

Lateral and vertical variations in contaminated sediments from the Tinto River area (Huelva, SW Spain): Incidence on tracemaker activity and implications of the palaeontological approach



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ABSTRACT

Geochemical and ichnological analyses of the Tinto River area (SW Spain) reveal the presence of macro-tracemakers colonizing highly contaminated soils. We found high concentrations of heavy metals and related elements, mainly accumulated in the upper parts (biofilms and thin salty crusts). Some concentrations, including those of Cu, Zn and As, exceed the intervention levels for natural areas and indicate potentially toxic conditions. Ichnological analysis reveals the presence of biogenic structures produced by the activity of the earthworm *Lumbricus terrestris* and the coleoptera *Platystethus*. Both alternatives for colonization can be correlated with those used by fossil tracemakers, and they serve to better understand biotic recovery after bio-events in the past.

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1. Introduction

Acid rock drainage — the natural process releasing acidity, sulfates, Fe, and other metals and metalloids — and in particular acid mine drainage from mining activities, are responsible for the extremely high levels of contamination registered in the Tinto River, located within the province of Huelva (SW Spain) (Olías et al., 2006; Cánovas et al., 2007). It has a length of approximately 100 km and a basin area of around 1700 km², draining the Iberian Pyrite Belt (IPB), which is one of the largest polymetallic massive sulfide regions in Europe (Fig. 1). Acid mine drainage causes acidity and high concentrations of trace elements (As, Cd, Cu, Co, Mo, Ni, Pb, Se, Zn, among others) carried in solution. Sulfide oxidation processes in the IPB determine the high pollution registered in the Tinto River, showing extremely low pH values and high metal and sulfate concentrations (Cánovas et al., 2010, and references therein).

The Tinto River has a long history of very high concentrations of toxic elements and acidity from its source, with massive sulfide deposits exploited nearby for some 5000 years (Davis et al., 2000). Important mining activities in the Iberian Pyrite Belt area having local and regional environmental impact began in the third millennium B.C., when copper was extracted. Activity continued during the Tartessian and Phoenician periods, until Roman domination; then, after a long period of inactivity,

mining reemerged in the 18th century and intensified during the 19th and 20th centuries, to obtain pyrite, silver, gold, zinc, lead and tin (see Davis et al., 2000 for a historical perspective). Contamination of the Tinto River continued even after the mines were abandoned, with huge quantities of acidic waters and heavy metals discharged into the river (Fernández-Caliani et al., 2009; see Romero et al., 2011 and references therein). This pollution is not only registered in river sediments, but also in marsh areas and estuarine sediments. Particular geochemical features are determined by the water and saline mixing processes, along with the superimposition of acid mixing processes due to the high acidity (pH < 3) of the river water (see López-González et al., 2012, and references therein), and very high concentration of heavy metals and metalloids (Martín et al., 2007).

Notwithstanding the high contamination, there is not an absence of life, but rather a particular microhabitat characterized by a high diversity of microorganisms, adapted to extreme life conditions (extremophile microbiology). More specifically, an iron-oxidizing, acidophilic, prokaryotic community of these waters is dominated by *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, with a minor presence of *Acidiphillum* spp. (e.g., López-Archilla and Amils, 1999; López-Archilla et al., 2001; Amaral Zettler et al., 2002; González-Toril et al., 2003a,b; Aguilera et al., 2006, 2007a,b; García-Moyano et al., 2007; Sánchez-España, 2008). These microorganisms are also found in pit lakes of the IPB, such as San Telmo (Sánchez-España et al., 2007). The diversity and abundance of the benthic microbial population were recently characterized, with the

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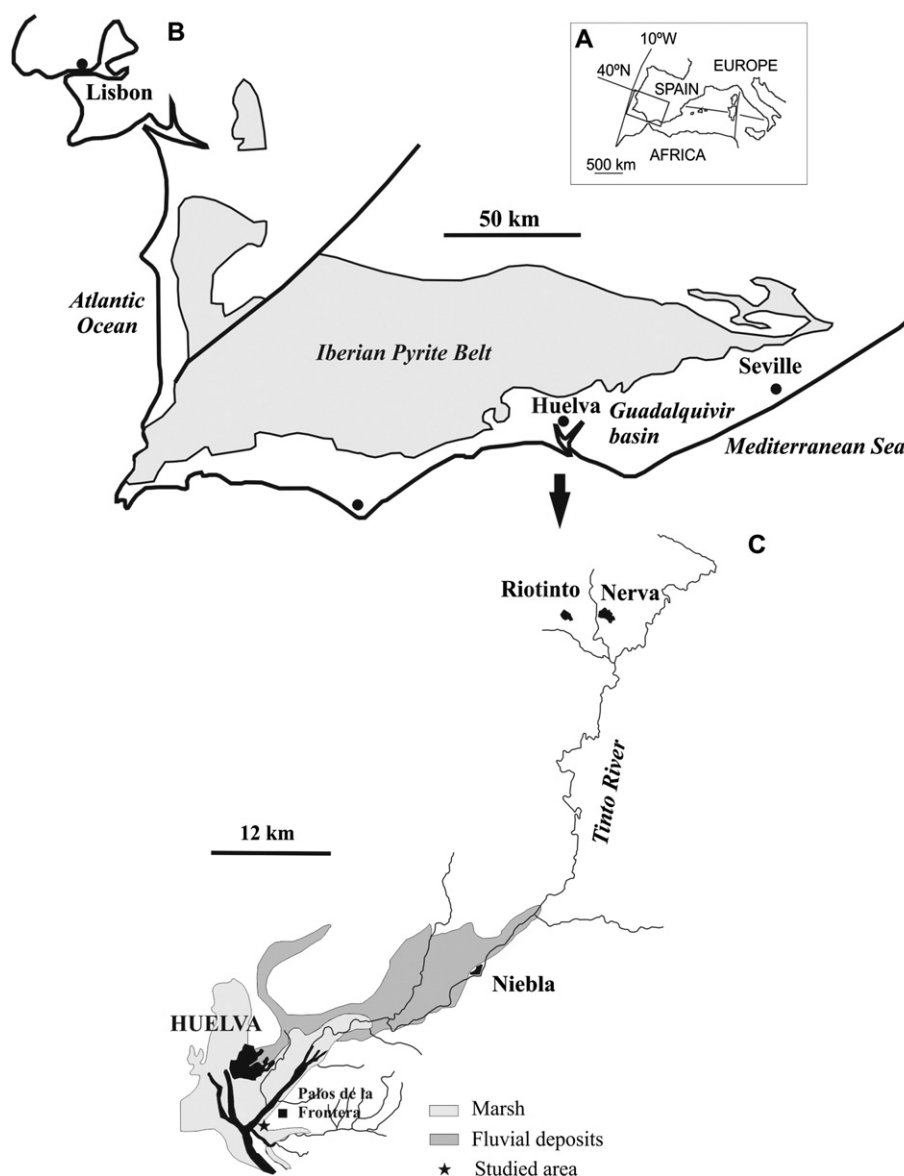


Fig. 1. General map of Spain (A), regional map of the southwestern Iberian margin showing distribution of the Iberian Pyrite Belt (B), simplified from Fernández-Caliani et al. (2009), and geological setting of the Tinto River basin (C) with indication of marshes and fluvial deposits and the studied area (from Cáceres et al., 2013).

identification of five phyla belonging to *Bacteria* and one order belonging to *Archaea*; and microorganisms involved in the iron, sulfur, and carbon cycles were identified (Sánchez-Andrea et al., 2011, 2012; Sanz et al., 2011; Sánchez-Andrea, 2012). Plants such as the endemic heather *Erica andevalensis* show a tolerance to the harshest edaphic conditions, through the selective absorption of essential elements and the accumulation of phytotoxic elements in the root epidermis (Monaci et al., 2011).

