187	747,9	230,8	404,8	Ni	20,3	29,1	32,1	24,704
Pb	7996	370,4	41,8	164,6	Sn	10,1	1	0,8	3,5043
Cu	1993	132,8	42,2	87,97	Y	7,6	18,3	16,1	14,071
As	3114	127	18,1	74,46	Be	0,8	1,8	1,9	1,471
Sb	699,8	27,7	2,2	12,77	U	2	1,7	1,8	1,481
Ba	639,9	292,1	292,9	292,3	Th	5	13,9	11,7	6,425
V	43,8	94,7	100,6	83,78	Sc	4	10,9	11,9	8,2285
Tl	54,2	2,1	0,5	1,305	Hg	3,3	0,4	0,4	0,0675
Cr	61	68,5	68,5	54,19	Mo	6,8	0,7	0,2	0,504
Co	47,3	15,9	14,4	13,29	In	2,4	0,2	0,1	0,0915
Bi	68,6	2,6	0,4	0,939	Se	3,1	0,5	0	0
					Au	-	-	-	0,1173

Table 5. Comparison between mean values obtained by Simón et al. (1988) and those obtained in our samples (SC: contaminated soils; SNC: non-contaminated soils; Mu: our samples).

Tabla 5. Comparación de valores medios de los valores obtenidos por Simon et al. (1988) y los de nuestras muestras de suelo.

	15 days	30 days	45 days	60 days	75 days	90 days
1.1	5	10	15	15	15	15
1.2	40	40	40	45	45	45
1.3	35	40	40	40	40	40
1.4	40	40	45	50	50	50
1.5	20	25	25	30	30	30
2.1	5	5	5	10	10	10
2.2	-	-	-	-	-	-
2.3	15	15	20	20	25	25
2.4	5	10	10	10	10	15
2.5	25	25	25	25	20	20
3.1	25	30	35	35	35	35
3.2	15	20	25	30	30	30
3.3	20	15	20	20	20	20
3.4	10	5	10	15	15	15
3.5	60	60	65	65	65	65
4.1	10	10	10	15	15	15
4.2	5	5	10	10	10	10
4.3	10	10	15	15	10	10
4.4	35	35	40	40	35	35
4.5	-	-	-	-	-	-

Table 6. Evolution of bryophytic cover (%) of the samples grouped in localities regarding culture days.

Tabla 6. Evolución de la cobertura briofítica (%) de las muestras agrupadas por localidades en relación a los días de cultivo.

values are higher than those found in non contaminated soils (42,2 ppm), they do not exceed the 100 ppm for intervention level in France (AFNOR 1985) and Germany (Barth & Hermite 1987), although the maximum value, which appears in the second locality, is slightly higher, these value are still over those found at non contaminated soils (18,8 ppm).

Immediately after the accident, Cd showed mean concentrations of 2,2 ppm, with a maximum value of 5,7 ppm. In the present study we found mean concentrations of around 1,29 ppm with a maximum value of 1,75 ppm, representing a decrease in concentration of 41,1 %. These soils are already below the intervention level of Belgium (2 ppm; Adriano et al. 1997), although they are still higher than the values found in non contaminated soils (0,5 ppm).

The same can be said in the case of Pb, for which the mean concentration after the accident was around 370,4 ppm, with a maximum value of 1785,5 ppm. The present study shows mean concentrations of around 164,6 ppm, with a maximum value of around 270,4 ppm. This represents a decrease in concentration of 55,5 % meaning that intervention levels for agricultural soils in Belgium are not reached (300 ppm; Adriano et al. 1997), although still above the

concentrations found in non contaminated soils (41,8 ppm).

The decrease in concentration of Bi (63,88 %), Sb (53,91 %) and Tl (37,86 %) mean that there has been a generalized decrease in the heavy metal concentration, except in the case of Mn (8,18 %) and Sn (250,4 %).

Cultures

The total bryophytic cover of the samples (Table 6, Figure 2) did not vary substantially during the three months that the cultivation experiment lasted, although the samples for the second and fourth locality showed a lower cover than the other samples.

