Environ Monit Assess (2012) 184:7141–7151
DOI 10.1007/s10661-011-2486-8

Environmental quality assessment combining sediment metal levels, biomarkers and macrobenthic communities: application to the Óbidos coastal lagoon (Portugal)

**Patrícia Pereira · Susana Carvalho ·
Fábio Pereira · Hilda de Pablo ·
Miguel B. Gaspar · Mário Pacheco · Carlos Vale**

Received: 4 August 2011 / Accepted: 8 December 2011 / Published online: 23 December 2011

Abstract Macroinvertebrate benthic communities are one of the key biological components considered for the assessment of benthic integrity in the context of the Water Framework Directive (WFD). However, under moderate contamination scenarios, the assessment of macrobenthic alterations at community level alone could be insufficient to discriminate the environmental quality of coastal and transitional waters. Keeping this in view, sediment quality of moderately contaminated sites in a coastal lagoon (Óbidos lagoon, Portugal) was assessed by the combination of sediment metal levels, *Carcinus maenas* biomarkers (accumulated metals and oxidative stress responses) and macrobenthic communities. Two sites were selected in confined inner branches (BS and BB) and a third one in the middle lagoon (ML). The site BB presented slightly higher levels of metals in sediment but biological variables calculated for macrobenthic data were not significantly different between sites. The biotic index M-AMBI that is being applied to assess environmental quality of transitional waters in the scope of the WFD pointed either to high (site ML) or good quality status (BS and BB) in the selected sites. However, crabs from BB site presented significantly higher levels of Ni in hepatopancreas than those from ML and macrobenthic community structure was significantly different between BB and ML. Additionally, spatial differences were obtained for oxidative stress parameters suggesting that BB site presented stressors for crabs (higher GST and lower GSH_t at BB site). Factor analysis (PCA) integrating sediment contamination, biomarkers in crabs and macrobenthic data also distinguished BB site as the most environmentally disturbed. On the other hand, at ML site, some macrobenthic variables (equitability and polychaetes' diversity) were found to be enhanced by current environmental conditions, suggesting the existence of a better sediment quality. Current results pointed to the usefulness of integrating macrobenthic community alterations with responses at organism level (bioaccumulation and biochemical endpoints) in order to increase the accuracy of

P. Pereira (✉) · H. de Pablo · C. Vale
Instituto Nacional de Recursos Biológicos
(INRB/IPIMAR),
Av. Brasília,
1449-006 Lisboa, Portugal
e-mail: patbio@ipimar.pt

S. Carvalho · F. Pereira · M. B. Gaspar
Instituto Nacional de Recursos Biológicos
(INRB/IPIMAR),
Av. 5 de Outubro,
8700-305 Olhão, Portugal

P. Pereira · M. Pacheco
Departamento de Biologia da Universidade de Aveiro,
CESAM,
Campus de Santiago,
3810-193 Aveiro, Portugal

environmental quality assessment in lagoon systems. Moreover, the application of different statistical methods was also found to be recommendable.

Keywords Sediment quality · Metals · Macrobenthic communities · *Carcinus maenas* · Oxidative stress · Óbidos lagoon

Introduction

In the frame of the Water Framework Directive (WFD), scientists are facing the need to develop and/or validate methodologies to assess the environmental quality of coastal systems. Transitional areas must be characterised in terms of chemical and biological elements in order to establish its baseline status, as well as the definition of homogenous classification criteria. Due to the complexity and variability of transitional ecosystems like estuaries and coastal lagoons, the development of adequate classification systems is one of the most difficult aspects of the WFD (Allan et al. 2006).

Benthic macroinvertebrates are a key component in the functioning of coastal and transitional ecosystems and several works have already used this biological component to assess the environmental quality of estuaries and coastal areas (Rosenberg et al. 2004; Salas et al. 2004; Carvalho et al. 2006; Blanchet et al. 2008). However, as adequacy is tested in coastal ecosystems within the context of the WFD, it was found that macrobenthic communities from naturally stressed areas often display similar features to those from anthropogenically stressed areas (Elliott and Quintino 2007). This may result from the natural variability in physical and chemical parameters like salinity, bottom nature, tidal currents, as well as availability of several contaminants (Mucha et al. 2004; Nunes et al. 2008; Specchiulli et al. 2010; Carvalho et al. 2011). Local communities may become resilient to environmental changes and, unless the anthropogenic stressor action is severe, communities have the ability to counterbalance it without showing adverse effects at this biological organization level (Elliott and Quintino 2007). Apparently, this may explain the discrepancies found in the assessment of ecological quality status based on macrobenthic biotic indices (Blanchet et al. 2008).