These particular conditions spark worldwide interest in the Tinto River area, as a natural experimental site to study life in extremely unfavorable environmental conditions, a geochemical terrestrial analogue of the planet Mars (e.g., Fernández-Remolar et al., 2003, 2005, 2008; González-Toril et al., 2003b; Amils et al., 2007, 2012; Puente-Sánchez et al., 2013).

In the past few years, ichnological analysis has been recognized as a very useful tool for interpreting palaeoenvironmental changes associated with past bio-events determining extreme environmental conditions. Some examples are the very low oxygen conditions associated with the Oceanic Anoxic Event (OAE-2) at the Cenomanian–Turonian boundary (Uchman et al., 2008, 2013a,b; Rodríguez-Tovar et al., 2009a,b; Monaco

et al., 2012) and with the Toarcian Oceanic Anoxic Event (T-OAE; Rodríguez-Tovar and Uchman, 2010; Rodríguez-Tovar and Reolid, 2013; Reolid et al., 2014); the severe global effects associated with a bolide impact at the Cretaceous–Palaeogene boundary (Rodríguez-Tovar and Uchman, 2004a,b, 2006, 2008; Rodríguez-Tovar et al., 2004, 2006, 2010, 2011b; Rodríguez-Tovar, 2005; Kędzierski et al., 2011); the palaeoenvironmental changes during the Danian–Selandian boundary interval (Rodríguez-Tovar et al., 2013), the Thermal Maximum associated with the Palaeocene–Eocene (Rodríguez-Tovar et al., 2011a); and the important sea-level changes associated with the Ypresian–Lutetian event (Ortiz et al., 2008; Molina et al., 2011). Especially relevant were the conclusions drawn regarding the Cretaceous–Palaeogene boundary, traditionally considered as a global mass-extinction event, with dramatic consequences for terrestrial and marine biota. Ichnological data revealed that the presumably unfavorable extreme environmental conditions, leading to an inhospitable habitat for colonization by organisms, had minor effects on particular tracemakers (Rodríguez-Tovar and Uchman, 2008). These relevant conclusions are supported by neoichnological research conducted around the recent environmental disaster next to Doñana National Park (southern Spain) caused by the failure of a tailing

pond adjacent to the pyrite mine at Aznalcóllar (Rodríguez-Tovar and Martín-Peinado, 2009; Martín-Peinado and Rodríguez-Tovar, 2010).

Given the proven importance of research on recent disasters when approaching the analysis of past bio-events, the aim of this research is to advance knowledge of the response of communities to extreme environmental conditions, such as those registered in the Tinto River area. In this case the focus is not on microorganisms, but on macrobiota. Based on an integrate ichnological and geochemical analysis, here we study the response of tracemakers, earthworms and coleoptera to contaminated soils of the Tinto River area. Different strategies for colonization of this “inhabitable” habitat can be compared to those used by organisms to recover from bio-events in the past.

2. Material and methods

The sampling zone is located in the marsh area of the Tinto River, close to the village of Palos de la Frontera (Huelva, SW Spain) (Figs. 1 and 2). Five sites were differentiated according to their distance from the Tinto River, at 50 cm (site 1), 5 m (site 2), 9 m (site 3), 15 m (site 4) and 23 m (site 5). It should be noted that site 5, while farthest from Tinto River, is the closest to an irrigation ditch at a lateral distance of 3.5 m. Ichnological and sedimentological observations were conducted in situ on the samples collected from all five sites.

Cores were sampled with a single gouge auger (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) from the soil material at depth,

directly from the surface and at 5-cm intervals to a depth of 30 cm. When cores were retrieved, the depth of the groundwater level was noted (Fig. 2).

Ichnological data were obtained in the field, focusing on burrow density (measured by counting the number of biogenic structures within a 1 m² standard quadrant), burrow configuration (mainly shape), and depth of penetration. Specimens of the organisms registered in the burrows were collected for observation and classification.

Sample preparation in the laboratory included air-drying and sieve to 2 mm. Total element concentrations were analyzed after very fine grinding and digestion in strong acids (perchloric acid + nitric acid + hydrochloric acid + hydrofluoric acid) in an international certified laboratory (Actlabs, ISO/IEC 17025, Activation Laboratories, 2013).

3. Results

Soils in the sampling site are classified as Gleyic Fluvisol (WRBSR, 2006), characterized by the absence of gravels (>2 mm), and the dominance of a silty-clay texture. The soils presented partially saturated conditions with groundwater, for a period that allows reducing conditions to occur.

In the studied sites, a superficial biofilm above the soil is especially well developed in sites 1 and 2, closer to the river, and then mostly in subaqueous conditions. Biofilms composed by algae, ciliates, flagellates, amoebas, and fungi have been extensively observed in the water

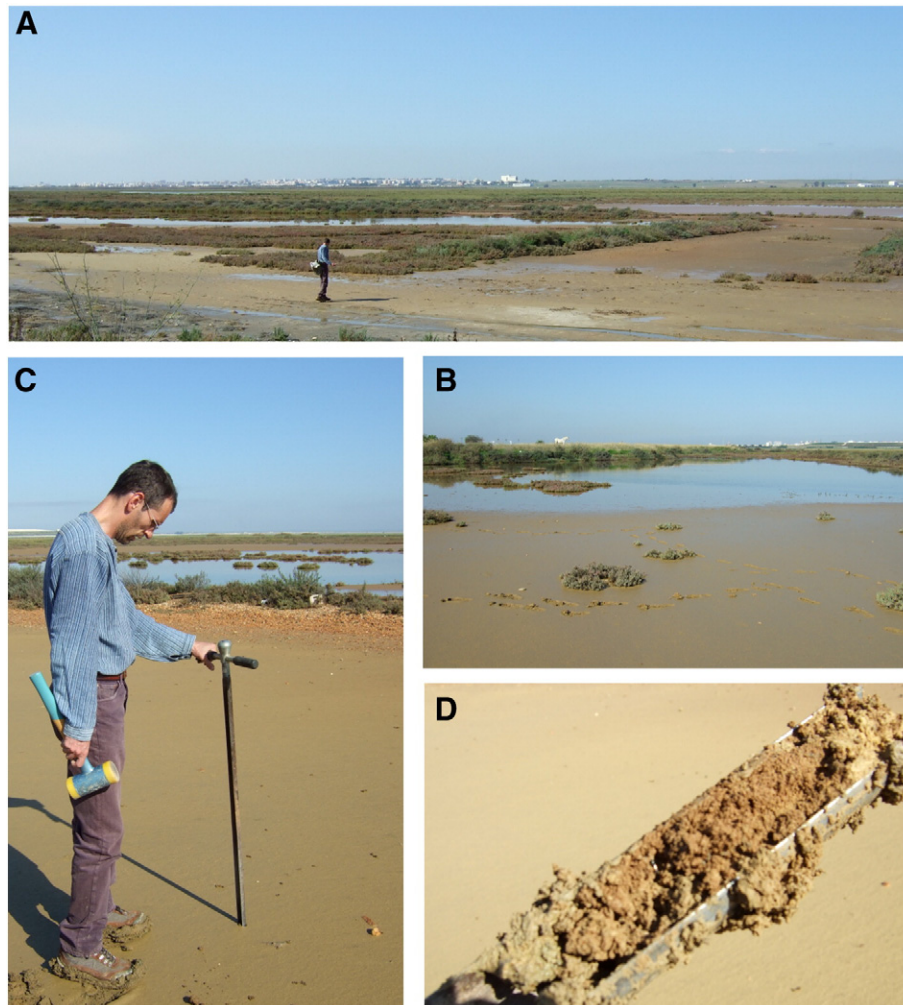


Fig. 2. (A) General view of the studied area in the marshes close to the Tinto River. (B) Close-up view of the area from the Tinto River. (C) Methodology used for core retrieval. (D) Close-up view of the soil core obtained.

column and throughout the beds of the Tinto River (Aguilera et al., 2006, 2007a; Baker et al., 2009; Sánchez-Andrea, 2012; Sánchez-Andrea et al., 2012). The other sites show no evident biofilm, only thin saline horizons in their upper parts. Increasing firmness is found from sites 1 to 4: from softground, unconsolidated sediment, to stiff but uncemented sediment. Site 5 shows decreasing cohesiveness (near softground), probably due to the proximity of the irrigation ditch. This observation agrees with variation in depth of the ground water level, from around 8-cm depth at site 1, to deeper than 20 cm in the rest of the sites.