Table 7 presents a list of the bryophyte species found and the trays in which each appear. The life strategy type according to During (1979) and the growth form are also mentioned.

The recovery process of a soil is divided into three succession stages by many authors (De Las Heras et al. 1994, Puche & Gimeno 2000). The first of them is identified as a colonization stage and usually begins to manifest itself by the appearance of species

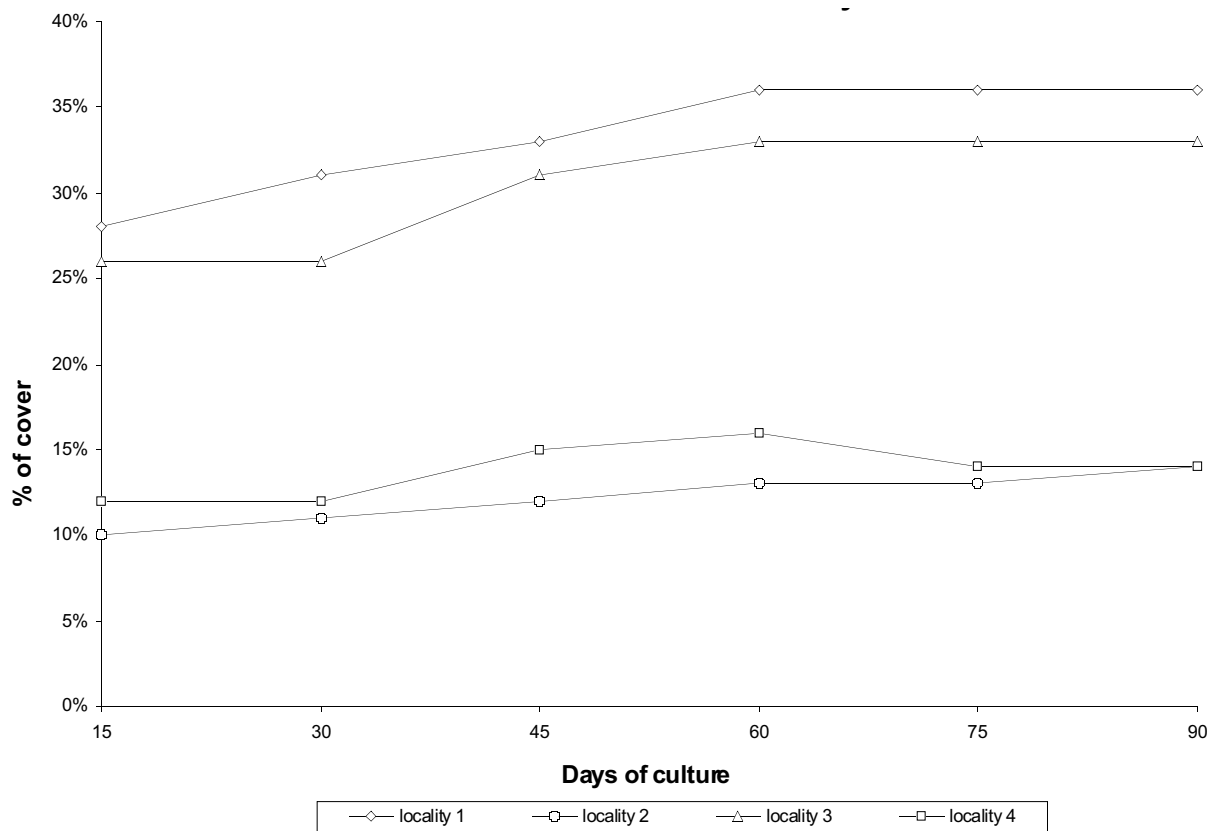


Figure 2. Mean variation of the cover along the study, in the different samples of the studied localities.

Figura 2. Variación media de la cobertura en las muestras de las diferentes localidades, a lo largo del estudio.

