



Depósito de Investigación
Universidad de Sevilla

Depósito de investigación de la Universidad de Sevilla

<https://idus.us.es/>

“This is an Accepted Manuscript of an article published by Elsevier in SCIENCE OF THE TOTAL ENVIRONMENT on 1 September 2011, available at: <https://doi.org/10.1016/j.scitotenv.2011.06.013>.”

Highlights

In this study we present the mineralogy of the Guadiana estuarine sediments.

The geochemical speciation and the mineralogy explain the element distribution.

We combine multiple techniques (XRD, SEM, BCR extraction and risks assessment)

The study reveals the environmental vulnerability of the Guadiana estuarine sediments.

1 **SPECIATION AND ECOLOGICAL RISK OF TOXIC ELEMENTS IN ESTUARINE SEDIMENTS**
2 **AFFECTED BY MULTIPLE ANTHROPOGENIC CONTRIBUTIONS (GUADIANA**
3 **SALTMARSHES, SW IBERIAN PENINSULA): I. SURFICIAL SEDIMENTS.**

4

5

6 **Joaquín Delgado ^{1*}, Cinta Barba-Brioso ², José Miguel Nieto ¹ & Tomasz Boski ³**

7

8 (1) Dpto. Geología, Facultad de Ciencias Experimentales, Universidad de Huelva. Av. 3 de Marzo, S/N, 21071, Huelva, Spain.

9 joaquin.delgado@dgeo.uhu.es.

10 (2) Dpto. Cristalografía, Mineralogía y Química agrícola, Facultad de Química, Universidad de Sevilla. 41012, Sevilla, Spain.

11 (3) CIMA, Centre for Marine and Environmental Research, University of Algarve, 8005-139 Faro, Portugal.

12

13 *** Corresponding author:**

14 Joaquín Delgado.

15 Address: Dpto. Geología, Facultad de Ciencias Experimentales, Universidad de Huelva. Av. 3 de Marzo,

16 S/N, 21071, Huelva, Spain.

17 Telephone: 0034 959 219826.

18 Fax: 0034 959 219810.

19 E-mail: joaquin.delgado@dgeo.uhu.es.

20

21

22

23

24

25

26

27

28

29

1 **SPECIATION AND ECOLOGICAL RISK OF TOXIC ELEMENTS IN ESTUARINE SEDIMENTS**
2 **AFFECTED BY MULTIPLE ANTHROPOGENIC CONTRIBUTIONS (GUADIANA**
3 **SALTMARSHES, SW IBERIAN PENINSULA): I. SURFICIAL SEDIMENTS.**

4
5
6 **Joaquín Delgado ^{1*}, Cinta Barba-Brioso ², José Miguel Nieto ¹ & Tomasz Boski ³**

7
8 (1) Dpto. Geología, Facultad de Ciencias Experimentales, Universidad de Huelva. Av. 3 de Marzo, S/N, 21071, Huelva, Spain.

9 joaquin.delgado@dgeo.uhu.es.

10 (2) Dpto. Cristalografía, Mineralogía y Química agrícola, Facultad de Química, Universidad de Sevilla. 41012, Sevilla, Spain.

11 (3) CIMA, Centre for Marine and Environmental Research, University of Algarve, 8005-139 Faro, Portugal.

12
13
14 *Recent studies have demonstrated that the Guadiana Estuary contains metal concentrations in*
15 *excess of background values. Therefore, this work aims to document the potential*
16 *environmental hazards associated with the availability of these metals in this environment of*
17 *high ecological value. Mineralogical analysis shows that the sediments are composed mainly of*
18 *quartz, albite, and clay minerals (illite, smectite, kaolinite, and vermiculite) along with several*
19 *small, reactive compounds (including soluble sulphated salts, Fe-Mn oxyhydroxides, organic*
20 *matter, and pyrite) capable of retaining metals, which can be subsequently released, causing*
21 *environmental degradation. BCR sequential extraction shows that As, Cd, Cu, Mn, Pb, and Zn*
22 *present mobile fractions with respect to the total metal content (41, 100, 57, 53, 70, and 69%,*
23 *respectively) in any of the described reactive phases (F1+F2+F3). Calculated environmental*
24 *risk indices demonstrate moderate to considerable ecological risk for almost the entire estuary,*
25 *associated mainly with acid mine drainage from the nearby Iberian Pyrite Belt. In addition, the*
26 *indices highlight several zones of extremely high risk, which are related to industrial and urban*
27 *dumps in the vicinity of the estuary and to heavy traffic on the international bridge.*

28
29 **Keywords:** Guadiana Estuary, sediment pollution, metal speciation, sequential extraction, ecological risk indices.

1 **1. Introduction**

2
3 The Guadiana River drains the western part of the Iberian Pyrite Belt (IPB), one of the world's
4 most important metallogenetic sulphide provinces (Figure 1), where mining dates back to the
5 Third Millennium B.C. (Nocete et al., 2005). Associated with these mining areas are acidic
6 leachates containing high concentrations of metals, metalloids, and sulphates, collectively
7 known as acid mine drainage (AMD). Extractions of minerals from the IPB were particularly
8 intense in the middle of the 19th century (Morral, 1990); since then, activity has declined with
9 only a small number of currently active mines. However, the environmental impact of AMD
10 still exists. There are several sources of AMD, including outcrops of polymetallic sulphide
11 deposits, the products related to the exploitation of these deposits (such as waste piles, residues
12 from the smelting process, ashes, and low-grade stockpiles), and the abandoned underground
13 stopes and chambers. Metals and metalloids leaching from abandoned, sulphide-rich mines are a
14 very important source of environmental pollution of water, soils, and sediments in the IPB
15 (Romero et al., 2006; Pérez-López et al., 2008). These leachates are responsible for the
16 present pollution and the degradation in water quality of the lower part of the Guadiana River
17 Basin (GRB) and, consequently, of the estuarine sediments, which act as the final continental
18 filters prior to fluvial discharge into the Gulf of Cádiz.

19 The consequences of these intense mining activities in the GRB have been documented in
20 various recent studies (e.g. Álvarez-Valero et al., 2008; Pérez-López et al., 2008; Delgado et
21 al., 2009). However, there has been only limited investigative effort made to evaluate the
22 consequences of these activities in the estuarine area. The estuarine area is recognised as having
23 high ecological value and contains the Natural Area “*Marismas de Isla Cristina*” in the Spanish
24 sector of the estuary and the Natural Reserve “*Sapal de Castro Marim y Vila Real de Sto.*
25 *António*” in the Portuguese sector. However, existing studies of the polluting agents in the
26 Guadiana Estuary (Morales, 1997; Ruiz et al., 2001; Delgado et al., 2009b; Delgado et al.,
27 2010) are not sufficient to evaluate their total environmental impact because the chemical state
28 in which elements are present in the sediments (easily exchangeable ions, metal carbonates,

1 oxides, sulphides, organometallic compounds, ions in crystal lattices of minerals, etc.) needs to
2 be known to evaluate their mobility, bio-availability, and toxicity (e.g. **Usero et al., 1998;**
3 **Weisz et al., 2000; Yu et al., 2001; Pérez-López et al., 2008**).

4 When environmental conditions change (pH, redox potential, organic matter, etc); some of the
5 sediment-bound metals can be remobilised and released back into the water, where they can
6 have adverse effects on living organisms (e.g. **Li et al., 2001; Morillo et al., 2002; Peng et al.,**
7 **2009**). The most crucial property of metal ions is that they are bioavailable and not
8 biodegradable in the environment and that their uptake by benthonic organisms depends largely
9 on their mobility, total concentration, and chemical form (**Morillo et al., 2007**). Several
10 analytical methodologies based on sequential extraction procedures (SEP) have been devised to
11 study metal mobility and the potential level of bioavailability (**Sundaray et al., 2011**) in
12 sediments based on the sum of the exchangeable and associated carbonate fractions (e.g.
13 **Kabala et al., 2001; Fuentes et al., 2004; Karbassi et al., 2005; Vanek et al., 2005**).

14 Sequential extractions provide quantitative information about the distributions of various
15 elements among operationally defined geochemical fractions in soils, sediments, and waste
16 materials. Numerous sequential extraction methods are used at present, and they differ
17 according to the types of reagent used, the experimental conditions applied, and the numbers of
18 steps involved (e.g. **Tessier et al., 1979; Gibson and Farmer, 1986; Dold, 2003**). One of the
19 most common methods is the BCR sequential extraction scheme, a simple three-stage procedure
20 that has been thoroughly tested by inter-laboratory trials (**Cappuyns et al., 2007**). The original
21 sequential extraction was proposed by the European Community Bureau of Reference (BCR) to
22 standardize various existing procedures, obtaining three separate fractions (acid soluble-
23 extractable, reducible, and oxidable) (**Ure et al., 1993**) and to achieve comparability when
24 sequential extraction is used (**Quevauviller, 1997**). This scheme has been improved in
25 subsequent studies (**Rauret et al., 1999; Sahuquillo et al., 1999; Arain et al., 2008**). The
26 digestion methods applied generally provide information on the total or pseudo-total content of
27 trace elements in sediments without giving a direct evaluation of anthropogenic contributions
28 and the consequent pollution level of the sites (**Perez-Santana et al., 2007**).

1 The objectives of the present study were to investigate the mobility of the most toxic elements
2 (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, S, and Zn) in the Guadiana saltmarsh sediments by
3 applying the BCR (modified) sequential extraction scheme to this environment affected by
4 multiple anthropogenic contaminants, to combine the sequential extraction data with
5 mineralogical analyses from X-ray diffraction (XRD) and scanning electron microscopy (SEM),
6 and to determine the mineral reactivity occurring in the estuary. The aforementioned techniques,
7 along with the calculation of ecotoxicity indexes, should allow us to establish monitoring
8 strategies for future action plans on this site of high ecological value. In addition, based on the
9 results obtained in this work, a detailed study of two boreholes through the Holocene infill of
10 the Guadiana Estuary is currently underway, using the same methodology and also examining
11 rare earth elements (REEs). The drilling is expected to reveal the evolution of the ecological
12 evolution of the Guadiana Estuary during the last 13,000 years and should improve our
13 understanding of metal fractionation in environments historically affected by AMD.