3.1. Biogenic structures and tracemakers

The biological activity of macroinvertebrates is revealed by the presence of different types of biogenic structures. Ichological analysis showed the presence of isolated holes/apertures without any pellets around them, holes surrounded by pellets with a mound appearance, or aggregations of pellets having no associated aperture (Fig. 3). Low substrate firmness impeded detailed observations of the morphology of the burrows in sediment. Simple, vertical or slightly curved (J-shape), uncompleted structures were observed

occasionally (2–5 mm in diameter and around 3 cm long); in any case the distal, terminal part was recognized.

Observed distribution of burrows in the studied area reveals a random arrangement, without evidence of patches or clusters of the biogenic structures in the different sites. Among the sites there are important variations in abundance, as well as in the depth of burrowing and shape. In general, an increase in the number of structures is registered from sites 1 to 5: a) Site 1 shows the lowest density in biogenic structures (<10 apertures \times 1 m²), with dominance of vertical structures; b) Site 2 shows an increase in the number of structures (20–30 apertures \times 1 m²), with a maximum depth of 15–20 cm; c) Site 3 reveals the highest density of traces (>50 apertures \times 1 m²), with a dominance of horizontal forms; d) in Site 4, decreasing abundance is registered (20–50 specimens \times 1 m²); and e) Site 5 shows an increase in abundance, with values similar to those of site 3 (>50 specimens \times 1 m²).

Two organisms were registered in the observed burrows: the earthworm *Lumbricus terrestris* and the coleoptera *Platystethus* (family Staphylinidae, subfamily Oxytelinae) (Fig. 4).

Lumbricus terrestris is a deep-burrowing anecic earthworm, which makes permanent vertical burrows in soils (Edwards and Bohlen,

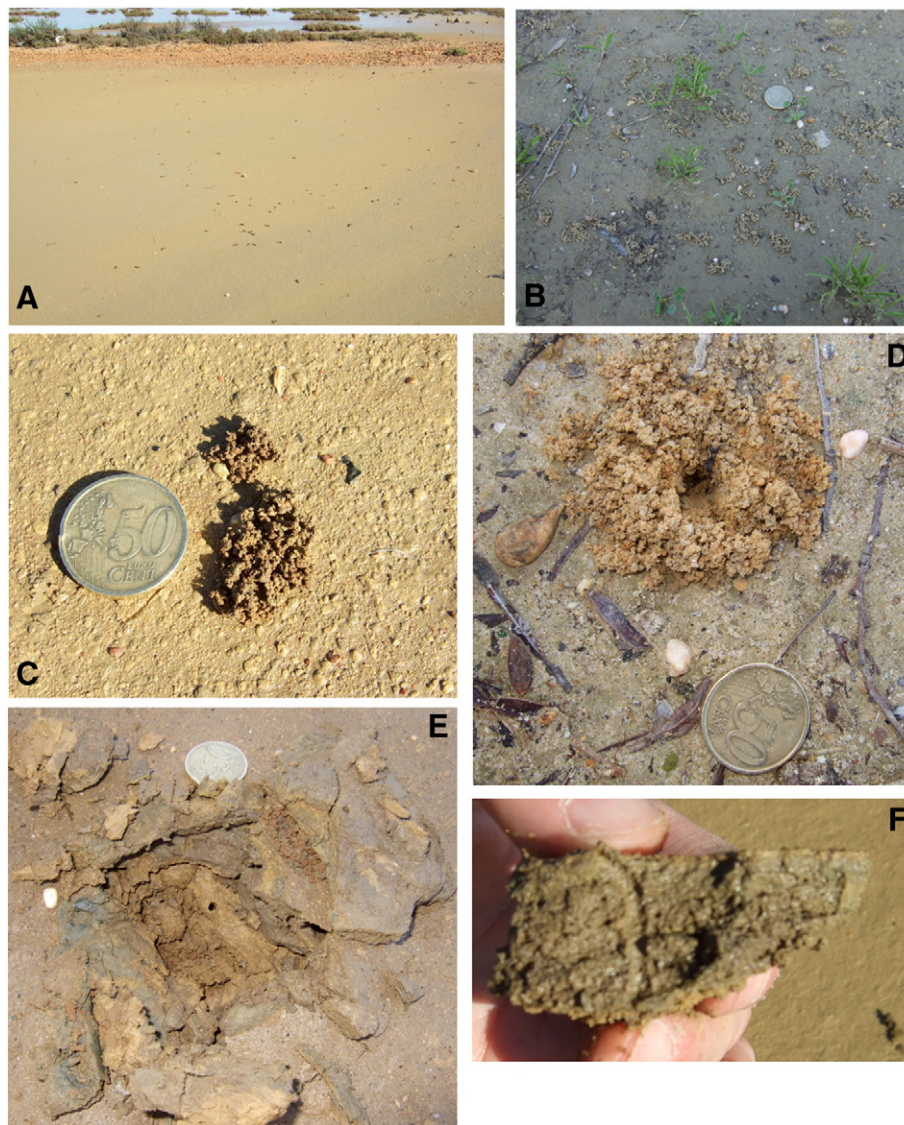


Fig. 3. (A) General view of site 1, showing dispersed mounds of pellets. (B) Close-up view of site 3 showing abundant mounds of pellets. (C) Close-up view of pellets conforming a mound. (D) Close-up view of one aperture surrounded by sediment from inside. (E) Hole of the burrow from the surface crossing the upper polluted levels of the soil. (F) Internal structure of the burrow just below the surface.



Fig. 4. The coleoptera *Platystethus* inside the core (A) and in surface sediment (B). (C) The earthworm *Lumbricus terrestris* a few centimeters below the surface.

1996). They feed on organic matter on the soil surface that they drag into their burrows, and also make piles of casts around the entrance of their burrows (i.e., Edwards and Bohlen, 1996; Canti, 2003). This organism produced permanent, normally single burrows, mainly characterized by nearly vertical to transversal tubes (Edwards and Bohlen, 1996), sometimes up to 3 m long or Y shaped (Shipitalo and Butt, 1999), though more branched and complex burrow systems have been also observed (e.g., Jégou et al., 1998, 1999; Langmaack et al., 1999). Burrowing activity by *L. terrestris* has been profusely studied, revealing important modifications of the physicochemical features of soils. Especially interesting is the incidence of *L. terrestris* not only on abiotic features of soils, but also on the microbial community (e.g., Thorpe et al., 1996; Tiunov et al., 2001; Aira et al., 2009; Nechitaylo et al., 2010).

