Application of a new integrated sediment quality assessment method to Huelva estuary and its littoral of influence (Southwestern Spain)

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HIGHLIGHTS:

- A new integrated sediment quality assessment method was satisfactorily applied to Huelva estuary.
- Bioavailability was assessed with protease K.
- Littoral samples were least degraded.
- Tinto estuary samples were the most degraded.

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Abstract

A new integrated sediment quality assessment method composed of several assays (particle size profile, total metal content, protease K extraction, total organic carbon, toxicity bioassay with *Photobacterium phosphoreum* and macrobenthic community alteration) that provides a single result, the environmental degradation index (EDI), has been developed. The new method was tested on the Huelva estuary (southwest of Spain), a highly polluted area where metals dissolved in the water of the Tinto and Odiel rivers precipitate after flowing through the Iberian Pyrite Belt, one of the largest metallogenic areas of massive sulphide deposits in the world.

The proposed method satisfactorily was able to reflect different degrees of pollution on the environmental degradation index. Thus, EDI categorized littoral samples as slightly degraded and all the Tinto and some of the Odiel as very highly degraded, emphasizing the lower zone of the Tinto estuary as the most deeply degraded of the entire study area.

Keywords

Integrated sediment quality assessment; Trace metals; Bioavailability; Sediment toxicity; Macrobenthic community; Huelva estuary.

1 Introduction

Aquatic ecosystems have suffered the discharge of large amount of waste for decades (Salomons et al., 1987), and pollution is reaching worrying levels in some areas (Soto-Jiménez and Páez-Osuna, 2001). Pollutants are present in aquatic ecosystems in both water and sediments, where they accumulate over time and can reach concentrations that are much higher than those in the water.

However, changes in pH, redox potential, or other environmental conditions can release a portion of the contaminants retained in the sediments and re-solubilize them in water (Ashokkumar et al., 2009). Thus, sediments may act as a sink or a source of contamination and, in the latter case, may affect aquatic life that live, feed and reproduce there, or even humans, through the food chain. Because of this mechanism, contaminants in the sediments of aquatic ecosystems have become one of the most important environmental problem at present (Chapman and Wang, 2001; Ruiz et al., 2008; Sainz and Ruiz, 2006).

In aquatic ecosystems, sediments are the main sink for trace elements, such as heavy metals, and can reach levels many times higher than water column concentrations (Ridgway and Shimmield, 2002). Trace elements are bioavailable and persistent in the environment causing bioaccumulation and toxicity effects in the biota. Typically, benthic invertebrates start bioaccumulation because of their close association with sediments and transfer them to higher trophic levels (Burgos and Rainbow, 2001). The uptake of trace metals by benthic organisms depends largely on their chemical forms (Morillo et al., 2008).

People's awareness of environmental issues, particularly the pollution of aquatic sediments, has led to the development of new methods for assessing their environmental quality, such as: chemical analysis, bioavailability, toxicity and alteration of the macrobenthic communities, etc. Nevertheless, each of these alternatives individually only provides partial information on the environmental quality of the studied ecosystem and is insufficient to give a clear idea of its general status. The latter requires to fully answer the three basic questions that arise in environmental assessment studies (Chapman, 1992): i) What kinds of contaminants are present in the ecosystem? ii) What are the levels of these pollutants? iii) What biological effects do they have on the ecosystem?

Integrated sediment quality assessment methods are used to answer these questions (Anderson et al., 2003; Chapman, 2002; Cherry, 2001; DelValls et al., 1998; Ghirardini et

al., 1999). These methods consist of a group of the above simple methods, ensuring that each provides as much information as possible and that they are used in such a way that they complement each other (Chapman, 1995). Thus, the correlation between the results obtained by different methods provides higher quality information than when they are employed separately (Chapman, 1995).

This research has two main objectives. First, to develop a new integrated sediment quality assessment method that can be implemented over a wide area: applicable to all kinds of sediments, simple, low-cost and with an easily understandable and comparable result. Second, to implement the proposed integrated sediment quality assessment method for the Huelva estuary and its littoral of influence, where natural and anthropogenic sources of pollutants and areas of high ecological and economic value coexist in a relatively small zone.

2 Huelva estuary

The Huelva estuary is located in the southwest of Spain, along the municipalities of Huelva, Punta Umbria and Palos de la Frontera. It is formed by the confluence of the rivers Tinto and Odiel and consists of estuaries of both rivers and the Padre Santo Canal (Figure 1). The estuary of the Odiel has a secondary outlet to the sea known as Punta Umbria estuary. Its waters are influenced by flows from the Tinto and Odiel rivers and by seawater from the Atlantic Ocean.

The Huelva estuary empties into the Atlantic Ocean in the area known as the Huelva littoral. This 145 km-long coast is bounded by the mouths of two major rivers, the Guadiana and the Guadalquivir. The prevailing currents in this area are from west to east and causes littoral drift, a process by which water and sediments are transported along the shoreline. Littoral drift has a high carrying capacity of materials whose deposition tends to fill any irregularities, leaving a smooth and straight coastline, interrupted only by river mouths.