Species	N° of trays	Life strategie	Growing form
<i>Bryum bicolor</i> Dicks.	17	Colonizer	Low turf
<i>Funaria hygrometrica</i> Hedw.	17	Fugitive	Open turf
<i>Didymodon fallax</i> (Hedw.) Zander	7	Colonizer	Low turf
<i>Barbula convoluta</i> Hedw.	7	Colonizer	Low turf
<i>Bryum argenteum</i> Hedw.	7	Colonizer	Low turf
<i>Barbula unguiculata</i> Hedw.	4	Colonizer	Low turf
<i>Bryum caespiticium</i> Hedw.	4	Colonizer	Low turf
<i>Bryum torquescens</i> De Not.	3	Colonizer	Low turf
<i>Dicranella howei</i> Ren. & Card.	2	Colonizer	Low turf
<i>Didymodon luridus</i> Hornsch.	1	Colonizer	Low turf
<i>Phascum cuspidatum</i> Hedw.	1	Annual shuttle	Open turf
<i>Microbryum starckeanum</i> (Hedw.) Zand.	1	Annual shuttle	Open turf
<i>Bryum donianum</i> Grev.	1	Colonizer	Low turf
<i>Tortula muralis</i> Hedw.	1	Colonizer	Low turf
<i>Trichostomum brachydontium</i> Bruch	1	Colonizer	Low turf

Table 7. Species found in the different samples (trays) in which they grow, life strategie and growing form.

Tabla 7. Especies encontradas en las diferentes muestras (bandejas), estrategias de vida y formas de crecimiento.

such as *Funaria hygrometrica*, *Bryum bicolor* and *Barbula convoluta* two years after the perturbation. The second one begins four years after perturbation and may last for up to ten years. Within this stage, two substages are recognized, the first a primary competition stage lasting up to ten years after the perturbation and characterized by the appearance of species as *Bryum capillare*, *Bryum torquescens* or *Barbula unguiculata* as well as such ephemeral species as *Bryum capillare*, *Bryum torquescens* or *Barbula unguiculata*. This is followed by a secondary competition stage which begins ten years after perturbation, during which pluriannual or perennial species like *Pleurochaete squarrosa* or *Homalothecium aureum* appear.

Of note in our study is the presence of *Bryum bicolor* and *Funaria hygrometrica* along with other species such as *Barbula convoluta* and *Bryum argenteum* for the frequency with which they occur. The propagation type and life strategy, as well as the growing form, may provide valuable information for identification of the recolonisation phase.

We observed a clear predominance of colonizers, mainly *Bryum bicolor*, *Funaria hygrometrica* and *Barbula convoluta*, which represents the initial phase of the colonisation of the bare soil, although we also found species which belong to a first phase of competition (*Barbula unguiculata* and *Bryum tor-*

quescens) and even some annual shuttle species such as *Phascum cuspidatum*, *Pottia starckeanum*.

As regards the growing forms, there was a predominance of turf, either open or low, growing forms that agree with the colonisation phase.

The distribution in cover values of each one of the species in the different samples is shown in Table 8.

It can be observed that *Bryum bicolor* and *Funaria hygrometrica* appear in almost all the soil samples and at least one of them is always present. These two species are followed in abundance by others such as *Didymodon fallax*, *Barbula convoluta* and *Bryum argenteum* which are present in seven of the twenty samples. The first of them appears in all the localities, while *Barbula convoluta* does not appear in the first locality and shows a clear preference for the third locality, where it is found in four out of the five samples. *Bryum argenteum* is more frequent in the first and third locality, where it grows in three of the five trays.

There is a group of species that appears in four or fewer samples, formed of *Bryum caespiticium*, *Barbula unguiculata*, *Bryum torquescens*, *Dicranella howei*. The first of them only appears in the samples of the first and third locality, with a higher frequency at the former. *Barbula unguiculata* appears in all