A recent catalog of rove beetles (Coleoptera, Staphylinidae) includes species belonging to the coleoptera *Platystethus* (family Staphylinidae, subfamily Oxytelinae) in Huelva province (Gamarrá et al., 2011). Most Staphylinidae (rove beetles) are predators of other insects (i.e., aphids and bark beetles, among others) and live on or in the soil, in ground litter, in moss or in decomposing organic matter. Some species of Oxytelinae feed on various organic substances and thus their gut contents include quantities of organic matter (Bohac, 1999). *Platystethus* live mostly in dung and on muddy river banks. Algal-feeding species are especially conspicuous in the sandy soils of beaches and stream-sides. Their burrows may be complex, with multiple entrances and chambers situated above and doubled back over their entrance burrows (Retallack, 2001). Rove beetles living in intertidal areas (or areas influenced by tides) build a vertical, wine-bottle shaped burrow in the sand with a living chamber about 5 mm in diameter into which they retreat when the tide comes in. The narrow burrow entrance prevents any water from entering. Other species excavate complex galleries in damp sand and clay along seashores, riverbanks and lakeshores. In some cases the female seals the entrance with mud during high tide. Some species are associated with mammal burrows, with ant and termite nests, or saline flats (Campbell and Davies, 1991). The oldest fossil record of staphylinids is from the upper Triassic (Capinera, 2008). Burrow morphology is similar to that of trace fossils such as the J-shaped burrows produced by fiddler crabs (i.e., de Gibert et al., 2013) or that of *Macanopsis plataniformis*, a banana-shaped trace fossil characterized by a short subvertical shaft, terminating in a wide, elongated, slightly curved basal, predominantly subhorizontal chamber (Muñiz and Mayoral, 2001).

3.2. Geochemical data

Detailed analysis of all the studied elements shows important variations in the concentration of some between (lateral) and inside (vertical) sites (Tables 1–3).

With regard to lateral variation among sites (Table 2), an important decreasing trend from sites 1 to 5 is observed for Cu, Cd, Cr, Sc, V, Ga, Tl, Sb, Ce, Th, and U. The most important variations occur in the case of Cd, with concentrations three times higher in site 1 than in

site 5; and Cu and Tl have double the concentrations in site 1 in comparison to site 5. Other elements do not show a clear trend, their values being either highly variable or similar in the five studied sites (Table 2).

Vertical variations within the different sites reveal different trends, downward or upward decreasing (Table 1 and Fig. 5). A decrease in the concentrations from the surface to 25–30 cm below is seen for Cd, U, Sr, Br, S, Mg, and Na; Cd and S are the elements with the strongest decline in concentration at depth, with respective 12 and 10-fold mean decreases. The opposite trend, with a general increase from surface downward, is observed for Cr, Ti, V, Mn, Ga, Sc, La, Ce, Sm, Eu, Yb, Th, Li, and Rb; in this case the elements having a stronger increase in concentrations are Rb, Sc and Mn, respectively multiplying in depth by 5, 2.4 and 2.3 the values of the surface samples. The rest of the selected elements do not show any particular trend. There may be similar values in the entire site or variations within a particular site, with maximum values in different intervals.

The vertical variations are usually registered in the uppermost part of the soils, not in the deeper intervals, with stronger differences when this uppermost part is represented by a biofilm (sites 1 and 2), or a thin saline horizon (sites 3 to 5) (Fig. 6). Thus, biofilms in sites 1 and 2 show statistically significant ($p < 0.05$) concentrations in Cd, Tl, U, Br, S and Na; meanwhile, surface saline horizons at sites 3, 4, and 5 have high concentrations of Cd, Sr, Br, S and Na.

4. Discussion

As presented by González et al. (2011) in their research on soil quality in Iberian Pyrite Belt areas affected by sulfide mining, some trace elements at low concentrations are essential for plant, animal and human growth (e.g., As, Co, Cr, Cu, Fe, Mn, Mo, Se, V, and Zn), and can be considered as essential micronutrients, yet toxic above certain concentrations. Of all the trace elements found in soils, those especially toxic and available at concentrations exceeding toxic levels are Ag, As, Bi, Cd, Co, Cu, Hg, Ni, Pb, Pd, Pt, Sb, Se, Sn, Te, Tl, and Zn (González et al., 2011). A comparison between the values obtained for selected elements (Cu, Zn, Cd, Pb, Tl, As, and Sb) in the Rio Tinto area and those proposed in a technical report by the Environmental Council of the Junta de Andalucía in 1999, as criteria to declare soil contaminated, serves to confirm the high levels of contamination in the studied area. The mean concentrations are higher than the reference values in most sites, with the exception of Pb. The intervention level for natural areas is exceeded in all sites for Cu, Zn and As (Table 3).

The statistically significant increase in the concentrations of Cd, Tl, U, Br, S and Na in the biofilms is highly correlated ($p < 0.05$, correlation coefficient > 0.648) with their concentrations in the surface samples (0–5 cm). Accordingly, elements such as Cd are readily available for plants from soil sources, and its concentration rapidly increases in plants growing in polluted areas (Kabata-Pendias, 2011). The capacity for accumulating metals and related elements in the tissues depends on the plant type. Kloke et al. (1984) estimated the phytotoxic concentrations of Cd to be 5–10 mg/kg in sensitive plant species, while the

normal concentrations in lichens range from 11 to 22 mg/kg in contaminated sites (Simon, 1977).

In the surface salt deposits, there is also a high correlation ($p < 0.05$; correlation coefficient > 0.605) with the concentration in the surface soil samples (0–5 cm), except in the case of Cd — its concentration in the salt deposits is three times the surface value. This accumulation of Cd in the surface would owe to its greater mobility in environments that alternate reducing and oxidant conditions (Asami, 1984).

4.1. Contamination levels and community dynamics

The highly diverse microscopic community colonizing the extremely acidic environment of Rio Tinto sediments (Sánchez-Andrea et al., 2011, 2012; Sánchez-Andrea, 2012), contrasts with the scarce macrobenthic community, especially that of endobenthic organisms. Ichthyological analysis in the study area reveals the presence of several types of biogenic structures associated with the burrowing activity of the earthworm *Lumbricus terrestris* and the coleoptera *Platystethus* (family Staphylinidae, subfamily Oxytelinae). The high levels of contamination registered in the study area overall (from sites 1 to 5), from the surface to the soil in depth, could preclude a “normal” colonization of the

substrate. Notwithstanding, the earthworm *L. terrestris* and the coleoptera *Platystethus* are observed in these extreme conditions. This leads us to the question: What particular features, behavior or strategy enables these organisms to colonize such extremely adverse habitats as in the Rio Tinto area?

Numerous studies focusing on the impact of metals in soils on earthworms (see Nahmani et al., 2007) document the capability of earthworms to survive and reproduce in anthropogenically metal-contaminated soils (Spurgeon et al., 1994), and to accumulate high concentrations of metals such as Cd, Cu, Zn and Pb within their bodies (Hobbelen et al., 2006; see Lionetto et al., 2012 and references therein). Recent experiments with several species of As-resistant earthworms, including *Lumbricus terrestris*, demonstrated an acquired resistance to As toxicity in the native earthworms living in soils highly contaminated with arsenic, as compared with earthworms from uncontaminated sites (Button et al., 2010).

The influence of earthworm burrowing activity on the chemistry of the metals in soil, in terms of availability and mobility, has been proven with *Lumbricus terrestris* (Sizmur and Hodson, 2009; Sizmur et al., 2011b,c). As anecic earthworms, *L. terrestris* can cause metals and metalloids to leach out of soils into surface waters or groundwaters (Sizmur et al., 2011a). Elements such as Pb and Sr can be incorporated into

Table 1

(a, b). Concentrations of selected studied elements showing important lateral (among sites) and vertical (within site) variations (above for Heavy metals and related elements and below for Rare elements and others). All data in ppm, except S, Fe, Ca, Mg, Na and P in %. Light gray for elements with a general decrease in abundance from surface to depth in sediment, middle gray for those increasing from surface to depth, and dark gray for those without any evident trend from surface to depth, but variable values within the sites.