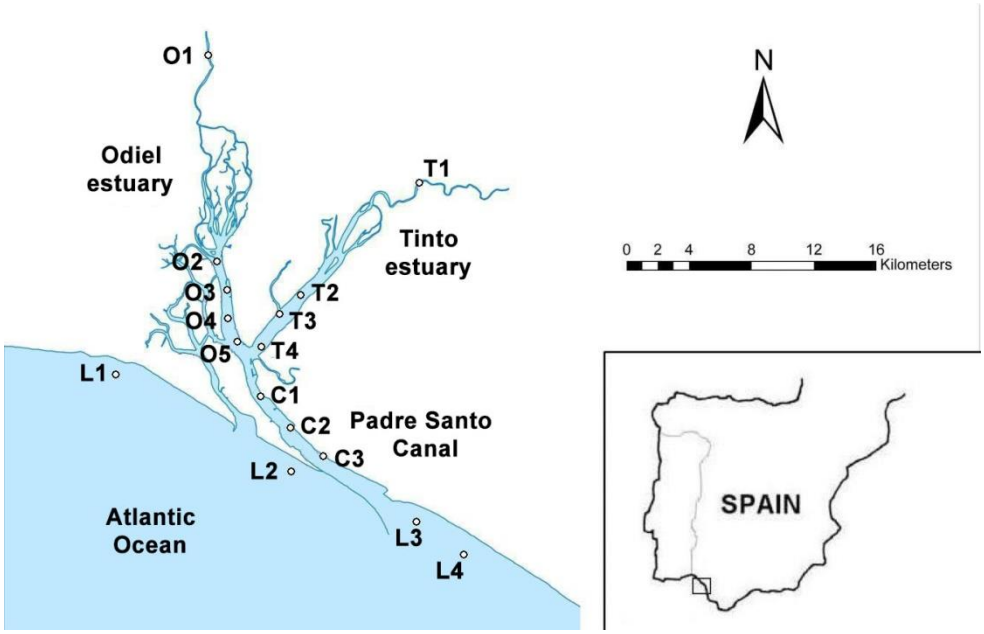

Figure 1. Location of the sampling points in the Huelva estuary.

The Tinto and Odiel rivers flow through the Iberian Pyrite Belt, one of the largest metallogenic areas of massive sulphide deposits in the world (Galán et al., 2003; Leistel et al., 1997). Thus, both waterways are characterised by low pH values (2–4) and contain large amounts of sulphates and sulphide-related metals due to pyrite oxidation (Morillo and Usero, 2008). Furthermore, the basins of the Tinto and Odiel rivers have hosted mining activity since Phoenician and Roman times because of the excellent conditions of the Iberian Pyrite Belt (Sánchez-Moyano and García-Asencio, 2010). Therefore, a great number of abandoned mine sites still contribute to high metal concentrations due to waters emanating from both surface and underground mining (Galán et al., 2003; Morillo and Usero, 2008).

Since the 1960's, Huelva estuary has been surrounded by heavily industrialised areas (phosphate fertilizer plant, power plants, chlor-alkali industry, petroleum refinery and other factories) and additional inputs of potentially toxic elements started to be discharged into the estuary. This waste contains high levels of sulphide-associated heavy metals. A third source of metals for the estuarine environment is the urban sewage from the city of Huelva (Ruiz, 2001).

In spite of this, many protected areas are located close to the Huelva estuary, such as the Odiel Marshes Nature Reserve and Doñana National Park, both wetlands where birds stop for their migrations and, thus, declared biosphere reserves by Unesco, Ramsar sites and

European Union Special Bird Protection Zones (Sánchez-Moyano and García-Asencio, 2010).

3 Materials and methods

3.1 Sampling and sample pre-treatment

16 sampling points were chosen to cover the Huelva estuary and its area of influence (the Huelva littoral), one of them located in a pollution free zone (Usero et al., 2008) to serve as a reference. According to these guidelines, 12 points were located in the Huelva estuary (see Table 1 and Figure 1): 5 in the Odiel River estuary (O1-O5), 4 in the Tinto River estuary (T1-T4) and 3 in the Padre Santo Canal (C1-C3). Four points correspond to the littoral (L1-L4). The reference point (L1) was placed west of the estuary to avoid its influence by prevailing west to east water currents.

Table 1. Coordinates of the sampling points.

Point	North	West
O1	37º22´50´´	6º58´41´´
O2	37º15´44´´	6º57´55´´
O3	37º14´45´´	6º57´24´´
O4	37º13´47´´	6º57´19´´
O5	37º12´35´´	6º56´53´´
T1	37º18´43´´	6º49´17´´
T2	37º14´41´´	6º54´14´´
T3	37º13´48´´	6º55´12´´
T4	37º12´50´´	6º55´51´´
C1	37º11´08´´	6º55´47´´
C2	37º10´06´´	6º54´27´´
C3	37º09´11´´	6º52´58´´
L1	37º11´41´´	7º02´04´´
L2	37º08´35´´	6º54´19´´
L3	37º07´01´´	6º48´51´´
L4	37º05´58´´	6º46´44´´

Sediment samples were taken in October 2013 with a Van Veen grab and in duplicate at each point. Only grabs that achieved adequate penetration (2/3 of total volume) to collect the first 5 cm of the sediment and that showed no evidence of leakage or surface disturbance were retained, transferred and stored in a dark cooler at 4 °C.

In the laboratory, a representative sample from each station was prepared by mixing and homogenizing the two samples taken at each sampling point. Then they were divided into two fractions as shown in Table 2: fraction 1, without any further treatment, to determine particle size profiles, and fraction 2, dried (60 ºC) and sieved (<63 µm), to perform metal analysis (totals and protease K), total organic carbon and the toxicity bioassay. All conventional sediment parameters were expressed as percentages of total dry sample weight (105 ºC).

Five replicate organism samples were collected with a 0.05 m^2 surface Van Veen grab in the same sampling points and dates as the sediment samples. Samples were sieved in situ through a 0.5-mm mesh and were preserved in a solution made of ethanol and bengal rose stain (0.4 g of bengal rose in 1 L of ethanol) for one week.