14

15 **2. Materials and methods**

16

17 ***2.1. Description of sampling sites and superficial sediments***

18 The physiographic and geological characteristics of the study area have been thoroughly
19 described in recent studies (**Boski et al., 2002; Boski et al., 2008; Delgado et al., 2009a;**
20 **2010**).

21 For sampling of surficial sediments, 20 representative sampling points in the estuarine area were
22 selected. The sampling points were positioned in the lower estuary within 30 km of the
23 shoreline, coinciding with the marine domain (Figure 1). The surface sediments correspond to
24 the margins of the principal channels in the estuary (Figure 1); these include the main channel of
25 the Guadiana River itself, the “Carrasqueira” and “Castro Marim” stream channels (Portuguese
26 basin), and the stream channels “La Canela”, “San Bruno”, and “Carreras” (Spanish basin).

27 The channels’ active margins are situated between the mean spring low-water and mean neap
28 high-water (**Borrego et al., 1995**). For this reason, they undergo tidal action (flooded during

1 high tide) and represent the current environmental state of the sedimentary system of interest
2 (Delgado et al., 2010). Further details about the sampling and sample pre-treatment are
3 described in Delgado et al. (2010).

4 5 **2.1.1. Sequential extractions procedure (SEP)**

6 The SEP applied in this study was the improved version of the initial BCR three-step scheme
7 (Quevauviller et al., 1997). The procedure is summarized below and full details are reported
8 elsewhere (e.g., Rauret et al., 1999; Sahuquillo et al., 1999).

9 **Step 1.** (water/acid soluble and exchangeable fraction/carbonate included): acetic acid (20 ml of
10 0.11 M solution) was added to 0.5 g of accurately weighed sample in 50 ml polyethylene
11 centrifuge tubes, and shaken for 16 h at room temperature. The extracts were then separated
12 from the residue by centrifuging for 20 min at 3000 rpm, decantation into polyethylene
13 containers and storage at 4° C for analysis. The residues were washed with 10 ml de-ionized
14 water by shaking for 15 min and centrifuged.

15 **Step 2.** (reducible fraction): 20 ml of 0.1 M hydroxylammonium chloride (adjusted to pH of
16 around 2 by adding HNO₃) were added to residues from Step 1. The extraction was performed
17 as described in Step 1.

18 **Step 3.** (oxidisable): 10 ml of 8.8M H₂O₂ (pH 2.0–3.0) was added drop-by-drop to the residues
19 from Step 2. The tubes were covered and the contents were digested for 1 h at room temperature
20 and 1 h at 85° C in a water bath. Volume was reduced to around 2–3 ml by evaporation. Step 3
21 was performed twice. Finally, 25 ml of 1M ammonium acetate (adjusted to pH 2 by adding
22 HNO₃) was added to the cool residues, which were separated and rinsed as described in Step 1.

23 **Step 4.** (residual fraction): The residue from Step 3 was treated by the procedure used for
24 determination of the pseudo-total trace elements content by *aqua regia* digestion (10 ml of a
25 mixture of 12M HCl and 15.8 M HNO₃ in a 3:1 ratio) in teflon reactors. Reactors were
26 maintained for 20 h in a fume hood and then simmered on a hot plate for 1 h at 100° C.

1 2.1.2. Analysis and quality control of the data

2 Total element concentrations were measured by Acme Analytical Laboratories Ltd (Vancouver,
3 Canada), accredited under ISO 9002, through its Italian affiliate (ERS Srl, Napoli), by
4 inductively coupled plasma atomic emission spectroscopy (ICP-AES) and inductively coupled
5 plasma mass spectrometry (ICP-MS). A total of 36 elements (Ba, Al, K, Mg, Mn, Si, Ag, As,
6 Be, Bi, Ca, Cd, Ce, Co, Cr, Fe, Cu, Li, Mo, Na, Ni, P, Pb, Sb, S, Se, Sn, Sr, Te, Ti, Tl, U, V, W,
7 Y, and Zn) were reported for a 0.1-g sample analysed by ICP-emission spectrometry following
8 a lithium metaborate/tetraborate fusion and dilute nitric acid digestion. The most polluting
9 elements form the basis for subsequent discussion: S, Fe, As, Cd, Cr, Co, Cu, Ni, Pb, Zn, and
10 Mn. Hereafter, the term “metal” includes both metals (e.g. Cd) and metalloids (e.g. As).
11 Although the sequential extraction procedure is usually applied for assessing metal mobility, S
12 is also discussed within this group because it is the most abundant element in AMD; Ca is also
13 discussed because of the important role of CaCO₃ in the incorporation of trace metals that are
14 released under acid conditions (Ayyamperumal et al. 2006). Total carbon and sulphur were
15 determined by loss on ignition (LECO) and have been reported in a recent paper (**Delgado et**
16 **al., 2010**).

17 Accuracy was calculated with respect to Acme’s in-house reference materials DS7 and SO-18.
18 These were calibrated for an *aqua regia* digestion/ICP-MS determination with published values
19 for concentrated HCl and HNO₃ digestion in the Canadian Certified Reference Materials Project
20 (CCRMP) TILL-4 and LKSD-2.

21 In addition, four replicates were analysed to check the quality of the analysis. The relative
22 percentage difference (% RPD) was calculated (Equation 1). The results for % RPD (Table 1)
23 were reasonably good and close to zero, the expected value. Most values were below 0.5%,
24 except for Cr at 2.14%. All RPD values were lower than 5%.

25 Evaluation of the analytical performance of the total concentration was made by analysing the
26 certified reference materials (CRMs) STSD-1 and STSD-2 (from stream sediments). The
27 measured concentrations of all analytes (Table 1) coincide with, or are very close to, the
28 standard reference values. Generally, the recovery ranges (Equation 2) of the principal elements

1 under study (Fe, Al, As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) were between 78.5 and 105%, except
2 those for Cd and Cr, for which there were no certified reference values.

3 For the studied elements, an internal check of the results was performed by comparing the sum
4 of the four steps from the SEP (acid-soluble + reducible + oxidisable + residual) with the
5 analysis of total element concentration (Acme Analytical Laboratories) reported in Table 3.
6 These calculations can be used to validate the results and for inter-laboratory comparisons
7 (**Pérez-López et al., 2008**). The recovery percentage of the sequential extraction was calculated
8 using Equation 3. The calculations revealed good percentage recoveries for As, Co, Cu, Fe, Mn,
9 Ni, Pb, and Zn, indicating the reliability of the data (except for Cd, because of its low
10 concentrations). The average recovery value for the studied major elements and metals was $90 \pm$
11 17% (Table 2).

12

$$13 \quad \% RPD = (S-D)/(S+D)/2 \times 100. \quad \text{Equation 1}$$

14 *Where: S = determined value of the samples, D = value of the duplicates.*

$$15 \quad \% Recovery = (Obtained\ value/Certified\ value) \times 100 \quad \text{Equation 2}$$

$$16 \quad \% Recovery = (F1 + F2 + F3 + R/ Total\ Concentration) \times 100 \quad \text{Equation 3}$$

17 *Where: Total concentration refers to the values obtained by Acme Laboratories.*

18

19 In addition, the results obtained for extractable concentrations were compared with indicative or
20 certified values following the procedures for the standard reference material (BCR-701), and
21 they showed that certified (or indicative) and obtained values were not significantly different
22 (Table 2). All these considerations provided an assurance of the quality of the analytical
23 extraction data.

24

25 **2.2. Mineralogical characterisation**

26 Mineralogical characterisation of the sediment was performed by X-ray diffraction (XRD)
27 techniques on a Bruker AXS model D8 Advance diffractometer. Working conditions were as
28 follows: slit fixed at 12 mm, CuK α monochromatic radiation, 20 mA, and 40 kV. Random

1 powders were scanned from 3 to 65° 2θ at 0.5 s counting time per step. For clay mineral
2 identification, oriented aggregates of the <2-μm fraction were obtained by sedimentation and
3 then saturated with ethylene glycol (EG) under vacuum for 48 h. This was followed by a
4 thermal treatment (TT) at 550°C for 2 h. Treated, oriented aggregates were scanned from 3 to
5 20° 2θ at 0.5 s counting time per step. Semi-quantitative mineralogical determination was
6 estimated with *XPowder* software (**Martin, 2004**).

7 In order to complete the mineralogical characterization, selected samples were examined on a
8 JEOL JSM-5410 scanning electron microscope (SEM) operating with an accelerating voltage of
9 20 kV, using back-scattered electron (BSE) imaging and energy dispersive X-ray (EDX)
10 microanalysis (Link ISIS system) to identify amorphous or poorly-crystallised phases and
11 accessory heavy minerals. Additionally, a sequential mineralogical study was developed on
12 each residue of the SEP to understand the reactivity of the metal in the different geochemical
13 fractions of the sediments.

14 **2.3. Statistical analysis**

15 Multivariate statistical analysis of the principal components (PCA) was performed using a
16 Pearson's correlation matrix (0.05 significance level) to evaluate the levels of association of the
17 variables (metals-metalloids) at the different steps of the SEP. This technique has been widely
18 used to distinguish the factors that control the geochemical behaviour of metals in the estuarine
19 system (e.g. Wu et al. 2007). The values of the factor matrix can be improved by using the
20 varimax rotation method, which maximizes factor variance (Kaiser et al., 1958), because it is an
21 orthogonal rotation that minimises the number of variables that have high loadings on each
22 factor, thereby simplifying the transformed data matrix and assisting interpretation.

23

24 **2.4. Quantification of sediment pollution**

25 A significant number of indicators designed to approximate the quality of sediments and soils
26 and the risk that these pose to aquatic ecosystems can be found in the literature (e.g. **Caeiro et**
27 **al., 2005**). To establish the environmental risk of the Guadiana Estuary sediments, the potential

1 ecological risk was estimated by calculating some of these frequently used pollution indices as
2 follows.