N° of tray	Site 1					Site 2					Site 3					Site 4				
	1.1	1.2	1.3	1.4	1.5	2.1	2.2	2.3	2.4	2.5	3.1	3.2	3.3	3.4	3.5	4.1	4.2	4.3	4.4	4.5
N° of species	4	2	5	5	6	2	0	2	2	4	5	3	5	6	5	3	4	6	5	0
<i>Bryum bicolor</i>	2	2	1	2	+	2	-	-	1	2	2	1	2	2	2	1	2	3	3	-
<i>Funaria hygrometrica</i>	1	2	1	1	2	1	-	2	2	2	2	+	1	1	1	2	-	1	+	-
<i>Didymodon fallax</i>	-	-	1	-	1	-	-	-	-	+	-	-	-	-	1	+	-	1	+	-
<i>Barbula convoluta</i>	-	-	-	-	-	-	-	-	-	+	1	1	+	-	1	-	+	1	-	-
<i>Bryum argenteum</i>	+	-	-	1	+	-	-	-	-	-	2	-	2	+	-	-	+	-	-	-
<i>Barbula unguiculata</i>	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	2
<i>Bryum caespitium</i>	-	-	1	2	3	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Bryum torquescens</i>	-	-	2	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dicranella howei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1
<i>Didymodon luridus</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
<i>Phascum cuspidatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
<i>Microbryum starckeanum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
<i>Bryum donianum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
<i>Tortula muralis</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trichostomum brachydontium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

Table 8. Species that appeared grouped into localities with their cover indexes. + = to 1%, 1 = 1 to 10 %, 2 = 10 to 25 %, 3 = 25 to 50 %.
 Tabla 8. Especies que aparecen, agrupadas por localidades; con sus índices de cobertura.

N° of tray	Greenhouse uncovered					Greenhouse covered					16:8 Photoperiod					12:12 Photoperiod				
	1.1	2.4	3.4	4.2	4.3	1.5	2.5	3.3	3.5	4.4	1.2	1.3	2.1	3.2	4.1	1.4	2.2	2.3	3.1	4.5
N° of species	4	2	6	4	6	6	4	5	5	5	2	5	2	3	3	5	0	2	5	0
<i>Bryum bicolor</i>	2	1	2	2	3	+	2	2	2	3	2	1	2	1	1	2	-	-	2	-
<i>Funaria hygrometrica</i>	1	2	1	-	1	2	2	1	1	+	2	1	1	+	2	1	-	2	2	-
<i>Didymodon fallax</i>	-	-	-	-	1	1	+	-	1	+	-	1	-	-	+	-	-	-	-	-
<i>Barbula convoluta</i>	-	-	-	+	1	-	+	+	1	-	-	-	-	1	-	-	-	-	1	-
<i>Bryum argenteum</i>	+	-	+	+	-	+	-	2	-	-	-	-	-	-	-	1	-	-	2	-
<i>Barbula unguiculata</i>	-	-	+	-	-	-	-	+	-	2	-	-	-	-	-	+	-	-	-	-
<i>Bryum caespitium</i>	-	-	+	-	-	3	-	-	-	-	-	1	-	-	-	2	-	-	-	-
<i>Bryum torquescens</i>	-	-	-	-	-	1	-	-	-	-	-	2	-	-	-	-	-	1	-	-
<i>Dicranella howei</i>	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-	-	-
<i>Didymodon luridus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Phascum cuspidatum</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microbryum starckeanum</i>	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Bryum donianum</i>	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tortula muralis</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trichostomum brachydontium</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 9. Species that appeared grouped into treatments with their cover indexes.
 Tabla 9. Especies que aparecen, agrupadas por tratamientos, con sus índices de cobertura.

localities except the second. *Bryum torquescens* only appears in first and second locality, with better representation in the first. *Dicranella howei* is found in the third and fourth locality, but only in one of the five samples of each locality, showing a slightly higher degree of cover in third locality.

Lastly, there is a group of species with a very scant representation, since they only appear in one of the twenty samples. These include *Didymodon luridus*, *Phascum cuspidatum*, *Pottia starckeana*, *Bryum donianum*, *Tortula muralis* and *Trichostomum brachydontium*. *Didymodon luridus* only appears in the third locality; *Phascum cuspidatum* appears in the fourth, along with *Pottia starckeana*, in the same sample; *Bryum donianum* also appears in the third locality; *Tortula muralis* appears in the first and *Trichostomum brachydontium* appears in the fourth locality.