A key step for the assessment of sediment quality is the measurement of contaminant levels, although these values do not directly indicate the bioavailability of pollutants to organisms (Depledge and Galloway 2005). Metals in sediments may be more or less bioavailable to organisms depending on their chemical form, but also on sediment physical characteristics, grain size and characteristics of local organisms. Consequently, depending on their bioavailability, the existence of similar levels of metals in sediment may result in different responses of macrobenthic communities, increasing vagueness in the relationship between anthropogenic activities and impacts on coastal systems. Therefore, the integration of biological responses of benthic organisms is being increasingly considered a relevant component of sediment quality assessment (Morales-Caselles et al. 2008, 2009). Among the wide range of effect biomarkers pointing to environmental contamination by metals, oxidative stress responses have been widely applied (Fernandes et al. 2008; Morales-Caselles et al. 2008, 2009). One of the benthic species that has been used as a bioindicator of environmental quality in impacted shallow estuaries and coastal lagoons is the shore crab (*Carcinus maenas*; Pereira et al. 2006; Martín-Díaz et al. 2007, 2008; Pereira et al. 2009a). *C. maenas* has favourable features as sentinel in shallow sediment-contaminated systems because it is an opportunist species that feeds on a large variety of prey items, from animal phyla (mainly Annelida, Crustacea and Mollusca) to plant and protest (Cohen et al. 1995), and displays biochemical responses to contaminants both in water and sediment (Martín-Díaz et al. 2007, 2008; Morales-Caselles et al. 2008).

In the current study, it was applied an integrated approach based on field data collected in the Óbidos lagoon (western Portuguese coast), specifically sediment metal contamination, macrobenthic communities, metal bioaccumulation and oxidative stress responses in *C. maenas*. This study used historical data [Carvalho et al. (2011); Pereira et al. (2011)] in order to provide new insights into the integration of different variables for the evaluation of sediment quality. Given the absence of major contaminant gradients in the Óbidos lagoon, it was investigated in what extent endpoints at lower organizational levels (i.e. metal bioaccumulation and oxidative responses in local organisms) could contribute to a more comprehensive perspective of sediment quality, particularly on the scope of the WFD.

Material and methods

Study area

The Óbidos lagoon is a shallow coastal ecosystem with a wet area of 7 km^2, permanently connected to the sea through a narrow inlet (Fig. 1). It comprises areas of sand banks and narrow channels in the lower/middle lagoon, and muddy bottom sediments in the two inner branches (Barrosa and Bom-Sucesso). The Barrosa branch is shallower (mean depth 0.5–1 m) than the Bom-Sucesso (mean depth 1–2 m) and water circulation is mostly driven by tides and by a small tributary (Cal River) that drains agricultural fields. At present, this branch exhibits high nutrient availability and is classified as eutrophic (Pereira et al. 2009b). Metal sources were identified to be diffusive and the Cal River the main vehicle. Metals are also remobilized from anoxic sediments in summer months (Pereira et al. 2009c). Accordingly, aquatic organisms living in Barrosa branch are exposed to metals provided by freshwater inputs and sediment remobilization. *Ulva* sp. and *Liza aurata* employed as sentinel species in the Óbidos lagoon exhibited higher metal levels at Barrosa than other lagoon areas (Pereira et al. 2009d, 2010a). Barrosa branch also presented compounds that stimulated the production of reactive oxygen species (ROS) in those target species and *C. maenas* (Pereira et al. 2009a). A previous study on temporal and spatial dynamics of macrobenthic communities pointed Barrosa branch as the most disturbed area in the lagoon (Carvalho et al. 2011). The Bom-Sucesso branch presents a higher water residence time (Malhadas et al. 2009) and low nutrient concentrations in the water column (Pereira et al. 2009b). The middle lagoon has a much lower residence time (2 days) and the bottom consists of muddy-sand sediments with reduced metal content (Pereira et al. 2009a). Previous studies identified the Bom-Sucesso branch as an intermediate hazard area based on biological responses of target species while the middle lagoon was considered as a reference area (Pereira et al. 2009a, 2010b). Both middle lagoon and Bom-Sucesso branch presented richest macrobenthic assemblages compared with Barrosa branch (Carvalho et al. 2011).