Table 2. Sample pre-treatment and related assays.

	Drying temperature	Grain size	Assays
Sediment sample (Fraction 1)	-	-	Particle size profile
Sediment sample (Fraction 2)	60 ºC	<63 µm	Total metal content Protease K extraction Total organic carbon Toxicity bioassay
Organism sample	-	>500 µm	Macrobenthic community

3.2 Sediment analysis

3.2.1 Particle size profiles

Particle size profiles were determined by wet sieving the sediments through a column of sieves with different mesh sizes according to the Wentworth classification (Wentworth, 1922) (<4 µm clay; 4-63 µm silt; 63–125 µm very fine sand; 125–250 µm fine sand; 250–500 µm medium sand; 500–1,000 µm coarse sand; 1,000–2,000 µm very coarse sand; 2,000–4,000 µm granulate gravel; 4,000-64,000 µm pebble gravel). Then, the selection coefficient, S_0, proposed by Trask (Trask, 1950) was determined by the equation:

$$S_0 = \sqrt{\frac{Q_{25}}{Q_{75}}}$$

Q_{25} and Q_{75} are 25 and 75 quartiles.

The selection coefficient indicates the homogeneity of the sediment (>2.75 bad; 2.75-1.87 poor; 1.87-1.35 moderate; 1.35-1.20 moderately good; 1.20-1.17 good; <1.17 very good). Thus, a low selection coefficient determines good homogeneity in grain size and a high coefficient corresponds to varied sizes or heterogeneous grains.

3.2.2 Total metal content

To determine total metal content, sediment underwent the method proposed by the BCR (Pueyo et al., 2001), in which 3 g of every sample are digested with 28 ml of aqua regia (commercial nitric and hydrochloric acids, 1:3 by volume) in a reaction flask with a reflux condenser and a sand bath. Then, the extract was centrifuged 20 min at 4,200 rpm (3,000 g) to separate the solid and liquid phases.

The analysis of Zn, Cu, Cd, Mn, Pb, Ni, Fe, and Cr in the solutions obtained following sample digestion was carried out by atomic absorption spectrophotometry (AAS) using a double-beam Perkin–Elmer 2380 AAS with deuterium background correction and, in the case of low concentrations, by graphite furnace atomic absorption spectrometry (GFAAS) using a Varian spectra 220 with Zeeman-effect background correction. The hydride-generation technique was used to determine As and Se, and the cold vapor technique was utilized for Hg, both employing a Perkin–Elmer MSH-10 connected to the Perkin–Elmer 2380 spectrophotometer.

Total metal content determination was tested using the reference material, BCR-701. The concentrations obtained for each of the elements analyzed demonstrated recoveries greater than 90%, which can be considered satisfactory for this type of analysis (Pueyo et al., 2001).

3.2.3 Protease K extraction

The bioavailable fraction was estimated using Protease K solutions (0.4 mg/ml) following a procedure based on those proposed by some authors (Ianni et al., 2010; Turner, 2006). Protease K was purchased lyophilized from Sigma-Aldrich (P-8044) and solved in pH 7.4 standard phosphate buffer solution prepared from solid salts: 1.179 g of potassium dihydrogen phosphate (KH_2PO_4) and 4.300 g of disodium hydrogen phosphate (Na_2HPO_4) in 1 L of distilled water.

Protease K extractable fraction was obtained by digesting 0.5 g of every sample with 25 ml of the Protease K solution for 3 h at room temperature with magnetic stirring (250 rpm). Then the suspensions were centrifuged for 20 min at 4,200 rpm (3,000 g). The supernatant was used to determine Zn, Cu, Cd, Mn, Pb, Ni, Fe and Cr as described for the total metal content.

3.2.4 Total organic carbon

The total organic carbon was determined in sediment samples using a Shimadzu TOC–VCSH analyzer connected to a solid sample module (Shimadzu, Model SSM-5000 A). For this purpose, 40 mg samples were pretreated with HCl 4M and heated on a hot plate to remove inorganic carbon that otherwise would have interfered with the measurement.

3.2.5 Toxicity bioassay with *Photobacterium phosphoreum*

The toxicity on the sediments samples was determined using a marine luminescent bacterium *(Photobacterium phosphoreum)* according to the procedure described by Svenson et al. (Svenson et al., 1996), in which a preassay allows one to estimate the toxicity prior to the main assessment. A suspension of 3 g of sediment sample was prepared in 30 ml of a 2% NaCl solution and was magnetically stirred for 10 min; then a series of dilutions were made and bacteria were exposed to these dilutions and to a blank (2% NaCl solution). Bioluminescence at 15 ^{o}C was measured with a Microtox luminescence meter (Microbics Corp., Carlsbad, CA) after 30 min of incubation. Inhibition was expressed as toxicity units per g of dry soil (TU_{50}), calculated as the inverse of EC_{50} multiplied by 100, instead of common half maximal effective concentration (EC_{50}). TU_{50} provides a more intuitive scale since the higher the toxicity the higher the value of TU_{50}, contrary to EC_{50} (Van den Brink and Kater, 2006).

3.3 Organism sample analysis

Macrobenthic organisms were sorted, numbered and identified to the lowest possible taxon, usually to family level as suggested by many authors (Estacio et al., 1997; Mucha et al., 2003; Sánchez-Montoya et al., 2010). Data on families were used to calculate the Shannon diversity index (H') (Shannon, 1948) and M-AMBI index (Borja et al., 2000).