3 **Potential ecological risk** was defined by **Hakanson et al., (1980)** and was used as a diagnostic
4 tool for water pollution control purposes: This empirical index provides a simple comparative
5 tool for assessing the level of heavy metal pollution (**Bhuiyan et al., 2010**) and allows
6 comparisons to be made between several estuarine systems.

7 The distribution of metal and metalloid pollution in the different phases of the modified BCR
8 procedure offers an indication of their availability, which, in turn, enables a risk assessment to
9 be made of the present pollutants in the aquatic environment (**Chen et al., 2010**). In this
10 context, the sediments were classified according to a **Risk Assessment Code (RAC)** used in
11 previous investigations of heavy metal pollution of sediments (e.g. **Perin et al., 1985**). The
12 RAC is assigned by taking into account the percentage of metal associated with the
13 soil/sediment in the exchangeable and carbonate fractions: there is no risk when the F1 BCR
14 fraction is lower than 1%; low risk for a range of 1–10%; medium risk for a range of 11–30%;
15 high risk from 31 to 50%; and very high risk for higher F1 percentages.

16

17 **3. Results and Discussion**

18

19 **3.1. Sediment characteristics**

20 Table 3 shows the total concentrations obtained for major oxides (%) and for the main metals
21 and metalloids (mg kg^{-1}) that can be hazardous to the environment. The mineral phases and the
22 semiquantitative mean percentages determined by “Xp powder” software from 20 sediment
23 samples are shown in Figure 2. The XRD study shows that the surface sediments are composed
24 mainly of quartz, feldspar type *albite*, and clay minerals in average proportions of 69.89, 6.67
25 and 23.44%, respectively (Figure 2A).

26 The clay-group minerals present in the sediments were determined by means of specific
27 treatments. The oriented aggregates allowed the distinction of *illite* in all samples, with a mean
28 of 6.67%. Subsequently, oriented aggregates were treated with EG to distinguish the presence of

1 *smectites* and *vermiculites* (Figure 2B, top). The results show that the *smectite*- and *kaolinite*-
2 type phyllosilicates dominate over the *vermiculite*-type in surface samples. Thus, the mean
3 percentage of vermiculite is 6.38, while *smectites-kaolinites* have higher values, with a mean of
4 10.49%. Moreover, oriented aggregates were subjected to a thermal treatment at 550°C, which
5 destroys *kaolinite*, thus allowing distinction between it and the chlorite-group minerals. As
6 shown in Figure 2B (bottom), a collapse of the 7Å peak is produced, which implies that
7 *kaolinite* is the detected mineral phase. The results for the peak located at 14Å are consistent
8 with the results of treatment with EG, because at that temperature the collapse of *vermiculite*
9 can also be seen.

11 3.2. SEM data

12 A scanning electron microscope was used to aid in characterizing the mineralogy of the
13 sediments, focusing on the main metallic phases that are unlikely to be distinguished by XRD.
14 The SEM spectra (Figures 3 and 4) indicate that the sediments are composed mainly of Si, Al,
15 Fe, Mg, Mn, and, to a lesser extent, Ca and K. In addition, Fe oxyhydroxides [probably low
16 crystalline or amorphous phases (Figure 3A)], fine-grained sulphide minerals, and native
17 sulphur were identified (Figure 3C). Pyrite appears in two neoformational habits: small
18 aggregated crystals occupying hollows or on other mineral surfaces (Figure 3B up) and
19 framboidal pyrite (Figure 3B down), both suggesting *in situ* secondary precipitation (**Sarmiento**
20 **et al., 2009**). In this case, perfectly spherical framboids could be identified in the sediments
21 (Figure 3B, down), characteristic of a slow-growing mineralization according to **Butler and**
22 **Rickard, (2000)**. In general, the framboids show a size distribution below 10 µm, suggesting
23 formation in an anoxic environment (**Hawkins and Rimmer, 2002**).
24 Various studies have attempted to explain the formation of framboidal pyrite (**Ohfuji and**
25 **Richard, 2005**). Some authors claim that this morphology is the result of the pyritisation (DOP)
26 of bacteria (**Love, 1957**); while others claim it is a process of DOP up to organic particles or
27 colloids (**Kribek, 1975**). More recent studies suggest another mineral (*greigite* - Fe₃S₄) as a
28 precursor to inorganic oxidation (**Butler and Rickard, 2000**). In summary, although the exact

1 mode of production of framboidal pyrite in sediment remains contentious, it obviously implies a
2 process of sulphide reduction.

3 Nevertheless, the pyrite/Fe-oxyhydroxide association could also point to their formation at the
4 expense of the amorphous or poorly crystallized Fe oxyhydroxides, which abound in these
5 sediments. Thus, the Fe oxyhydroxides' reactivity (**Haese, 2001**) depends on their crystal-
6 chemical parameters as well as on other factors like the selectivity of the organisms involved in
7 the reduction process. As an example, according to **Lovely and Phillips (1986)**, *Geobacter*
8 *metallireducens* are capable of reducing amorphous and poorly crystalline Fe phases but not
9 crystalline ones.

10 In recent studies (**Otero and Macías, 2002a, b**), the presence of Fe oxyhydroxides in anoxic
11 environments is argued based on two modes: I) sediment is colonized by vascular plants
12 (mainly *Spartina maritima*) and high pyrite concentrations, and part of the Fe is mobilized to
13 the surface, where it is oxidised and precipitates, generating high quantities of reactive Fe; and
14 II) the Fe²⁺ generated by amorphous Fe oxyhydroxides is adsorbed on the surface of the
15 crystalline Fe oxyhydroxides, thus preserving their structure.

16 The presence of lanthanide group elements (La and Ce) was detected, associated with P (Figure
17 3A bottom) and with other major constituents, which points to the precipitation of secondary
18 minerals such as *monazite*, whose presence may be controlled by P inputs related to the
19 extended use of phosphate fertilizers in the inner zones of the basin. In this case, REE
20 fractionation is probably attributable to the presence of P in the estuarine waters, although
21 recent studies in the source area show MREE enrichment of the leachates originating from the
22 massive sulphides (**Pérez-López et al., 2010**).

23 The presence of Cu associated with S (Figure 4A) enables the existence of secondary sulphated
24 salts capable of retaining heavy metals that can be subsequently liberated, causing estuarine
25 degradation when environmental conditions, such as pH and salinity, change (**Pérez et al.,**
26 **1991**).

27 The sequential mineralogical study (Figure 4) allowed the reactivity of the metal phases to be
28 determined. Therefore, the resistance of the Fe-Mn oxyhydroxides and pyrite to neutral or acetic

1 acid (0.11 mol/l) solutions (F1) was checked (see Figure 4A for an example). The SEM images
2 confirmed that the Fe-Mn oxyhydroxides were dissolved in step 2 and pyrite in step 3 associated
3 with the oxidisable fraction. *Monazite*, as previously described, was still observed after step 2
4 (Figure 4B, sample CAN-1). According to the literature (Oelkers et al, 2002), monazite
5 dissolution rates are relatively low, as they are controlled by the breaking of strong O-REE
6 links.

7 Also of note is the association between Ca and elements such as S and Fe (Figure 4A, top), as
8 previously described for the Holocene sediments of the Guadiana Estuary (**Boski et al., 2008**).
9 This association could explain pyrite oxidation processes under locally acidic conditions in the
10 presence of shell fragments that release Ca, favouring the precipitation of secondary carbonates
11 like *siderite* (FeCO₃) that usually incorporate metals such as As, Cd, or Cu.

12 Finally, heavy accessory minerals resistant to the sedimentary cycle (*zircon* and *titanite*) were
13 identified, according to previous studies of the Huelva littoral sediments (**Fernández-Caliani et**
14 **al., 1997**), as well as other accessory minerals originating from the source area, such as *apatite*,
15 preserving its typical prismatic habit (Figure 4C).

16 The progressive removal of metallic phases is shown in Figure 4D, which depicts the sequence
17 of the treatment from F1 to F4 (attack with *aqua regia*, pseudo-total concentrations). Most of
18 the bright metallic elements (images of back-scattered electrons) present in F1 disappear after
19 F4 treatment because of the progressive dissolution of soluble salts, Fe-Mn oxyhydroxides,
20 authigenic sulphides, and, lastly, silicate minerals strongly resistant to the steps of the treatment.

21

22 **3.3. Geochemical fractionation**

23 To determine the reactivity of the mineral phases capable of incorporating metals and
24 metalloids, the leachates from each step of the SEP of the sediments of the Guadiana Estuary
25 were analysed and are shown in Figure 5 as percentages of As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni,
26 Pb, S, and Zn.

27 Considering the mobile fraction (F1+F2+F3) of surficial sediments (Pérez-López et al.,
28 2008), high percentages of As, Cd, Co, Cu, Mn, Pb, S, and Zn (41, 100, 51, 57, 53, 70, 96, and

1 69%, respectively) were recovered. Among the considered elements, Fe, Cr, and Ni have similar
2 distributions, with recovery percentages below 30%. These elements show natural
3 concentrations and are associated with the finer fraction of the sediments and with Al content
4 (**Delgado et al., 2010**). On the other hand, the distribution of Fe (Figure 5A) is important, as the
5 higher proportions (average concentrations above 5000 ppm) were extracted in the reducible
6 fraction (F2), associated with Fe-Mn oxyhydroxides, and thus it plays an important role in the
7 leaching of metals into the environment. Such metals are still labile and may be released upon
8 decomposition of the oxides under the suboxic conditions produced during high tide periods;
9 consequently, they could present a significant risk to soil quality and biota (**Chlopecka, 1996**).

10 Chromium (36 ppm) and Ni (29 ppm) concentrations (Table 3) are low, similar to that of Co,
11 which, in spite of extraction percentages above 51%, has low concentrations (average 18 ppm).
12 These three elements are at close to the background values determined by **Delgado et al.,**
13 **(2009b)** and do not pose overall risks for the estuary. However, recent studies (**Delgado et al.,**
14 **2010**) have demonstrated that, in some areas (e.g. *Castro Marim tidal channel*), Ni can
15 represent an environmental risk, reaching values 12 times above the background of the area.
16 Preferable extraction of Cr in the oxidisable fraction has been previously reported in estuarine
17 environments (e.g. **Pazos-Capeans et al., 2005**), associated with sulphides or with organic
18 matter. The similar extraction of Co, Cr, and Ni in the F2 and F3 stages indicates that Fe oxide
19 (**Sakan et al., 2009**) and sulphide/organic matter, respectively, are the most significant phases
20 for binding these elements.