Database

Data used in this study were obtained in summer 2006 at three sites with different environmental characteristics: middle lagoon (ML) and the upper branches Bom-Sucesso (BS) and Barrosa (BB). Data consisted of sediment chemistry (metal levels, organic matter content—OM), macrobenthic communities (abundance, number of species, biotic indices) and biomarkers in *C. maenas*

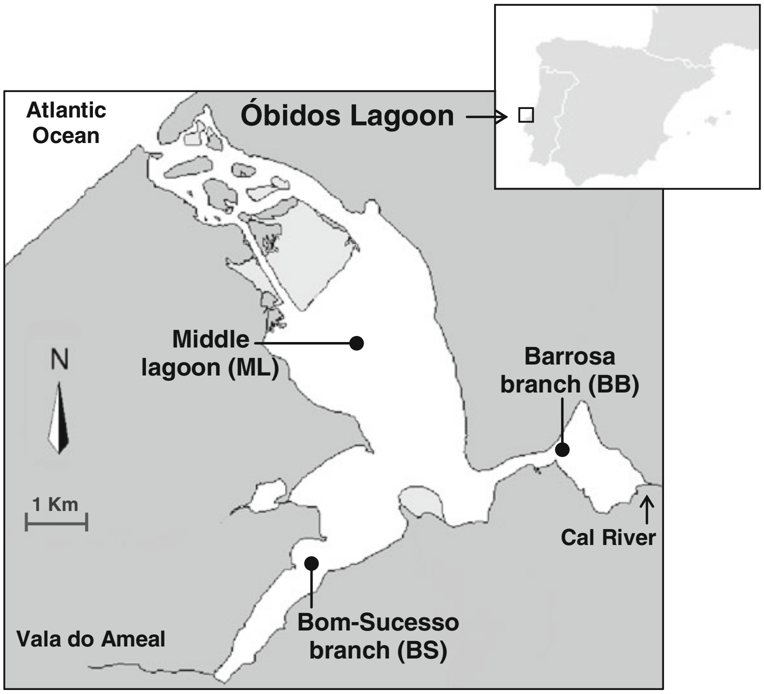

Fig. 1 Map of Óbidos lagoon (Portugal) with location of the sampling sites: *ML* in the middle lagoon; *BS* and *BB* in the inner branches (Bom-Sucesso and Barrosa, respectively)

hepatopancreas (including metal levels and oxidative stress endpoints). Only data of *C. maenas* females were considered in the current work. Detailed information on the sampling, analytical methodologies and results can be found in the following works: Carvalho et al. (2011) and Pereira et al. (2011). Briefly, macrobenthic communities (triplicates per site) and sediments (one sample per site) were collected with a Van-Veen grab (0.05 m^2). Samples were washed through a 0.5-mm mesh sieve and the retained material was preserved in 4% buffered formalin stained with Rose Bengal. In the laboratory, animals were hand sorted into major taxonomic groups, identified to the lowest practical taxonomic level and counted.

Sediment samples (100 mg) were mineralized completely with a mixture of acids in closed Teflon bombs (100°C for 1 h), evaporated and redissolved following the methodology described in Carvalho et al. (2011). The concentrations of Mn, Cu, Cr, Ni, Pb and Cd were determined by ICP-MS (Thermo Elemental, X-Series) and the levels of Al, Fe and Zn by AAS (Perkin Elmer, AAnalyst 600). The accuracy of the analytical procedures was assessed by the analysis of Certified Reference Materials (MESS-3, BCSS-1, 1646a). Metal levels obtained in the reference materials were consistently within the ranges of certified values, with a percentage of recovery ranging between 95% and 100%. Procedural blanks always accounted for less than 1% of the total metal counts in samples. Organic matter content in sediment was estimated by loss on ignition (4 h at 500°C).