In general, samples from second locality showed a lower degree of diversity and cover. If, instead of comparing each locality, we compare the different photoperiods we applied to each group of samples, the results reflected in Table 9 are obtained.

In this case *Bryum bicolor* and *Funaria hygrometrica* are still the most frequent and abundant, but less so in the culture chamber with the 12:12 photoperiod, where we also found two samples with no bryophytes at all. Following the above species we find *Didymodon fallax*, *Barbula convoluta* and *Bryum argenteum*. The first not appears in photoperiod 12:12, but where it has the highest presence is in the greenhouse, in the covered trays, where it appears in four out of the five trays. Although it also appears under different conditions, *Barbula convoluta* shows greater presence in the greenhouse, especially in the covered trays. *Bryum argenteum* however, does not appear under the 16:8 photoperiod but does so under the 12:12 photoperiod. Its greatest presence is shown in the uncovered greenhouse trays, where it appears in three of the five trays.

Barbula unguiculata behaves in a similar way to *Bryum bicolor*, appearing in all conditions except the 16:8 photoperiod. However, where it seems to have the highest presence, though the difference is just one tray, is in the covered greenhouse trays. *Bryum caespitium*, on the other hand, appears regardless of the conditions the tray is exposed to, appearing in one of the trays of each condition. *Bryum torquescens* grows under all conditions but the uncovered greenhouse trays, while *Dicranella howei* only appears in the covered greenhouse trays.

Finally, *Didymodon luridus*, *Phascum cuspidatum*, *Pottia starckeana*, *Bryum donianum*, *Tortula muralis* and *Trichostomum brachydontium*, as previously mentioned, appear in only one of the twenty trays.

Didymodon luridus appears in the trays kept in the 12:12 culture chamber; *Pottia starckeana* along with *Phascum cuspidatum* appears in the uncovered greenhouse trays, the same as *Bryum donianum*, *Tortula muralis* and *Trichostomum brachydontium*.

In general, it can be observed that the samples showing the lowest cover are those from the culture chamber, especially those kept under the 12:12 photoperiod. On the other hand, the samples showing the highest cover were those placed in the greenhouse, where a further differentiation may be made, those that were left uncovered with a higher number of species and those that were left covered. Although the later produce a lower number of species, they still have a higher cover than that observed under the other conditions assayed.

Discussion

The fact that the samples from the second locality show a lower plant cover, regardless of the culture conditions, seems to be related with the characteristics of the soil, since these soils have a significantly lower pH value and a lower carbonate content than the others. This may imply a certain degree of toxicity on the part of other elements, which, in our case, are present in the soil in large quantities due to the spill, such as Mn (732,9 ppm), Zn (417,6 ppm) or Ni (25,17 ppm). For this reason, too, it is not unexpected that one of the samples from this site produced no bryophyte vegetation. Moreover, in the trays where some growth could be observed, this growth is solely due to five species *Bryum bicolor*, *Funaria hygrometrica*, *Didymodon fallax*, *Barbula convoluta* or *Bryum torquescens*, of which, only the first two appear in most of the samples (*Bryum bicolor* appears in three of the five trays and *Funaria hygrometrica* appears in four), while the other three species only appear in one sample. So, it is clear that the second locality soils remains severely affected by the spill, due to the their pH, which renders some ions toxic that under other conditions would not be toxic.

As regards the soils of the fourth locality, the higher pH than that of the other soils is probably due to the high carbonate content, but this does not explain the presence of Na and Mg. The presence of Na would be explained by a high electrical conductivity, which does not occur, meaning that the high pH is due to the presence of Mg in the soil. However, the pH value is very high, which would make this soil toxic for some plants. This would explain the low cover found in this locality and the fact that one of the samples from this site produced no bryophyte vegetation. Despite this, a great diversity is observed in this lo-

cality (up to ten different species). Another peculiarity of this locality is the low electrical conductivity of the soils, much lower than in others, but this seems to have no special repercussion on bryophyte growth; in fact there are some species such as *Trichostomum brachydontium* or *Phascum cuspidatum* that only appear in these soils.