Site	Cu	Zn	Cd	Pb	Co	Ni	Cr	Ti	V	Mn	Ga	Tl	As	Sb
a)														
RT1 C	1110	1650	11.7	97	36	46	72	0.30	106	398	11.0	3.3	116	6.5
RT1 0–5	1330	1870	13.6	121	35	49	84	0.34	112	313	12.1	3.4	128	8.2
RT1 5–10	598	859	0.8	162	26	49	93	0.44	135	710	15.9	0.7	124	10.1
RT1 10–15	397	648	0.6	163	17	44	86	0.31	111	363	16.2	0.7	89	8.6
RT1 15–20	573	380	0.3	146	22	47	100	0.51	153	759	16.2	0.6	79	9.2
RT1 20–25	877	365	0.3	202	30	53	100	0.48	150	1300	16.5	0.7	102	12.8
RT1 25–30	1180	350	<0.3	257	38	40	99	0.45	147	1840	16.7	0.7	125	16.4
RT2 C	770	1290	5.4	98	30	47	57	0.29	92	401	9.9	1.3	78	5.5
RT2 0–5	964	1650	5.8	121	35	55	74	0.34	106	387	12.4	1.4	108	6.7
RT2 5–10	674	2460	0.4	155	50	49	70	0.31	101	382	11.0	0.6	174	7.7
RT2 10–15	633	1970	0.4	186	35	44	86	0.39	109	395	12.5	0.6	165	8.8
RT2 15–20	441	872	0.3	170	18	44	95	0.40	118	514	14.7	0.6	84	10.0
RT2 20–25	333	358	0.8	142	16	44	94	0.47	127	500	14.9	0.6	54	9.8
RT2 25–30	298	224	0.5	139	17	31	101	0.44	124	493	15.5	0.6	55	9.2
RT3 C	386	1030	3.1	65	26	40	52	0.23	68	483	8.2	0.4	57	3.3
RT3 0–5	466	1440	1.4	73	34	49	67	0.29	91	406	9.9	0.5	85	5.0
RT3 5–10	547	1930	1.0	148	41	49	74	0.33	102	415	10.2	0.6	147	7.6
RT3 10–15	476	1820	1.0	168	34	49	82	0.35	107	419	10.7	0.6	162	9.0
RT3 15–20	422	1850	0.9	241	23	45	76	0.41	114	490	13.1	0.7	170	9.8
RT3 20–25	376	1580	0.9	184	22	47	102	0.30	100	746	14.9	0.7	104	7.6
RT3 25–30	341	771	0.7	132	20	32	99	0.44	127	957	15.9	0.7	65	7.1
RT4 C	382	1120	2.2	68	22	53	41	0.23	68	459	7.4	0.4	66	2.8
RT4 0–5	620	2150	0.7	141	44	57	74	0.33	99	487	10.2	0.6	141	8.6
RT4 5–10	680	3020	1.1	251	40	48	87	0.42	118	604	13.1	0.8	207	11.8
RT4 10–15	463	1750	1.1	170	26	50	86	0.47	126	691	15.1	0.8	140	8.3
RT4 15–20	429	1500	1.1	155	26	48	92	0.42	119	683	14.2	0.8	118	7.8
RT4 20–25	440	1530	0.9	148	27	49	91	0.44	118	674	13.5	0.8	116	7.4
RT4 25–30	298	701	0.9	152	21	25	98	0.49	135	890	15.4	0.8	74	6.1
RT5 C	272	774	2.7	83	15	38	42	0.25	65	395	6.7	0.4	56	3.1
RT5 0–5	404	1190	0.6	114	24	43	81	0.37	99	528	10.5	0.5	101	5.7
RT5 5–10	453	1800	1.0	151	26	44	76	0.40	110	615	12.4	0.7	126	7.1
RT5 10–15	516	2110	0.9	192	30	45	78	0.37	103	622	11.2	0.7	172	8.9
RT5 15–20	451	1950	1.1	158	30	45	86	0.38	107	605	11.5	0.7	140	7.9
RT5 20–25	280	1010	1.3	106	21	48	96	0.28	105	681	14.1	0.9	86	6
RT5 25–30	249	345	0.7	92	18	46	108	0.44	131	771	15.9	0.7	52	6

(continued on next page)

Table 1 (continued)