21 The rest of the studied elements (**As, Cd, Cu, Pb, Zn, and S**) are generally associated with
22 sulphide mineral oxidation processes and the generation of AMD. These elements have the
23 highest extraction percentages, reaching values near 100% in the water-soluble fraction (F1) for
24 Cd as well as rather high percentages of Zn, S, and Mn. This fraction poses the main
25 environmental risk for aquatic ecosystems (**Morillo et al., 2007**) because the metals contained
26 are easily leached in neutral or slightly acidic waters and are thus amenable to assimilation by
27 organisms. For this reason, numerous authors have proposed F1 as the more bio-available
28 fraction (e.g. **Kabata-Pendias, 1993; Alvarez-Valero et al., 2009**) in the environment and

1 could therefore be used to assess the potential ecological risk. These weakly absorbed metals,
2 retained on the sediment surface by relatively weak electrostatic interaction (such as within clay
3 minerals, organic matter, and hydrous oxides), can be released by ion-exchange processes and
4 precipitated or co-precipitated with the carbonates present in many soils (**Marín et al., 1997;**
5 **Filgueiras et al., 2004**).

6 The hazardousness of Cd should be highlighted, because it was recovered in the F1 fraction
7 up to 88% in all samples of the estuary (mean value of 0.15 ppm), with particularly high
8 concentrations in samples such as CAN-1 (0.5 ppm), S-00-15 (0.7 ppm), and LEZ-6 (1.4 ppm).

9 The high percentages of Cd obtained from F1 leaching could be related to the selective
10 oxidisation of the pyrite and to the formation of secondary carbonates (siderite) capable of
11 retaining heavy metals. This notion is supported by its high affinity with Ca under alkaline and
12 oxidising conditions; Cd with Ca, which showed high concentrations in this phase, were
13 released from carbonates. Apart from this source, which is clearly related to AMD, Cd can also
14 be released from several sources in the area, including fuel combustion, phosphate fertilizers,
15 road traffic, sewage sludge, and waste disposal (**Hutton, 1983**), all related to demographic
16 pressure in the vicinity of the mouth of the Guadiana River.

17 The mean recovery percentages for Mn in F1 were 25% (around 124 ppm), but they reached
18 maximum values of 45% in samples RG-17, CARR-11, and CAR-11. High values of Mn
19 associated with F1 have been reported in other sediment studies (**Sakan et al., 2009; Chen et**
20 **al., 2010**). In this case, Mn^{2+} can be more easily adsorbed on the surfaces of fine granules than
21 can other ions, and it can be precipitated under higher pH conditions ranging from 8.5 to 10
22 (**Lui et al., 2005**), a range that has been determined in the study area by **Delgado et al.,**
23 **(2009a)**. Furthermore, it could interact with CO_3^{2-} to form $MnCO_3$ (*rhodochrosite*).

24 Zinc was extracted in F1 in average proportion of 36% of the total concentration (66 ppm)
25 and S in mean proportion of 42%. These elements are likely related to water or acid-soluble
26 sulphidic compounds, typical of metal-enriched environments (e.g. **Cánovas et al., 2007;**
27 **Barba-Brioso et al., 2009**), and to the formation of organo-metallic compounds associated with
28 the F3 fraction, as previously documented in the IPB (**Morillo et al., 2008**).

1 Lead and Cu are mainly associated with the reducible fraction (F2), accompanied by
2 noticeable proportions of Zn, attaining mean values of 65, 35 and 26%, respectively.
3 Concentrations of these elements as high as 22, 20 and 50 ppm, respectively, were extracted,
4 similar to the results reported in several previous studies (e.g., **Morillo et al., 2008; Wang et**
5 **al., 2009**). The accumulation of hazardous metals in the reducible fraction is in accordance with
6 many studies showing that Fe-Mn-oxyhydroxides are important scavengers of these metals in
7 soils and that they play an important role in controlling their mobility in the environment (**Burt**
8 **et al., 2003; Kaasalainen and Yli-Halla, 2003; Alvarez et al., 2006; Davidson et al., 2006**).
9 Arsenic also appears to be associated with F2, probably related to Fe-Mn oxyhydroxides, as
10 arsenate is commonly adsorbed onto Fe (III) hydroxides (**Dold and Fontboté, 2001**). This
11 element shows slightly higher mean values in the oxidisable fraction (F3) (12% of the total
12 concentration, 7 ppm), mainly associated with S (mean values 52%, 29 ppm). These
13 concentrations are probably related to the authigenic Fe-Cu sulphide formation and organic
14 matter, which play an important role in controlling the mobilization of As (**Bhuiyan et al.,**
15 **2010**).

16 The elements associated with AMD processes in the inner zones of the GRB (**Delgado et al.,**
17 **2009**) occur in the same association in the Guadiana sediments (**Delgado et al., 2010**).
18 Moreover, recent isotopic studies (**Company et al., 2008**) confirm their association with
19 polymetallic, massive sulphides upstream. Nonetheless, there exist other discrete sources, such
20 as port activities, that are likely to be adding Zn and Pb (paint and fuel), or traffic on the
21 international bridge, contributing to levels of Pb (**Delgado et al., 2010**) and to levels of other
22 traffic-related elements such as As and Cd.

23

24 ***3.4. Relationship between variables: PCA***

25 Principal components analysis of the association of metal-metalloids with different reactive
26 phases showed that the variables analysed account for 62, 66, 66, and 73% of the total sample
27 variability from steps F1, F2, F3, and F4, respectively (Figure 6). In fact, the PCA simulation
28 shows two groups of elements with a high level of association (correlation coefficient > 0.5) in

1 step 1 of SEP (F1 fraction, water-soluble fraction and carbonates) based on the factor loading
2 obtained. Group I is characterized by elements such as As, Cd, Co, Cr, Cu, Ni, and Zn
3 preferentially associated with S, suggesting the association of metal-metalloid elements with
4 sulphated salts precipitated during dry periods (Figure 6). Group II is characterized by an
5 association of Fe, Ca, Mn, and Pb, which confirms the presence of secondary carbonate
6 precipitates, such as *siderite* (Fe carbonate) or *rhodochrosite* (Mn carbonate), capable of
7 retaining metallic elements.

8 The analysis for step 2 (F2, reducible fraction) of SEP is characterized by the distribution of two
9 groups of elements. Group I, composed of As, Cd, Cr, Cu, Zn, and Pb associated with Fe,
10 confirms the important role of Fe oxyhydroxides in the retention of the metallic elements in the
11 F2 fraction. Group II contains elements not associated with the Fe oxyhydroxides, such as Co,
12 Ni, Ca, and Mn.

13 The analysis for F3 (oxidisable fraction) seems to show the relationship between the trace
14 elements and sulphides/organic matter. Figure 6 shows a concentrated group of elements (S, Fe,
15 Cu, and Zn) that confirm this association. Finally, for F4 (residual fraction), there is no clear
16 relationship evident between the elements. However, a visual assessment of the distribution of
17 elements in Figure 6 shows that a group of elements (Mn, Fe, Cu, Co, Cr, Ni, and Zn)
18 characterized by their affinity to the silicate minerals can be distinguished.

20 ***3.5. Ecological risk assessment***

21 It should be noted that the RAC does not take into account the total metal concentration
22 (Keller and Hammer 2004). However, the RAC code may be useful in assessing the
23 environmental risk using sequential extractions as a characterization method (**Jain, 2004; Singh
24 et al., 2005; Liu et al., 2009; Rodriguez et al., 2009**). Based on this premise, the RAC results
25 show that Cd (FI = 88.01%) represents a very high ecological risk, and Zn (FI = 36.13%) and
26 Mn (FI = 25%) a medium risk. The rest of the metals (except Pb with FI = 0.19%, no risk)
27 represent a low environmental risk in the Guadiana Estuary (Figure 5 and Table 4).

1 Other parameters have been used in the literature to estimate the risk associated with metals
2 and metalloids in aquatic environments. The **pollution load index (PLI)** (**Wilson and Jeffrey,**
3 **1987; S. Caeiro et al., 2005; Bhuiyan et al., 2010**) and the **degree of contamination (Dc)**
4 (**Hakanson et al., 1980; Chen et al., 2010**) are based on the sum of the contamination factors
5 (C_f^i), equivalent to the enrichment factors (EF). In general, the highest values of PLI (Table 4)
6 are indicative of the highest environmental risk for the Guadiana Estuary and are particularly
7 high in areas close to coastal populations (S-00-15; CAN-1; CAN-9; CARR-4), near the
8 international bridge (S-00-5), and in the Castro Marim tidal channel (LEZ-6; RG-3), all sites
9 that have been proposed by **Delgado et al. (2010)** as ecologically vulnerable. Similarly, the high
10 Dc values present in most of the estuary correspond to the most vulnerable areas identified
11 using the PLI, with the risk ranges established varying between moderate and very high in all
12 cases.

13 In spite of the good quality of these indexes for representing environmental risks, they do not
14 take into consideration the toxic response to the elements (T_r^i), based mainly on the effects of
15 bio-production and of lipophilic substances engaged in the pattern of metals (**Hakanson et al.,**
16 **1980**). For this reason, the potential ecological risk (Table 4) should be taken into consideration
17 for sustainable development (**Chen et al., 2010**). The **RI (Hakanson et al., 1980)** provides
18 information regarding the potential ecological risk that the sediments could pose in aquatic
19 environments resulting from the release of metals. For this reason, RI has been used frequently
20 as a diagnostic tool for water pollution control purposes, but the RI considers that the source of
21 contamination in water is the sediments and has therefore been used in a wide range of
22 ecological risk assessments of heavy metals in sediments. The calculations of RI values in the
23 present study have identified some environmental risk ranges lower than those for the other
24 reported indexes, although most of the vulnerable sites listed above remain consistent in the new
25 calculations. Samples such as S-00-5, S-00-12, and S-00-15 continue to indicate high ecological
26 risk values, while samples such as LEZ-6, CAN-1, and CARR-4 decrease from very high to
27 considerable risk, and others such as CAN-9 and RG-3 change from very high to moderate risk.