3.4 Data analysis

3.4.1 Pollution indices

The indices are based on those proposed by Usero et al. (Usero et al., 2008) and, as shown in Table 3, consist in 5 pollution indices related to one assessment each: inorganic contamination index (ICI, total metal content), bioavailability index (BI, protease K extraction), organic contamination index (OCI, total organic carbon), toxicity index (TI, bioassay with *Photobacterium phosphoreum*) and macrobenthic alteration index (MAI, 1/M-AMBI index). Pollution indices are higher the higher the levels of contamination. Thus, the inverse of M-AMBI has been used to calculate MAI, as M-AMBI is inversely proportional to the alteration degree of the macrobenthos.

Table 3. Calculation description of the environmental degradation index in every sampling point.

Assay	Assay output	NVs	Pollution indices	
Total metal content	$Metal_1$ (mg/kg) … $Metal_{11}$ (mg/kg)	$NV_{ICI,1}$ … $NV_{ICI,11}$	Inorganic contamination index (ICI)	Environmental degradation index (EDI)
Protease K extraction	$Metal_1$ (mg/kg) … $Metal_8$ (mg/kg)	$NV_{BI,1}$ … $NV_{BI,8}$	Bioavailability index (BI)	
Total organic carbon	%	NV_{OCI}	Organic contamination index (OCI)	
Toxicity bioassay	TU/g	NV_{TI}	Toxicity index (TI)	
Macrobenthic community	1/M-AMBI	NV_{MAI}	Macrobenthic alteration index (MAI)	

To determine pollution indices at each of the sampling points, first, normalized values (NVs) related to the reference station (L1) are calculated with the following equation:

$$NV_i = \frac{P_{iE}}{P_{iR}}$$

NV_i, P_{iE} and P_{iR} respectively refer to the normalized value of the parameter i, the parameter i in the sampling station E and the parameter i in the reference station R. Next, the indices mentioned above are calculated as the NV's geometric mean in the corresponding assessment according to the expression:

$$X = \sqrt[n]{NV_1 \times NV_2 \times \ldots \times NV_n}$$

X refers to the ICI, BI, OCI, TI, and MAI indices.

In cases when the index is composed of a single analytical result (OCI, TI and MAI), there is only one NV and it matches the index value. However, in the case of ICI and BI, there are several NVs (one for each metal) and the index and the NV's differ.

3.4.2 Environmental degradation index

The environmental degradation index (EDI) at each station is calculated as the geometric mean of the indices calculated in the previous section. It could therefore be called an index of indices, which summarizes the main characteristics of the environmental situation of each sampling point.

$$\mathrm{EDI} = \sqrt[5]{\mathrm{ICI} \times \mathrm{BI} \times \mathrm{OCI} \times \mathrm{TI} \times \mathrm{MAI}}$$

The EDI has a special interest because it summarizes in a single number the main environmental quality features of the sediment and allows for an unequivocal comparison of two samples, unlike a simple set of analytical results, in which not all of a station's results are greater than the other.

4 Results and discussion

4.1 Sediment analysis

4.1.1 Particle size profiles

The types of sediments found in the study area are quite diverse. As shown in Table 4, the littoral is dominated by coarse particles (sands). However, in the estuary of the Odiel are more abundant fine textures, mainly silt. In the Tinto estuary very fine sands prevail and in the Padre Santo Canal grain size is variable.

Padre Santo Canal holds the most heterogeneous grain size distribution, where samples are categorized as moderate, poor and bad in terms of homogeneity. In contrast, the most homogeneous sediments are on the littoral and in the Odiel estuary, where moderate and moderately good homogeneity predominate. In the estuary of the Tinto homogeneity varied from poor to moderately good.

Table 4. Results of the tests conducted in the laboratory.

	T1	T2	T3	T4	O1	O2	O3	O4	O5	C1	C2	C3	L1	L2	L3	L4
Particle size profiles																
Sediment type*	CS	VFS	S	VFS	S	FS	S	S	FS	CS	S	MS	FS	CS	VFS	VFS
Type of selection**	M	P	MG	M	MG	P	MG	MG	M	B	M	P	MG	M	M	M
Total metal content (mg/kg)																
Zn	968	1420	1640	2920	200	1050	2750	1910	1770	2170	2110	1800	88	171	243	212
Cu	1880	1930	1940	2420	363	1310	2190	1790	1820	2020	1740	1480	18	46	106	89
Cd	2.30	2.40	2.60	5.50	0.60	0.60	2.00	2.40	2.80	1.60	1.40	1.30	0.06	0.09	0.13	0.10
Pb	788	1370	1370	2420	610	290	454	475	548	584	538	533	19	24	57	37
Fe ***	154	120	120	158	98	86	77	71	101	76	82	75	14	17	18	16
As	578	794	857	1310	503	202	350	297	702	440	368	358	12	18	39	29
Se	4.2	6.4	5.8	13.4	3.2	2.5	3.2	3.7	3.9	3.1	2.7	3.0	0.1	0.1	0.1	0.1
Hg	1.8	3.2	3.5	8.3	4.2	2.5	2.4	3.4	4.6	3.4	3.1	2.9	0.2	0.4	0.7	0.6
Mn	121	180	247	274	215	341	275	301	296	416	396	352	190	237	220	178
Ni	18	33	30	27	17	25	33	40	31	40	39	31	8	10	14	9
Cr	85	105	118	117	74	72	99	96	106	98	84	86	24	28	29	24
Protease K extraction (mg/kg)																
Zn	4.29	5.28	4.09	10.53	3.94	4.19	9.99	7.35	6.38	7.82	8.39	6.25	2.87	3.13	4.17	3.65
Cu	120	81	87	55	26	47	77	70	73	67	71	72	6	15	22	21
Cd	0.10	0.03	0.03	0.11	0.01	0.03	0.03	0.04	0.05	0.02	0.03	0.03	0.01	0.01	0.01	0.01
Pb	5.07	8.89	7.86	10.00	3.16	3.40	3.68	3.15	3.66	4.69	4.45	4.53	1.04	1.56	2.08	2.60
Fe	275	151	117	184	254	114	118	131	149	105	102	112	41	22	34	51
Mn	1.57	1.05	1.62	2.63	2.63	4.19	2.63	2.73	2.51	4.17	5.77	4.43	3.13	2.76	1.56	3.23
Ni	0.34	0.52	0.93	0.77	1.33	0.79	1.00	1.23	1.01	0.63	0.58	0.52	0.26	0.29	0.42	0.20
Cr	0.07	0.09	0.08	0.10	0.06	0.06	0.09	0.09	0.10	0.09	0.08	0.08	0.03	0.03	0.04	0.03
Total organic carbon (%)																
	3.43	2.13	1.67	1.74	2.94	2.79	2.19	1.70	1.68	1.57	1.67	1.69	0.10	0.12	0.20	0.18
Toxicity bioassay (TU/g)																
	2500	5300	6400	8700	2540	3500	8000	6300	5200	2900	2500	2100	54	67	230	210
Macrobenthic community																
Number of families	4	5	6	9	4	5	7	6	10	7	8	7	15	18	13	17
Shannon-Wiener index	0.41	0.32	1.03	1.48	1.00	1.00	1.20	0.84	1.52	1.51	1.68	1.46	1.89	1.88	1.92	2.04
M-AMBI index	0.32	0.33	0.35	0.38	0.40	0.45	0.55	0.46	0.64	0.66	0.70	0.69	0.95	0.94	0.78	0.84