Site	Sc	La	Ce	Nd	Sm	Eu	Yb	Lu	Th	U	Li	Rb	Sr	Br	S (%)	Fe (%)	Ca (%)	Mg (%)	Na (%)	P (%)
b)																				
RT1 C	9.8	25.3	43	<5	4.9	0.8	1.8	0.29	8.7	6.2	47.9	58	130	272	1.45	3.55	2.43	2.40	5.55	0.103
RT1 0–5	11.6	30.0	55	<5	5.9	1.0	2.2	0.40	10.2	5.4	53.8	110	123	216	0.77	4.18	3.66	2.04	3.80	0.116
RT1 5–10	15.0	34.2	62	23	6.6	1.2	2.8	0.37	11.7	3.6	74.6	116	105	126	0.25	4.76	1.90	1.42	2.46	0.093
RT1 10–15	14.7	35.0	64	24	6.7	1.3	2.8	0.42	13.5	3.3	69.9	115	96	102	0.17	4.34	0.77	1.29	2.01	0.064
RT1 15–20	16.8	36.4	66	28	7.0	1.3	2.8	0.44	13.7	2.8	61.9	110	61	117	0.16	4.98	0.36	0.93	2.28	0.056
RT1 20–25	17.0	35.7	66	26	6.9	1.3	2.8	0.41	13.4	3.0	68.2	125	73	125	0.18	5.18	0.38	1.07	2.33	0.060
RT1 25–30	17.2	35.0	66	24	6.7	1.3	2.8	0.37	13.0	3.2	74.5	140	85	132	0.20	5.38	0.39	1.21	2.38	0.063
RT2 C	8.7	23.0	37	16	4.6	0.8	1.9	0.26	7.6	6.7	44.9	82	138	294	1.44	3.19	2.78	2.60	5.33	0.101
RT2 0–5	10.6	28.6	48	20	5.8	1.2	2.0	0.24	8.8	4.2	52.8	74	122	214	0.73	4.07	3.98	2.20	3.69	0.147
RT2 5–10	10.4	31.7	53	22	6.2	1.3	2.4	0.38	10.6	4.0	50.7	72	139	155	0.29	4.25	6.04	1.89	2.74	0.208
RT2 10–15	12.2	33.2	64	18	6.6	1.1	2.6	0.42	10.7	2.8	54.9	75	119	116	0.23	4.60	3.65	1.60	2.27	0.156
RT2 15–20	15.1	36.5	67	29	7.0	1.4	3.0	0.48	13.3	3.4	69.2	110	93	97	0.15	4.31	0.51	1.24	2.06	0.061
RT2 20–25	16.3	40.0	71	35	7.8	1.4	3.2	0.49	14.0	2.7	72.6	91	98	87	0.12	4.21	0.44	1.23	1.88	0.051
RT2 25–30	16.6	42.0	78	34	8.0	1.7	3.4	0.58	14.5	3.4	74.9	130	94	84	0.11	4.46	0.43	1.18	1.85	0.048
RT3 C	6.7	17.2	32	<5	3.6	<0.2	1.6	0.18	4.9	1.8	32.6	<15	221	245	1.60	2.38	3.26	2.44	8.35	0.100
RT3 0–5	9.8	27.0	47	18	5.5	1.2	2.3	0.20	8.5	3.6	40.2	<15	125	227	0.76	3.46	4.26	2.70	4.24	0.137
RT3 5–10	11.2	32.3	54	22	6.6	1.6	2.4	0.42	10.1	4.1	47.7	122	118	157	0.34	4.16	5.14	1.98	2.92	0.154
RT3 10–15	12.0	32.5	61	26	6.6	1.3	2.5	0.44	10.4	3.6	55.4	107	102	145	0.32	4.47	3.76	1.76	3.01	0.136
RT3 15–20	13.1	31.6	59	19	6.4	1.2	2.6	0.40	10.8	3.7	64.2	98	92	100	0.23	4.66	0.90	1.43	2.15	0.103
RT3 20–25	15.8	36.8	67	22	7.2	1.3	2.9	0.49	13.8	3.6	67.4	101	92	95	0.18	4.92	0.54	1.39	1.99	0.064
RT3 25–30	17.4	38.5	67	25	7.4	1.3	3.2	0.47	13.1	3.8	69.6	90	92	89	0.14	4.82	0.39	1.29	1.88	0.056
RT4 C	6.5	17.6	24	16	3.5	<0.2	1.4	0.17	5.4	2.7	31.2	<15	173	218	1.22	2.33	2.84	2.57	9.54	0.099
RT4 0–5	10.4	29.3	52	24	6.0	1.3	2.4	0.40	9.4	3.2	49.5	79	129	205	0.65	4.01	3.26	2.92	3.91	0.163
RT4 5–10	13.3	33.6	60	19	6.6	1.3	2.8	0.34	11.9	3.9	62.8	96	101	122	0.31	5.51	1.96	2.21	2.57	0.125
RT4 10–15	15.2	34.0	60	20	6.6	1.3	2.9	0.43	12.8	4.4	69.3	95	93	97	0.22	5.40	1.38	1.88	1.98	0.082
RT4 15–20	15.1	34.1	64	25	6.6	1.2	2.9	0.38	12.6	3.2	64.2	84	97	98	0.21	5.12	1.69	1.88	1.89	0.081
RT4 20–25	14.1	32.8	62	22	6.4	1.2	2.5	0.38	11.7	3.3	61.3	90	91	103	0.23	4.98	2.05	1.78	2.05	0.092
RT4 25–30	16.6	35.4	65	31	6.8	1.3	3.0	0.35	13.4	3.2	71.0	110	92	90	0.16	4.98	1.07	1.58	1.80	0.068
RT5 C	6.1	16.1	29	<5	3.2	0.5	1.1	0.11	6.2	<0.5	32.8	<15	220	227	1.54	2.34	2.16	1.85	9.03	0.066
RT5 0–5	10.5	28.2	52	19	5.8	1.2	2.3	0.28	10.0	3.5	49.9	68	102	163	0.42	3.87	3.03	1.98	3.06	0.091
RT5 5–10	12.1	30.8	55	22	6.0	1.1	2.4	0.40	10.4	4.1	54.5	63	101	124	0.30	4.68	2.49	2.03	2.31	0.096
RT5 10–15	11.8	31.1	56	20	6.4	1.3	2.8	0.41	10.6	3.5	47.5	66	103	129	0.25	4.94	3.27	1.97	2.33	0.102
RT5 15–20	13.1	32.6	61	18	6.6	1.3	2.6	0.40	11.1	2.6	57.3	99	101	112	0.25	5.11	2.63	1.97	2.19	0.082
RT5 20–25	15.7	34.1	64	25	7.1	1.1	2.6	0.43	12.4	<0.5	69.8	108	99	80	0.17	5.24	2.01	1.86	1.72	0.057
RT5 25–30	17.8	38.0	70	23	7.2	1.3	3.0	0.42	13.9	2.5	76.3	125	95	75	0.13	4.91	1.13	1.66	1.66	0.056

biomineralized calcium carbonate granules secreted by *L. terrestris* (Fraser et al., 2011; Brinza et al., 2013).

Several studies describe the role of earthworms as early colonizers of harsh environments, including contaminated soils, reclaimed mine areas, colliery mine waste heaps, ash deposits and refuse deposits (see Eijsackers, 2010 and references therein for a detailed review). Eijsackers (2010) concludes that the environmental flexibility of earthworms towards pH, desiccation, temperature, or contaminants (as heavy metals) makes them successful colonizers, regardless of the eco-type or r-K strategy.

Staphylinidae are effective predators in agro-ecosystems, because they are dominant in terms of activity-density (the average number of individuals per pitfall trap) (Balog et al., 2011). The structure of communities of staphylinids living near running waters depends on various abiotic and biotic factors (soil type, relief of landscape, plant cover and water pollution, among others; see Bohac, 1999 and references therein). Communities of staphylinids can therefore be used as bioindicators of the environmental status, in that they are bioconcentrators of heavy metals (Bohac, 1989, 1999) and are known to recolonize metalliferous tailings and mine soils (Good, 1999).

In light of the above considerations, the presence of macrobenthic tracemakers such as the earthworm *Lumbricus terrestris* and the coleoptera *Platystethus* in the extremely polluted soils around Rio Tinto can be attributed to two characteristics: a) a great resistance to high concentrations of certain elements, allowing for the colonization and development in anthropogenically metal-contaminated soils of the earthworm *L. terrestris*, and b) the relative independence from substrate features of the coleoptera *Platystethus*, together with its possibility to bioconcentrate heavy metals, and therefore inhabit highly polluted, “unfavorable” habitats.

Even so, and considering the possibility that both tracemakers may inhabit highly contaminated areas, the increase in abundance of burrows from sites 1 to 5 could be associated with the decreasing levels of some elements registered in the same direction, revealing a more abundant community in better substrate conditions. Analysis of abundance of biogenic structures between sites (given the increase from sites 1 to 5), reveals no close relationship with substrate cohesiveness, which could be an indirect proxy of the depth of the groundwater level. The higher density in sites 3 and 5 corresponds to stiff and softground conditions, respectively. Thus, substrate consistency – or

Table 2

Mean values of selected elements in the studied sites. Gray color for those elements showing a general decrease from site 1 to the rest.

Element	Sites				
	1	2	3	4	5
Cu	866.4	587.6	430.6	473.1	375.0
Zn	874.6	1260.6	1488.7	1681.6	1311.3
Cd	3.9	1.9	1.3	1.1	1.2
Pb	163.9	144.4	144.4	155.0	128.0
Co	29.1	28.7	28.6	29.4	23.4
Ni	48.3	46.1	44.3	48.1	41.1
Cr	90.5	82.4	78.9	81.3	81.0
Sc	14.6	12.8	12.3	13.0	12.4
V	130.6	111.0	101.3	111.9	102.9
Mn	811.8	438.9	559.4	641.1	602.4
Ga	14.9	13.0	11.8	12.7	11.8
Tl	1.4	0.8	0.6	0.7	0.7
As	109.0	102.6	112.8	123.1	104.5
Sb	10.3	8.2	7.1	7.5	6.4
La	33.1	33.6	30.8	31.0	30.1
Ce	60.3	59.7	55.3	55.3	55.3
Nd	19.3	24.9	19.6	22.4	18.9
Sm	6.38	6.57	6.19	6.07	6.04
Eu	1.17	1.27	1.16	1.11	1.11
Yb	2.57	2.64	2.50	2.56	2.40
Lu	0.39	0.41	0.37	0.35	0.35
Th	12.0	11.4	10.2	11.0	10.7
U	3.93	3.89	3.46	3.41	2.46
Sr	96.1	114.7	120.3	110.9	117.3
S	0.45	0.44	0.51	0.43	0.44
Fe	4.62	4.16	4.12	4.62	4.44
Ca	1.41	2.55	2.61	2.04	2.39
Mg	1.48	1.71	1.86	2.12	1.90
Na	2.97	2.83	3.51	3.39	3.19

indirectly the depth of the groundwater level – can be discarded as a major physical parameter determining colonization by tracemakers in the studied area, although we cannot discard a secondary influence.