1 Sequential extraction investigations, widely described and used in the literature, normally
2 estimate the potential risk of a waste-soil-sediment based on relative comparison. However,
3 these indexes are an approach to environmental risk associated with metals and the effects they
4 may cause in this environment of high ecological value. These effects are currently not
5 significant, but they could be more evident with the human impacts on the environment such as
6 the recent proposal to dredge the main channel of the estuary to increase tourism in the
7 emergent coastal area. Nevertheless, the results obtained using the most relevant potential
8 toxicity indexes suggest that a toxicological study should be made for a better characterization
9 of the total bioavailability of pollutants in the study area.

11 **4. Conclusions**

13 This research has examined the ecological risks posed by toxic elements in the surficial
14 sediments of the Guadiana Estuary on the border between Spain and Portugal. A mineralogical
15 study of the sediments showed that the main components of the sediments are quartz, albite,
16 illite, smectite-kaolinite, and vermiculites. Although XRD did not identify reactive phases
17 capable of containing potentially mobilised metals, the SEM analysis showed the presence of
18 soluble sulphated salts, low crystalline Fe-Mn oxyhydroxides, and authigenic pyrite, all capable
19 of retaining elements such as As, Cd, Cu, Pb, and Zn.

20 The association of Fe, Ca, Mn, and Pb obtained in the F1 (mobile fraction) of the PCA points
21 to the possible presence of secondary carbonate precipitates, such as *siderite* or *rhodochrosite* ,
22 capable of retaining metallic elements. The analysis also revealed a relationship between metal-
23 metalloids and Fe oxyhydroxides in F2 (reducible fraction) and between trace elements and
24 sulphides/organic matter in F3 (oxidisable fraction).

25 Based on the BCR F1 extraction percentages, the elements can be ordered in terms of their
26 potential hazards: Cd > Zn > Mn > Cu > As > Pb. Elements such as Co, Cr, Ni, and Fe are
27 present in sediments at natural (background) concentrations and do not pose any environmental

1 risk, although the pattern of Fe oxyhydroxides may play an important role in the release of
2 metals.

3 Because of the high F1+F2+F3 extraction percentages of Cd, Zn, Mn, Cu, As, and Pb, these
4 elements could pose a potential environmental risk, especially for biota. Cadmium is of
5 particular concern because it is highly toxic, while Zn and Mn have very high concentrations in
6 the mobile and consequently more available fraction (F1) of the sediments.

7 Overall, environmental risk analysis for the metals investigated shows a moderate to
8 considerable ecological risk over almost the entire estuary, associated mainly with acid mining
9 drainage processes in the nearby Iberian Pyrite Belt. In addition, the analysis reveals the
10 existence of discrete zones of extremely high risk, particularly related to high concentrations of
11 As, Cd, Zn, Pb, and Hg, probably derived from industrial and urban dumps in the vicinity of the
12 estuary and from heavy volumes of traffic on the international bridge. The results of this
13 research should provide a reference point for future activities affecting the sediments of the
14 Guadiana estuary, such as construction of new bridges or dredging of the main channel to
15 improve its navigability.

16

17 **Acknowledgements**

18

19 This study was developed within the framework of the project INTERREG IIIA MEGASIG:
20 "Monitoring and environmental management of the Guadiana estuary wetlands". J. Delgado
21 acknowledges a Ph.D. grant from the Huelva University. We also thank the editor and reviewers
22 for their help in improving the manuscript.

23

24

25

26

27

28

1 **References**

- 2
- 3 Álvarez JM, López-Valdivia LM, Novillo J, Obrador A, Rico MI. Comparison of EDTA and
4 sequential extraction tests for phytoavailability prediction of manganese and zinc in
5 agricultural alkaline soils. *Geoderma* 2006;132:450–463.
- 6 Álvarez-Valero AM, Pérez-López R, Matos J, Capitán M A, Nieto J M, Sáez R, Delgado J,
7 Caraballo M. Potential environmental impact at São Domingos mining district (Iberian
8 Pyrite Belt, SW Iberian Peninsula): Evidence from a chemical and mineralogical
9 characterization. *Environmental Geology* 2008;55(8):1797-1809.
- 10 Álvarez-Valero AM, Sáez R, Pérez-López R, Delgado J, Nieto JM. Evaluation of heavy metal
11 bio-availability from Almagrera pyrite-rich tailings dam (Iberian Pyrite Belt, SW Spain)
12 based on a sequential extraction procedure. *Journal of Geochemical Exploration*
13 2009;102(2):87-94.
- 14 Arain MB, Kazi TG, Jamali MK, Afridi HI, Jalbani N, Sarfraz RA, Baig JA, Kandhro GA,
15 Memon MA. Time saving modified BCR sequential extraction procedure for the
16 fraction of Cd, Cr, Cu, Ni, Pb and Zn in sediment samples of polluted lake. *Journal of*
17 *Hazardous Materials* 2008;160(1):235-239.
- 18 Ayyamperumal T, Jonathan MP, Srinivasalu S, Armstrong-Altrin JS, Ram-Mohan V.
19 Assessment of acid leachable trace metals in sediment cores from River Uppanar,
20 Cuddalore, Southeast coast of India. *Environmental Pollution* 2006;143(1):34-45.
- 21 Barba-Brioso C, Fernández-Caliani JC, Miras A, Galán E. Evaluation of labile metal pools in
22 soils from a highly industrialised wetland area of southwestern Spain by single and
23 sequential extraction methods. *Geochimica et Cosmochimica Acta Supplement*
24 2009;73:A85.
- 25 Bhuiyan MAH, Parvez L, Islam MA, Dampare SB, Suzuki S. Heavy metal pollution of coal
26 mine-affected agricultural soils in the northern part of Bangladesh. *Journal of*
27 *Hazardous Materials* 2010;173(1-3): 384-392.
- 28 Borrego J, Morales JA, Pendón JG. Holocene estuarine facies along the mesotidal coast of
29 Huelva, south-western Spain. In Flemming, B.W. and Bartholoma, A., editors, *Tidal*
30 *signatures in modern and ancient sediments*, Oxford: International Association of
31 *Sedimentologists Special Publication* 1995;24: 151-69.
- 32 Boski T, Camacho S, Moura D, Fletcher W, Wilamowski A, Veiga-Pires C, Correia V, Loureiro
33 C, Santana P. Chronology of the sedimentary processes during the postglacial sea level
34 rise in two estuaries of the Algarve coast, Southern Portugal. *Estuarine, Coastal and*
35 *Shelf Science* 2008;77(2): 230-244.

- 1 Boski T, Moura D, Veiga-Pires C, Camacho S, Duarte D, Scott DB, Fernandes SG. Postglacial
2 sea-level rise and sedimentary response in the Guadiana Estuary, Portugal/Spain border.
3 *Sedimentary Geology* 2002;150(1-2):103-122.
- 4 Burt R, Wilson MA, Keck TJ, Dougherty BD, Strom DE, Lindahl JA. Trace element speciation
5 in selected smelter-contaminated soils in Anaconda and Deer Lodge Valley, Montana,
6 USA. *Adv. Environ. Res.* 2003;8: 51–67
- 7 Butler IB, Rickard D. Framboidal pyrite formation via the oxidation of iron (II) monosulfide by
8 hydrogen sulphide. *Geochim Cosmochim Acta* 2000;64:2665–72.
- 9 Caeiro S, Costa MH, Ramos TB, Fernandes F, Silveira N, Coimbra A, Medeiros G, Painho M.
10 Assessing heavy metal contamination in Sado Estuary sediment: An index analysis
11 approach. *Ecological Indicators* 2005;5(2): 151-169.
- 12 Cánovas CR, Olías M, Nieto JM, Sarmiento AM, Cerón JC. Hydrogeochemical characteristics
13 of the Tinto and Odiel Rivers (SW Spain). Factors controlling metal contents. *Science*
14 *of the Total Environment* 2007;373(1): 363-382.
- 15 Cappuyns V, Swennen R, Nielaes M. Application of the BCR sequential extraction scheme to
16 dredged pond sediments contaminated by Pb-Zn mining: A combined geochemical and
17 mineralogical approach. *Journal of Geochemical Exploration* 2007;93(2): 78-90.
- 18 Company R, Serafim A, Lopes B, Cravo A, Shepherd TJ, Pearson G, Bebianno MJ. Using
19 biochemical and isotope geochemistry to understand the environmental and public
20 health implications of lead pollution in the lower Guadiana River, Iberia: A freshwater
21 bivalve study. *Science of the Total Environment* 2008;405(1-3):109-119.
- 22 Chen C, Lu Y, Hong J, Ye M, Wang Y, Lu H. Metal and metalloid contaminant availability in
23 Yundang Lagoon sediments, Xiamen Bay, China, after 20 years continuous
24 rehabilitation. *Journal of Hazardous Materials* 2010;175(1-3):1048-1055.
- 25 Chlopecka A. Assessment of form of Cd, Zn and Pb in contaminated calcareous and gleyed
26 soils in southwest Poland. *Science of the Total Environment* 1996;188:253–262.
- 27 Davidson CM, Urquhart GJ, Ajmone-Marsan F, Biasioli M, da Costa Duarte A, Díaz-Barrientos
28 E, Grčman H, Hossack I, Hursthouse AS, Madrid L, Rodrigues S, Zupan M.
29 Fractionation of potentially toxic elements in urban soils from five European cities by
30 means of a harmonised sequential extraction procedure. *Anal. Chim. Acta*
31 2006;565:63–72.
- 32 Delgado J, Sarmiento A, Condesso de Melo M, Nieto J. Environmental Impact of Mining
33 Activities in the Southern Sector of the Guadiana Basin (SW of the Iberian Peninsula).
34 *Water, Air, & Soil Pollution* 2009;199(1): 323-341.
- 35 Delgado J, Nieto JM, Boski T. Mine-related pollution in the Guadiana Estuary (SW Iberia).
36 *Geochimica et Cosmochimica Acta* 2009;73(Number 13S):A276.