* CS: coarse sand. MS: medium sand. FS: fine sand. VFS: very fine sand. S: silt.

** MG: moderately good. M: moderate. P: poor. B: bad.

*** Fe total content is expressed in g/kg.

4.1.2 Total metal content

Metal concentrations found in the sediments of the Huelva estuary (Table 4) are generally very high compared to background levels and quality criteria in Table 5, especially considering the metals of pyritic origin (Zn, Cu, Cd, Pb, Fe, As and Se). The waters of the Tinto and Odiel rivers are enriched in these metals (Morillo and Usero, 2008; Morillo et al., 2005) when they flow through the so-called "Iberian Pyrite Belt" (Leblanc et al., 2000; Sánchez España et al., 2005) and much of the metals dissolved precipitate in areas of tidal influence (estuaries and Padre Santo Canal) due to the strong increase of pH (changes from remarkably acid in the upper and middle basins to close to 8 near the mouth) (Galán et al., 2003; Morillo et al., 2008, 2002; Ruiz, 2001; Sainz and Ruiz, 2006; Santos Bermejo et al., 2003; Usero et al., 2008, 2005). In addition, precipitation is favoured by the precipitation of $Fe(OH)_3$, to which other metals are adsorbed (Baruah et al., 1996; Panda et al., 1995).

Metal concentrations found in sediments of the Huelva littoral are significantly lower than those of the Huelva estuary. The majority of the metals present in the waters of the Tinto and Odiel rivers precipitate in the estuary before reaching the mouth (Galán et al., 2003; Morillo et al., 2008, 2002; Ruiz, 2001; Sainz and Ruiz, 2006; Santos Bermejo et al., 2003; Usero et al., 2008, 2005).

Table 5. Background levels and several quality criteria for metal concentrations in sediments.

Metal	Background levels (Turekian and Wedepohl, 1961)	MacDonald (MacDonald et al., 2000)		Long (Long et al., 1995)		Crommentuijn (Crommentuijn et al., 2000)	Canada (Bray, 2008)	Spain (CEDEX) (DelValls et al., 2004)	
		TEC[1]	PEC[2]	ERL[3]	ERM[4]	MPC[5]	HWT[6]	AL1[7]	AL2[8]
Zn	95	121	459	150	410	620	820	500	3000
Cu	45	31.6	149	34	270	73	110	100	400
Cd	0.3	0.99	4.98	1.2	9.6	12	10	1.0	5.0
Pb	20	35.8	128	46.7	218	530	250	120	600
Fe	47000	-	-	-	-	-	-	-	-
As	13	9.79	33	8.2	70	55	33	80	200
Se	0.6	-	-	-	-	2.9	-	-	-
Hg	0.4	0.18	1.06	0.15	0.71	10	2	0.6	3.0
Mn	850	-	-	-	-	-	1100	-	-
Ni	68	22.7	48.6	20.9	51.6	44	75	100	400
Cr	90	43.4	111	81	370	380	110	200	1000

[1] TEC: threshold effect concentration.
[2] PEC: probable effect concentration.
[3] ERL: effects range-low.
[4] ERM: effects range-median.
[5] MPC: maximum permissible concentration.

[6] HWT: hazardous waste threshold.
[7] AL1: action level 1.
[8] AL2: action level 2.

Table 4 shows that metal concentrations in sediments of the Tinto estuary increase seawards and the highest concentrations of all samples studied in several metals (Zn, Cu, Cd, Pb, Fe, Hg, Se and As) are reached at the end of the estuary (point T4), especially those with a pyritic origin. The Tinto River is more affected by the Iberian Pyrite Belt than the Odiel and therefore the waters of the Tinto River have higher concentrations of metals and lower pH levels than those found in the Odiel River. In the estuary of the Odiel, different trends are observed depending on the metal. In the Padre Santo Canal a general decline in the concentrations of metals takes place seawards. In the Huelva littoral, point L3 (closest to the mouth of the Huelva estuary) usually has the highest metal concentrations.