The obtained results point to similarities and differences with respect to previous research conducted on contaminated soils from Vado del Quema (Sevilla, southern Spain) produced by the environmental

Table 3

Mean/maximum values (ppm) obtained from the studied sites compared with reference and intervention values.

Element	Reference	Studied sites					Intervention
	value*	1	2	3	4	5	level*
Cu	<100	866/1330	588/964	431/547	473/680	375/516	>500
Zn	<300	875/1870	1261/2460	1489/1930	1682/3020	1311/2110	>1000
Cd	<3	3.9/13.6	1.9/5.8	1.3/3.1	1.1/2.7	1.2/2.7	>10
Pb	<200	164/257	144/186	144/241	155/251	128/192	>500
Tl	<1	1.4/3.4	0.8/1.4	0.6/0.7	0.7/0.8	0.7/0.9	>5
As	<20	109/128	103/174	113/170	123/207	105/172	>50
Sb	–	10.3/16.4	8.2/10.0	7.1/9.8	7.5/11.8	6.4/8.9	–

* Criteria and standards to declare contaminated soil in Andalucía (Spain). Technical report. Environmental Council. Junta de Andalucía (Aguilar et al., 1999).

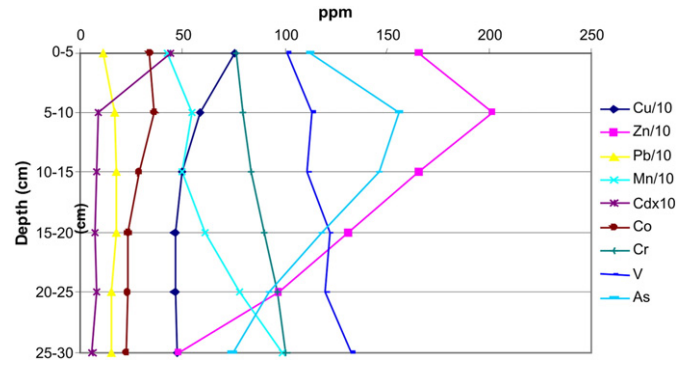


Fig. 5. Mean concentrations in depth at Site 1 of selected heavy metals and As.

disaster that took place next to Doñana National Park (caused by the tailing pond failure of the pyrite mine at Aznalcóllar). Neoichnological and geochemical analyses of Vado del Quema soils revealed a rapid initial recovery in biota colonizing the contaminated substrate; nesting of the ant *Tapinoma nigerrima* (Nylander) has taken place through the tailing layer, with arranged particles from inside the soils showing similar values in pollutants (Rodríguez-Tovar and Martín-Peinado, 2009; Martín-Peinado and Rodríguez-Tovar, 2010). This rapid colonization was associated with the aggressive life habits and opportunistic behavior of *T. nigerrima*, together with its independence regarding features of the polluted substrate. In the case studied here, colonization by the coleoptera *Platystethus* could be explained by a similar strategy on the part of an effective predator with a high independence from substrate features. However, in the case of the earthworm *Lumbricus terrestris*, highly dependent upon soil features, and considered an ecosystem engineer with an important role in organic matter degradation, nutrient cycling and hydrology (e.g., Sizmur et al., 2011a), the colonization would be facilitated by its high environmental flexibility with regard to the physicochemical parameters of the sediments, including pH, redox potential and heavy metal concentrations.

4.2. A paleontological perspective

Biotic recovery after mass-extinction events is an outstanding unsolved question, especially in relation with the incidence of the “Big Five” mass extinctions traditionally recognized by paleontologists (e.g., Erwin, 2001 for a review). Thus, there is much debate about the time of recovery after mass extinctions. Some suggest that re-establishment of biodiversity is in the range of millions of years, while others propose a fast recovery in the range of 10^{3-4} years. This vast disagreement depends, in part, on the particular mass-extinction event in question, the organisms involved, and the concept and models of recovery, which may imply the return of community-level diversity to pre-extinction levels or the re-emergence of all trophic levels (see Chen and Benton, 2012 for the end-Permian mass extinction).

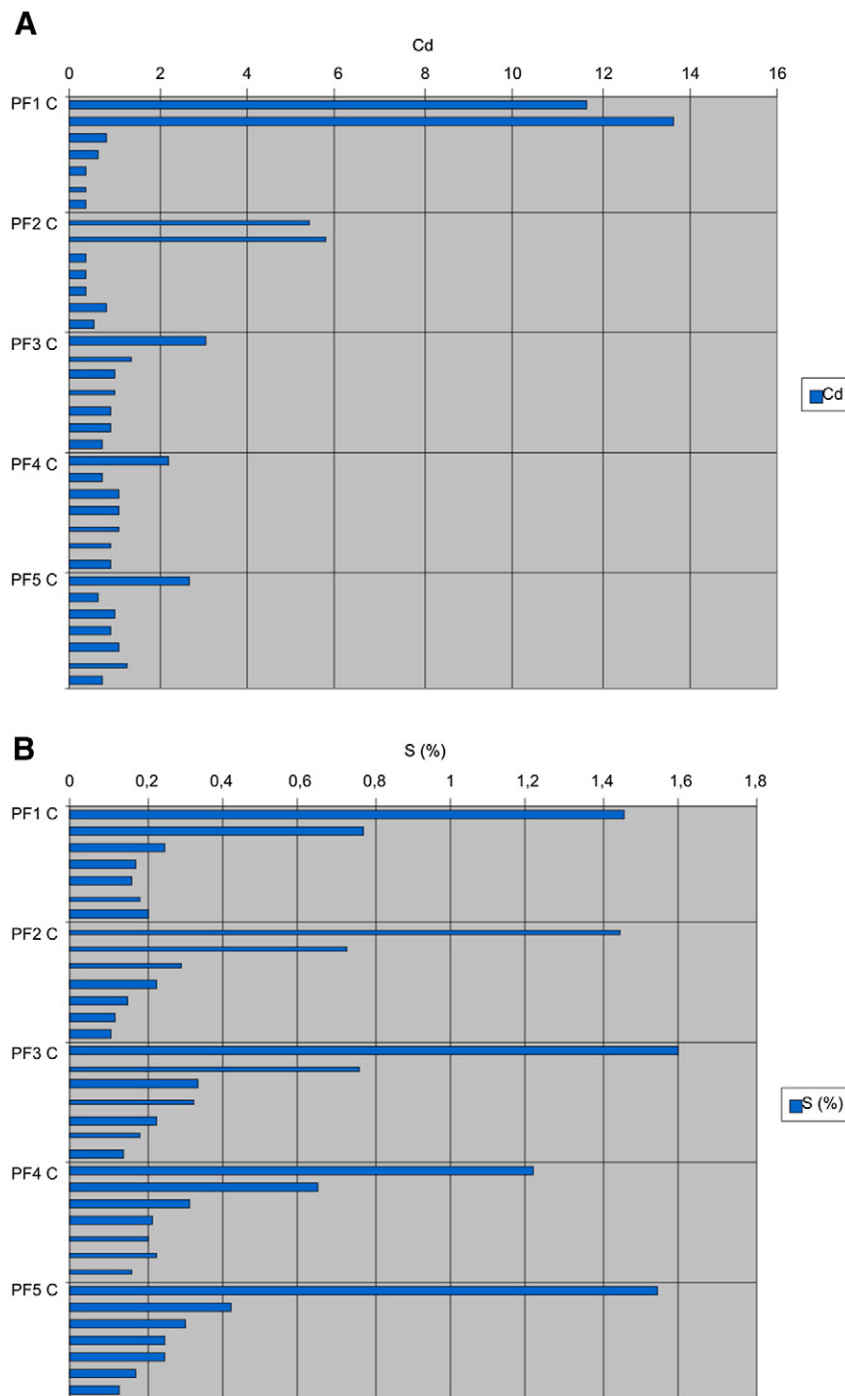


Fig. 6. Vertical variations in concentrations at the studied sites in Cd (A) and S (B), showing an important relationship with the presence/absence of biofilm/salt horizon in the uppermost part of the soil.