- 1 Delgado J, Nieto J M, Boski T. Analysis of the spatial variation of heavy metals in the Guadiana
2 Estuary sediments (SW Iberian Peninsula) based on GIS-mapping techniques.
3 Estuarine, Coastal and Shelf Science 2010;88(1):71-83.
- 4 Dold B, Fontboté L. Element cycling and secondary mineralogy in porphyry copper tailings as a
5 function of climate, primary mineralogy, and mineral processing. Journal of
6 Geochemical Exploration 2001;74(1-3):3-55.
- 7 Dold B. Speciation of the most soluble phases in a sequential extraction procedure adapted for
8 geochemical studies of copper sulfide mine waste. Journal of Geochemical Exploration
9 2003;80(1):55-68.
- 10 Fernández-Caliani JC, Munoz FR, Galán E. Clay mineral and heavy metal distributions in the
11 lower estuary of Huelva and adjacent Atlantic shelf, SW Spain. Science of The Total
12 Environment 1997;198(2):181-200.
- 13 Fernández-Caliani, JC, Barba-Brioso C, González I, Galan E. Heavy metal pollution in soils
14 around the abandoned mine sites of the Iberian Pirite Belt (Southwest Spain). Water
15 Air and Soil Pollution 2009;200:211-226.
- 16 Filgueiras AV, Lavilla I, Bendicho C. Evaluation of distribution, mobility and binding
17 behaviour of heavy metals in surficial sediments of Louro River (Galicia, Spain) using
18 chemometric analysis: a case study. Sci Total Environ 2004;330:115–129.
- 19 Fuentes A, Lloréns M, Sáez J, Soler A, Aguilar MI, Ortuño JF, Meseguer VF. Simple and
20 sequential extractions of heavy metals from different sewage sludges. Chemosphere
21 2004;54(8):1039-1047.
- 22 Gibson MJ, Farmer JG. Multi-step sequential chemical extraction of heavy metals from urban
23 soils. Environmental Pollution Series B: Chemical and Physical 1986;11(2):117-135.
- 24 Guillén MT, Delgado J, Albanese S, Nieto JM, Lima A, De Vivo B. Environmental
25 geochemical mapping of Huelva municipality soils (SW Spain) as a tool to determine
26 background and baseline values. Journal of Geochemical Exploration 2011;109:59-69.
- 27 Haese RR. The iron reactive. En H.D. Schulz y M Zabel, Marine geochemistry. Springer-
28 Verlag, Berlin-Heidelberg; 2001. p. 233-261.
- 29 Håkanson L. An ecological risk index for aquatic pollution control. A sedimentological
30 approach. Water Research 1980;14(8):975-1001.
- 31 Hawkins S, Rimmer SM. Pyrite framboid size and size distribution in marine black shales: a
32 case study from the Devonian–Mississippian of central Kentucky. GSA Joint Annual
33 Meeting (April 3–5), Lexington, Kentucky; 2002.
- 34 Hutton M. Sources of Cadmium in the Environment. Ecotoxicology and Environmental Safety
35 1983;7:9-24.
- 36 Jain CK. Metal fractionation study on bed sediments of river Yamuna, India. Water Res
37 2004;38:569–578.

- 1 Kaasalainen M, Yli-Halla M. Use of sequential extraction to assess metal partitioning in soils.
2 Environ Pollut 2003;126:225–233.
- 3 Kabala C, Singh BR. Fractionation and mobility of copper, lead and zinc in soil profiles in the
4 vicinity of a copper smelter. J. Environ. Qual. 2001;30:485–492.
- 5 Kabata-Pendias A. Behavioural properties of trace metals in soils. Applied Geochemistry
6 1993;(Suppl. 2):3-9.
- 7 Kaiser H. F. The varimax criterion for analytic rotation in factor analysis, Psychometrika
8 1958;23:187-200.
- 9 Karbassi AR, Shankar R. Geochemistry of two sediment cores from the west coast of India, Intl.
10 J. Environ. Sci. Technol. 2005;1:307–316.
- 11 Keller C, Hammer D. Metal availability and soil toxicity after repeated croppings of *Thlaspi*
12 *caerulescens* in metal contaminated soils. Environ Pollut 2004;131:243–254.
- 13 Kribek B. The origin of framboidal pyrite as a surface effect of sulphur grains, Miner. Deposita
14 1975;10:389–396.
- 15 Li X, Shen Z, Wai O WH, Li YS. Chemical Forms of Pb, Zn and Cu in the Sediment Profiles of
16 the Pearl River Estuary. Marine Pollution Bulletin 2001;42(3);215-223.
- 17 Liu J, Li Y, Zhang B, Cao J, Cao Z, Domagalski J. Ecological risk of heavy metals in sediments
18 of the luan river source water. Ecotoxicology 2009;18(6):748-758.
- 19 Love LG. Micro-organisms and the presence of syngenetic pyrite: Quart. Jour. Geol. Soc. Lond.
20 1957;113:429-440.
- 21 Lovley DR, Phillips EJP. Availability of ferric iron for microbial reduction in botton sediments
22 of the freshwater tidal Potomac river. Appl Environ Microbiol 198;652:751-757.
- 23 Marín B, Valladón M, Polve M, Monaco A. Reproducibility testing of a sequential extraction
24 scheme for the determination of trace metal speciation in a marine reference sediment
25 by inductively coupled plasma-mass spectrometry. Anal Chim. Acta 1997;342:91–112.
- 26 Martin JD. X Powder. Quantitative and Qualitative Powder X-ray Diffraction Analysis, version
27 2004.04. Orion Network Communication: Granada. 2004. Available from:
28 <http://www.XPowder.com>.
- 29 Morales JA. Evolution and facies architecture of the mesotidal Guadiana River delta (S.W.
30 Spain-Portugal). Marine Geology 1997;138(1-2):127-148.
- 31 Morillo J, Usero J, Gracia I. Partitioning of Metals in Sediments From the Odiel River (Spain).
32 Environment International 2002;28(4):263-271.
- 33 Morillo J, Usero J, Gracia I. Potential Mobility of Metals in Polluted Coastal Sediments in Two
34 Bays of Southern Spain. Journal of Coastal Research 2007;23(2):352-361.
- 35 Morillo J, Usero J, Rojas R. Fractionation of metals and As in sediments from a biosphere
36 reserve (Odiel salt marshes) affected by acidic mine drainage. Environmental
37 Monitoring and Assessment 2008;139(1-3):329-337.

- 1 Morral FR. A mini-history of the Rio Tinto (Spain) region. *Can Inst Min Metall Bull*
2 1990;83:150–154.
- 3 Nieto JM, Sarmiento AM, Olías M, Cánovas CR, Riba I, Kalman J, Del Valls TA. Acid mine
4 drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and
5 bioavailability of the transported metals to the Huelva Estuary. *Environment*
6 *International* 2007;33:445–455.
- 7 Nocete F, Alex E, Nieto JM, Sáez R, Bayona MR. An archaeological approach to regional
8 environmental pollution in the south-western Iberian Peninsula related to Third
9 millennium BC mining and metallurgy. *Journal of Archaeological Science*
10 2005;32(10):1566-1576.
- 11 Oelkers EH, Poitrasson F. An experimental study of the dissolution stoichiometry and rates of a
12 natural monazite as a function of temperature from 50 to 230 °C and pH from 1.5 to 10.
13 *Chem Geol* 2002;191:73-87.
- 14 Ohfuji H, Rickard D. Experimental syntheses of framboids--a review. *Earth-Science Reviews*
15 2005;71(3-4):147-170.
- 16 Otero XL, Macías F. Variation with depth and season in metal sulfides in salt marsh soils.
17 *Biogeochemistry* 2002;62:59-86.
- 18 Otero XL, Macías F. Seasonal variation in heavy metals in interstitial water. *Environ. Pollut*
19 2002;120:183-190.
- 20 Pazos-Capeáns P, Barciela-Alonso MC, Bermejo-Barrera A, Bermejo-Barrera P. Chromium
21 available fractions in arousa sediments using a modified microwave BCR protocol
22 based on microwave assisted extraction. *Talanta* 2005;65(3):678-685.
- 23 Peng J-f, Song Y-h, Yuan P, Cui X-y, Qiu G-l. The remediation of heavy metals contaminated
24 sediment. *Journal of Hazardous Materials* 2009;161(2-3):633-640.
- 25 Pérez M, Usero J, Gracia I, Cabrera F. Trace metals in sediments from the Ria de Huelva,
26 *Toxicol Environ Chem* 199;131:275–283.
- 27 Pérez-López R, Álvarez-Valero AM, Nieto JM, Sáez R, Matos JX. Use of sequential extraction
28 procedure for assessing the environmental impact at regional scale of the São Domingos
29 Mine (Iberian Pyrite Belt). *Applied Geochemistry* 2008;23(12):3452-3463.
- 30 Pérez-López R, Delgado J, Nieto JM, Márquez-García B. Rare earth element geochemistry of
31 sulphide weathering in the São Domingos mine area (Iberian Pyrite Belt): A proxy for
32 fluid–rock interaction and ancient mining pollution. *Chemical Geology* 2010;276:29-
33 40.
- 34 Pérez-Santana S, Pomares AM, Villanueva TM., Peña Icart M, Brunori C, Morabito R. Total
35 and partial digestion of sediments for the evaluation of trace element environmental
36 pollution. *Chemosphere* 2007;66(8):1545-1553.