4.1.3 Protease K extraction

Overall, the bioavailability of the metals that precipitate in the sediment is higher than that of those whose source is a longer-term process. Therefore, heavier precipitation implies that total and bioavailable amounts of metal in the sediment are greater. Thus, the spatial distribution of the total metal content is reproduced in bioavailable concentrations. For example, Zn extracted by protease K (Table 4) shows that the bioavailable fractions are closely related to the total metal content, replicating the rise towards the sea at the Tinto estuary, the ascent in the estuary of the Odiel up to point O3 and descent downstream at this point, the decline in the Padre Santo Canal and the variations on the littoral. In the case of Cu, patterns of total metal content are also reproduced in bioavailable fractions, however, point T1 (Tinto estuary) has significantly higher bioavailable metal concentrations than expected. The rest of the metals assessed reproduce the spatial distribution of the total metal content in bioavailable concentrations. Besides in the case of Cu, point T1 reaches higher bioavailable concentrations than expected for Cd, Fe, Mn and Ni.

Protease K extraction is different in every metal assessed as a percentage of the metal content. The means in all the samples studied show that Cu is the metal with the highest percentage (10.0%), followed by Cd (4.5%), Ni (2.9%), Pb (2.0%), Mn (1.1%), Zn (0.9%), Fe (0.2%) and Cr (0.1%).

4.1.4 Total organic carbon

The total organic carbon average in sediment samples from the Huelva littoral (0.15%) is significantly lower ($p<0.05$) than the mean values obtained in the Padre Santo Canal (1.64%), Odiel estuary (2.26%) and Tinto estuary (2.24%). The highest percentages of total organic carbon correspond to samples taken at the upper part of the estuaries of the Tinto (point T1 with 3.43%) and Odiel (point O1 with 2.94%) and decrease moving down the Padre Santo Canal. These results agree with those previously obtained by López-González et al. (López González, 2006).

4.1.5 Toxicity bioassay with *Photobacterium phosphoreum*

The lowest average toxicity is found in the littoral (140 TU/g; $p<0.05$), with a minimum value of 54 TU/g at point L1 and a maximum of 230 TU/g at the point L3.

Following the coast, the second zone with the lowest toxicity average in sediments is the Padre Santo Canal (2,500 TU/g), where the toxicity decreases towards the sea (from 2,900 TU/g in point C1 to 2,100 TU/g at C3).

The areas with the highest average toxicities are the estuaries of the Tinto (5,725 TU/g) and Odiel (5,108 TU/g). In the case of Tinto, toxicity increases downstream, until the T4 (8,700 TU/g), where the maximum value of all surveyed points is reached. In the estuary of the Odiel, toxicity increases from O1 (2,540 TU/g) to O3 (8,000 TU/g), where it decreases to point O5 (5,200 TU/g).

4.2 Organism sample analysis

The analyses of the macrobenthic community in sediments have identified a total of 8 taxa, three of which are well represented: annelids (17 families), crustaceans (11 families) and molluscs (11 families). Also present, to a lesser extent, are insects (4 families) and 4 other groups with only one family each (Nemertea, Phoronida, Chaetognatha and Platyhelminthes).

The predominant group corresponds to the annelids, which make up more than 50% of all individuals at all points, except for the L2, O1 and T1. Within the group of annelids, polychaetes are the most abundant class, mainly detritivores organisms belonging to the families Spionidae, Capitellidae and Nephtyidae. These families are composed of

opportunistic species or primary colonizers who take advantage of any alteration of the environment to occupy a large ecological niche.

Crustaceans are the next largest group, with percentages between 0-25% in all points, with the exception of point L2, where 54% of crustaceans is achieved due to the abundant presence of members of the Diogenidae family. Molluscs are the next largest group and they are especially important in the littoral, where they reaches between 10% and 40% of the total. However, in the Huelva estuary they do not exceed 8%. In this group, the Veneridae family is the most abundant. The group called "other" often assumed between 1% and 5% of the total, except at the points O1 and T1, where insect families composed by detritivores species benefit from organic inputs from the river (Ceratopogonidae and Chironomidae, O1; Corixidae, T1). It is also relevant in the Channel, where members of the Sagittidae family reach almost 50% in the C3 and 15% in the C1.

Regarding the number of families and the Shannon-Wiener diversity index, the results show that the macrobenthic community is more diverse in the littoral than in the Huelva estuary, which agrees with the lower toxicity and lower total and bioavailable metal content in the littoral. As shown in Table 4, sampling points with the highest number of families are concentrated in the littoral, and point L3 reaches the highest value of them all. In the other study areas (Tinto and Odiel estuaries and Padre Santo Canal) the number of families is lower, as corresponds to contaminated areas.

The Shannon-Wiener diversity index (Table 4) shows a greater diversity in the littoral, followed by the Padre Santo Canal and the lower areas of the estuaries of the Tinto and Odiel rivers. These results agree with those reported by Sánchez-Moyano and García-Asencio (Sánchez-Moyano and García-Asencio, 2010). The lower areas of Tinto and Odiel estuaries have both greater diversity of macrobenthic organisms and a higher concentration of metals than higher areas. These results can be explained because the waters in the higher areas have a low pH, high metal concentration (Santos Bermejo et al., 2003) and a lower salinity than marine levels (Mucha et al., 2003; Sánchez-Moyano et al., 2009; Ysebaert and Herman, 2002), which reduces life to species adapted to these extreme conditions.