From these Big Five, the Cretaceous–Palaeogene (K/Pg) boundary event, occurring around 65.5 million years ago, is one of the most important mass extinction events in the Phanerozoic, determining the disappearance of about 70% of marine and continental species (D'Hondt, 2005; Labandeira et al., *in press* for a recent review). The hypothesis that an extraterrestrial bolide impact caused the K/Pg event is now widely accepted, determining important global environmental changes, including earthquakes of magnitude >11, tsunamis 100–300 m in height that swept over 300 km, shock waves and air blasts that radiated across the landscape, and high temperatures that generated fires as far away as 1500 to 4000 km from the crater (Sosa-Montes de Oca et al., 2013). Of the numerous marine and continental K/Pg boundary sections

worldwide, those from Agost and Caravaca in southeast Spain have been extensively studied, supporting detailed geochemical and ichnological data (Rodríguez-Tovar et al., 2004, 2006; Sosa-Montes de Oca et al., 2013). An ultra-high-resolution geochemical analysis at the millimeter scale conducted across the K/Pg boundary in the Caravaca section (southeast Spain), one of the best-preserved and most continuous distal sections worldwide, evidences a rapid return to pre-impact conditions in terms of oxygenation after this major catastrophe of some 10^2 years, several orders shorter than traditionally proposed (Sosa-Montes de Oca et al., 2013). This agrees with the rapid recovery interpreted for the macrobenthic tracemaker community, based on detailed ichnological analysis across the K/Pg boundary interval, at the

Agost and Caravaca sections (Rodríguez-Tovar, 2005; Rodríguez-Tovar and Uchman, 2008).

The rusty, red boundary layer (also called ejecta layer) is characterized by the presence of spherules and high iridium anomalies, together with those in other platinum group elements. This K/Pg boundary layer was considered unbioturbated, and traditionally interpreted as revealing unfavorable environmental conditions that were inhospitable for colonization by organisms. However, ichnological analysis at the Caravaca and Agost sections revealed a relatively diverse trace fossil assemblage.

At the Caravaca section, the lower Danian dark boundary layer assemblage is composed mainly by *Zoophycos*, *Chondrites*, *Thalassinoides*, *Planolites* and *Alcyonidiopsis* (Rodríguez-Tovar and Uchman, 2006, 2008). *Zoophycos*, *Chondrites*, and *Planolites* disturb the rusty layer, with *Zoophycos* and *Chondrites* vertically cross-cutting the boundary layer, supplemented by lateral crossing of the interval by *Chondrites* (Rodríguez-Tovar and Uchman, 2008). Colonization of the “inhabitable” ejecta layer by *Zoophycos* and *Chondrites* would be associated with the independence of tracemakers from substrate features and their adaptation to lower oxygenation and food content; *Zoophycos* and *Chondrites* tracemakers constructed open or partly ventilated burrows that accommodated various substrate features, followed by infaunal colonization of sediment poor in oxygenated pore waters and food (Rodríguez-Tovar and Uchman, 2008).

At the Agost section, the lower Danian dark boundary layer assemblage is similar to that from the nearby Caravaca section, mainly consisting of *Zoophycos*, *Chondrites*, *Thalassinoides*, *Planolites* and *Alcyonidiopsis* (Rodríguez-Tovar, 2001; Rodríguez-Tovar and Uchman, 2004a,b). In this case, the material infilling *Thalassinoides* reveals the presence of dark infill material that includes iron oxide spherules, similar to those registered in the ejecta layer. *Thalassinoides* is interpreted as a domichnial/fodinichnial structure, passively infilling, produced by crustaceans, mostly decapods. Thus, the passive filling of the material into the *Thalassinoides* allows interpretation of a rapid colonization by *Thalassinoides* tracemakers, almost contemporaneous with the spherule layer deposit (Rodríguez-Tovar, 2005). This behavior may be comparable with that of the coleoptera *Platystethus* registered in Rio Tinto, a second colonizer after the r-strategy organisms regardless of substrate features.

In both sections, Caravaca and Agost, *Planolites* is registered in the lower Danian dark boundary layer assemblage. *Planolites* is interpreted as a pascichnion, reflecting a combination of locomotion and feeding, probably produced by a number of different organisms, but representing the activity of mobile, infaunal deposit feeders or epifaunal detritus-feeding organisms. Accordingly, *Planolites* tracemaker is highly dependent on substrate characteristics and so colonization of the ejecta layer must depend on features other than those interpreted for *Zoophycos*, *Chondrites* and *Thalassinoides* producers. In this case, *Planolites* could be produced by organisms with high environmental flexibility regarding the physicochemical parameters of sediments. This case could be comparable to that of *Lumbricus terrestris*, showing a great resistance to high concentrations of certain elements, allowing it to colonize and develop anthropogenically metal-contaminated soils such as those by Rio Tinto. Recently, Chin et al. (2013) calibrated the occurrence of *Planolites*, interpreted as produced by extent earthworms, less than ten thousand years after the end of the Cretaceous impact.

5. Conclusions

Sites in the marsh area of the Tinto River, close to Palos de la Frontera village (Huelva, SW Spain), show high concentrations of toxic elements in soils (mainly Cu, Zn and As, exceeding the intervention levels for natural areas), with some lateral and vertical variations. However, these extreme conditions do not preclude colonization by particular macroinvertebrates. Variable strategies for colonization can

be compared with those used by past tracemakers after the impact of bio-events.

1. Lateral and vertical variations in concentrations of several elements are registered within the different study sites. The uppermost part of the soil shows differentiation when represented by a biofilm (sites 1 and 2, with significant concentrations in Cd, Tl, U, Br, S, Na, and Mg), or by a thin saline horizon (sites 3 to 5, with high levels in Cd, Sr, Br, S and Na).
2. Ichnological analysis shows the presence of isolated holes/apertures without any pellets around them, holes surrounded by pellets with a mound appearance, or aggregations of pellets having no associated aperture. These biogenic structures are produced by the activity of the earthworm *Lumbricus terrestris* and the coleoptera *Platystethus*.
3. Minor differences in the abundance of biogenic structures, with a general increase from sites 1 to 5, could be associated with decreasing levels of contamination, hence more favorable living conditions.
4. Colonization of this highly contaminated habitat is possible due to particular features of the tracemakers. The earthworm *Lumbricus terrestris* is characterized by a great resistance to high concentrations of a number of elements, allowing its colonization and development in anthropogenically metal-contaminated soils. The coleoptera *Platystethus* is relatively independent from substrate features, and some staphylinids moreover have the ability to bioconcentrate heavy metals.
5. Particular means of colonization of the contaminated Tinto River area can be correlated with those used during the recovery of past tracemakers after the Cretaceous–Palaeogene event. *Planolites*, a pascichnion, probably reflects the activity of mobile, infaunal deposit feeders or epifaunal detritus-feeding organisms, highly dependent on substrate characteristics. This may be associated with a high resistance of the producer to heavy concentrations of certain elements, as occurs with *Lumbricus terrestris*. *Thalassinoides*, a domichnial/fodinichnial structure, passively infilling, produced by crustaceans (mostly decapods) could reflect a high independence from substrate features, as occurs with the coleoptera *Platystethus*.

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