- 1 Perin G, Craboledda L, Lucchese M, Cirillo R, Dotta L, Zanette ML, Orio AA. Heavy metal
2 speciation in the sediments of Northern Adriatic Sea – a new approach for
3 environmental toxicity determination. In: Lekkas, T.D. (Ed.), Heavy Metal in the
4 Environment. 1985. p 454–456.
- 5 Quevauviller P, Rauret G, López-Sánchez JF, Rubio R, Ure A, Muntau H. Certification of trace
6 metal extractable contents in a sediment reference material (CRM 601) following a
7 three-step sequential extraction procedure. *Science of the Total Environment*
8 1997;205(2-3):223-234.
- 9 Rauret G, López-Sánchez JF, Sahuquillo A, Rubio R, Davidson C, Ure AM, Quevauviller Ph.
10 Improvement of the BCR three step sequential extraction procedure prior to the
11 certification of new sediment and soil reference materials. *J Environ Monitor*
12 1999;1:57–61.
- 13 Rodríguez L, Ruiz E, Alonso-Azcárate J, Rincón J. Heavy metal distribution and chemical
14 speciation in tailings and soils around a Pb-Zn mine in Spain. *Journal of Environmental*
15 *Management* 2009;90(2):1106-1116.
- 16 Romero A, González I, Galán E. Estimation of potential pollution of waste mining dumps at
17 Peña del Hierro (Pyrite Belt, SW Spain) as a base for future mitigation actions. *Applied*
18 *Geochemistry* 2006;21(7):1093-1108.
- 19 Ruiz F. Trace Metals in Estuarine Sediments from the Southwestern Spanish Coast. *Marine*
20 *Pollution Bulletin* 2001;42(6):481-489.
- 21 Sundaray SK, Nayak BB, Lin S, Bhatta D. Geochemical speciation and risk assessment of
22 heavy metals in the river estuarine sediments-A case study: Mahanadi basin, India.
23 *Journal of Hazardous Materials* 2011;186(2-3):1837-1846.
- 24 Sahuquillo A, López-Sánchez JF, Rubio R, Rauret G, Thomas RP, Davidson CM, Ure AM. Use
25 of a certified reference material for extractable trace metals to assess sources of
26 uncertainty in the BCR three-stage sequential extraction procedure. *Analytica Chimica*
27 *Acta* 1999;382(3):317-327.
- 28 Sakan SM, Đorđević DS, Manojlović DD, Predrag PS. Assessment of heavy metal pollutants
29 accumulation in the Tisza river sediments. *Journal of Environmental Management*
30 2009;90(11):3382-3390.
- 31 Sarmiento AM, Olías M, Nieto JM, Cánovas C, Delgado J. Natural attenuation processes in two
32 water reservoirs receiving mine acid drainage. *Science of the Total Environment*
33 2009;407:2057-2062.
- 34 Siivola J, Schmid R. A systematic nomenclature for metamorphic rocks: 12. List of mineral
35 abbreviations. Recommendations by the IUGS Subcommission on the Systematics of
36 Metamorphic Rocks. 2007. Recommendations, web version of 01.02.2007.

- 1 Singh KP, Mohan D, Singh VK, Malik A. Studies on distribution and fractionation of heavy
2 metals in Gomti river sediments – a tributary of the Ganges, India. *J Hydrol*
3 2005;312:14–27.
- 4 Tessier A, Campbell PGC, Bisson M. Sequential extraction for the speciation of particulate
5 trace metals. *Anal Chem* 1979;51:844–851.
- 6 Usero J, Morillo J, Gracia I. Comparative Study of Three Sequential Extraction Procedures for
7 Metals in Marine Sediments. *Environment International* 1998;24(4):487-496.
- 8 Usero J, Morillo J, el Bakouri H. A General Integrated Ecotoxicological Method for Marine
9 Sediment Quality Assessment: Application to Sediments From Littoral Ecosystems on
10 Southern Spain's Atlantic Coast. *Marine Pollution Bulletin* 2008;56(12):2027-2036.
- 11 Ure AM, Quevauviller Ph, Muntau H, Griepink B. Speciation of heavy metal in soils and
12 sediments. An account of the improvement and harmonisation of extraction techniques
13 undertaken under the auspices of the BCR of the Commission of the European
14 Communities. *Int J Environ An Ch* 199;351:135–151.
- 15 Vanek A, Boruvka L, Drabek O, Mihaljevic M, Komarek M. Mobility of lead, zinc and
16 cadmium in alluvial soils heavily polluted by smelting industry, *Plant Soil Environ*.
17 2005;51(7):316–321.
- 18 Wang L, Yu R, Hu G, Tu X. Speciation and assessment of heavy metals in surface sediments of
19 Jinjiang River tidal reach, southeast of China. *Environmental Monitoring and*
20 *Assessment* 2009;1-9.
- 21 Weisz M, Polyák K, Hlavay J. Fractionation of elements in sediment samples collected in rivers
22 and harbors at lake Balaton and its catchment area. *Microchem J* 2000;67:207–217.
- 23 Wilson JG, Jeffrey DW. Europe-wide indices for monitoring estuarine quality. In: Kramer,
24 K.J.M. (Ed.), *Biological Indicators of Pollution*. Royal Irish Academy, Dublin, Ireland.
25 1987. p 225–242.
- 26 Wu Y, Hou X, Cheng X, Yao S, Xia W, Wang S. Combining geochemical and statistical
27 methods to distinguish anthropogenic source of metals in lacustrine sediment: A case
28 study in Dongjiu Lake, Taihu Lake catchment, China. *Environmental Geology*
29 2007;52(8):1467-1474.
- 30 Yu R, Hu G, Wang L. Speciation and ecological risk of heavy metals in intertidal sediments of
31 Quanzhou Bay, China. *Environmental Monitoring and Assessment* 2001;163(1):241-
32 252.

	CAN STSD-1			CAN STSD-2			% RPD
	Obtained value	Certified value	% Recovered	Obtained value	Certified value	% Recovered	
Al ₂ O ₃	8.67	9.00	96.3	16.0	16.1	99.4	1.49
Fe ₂ O ₃	6.28	6.50	96.6	7.35	7.50	98.0	0.34
CaO	3.66	3.60	102	4.18	4.00	105	0.66
MnO	0.47	0.50	94.0	0.13	0.10	130	0.06
TiO ₂	0.64	0.80	80.0	0.73	0.80	91.3	0.45
As	19.8	23.0	86.1	33.3	42.0	79.3	0.60
Cd	0.90	<i>not data</i>	-	0.80	<i>not data</i>	-	0.10
Co	16.3	17.0	95.9	20.0	19.0	105	0.40
Cr	54.4	<i>not data</i>	-	102	<i>not data</i>	-	2.14
Cu	34.6	36.0	96.1	41.2	47.0	87.7	0.90
Ni	19.3	24.0	80.4	49.2	53.0	92.8	0.50
Pb	37.4	35.0	107	68.5	66.0	104	0.50
Zn	150	178	84.3	193	246	78.5	0.90

Mayor in %, mirrors in ppm. % RPD (Relative Percentage Difference)

Table 1. Evaluation of the performance of the analysis using the % RPD and comparison with certified values.

STEPS / ELEMENT	As	Cd	Co	Cr	Cu	Ni	Fe	Mn	Pb	S	Zn	Ca	
F1	Obtained value	-	7.05 ± 0.09	-	2.10 ± 0.15	48.0 ± 1.5	14.1 ± 0.1	-	-	2.55 ± 0.01	-	184 ± 7	-
	Certified value	-	7.34 ± 0.35	-	2.26 ± 0.16	49.3 ± 1.7	15.4 ± 0.9	-	-	3.18 ± 0.21	-	205 ± 6	-
		-	-	-	-	-	-	-	-	-	-	-	-
F2	Obtained value	-	3.21 ± 0.02	-	47.0 ± 0.9	138 ± 3	27.5 ± 1.1	-	-	121 ± 3	-	98 ± 6	-
	Certified value	-	3.77 ± 0.28	-	45.7 ± 2.0	124 ± 3	26.6 ± 1.3	-	-	126 ± 3	-	114 ± 5	-
		-	-	-	-	-	-	-	-	-	-	-	-
F3	Obtained value	-	0.09 ± 0.07	-	129 ± 13	46.9 ± 4.9	13.8 ± 3.7	-	-	9.5 ± 1.8	-	43.3 ± 13.3	-
	Certified value	-	0.27 ± 0.06	-	143 ± 7	55.2 ± 4.0	15.3 ± 0.9	-	-	9.3 ± 2.0	-	45.7 ± 4.0	-
		-	-	-	-	-	-	-	-	-	-	-	-
R	Obtained value	-	0.05 ± 0.01	-	52.3 ± 7.7	32.6 ± 3.8	31.3 ± 0.8	-	-	12.0 ± 0.7	-	69.6 ± 0.1	-
	Indicative value	-	0.125 ± 0.075	-	62.5 ± 7.4	38.5 ± 11.2	41.4 ± 4.0	-	-	11.0 ± 5.2	-	94.6 ± 12.2	-
		-	-	-	-	-	-	-	-	-	-	-	-
% Recovery (Eq. 3)	104	47	83	90	91	89	95	89	88	116	86	105	

Certified BCR-701 References Material Values

Table 2. Quality control of data using: (1) measured, certified, and indicative values for extractable amounts in certified reference material BCR-701; and (2) comparative results (% recovery) calculated using Equation 3.