Width and weight of crabs varied within comparable intervals for the three sampling sites: 4.9–5.0, 5.0–5.5 and 5.6–5.9 cm for ML, BS and BB, respectively; 21–29, 21–30 and 28–35 g for ML, BS and BB, respectively. Crabs (n=6) were sacrificed and their hepatopancreas were excised, divided in two parts. Metal determinations were made in individual freeze-dried and grounded hepatopancreas sub-samples. Approximately 50 mg were digested with a mixture of HNO_3 and H_2O_2 at 60°C for 12 h, 100°C for 1 h and at 80°C for 1 h, according to the method described in Pereira et al. (2011). The concentrations of Mn, Cu, Cr, Ni, Pb and Cd were determined by ICP-MS. The accuracy of the analytical procedures was assessed by the analysis of Certified Reference Materials (TORT-1, TORT-2, DOLT-3). Metal levels obtained in the reference materials were consistently within the ranges of certified values. Metal levels obtained in the reference materials were consistently within the ranges of certified values, with a percentage of recovery ranging between 95% and 98%. Procedural blanks always accounted for less than 1% of the total metal counts in samples. Biochemical analyses were made in individual sub-samples of hepatopancreas. Tissue samples were homogenized in an appropriate buffer (1 g of tissue/10 ml buffer; (Pereira et al. 2011)). An aliquot of homogenate (150 μL) was taken for thiobarbituric acid reactive substances measurement and stored at −80°C after adding 10 μL BHT (1-1 butylated hydroxytoluene) to prevent oxidation, and the rest was centrifuged (first at 12,000×*g* for 20 min; the supernatant was recentrifuged at 135,000×*g* for 75 min). Biochemical measurements were carried out in the cytosolic fraction (at 25°C) as described detailed in Pereira et al. (2011), namely: total glutathione content (GSH_t), glutathione-*S*-transferase (GST) activity (Habig et al. (1974)), catalase (CAT) activity (Claiborne 1985), glutathione peroxidase (GPx) activity (Mohandas et al. 1984) and lipid peroxidation (LPO; (Filho et al. 2001)). Protein concentrations were determined according to the Biuret method (Gornall et al. 1949) using bovine serum albumin (E. Merck-Darmstadt) as standard.

Data analysis

Macrobenthic community descriptive statistics such as number of taxa (S), abundance (N), Pielou's equitability (J) and Margalef diversity (d), as well as the relative abundance of the major taxonomic groups (Polychaeta, Mollusca and Amphipoda) were determined for each sample. Hereafter, number of taxa and abundance when used alone refer to the all community data. The biotic indices AMBI (Borja et al. 2000) and M-AMBI (Muxika et al. 2007) were also calculated. AMBI is based on the classification of species into five ecological groups, reflecting the level of sensitivity of a species to contamination (see Borja et al. 2000 for details), while M-AMBI was calculated by factor analysis of AMBI, number of taxa and Shannon–Wiener diversity.

Differences between sites for all biological variables and environmental parameters were tested by analysis of variances (one-way ANOVA) or using the non-parametric test of Kruskal–Wallis (K-W) ANOVA on ranks. Prior to ANOVA, data were analyzed to test for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Cochran's test) among treatments using the statistical package Statistica software v6. Multiple comparisons were performed using the Tukey test. In order to test for the null hypothesis of no significant spatial differences on the multivariate structure and composition of macrobenthic assemblages, one-way PERMANOVA, employing the PERMANOVA+ add-on in PRIMER v6 (Anderson et al. 2008), was performed on the Bray-Curtis similarity matrix after square-root transformation of abundance data. Significant terms of factor "SITE" were investigated using a posteriori pairwise comparisons between sampling sites with the PERMANOVA t-statistic and permutations under a reduced model (Anderson et al. 2008). Whenever there were not enough possible permutations to get a reasonable test, the Monte Carlo *P* values were used instead.