In the first and third locality soils, the most exceptional characteristics are the high electrical conductivity shown by both and the large amount of organic matter in the first locality. These soils show the highest values of cover and the lowest diversity. Indeed, species that are not present in the other two localities, such as *Bryum caespiticium*, *Didymodon luridus*, *Bryum donianum* or *Tortula muralis*, appear in these two localities

Acknowledgements

We thank María Ángeles López Fernández for the soil sample analysis and the whole Soils Science and Technology research group for their help. We are also grateful to the ICP-Masas Unity from the Radiometric Dating and Isotopic Geology Service of the Scientific Instrumentation Service of the University of Granada for the heavy metal analysis.

References

- Adriano DC, Chlopecka A, Kaplan DI, Clijsters H, Vangronsveld J & Impens I. 1997. Soil contamination and remediation: philosophy, science and technology. In: Contaminated soils: Proceedings of the 3rd International Conference on the Biogeochemistry of Trace Elements in Soils (Prost R, ed.). Paris: INRA editions, pp. 465-504.
- AFNOR. 1985. Qualité des sols. Recueil de normes françaises. In: Teneurs totales en éléments traces métalliques dans les sols (France). Références et stratégies d'interprétation. (Baize D, ed.). Paris: INRA editions.
- Almodóvar GR, Sáez R, Pons JM & Maestre A. 1998. Geology and genesis of the Aznalcóllar massive sulphide depots, Iberian Pyrite Belt, Spain. *Mineralium Deposita* 33: 111-136.
- Anne P. 1945. Dosage rapide du carbone organique dans les sols. *Annales Agronomiques* 15: 161-172.
- Barth H & L'Hermite P. 1987. Scientific basis for soil protection in the European Community. London: Commission of the European Communities/Elsevier Applied Science.
- Bisang I. 1996. Quantitative analysis of the diaspore banks of bryophytes and ferns in cultivated fields in Switzerland. *Lindbergia* 21: 9-20.
- Bower CA & Wilcox LV. 1965. Soluble salts. In: *Methods of Soil Analysis*, part 2 (Black CA, ed.). Wisconsin: American Society of Agronomy/Inc. Madison, pp. 933-940.
- Brasell HM, Davies SK & Mattay JP. 1968. Nitrogen fixation associated with bryophytes colonizing burnt sites in Southern Tasmania, Australia. *Journal of Bryology* 14: 139-149.
- Chapman HD. 1965. Cation exchange capacity. In: *Methods of Soil Analysis*, part 2 (Black CA, ed.). Wisconsin: American Society of Agronomy/Inc. Madison, pp. 811-900.
- De Las Heras J, Guerra J & Herranz JM. 1990. Bryophyte colonization of soils damaged by fire in south-east Spain: a preliminary report on dynamics. *Journal of Bryology* 16: 275-288.
- De Las Heras J, Guerra J & Herranz JM. 1991. Changes in floristic diversity and fugacity of bryophytes in burnt sites of SE Spain. *Lindbergia* 17: 11-16.
- De Las Heras J, Guerra J, Hernández-Bastida J & Herranz JM. 1992. Synchronic study of the bryophytic vegetation of five burnt zones in SE Spain. *Vegetatio* 102: 97-105.
- De Las Heras J, Guerra J & Herranz JM. 1994. Stages of bryophyte succession after fire in mediterranean forests (SE Spain). *International Journal of Wildland Fire* 4: 33-44.
- Duchauffour, Ph. 1970. *Précis de Pédologie*. Paris: Masson.
- During HJ. 1979. Life strategies of bryophytes: a preliminary review. *Lindbergia* 5: 2-18.
- During HJ. 1997. Bryophyte diaspore banks. *Advances in Bryology* 6: 103-134.
- During HJ, Brugués M, Cros RM & Lloret F. 1987. The diaspore banks of bryophytes and ferns in the soil in some contrasting habitats around Barcelona, Spain. *Lindbergia* 13: 137-149.
- During HJ & Lloret F. 2001. The species-pool hypothesis from a bryological perspective. *Folia Geobotanica* 36: 63-70.
- Gloaguen JC. 1990. Post-burn succession on Brittany heathlands. *Journal of Vegetation Science* 1: 147-152.
- Guerra J, Cano MJ, Gallego MT, Ros RM & Jiménez JA. 2002. Bryophyte diversity in the Guadamar river basin (SW of Spain). *Anales de Biología* 24: 97-106.
- Hébrard JP. 2001. La banque édaphique de diaspores de bryophytes de l'écosystème du chêne vert au bois d'Astros (commune de Vidauban, Var, France). *Nova Hedwigia* 70: 323-337.
- Herranz JM, De Las Heras J & Martínez-Sánchez JJ. 1991. Efecto de la orientación sobre la recuperación de la vegetación natural tras el fuego en el valle del río Tus (Yeste, Albacete). *Ecología* 5: 111-123.