The points with the highest M-AMBI index values are concentrated along the littoral (indicating a minor alteration of the macrobenthic community), followed by the Padre Santo Canal and the estuaries of the Odiel and Tinto rivers (Table 4). The point with the highest M-AMBI of all those studied is the L1 (0.95) and the lowest is the T1 (0.32). The

values of M-AMBI found on the Huelva littoral are similar to those found on the coast of the Basque Country (Borja et al., 2009) which is also affected by urban, industrial and mining effluents. The results obtained in the estuaries of the Odiel and Tinto rivers are similar to those of other contaminated areas, such as the Indian River Lagoon, Florida, USA (Borja and Tunberg, 2011), Chesapeake Bay, Maryland, USA (Borja et al., 2008), and the estuaries of the Basque Country (Borja et al., 2009).

4.3 Data analysis

4.3.1 Pollution indices

The indices are lower on the Huelva littoral than in the other study areas (Figure 2). Thus, the coast is the area with the lowest contamination of all those studied. Within the coastline, most indices reach their highest values in point L3, followed by L4, L2 and L1. These results, together with the effect of predominant currents from west to east, indicate that effluent from the Padre Santo Canal and to a lesser extent, from the Punta Umbria Estuary (Huelva Estuary's secondary outlet), are responsible for the alteration differences in the coast samples.

Within the Huelva estuary, most indices reach lower values in the Padre Santo Canal than in the Tinto and Odiel estuaries. Within the Padre Santo Canal, the inorganic contamination index (ICI) and toxicity index (TI) decrease seaward (C1 to C3), while little variation is observed in the remaining indicators.

Pollution indices are higher in the estuary of the Tinto than in that of the Odiel. The inorganic contamination index (ICI) increases in both estuaries seaward, as does the pH, which indicates that the precipitation of metals caused by increased pH is the main cause of the increase in ICI. The bioavailability index (BI) also rises as it goes down the estuaries of the two rivers, although in this case the increase is lower and the variation between some points and others is less marked. The index of organic pollution (OCI) has its maximum values at points T1 and O1 (34 and 29 respectively), and then decreases in both estuaries seaward up to points T3 and O4, where it reaches a value of 17 and this value remains almost constant in the rest of the points up to the mouth of the estuary.

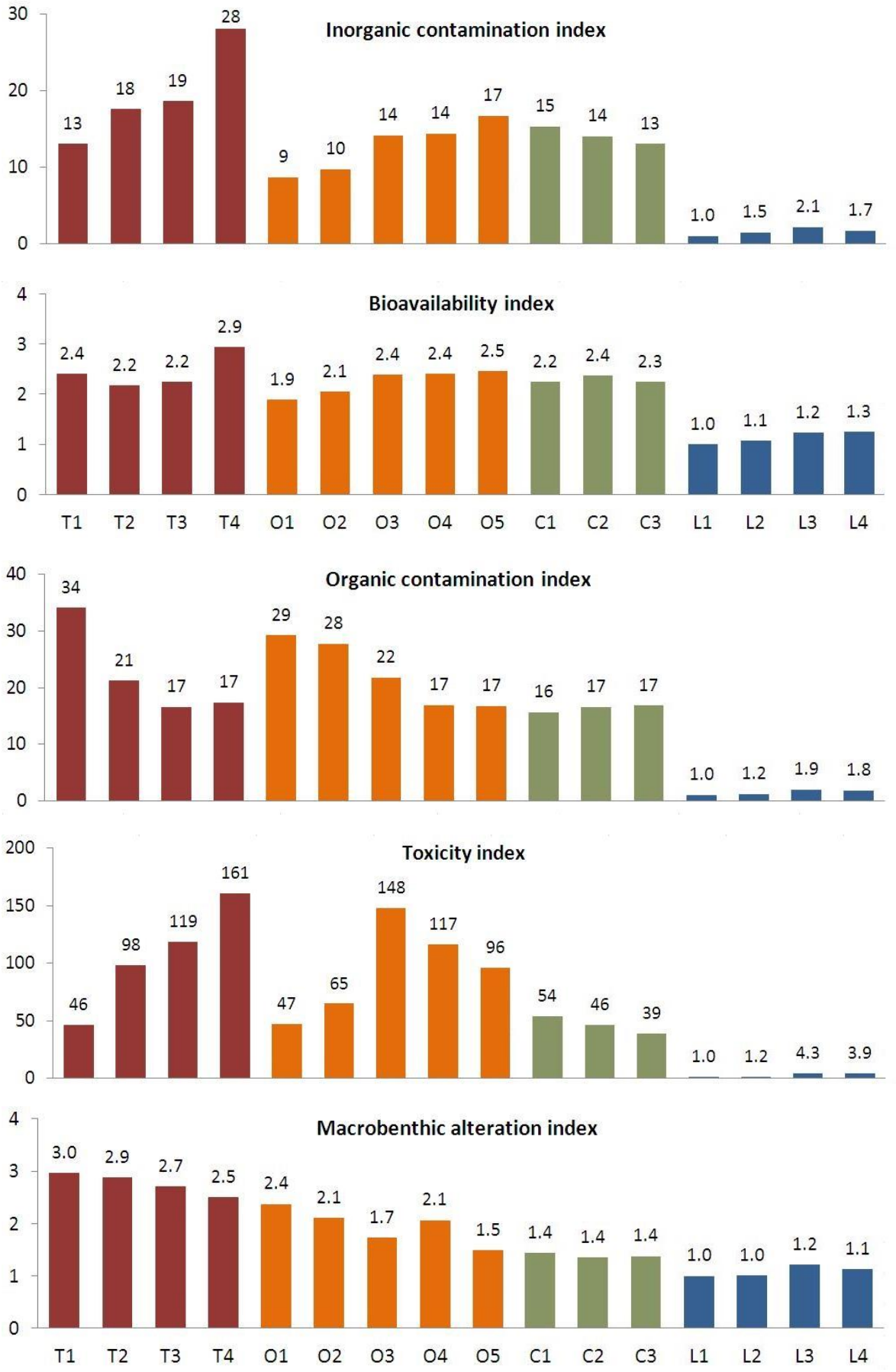

Figure 2. Values of pollution indices in the Huelva estuary and its littoral of influence.