Data gathered within this multidisciplinary study were integrated by means of a factor analysis using the principal component analysis (varimax normalized rotation) as the extraction procedure. Specifically, the analysis was based on the sediment descriptors (concentrations of Al, Fe, Mn, Zn, Cu, Cr, Ni, Pb, Cd and organic matter content), metals' bioaccumulation on *C. maenas* females (concentrations of Mn, Cu, Cr, Ni, Pb and Cd in hepatopancreas), biological responses (CAT, GPx, GST, GSH_t and LPO) and biological descriptors (S, N, *d* and *J*, as well as mean number of species and abundance of Polychaeta, Mollusca and Amphipoda). This approach aimed to assess the relationship between all variables for the three selected sites and was already applied with similar purposes (Choueri et al. 2009; Morales-Caselles et al. 2009). Based on the correlations between the set of the original variables and their significance in each study site, it was inferred about the environmental quality of the sampled areas. The analysis was performed using the Statistica software v6.

Results

Sediment characteristics, benthic community structure and response by *C. maenas*

Data on sediment physical–chemical characteristics, metal concentrations and biological responses in crab hepatopancreas, as well as macrobenthic community descriptors are summarized in Table 1. Levels of Zn, Cu, Pb and Cd were slightly higher in sediments of site BB while OM was maxima at BS.

The lowest number of taxa and diversity of the whole macrobenthic community, as well as of both the abundance and number of molluscan taxa, was found at site BB (Table 1). Amphipods were the dominant taxonomic group, both in abundance and number of taxa (Fig. 2). In general, the highest values of number of taxa and abundance considering both all community and the most representative taxonomic groups were observed at site BS. Herein, polychaetes dominated in terms of abundance although not in the number of taxa (Fig. 2). Conversely, ML site showed the highest Margalef species richness, polychaete number of species and evenness, evidencing the occurrence of less dominated assemblages (Table 1). The composition of the benthic assemblages per site was very similar considering either the number of taxa or abundance (Fig. 2). Due to the high variability among replicates no significant differences were found for any of the macrobenthic descriptors. PERMANOVA analyses showed a significant effect of factor "Site" ($F=2.9494$, $P<0.01$) on the composition and structure of macrobenthic communities. Post-hoc comparisons showed that significant differences were only detected between ML and BB ($t=1.8259$, $P<0.05$). The biotic index AMBI pointed to a slight (ML, BS) to moderately (BB) disturbed conditions, while M-AMBI classified the environmental status between high (ML) and good (BB, BS). However, statistical analysis did not indicate any significant difference among sites.

In general, metal levels in hepatopancreas were higher in crabs from both BS and BB, reaching Mn, Cr, Ni and Pb higher values at BB. However, statistical differences were only found for Ni with levels being significantly higher at BB in comparison with ML ($q=3.747$, $P<0.05$). Significant differences between sites were also recorded for oxidative stress parameters, namely: increased activity of GST in crabs from BB in relation to those from BS ($q=5.123$, $P<0.05$); lower GSH_t content in crabs from BB comparing with BS ($q=3.594$, $P<0.05$).

Multivariate analysis

The factor analysis revealed that all the original variables could be grouped into two principle components (factors) that explained 100% of the total variance (Table 2). Factor 1 explained 60.2% of the variance and related positively sediment levels of Cr, Ni and Pb with those accumulated in crabs (Table 2). Additionally,

Table 1 Sediment physical–chemical characteristics in three sites of the Óbidos Lagoon [middle lagoon (ML) and inner branches (Bom-Sucesso—BS, Barrosa—BB)], mean (±SD) of accumulated metal levels, biological responses in *Carcinus maenas* and benthic community descriptive variables