- Kilmer VJ & Alexander LT. 1949. Methods of making mechanical analyses of soils. *Soil Science* 68: 15-24.
- Kloke, A. 1980. Richtwerte 1980-Orientierungsdaten für tolerierbare Gesamtgehalte einiger Elemente in Kulturböden. In: *Mitteilungen VDLUFA*. Brunswick: Biologische Bundesanstalt für Land- und Forstwirtschaft 1-3: 9-11.
- López Ritas J & López Melida J. 1990. El diagnóstico de suelos y plantas (métodos de campo y laboratorio). Madrid: Mundi-Prensa.
- Malone CR. 1967. A rapid method for enumeration of viable seeds in soil. *Weeds* 15: 381-382.
- Martínez ML & Maun MA. 1999. Responses of dune mosses to experimental burial by sand under natural and greenhouse conditions. *Plant Ecology* 145: 209-219.
- Milberg P & Persson TS. 1994. Soil seed bank and species recruitment in road verge grassland vegetation. *Annales Botanici Fennici* 31: 155-162.
- NMHPPE. 1991. Netherlands Ministry of Housing, Physical Planning and Environment. Leidschendam, Netherlands.
- Peech M. 1965. Hydrogen-ion activity. In: *Methods of soil analysis part 2* (Black CA, ed.). Wisconsin: American Society of Agronomy/Inc Madison, pp. 914-916.
- Prost R. 1997. Contaminated soils. 3rd International Conference on the Biogeochemistry of Trace Elements. Paris: INRA editions.
- Puche F & Gimeno C. 2000. Dynamics of the early stages of bryophyte colonization of burnt Mediterranean forests. *Nova Hedwigia* 70: 523-536
- Roberts HA. 1981. Seed banks in soil. *Advances in Applied Biology* 26: 1-55
- Sheppard SC, Gaudet C, Sheppard PI, Cureton PM & Wong MP. 1992. The development of assessment and remediation guidelines for contaminated soils—a review of the science. *Canadian Journal of Soil Science* 72: 359-394.
- Simón M, Ortiz I, García I, Fernández E, Fernández J, Dorronsoro C & Aguilar J. 1998. El desastre ecológico de Doñana. *Edafología* 5: 153-161.
- Stringer DA. 1990. Hazard assessment of chemical contaminants in soil. European Center for Ecotoxicology and Toxicology of Chemicals, Technical Report Number 40. Brussels: ECETOC.
- Sundberg S & Rydin H. 2000. Experimental evidence for a persistent spore banks in Sphagnum. *New Phytologist* 148: 105-116.
- Trabaud L, Martínez-Sánchez JJ, Ferrandis P, González-Ochoa AI & Herranz JM. 1997. Végétation épigée et banque de semences du sol: leur contribution à la stabilité cyclique des pinèdes mixtes de *Pinus halepensis* et *P. pinaster*. *Canadian Journal of Botany* 75: 1012-1021.
- Thompson K, Grime JP & Mason G. 1977. Seed germination in response to diurnal fluctuations of temperature. *Nature* 267: 147-149.