Sample	Al ₂ O ₃	Fe ₂ O ₃	MnO	CaO	TOT/S	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	Zn
BRU 2	15.39	6.13	0.07	0.94	0.07	21.8	0.20	17.4	37	48.5	0.33	26.8	30.5	136
BRU 5	16.46	5.95	0.04	0.63	0.42	29.4	0.40	21.0	40	69.3	0.40	32.1	42.5	234
CAN 1	16.26	6.12	0.05	0.84	0.23	44.6	0.50	20.5	35	64.8	0.57	28.9	47.9	271
CAN 9	16.35	6.31	0.06	0.99	0.53	26.1	0.40	20.9	38	65.8	0.47	30.5	43.4	205
CAR 4	15.78	5.86	0.05	0.81	0.09	21.2	0.20	14.1	32	39.4	0.31	22.7	25.6	123
CAR 12	15.88	6.33	0.11	0.62	0.23	39.6	0.20	16.8	39	49.5	0.24	30.9	39.0	182
CARR 4	13.59	5.04	0.04	0.92	1.22	27.4	0.40	15.1	31	43.3	0.45	23.0	32.3	173
CARR 11	15.29	6.07	0.06	0.84	0.08	30.8	0.10	17.1	37	64.9	0.42	26.0	41.4	181
CARR 15	16.59	6.12	0.04	0.69	0.33	28.8	0.20	16.1	41	66.6	0.53	28.9	46.8	215
LEZ 6	16.82	6.77	0.05	0.85	0.50	55.6	1.40	21.8	36	71.9	0.53	30.8	45.6	483
LEZ 11	16.02	6.20	0.06	0.91	0.18	25.2	0.30	18.5	31	48.0	0.34	28.0	34.0	173
RG 3	14.74	5.24	0.04	0.74	0.38	25.8	0.20	12.6	32	57.6	0.34	24.1	30.2	102
RG 5	16.11	5.52	0.05	0.88	0.13	25.5	0.30	15.3	42	65.3	0.39	29.7	36.4	207
RG 12	16.76	6.45	0.14	1.00	0.06	23.8	0.10	20.8	49	51.5	0.19	41.6	30.0	147
RG 15	17.89	7.18	0.19	0.86	0.05	19.1	0.30	22.6	46	47.5	0.16	38.1	23.9	119
RG 17	18.08	7.06	0.15	0.61	0.03	25.7	0.10	19.9	43	56.6	0.21	34.3	32.1	169
S-00-5	14.08	5.32	0.05	1.41	0.19	16.1	0.20	16.2	31	34.5	4.43	22.7	26.6	108
S-00-12	14.14	5.23	0.06	1.15	0.28	23.4	0.40	18.2	31	49.2	3.76	25.1	29.2	161
S-00-15	14.71	6.97	0.09	1.02	0.13	81.8	0.70	17.5	36	68.9	2.97	29.8	28.1	311
S-4	12.82	4.61	0.06	1.75	0.20	19.4	0.20	13.7	14	32.5	0.27	20.8	22.9	147

Mayor Oxides and total sulfur (TOT/S) in %, Trace Elements in ppm

Table 3. Total concentrations of major elements (presented as oxides) and the main metals and metalloids (presented in mg kg⁻¹) in surficial sediments of the Guadiana Estuary.

Sample	40.00		10.00		30.00		2.00		5.00		5.00		5.00		1.00		PLI ^b	Dc ^c	Risk Range	RI ^d	Risk Range		
	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ	C _r ⁱ	E _r ⁱ							
CAR 4	2.10	84.04	1.49	14.94	1.97	59.15	0.89	-	0.90	1.81	1.47	7.33	0.85	4.24	1.44	7.20	1.81	13.15	12.92	MODERATE	181	MODERATE	
CAR 12	1.62	64.66	2.77	27.73	1.96	58.78	1.05	-	0.98	1.96	1.83	9.15	0.91	4.56	2.18	10.9	2.65	16.45	14.98	CONSIDERABLE	178	MODERATE	
LEZ 6	3.37	134.8	3.68	36.75	12.95	388.5	1.29	-	0.92	1.85	2.51	12.5	1.05	5.27	2.41	12.0	6.65	38.53	33.91	VERY HIGH	597	CONSIDERABLE	
LEZ 11	2.27	90.80	1.75	17.49	2.91	87.40	1.15	-	0.97	1.94	1.76	8.80	0.96	4.78	1.88	9.42	2.50	16.58	15.18	CONSIDERABLE	221	MODERATE	
CAN 1	3.75	150.0	3.05	30.50	4.78	143.5	1.25	-	0.96	1.91	2.34	11.7	0.90	4.48	2.62	13.1	3.86	25.16	22.55	VERY HIGH	357	CONSIDERABLE	
CAN 9	3.07	123.0	1.77	17.75	3.81	114.2	1.27	-	1.03	2.06	2.36	11.8	1.00	5.02	2.36	11.8	2.90	20.42	18.55	VERY HIGH	286	MODERATE	
BRU 2	2.29	91.73	1.57	15.75	2.02	60.65	1.12	-	1.01	2.02	1.85	9.25	1.00	5.00	1.76	8.80	2.05	14.82	13.67	CONSIDERABLE	193	MODERATE	
BRU 5	2.60	104.0	1.99	19.86	3.78	113.4	1.27	-	0.87	1.73	2.47	12.4	1.02	5.08	2.29	11.5	3.29	20.60	18.71	VERY HIGH	269	MODERATE	
S-00-5	33.65	1346	1.10	10.97	2.21	66.30	1.14	-	1.01	2.02	1.44	7.19	0.82	4.09	1.68	8.39	1.78	50.41	43.81	VERY HIGH	1445	VERY HIGH	
S-00-12	28.44	1138	1.47	14.69	4.40	132.0	1.28	-	1.01	2.02	2.04	10.2	0.75	3.77	1.83	9.17	2.64	49.12	42.86	VERY HIGH	1310	VERY HIGH	
S-00-15	21.59	863.8	6.58	65.76	7.40	222.1	1.18	-	0.88	1.76	2.75	13.7	1.04	5.22	1.70	8.48	4.90	54.30	47.14	VERY HIGH	1184	VERY HIGH	
CARR 4	3.54	141.7	2.24	22.42	4.58	137.4	1.10	-	0.86	1.72	1.87	9.35	0.85	4.25	2.11	10.6	2.95	21.43	19.25	VERY HIGH	329	CONSIDERABLE	
CARR 11	2.94	117.5	2.24	22.40	1.02	30.52	1.11	-	1.02	2.03	2.49	12.5	0.83	4.16	2.40	12.0	2.74	17.33	15.78	CONSIDERABLE	202	MODERATE	
CARR 15	3.42	136.7	1.93	19.30	1.88	56.27	0.96	-	0.94	1.87	2.36	11.8	0.91	4.55	2.50	12.5	3.00	18.90	16.96	CONSIDERABLE	244	MODERATE	
RG 3	2.47	98.68	1.95	19.46	2.11	63.33	0.85	-	0.88	1.76	2.29	11.5	12.24	61.2	1.82	9.10	1.60	28.92	25.33	VERY HIGH	265	MODERATE	
RG 5	2.59	103.6	1.76	17.60	2.90	86.91	0.94	-	1.05	2.09	2.38	11.9	1.09	5.43	2.01	10.0	2.98	18.54	16.64	CONSIDERABLE	238	MODERATE	
RG 12	1.21	48.50	1.58	15.79	0.93	27.85	1.23	-	0.93	1.85	1.80	9.02	1.32	6.58	1.59	7.95	2.03	12.36	11.69	MODERATE	118	LOW	
RG 15	0.96	38.26	1.19	11.87	2.61	78.27	1.26	-	1.01	2.03	1.56	7.79	1.28	6.40	1.19	5.93	1.54	12.19	11.57	MODERATE	150	MODERATE	
RG 17	1.24	49.69	1.58	15.80	0.86	25.81	1.09	-	0.93	1.86	1.84	9.19	1.10	5.52	1.58	7.88	2.17	12.25	11.46	MODERATE	116	LOW	
S-4	2.25	90.10	1.50	15.02	2.43	72.81	1.06	-	0.91	1.82	1.49	7.44	0.69	3.43	1.59	7.93	2.66	14.88	13.66	MODERATE	199	MODERATE	
RAC ^a			1.63		86.01				8.96				4.80		0.19		36.13						
Risk Range			LOW		VERY HIGH			LOW		LOW		LOW	LOW		LOW		MEDIUM						

^a Risk assessment code F1 < 1%, no risk; F1 = 1-10%, Low risk; F1 = 11-30% medium risk; F1 = 31-50%, high risk; F1 > 50%, very high risk (Perin, 1985)

^b Pollution load index (Wilson and Jeffrey, 1987)

^c Degree of contamination (Hakanson, 1980)

^d Potential ecological risk (Hakanson, 1980)

C_rⁱ contamination factor

E_rⁱ Potential ecological risk factor

T_rⁱ Toxic response factor

Table 4. Risk indexes and grades of potential ecological risk assessment of sediments polluted by As, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn in the Guadiana Estuary.

Figure Captions

Fig. 1. Map of the study area (Guadiana saltmarshes, SW Iberian Peninsula), showing the different sub-systems of the area and the locations of sample points.

Figure 2. A. Average percentages obtained by “*Xpowder*” semi-quantitative analysis for major minerals and clays in the sediments. **B.** Examples of solvation with Ethylene glycol (sample RG-17) and thermal treatment (sample S-00-15) for distinguishing clay mineral phases. Abbreviations of mineral names recommended by SCMR (Siivola and Schmid, 2007). Qtz, quartz; Ab, albite; Ill, Illite; Sme, smectite; Kln, kaolinite; Chl, Chlorite; Vrm, vermiculite.

Figure 3. Mineralogical characterisation (by SEM) of the surficial samples. **A. Top:** Example of presence of Fe-Mn oxyhydroxides (sample CAN-1), **Bottom:** Phosphorous and lanthanides in surficial sediments; Mnz, monazite (sample CAN-1). **B.** Example of the presence of sulphide minerals in surficial sediments (CAN-1 and LEZ-6 samples); Py (N), newly-formed pyrite and Py (NF) newly-formed framboidal pyrite. **C.** Native sulphur (sample LEZ-6). **D.** Example of accessory minerals: Zrn, zircon and Ttn, titanite.

Figure 4. Mineralogical characterisation (by SEM) of the different steps to the SEP. **A.** Example of the presence of sulphide minerals Py (N), new-formed pyrite and Py (NF) newly-formed framboidal pyrite, associated with Fe-Mn oxyhydroxides (S-00-15). **B.** Phosphorous and lanthanides association; Mnz, monazite (CAN-1). **C.** Accessory minerals in surficial sediments (S-00-5) and main constituents of sediments. **D.** Evolution between steps 1 and 4, progressive destruction of the metallic mineral phases.

Figure 5. Percentages of As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, S, and Zn extracted in each step of the sequential extraction procedure for the surficial sediments of the Guadiana Estuary.

Figure 6. Representation of the principal component analysis (PCA) for the different steps recovered in the SEP. F1, water-soluble fraction and carbonates; F2, reducible fraction; F3, oxidisable fraction; F4, residual fraction. Alpha significance level 0.05.