The toxicity index (TI) behaves differently in the estuaries of the Tinto and Odiel. In the Tinto estuary it increases downstream from point T1 up to point T4 (161), where the maximum value of all points studied is reached. However, in the estuary of the Odiel the values of the TI have a bell-shaped structure, i.e. they increase downstream up to point O3, where the maximum value of this estuary is obtained (148) and, from this point on, they decrease downstream.

The macrobenthic alteration index (MAI) decreases downstream of both estuaries and the Padre Santo Canal, unlike the ICI and BI. This can be explained mainly by the increase in the pH of water (from very acidic values to neutrality) that occurs when descending estuaries.

4.3.2 Environmental degradation index

Figure 3 shows that the lowest average EDI of all study areas is on the coast ($p<0.05$), followed by the Padre Santo Canal and the estuaries of the Odiel and Tinto rivers.

The lowest value of EDI (1.00) is obtained in L1 (farthest from the mouth of Huelva estuary), followed by the L2 (1.19), influenced by the estuary of Punta Umbria (Huelva Estuary's secondary outlet) and, L3 (1.92) and L4 (1.77), both influenced by the Padre Santo Canal. In the Padre Santo Canal, EDI shows a slight lowering tendency as it approaches the sea, from point C1 (8.38) to point C3 (7.66). In the Odiel, EDI values follow a bell-shaped evolution: it rises from point O1 (8.82) as it moves down the towards the sea, reaching the maximum value of the area in point O3 (11.4), followed by a decline to 9.95 in O5.

In the estuary of the Tinto (where the highest EDI averages are recorded), this index increases seaward, from point T1 (10.8) to T4 (14.2), the point with the maximum EDI of all points studied.

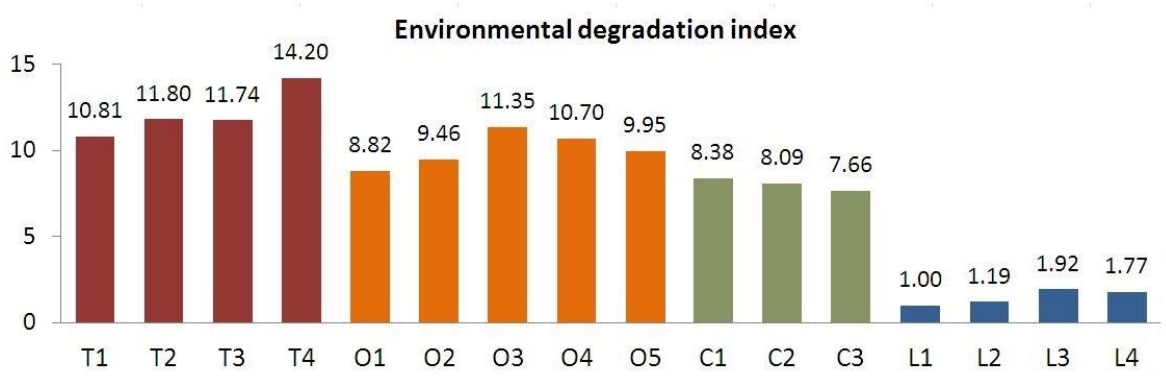

Figure 3. Values of the environmental degradation index in the Huelva estuary and its littoral of influence.

The samples studied can be classified on the basis of their environmental degradation in the following groups:

- Low degradation (EDI<2): samples taken on the littoral of Huelva, which are those with the lowest values of all contamination indices: inorganic contamination (ICI), bioavailability (BI), organic concentration (OCI), toxicity (TI) and alteration of macrobenthos (MAI).
- Average degradation: (2<EDI≤5): none of the samples are included in this group.
- High degradation (5<EDI≤10): Padre Santo Canal samples and those taken in the upper area of the estuary of the Odiel (O1 and O2) and the lowest (O5).
- Very high degradation (EDI>10): this group consists of all samples in the Tinto estuary and those in the middle and lower zones of the estuary of the Odiel (O3 and O4). The sample T4 has the highest values of ICI, BI and TI in all areas studied and T1 has the highest values of OCI and MAI.

5 Conclusions

A new integrated sediment quality assessment method composed of several tests (particle size profile, total metal content, protease K extraction, total organic carbon, toxicity bioassay with *Photobacterium phosphoreum* and macrobenthic community alteration) has been applied to sediment samples from the Huelva estuary and its littoral of influence.

The proposed method has satisfactorily complied with the features to be implemented on a wide area: applicable to all kinds of sediments, simple, low cost and with an easily understandable and comparable result.

The sediment samples were classified on the basis of their environmental degradation index in four groups: i) low degradation: the samples belonging to the littoral, ii) average degradation: none of them, iii) high degradation: Padre Santo Canal and samples taken in the upper area (O1 and O2) and the lowest (O5) of the estuary of the Odiel and iv) very high degradation: all samples in the Tinto estuary and those in the middle and lower zones of the estuary of the Odiel (O3 and O4). Point T4, located on the estuary of the Tinto River near the junction with the estuary of the Odiel, has the maximum environmental degradation index of all points studied.

Acknowledgements

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