			Site		
			ML	BS	BB
Sediment characteristics	Al	(%)	10	11	9.4
	Fe		4.8	4.7	5.2
	Mn	(μg g^{-1})	297	285	284
	Zn		110	104	130
	Cu		43	46	58
	Cr		69	56	72
	Ni		29	24	30
	Pb		39	33	48
	Cd		0.22	0.23	0.29
	OM	(%)	6.9	7.3	7.0
Accumulated metal levels in *C. maenas*	Mn	(μg g^{-1})	17±6.1	20±10	57±59
	Cu		205±196	271±306	257±167
	Cr		0.58±0.34	0.38±0.20	0.89±0.28
	Ni		2.7±1.8	4.7±3.2	6.8±1.9[a]
	Pb		0.26±0.04	0.05±0.07	0.50±0.28
	Cd		0.12±0.05	0.35±0.27	0.24±0.13
Biological responses in *C. maenas*	CAT (μmol H_2O_2/min/mg prot.)		20±11	20±14	12±7.9
	GPx (nmol NADPH/min/mg prot.)		6.0±3.8	5.6±3.3	7.2±4.1
	GST (nmol CDNB/min/mg prot.)		59±11	38±14	88±35[b]
	GSH$_t$ (nmol TNB/min/mg prot.)		9.8±1.6	10±1.7	7.1±1.2[b]
	LPO (nmol TBARS/mg F.W.)		6.7±5.1	4.8±1.6	8.6±2.0
Benthic community descriptors	S		13±5.9	16±8.7	9.3±9.5
	N		45±30	161±90	105±159
	d		3.3±1.0	3.0±1.8	1.9±1.5
	J		0.9±0.04	0.5±0.3	0.6±0.1
	Polychaeta	N	27±22	79±86	53±89
		S	6.7±1.5	2.3±0.6	3.3±3.5
	Mollusca	N	6.7±3.1	20±18	6.0±9.5
		S	3.0±2.0	3.7±3.1	1.7±2.1
	Amphipoda	N	5.7±5.5	47±65	43±56
		S	1.3±1.2	3.0±2.6	2.7±1.2
Biotic indices	AMBI		2.9±0.15	3.0±0.04	3.4±0.91
	M-AMBI		0.91	0.67	0.76

See text for abbreviations

[a] Significant different between BB and ML ($p<0.05$)

[b] Significant differences between BB and BS ($p<0.05$)

GST, GPx and LPO in crabs were also positively related with factor 1, while CAT and GSH$_t$ were negatively associated with this factor (Table 2). The previous metals, as well as those biological responses were connected to macrobenthic changes, namely the decrease of number of taxa, Margalef diversity and both the abundance and number of molluscan taxa (Table 2). Factor 1 scores only positively for BB (Fig. 3). Factor 2 explained 39.8% of

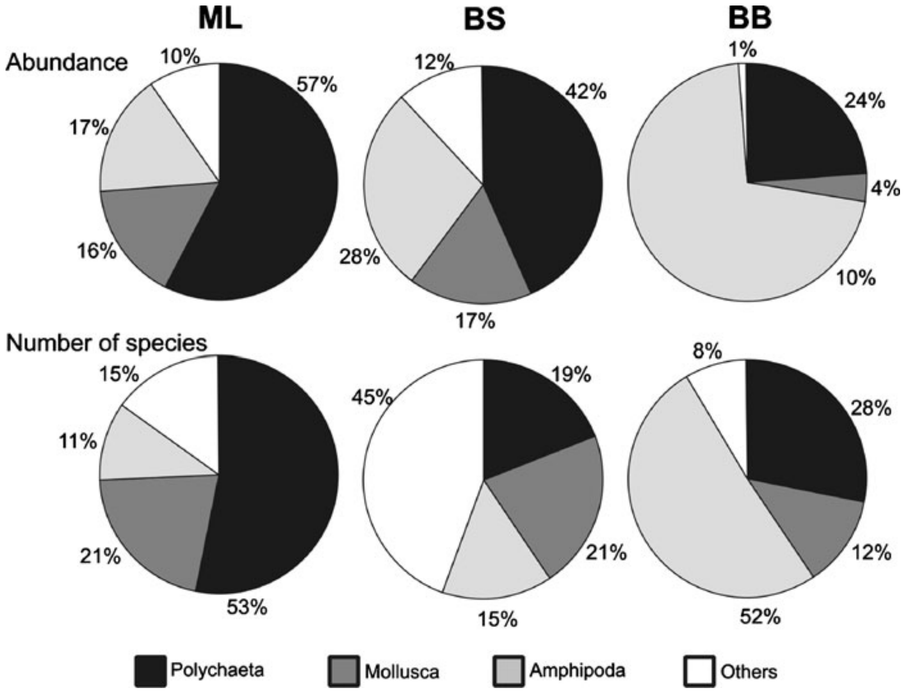

Fig. 2 Dominance distribution in terms of abundance and number of species of the main taxonomic groups for each sampling site

the variance and related negatively organic matter content in sediment, accumulated Cu and Cd in crabs and benthic alterations. This factor had a positive score only at ML site (Fig. 3). The conditions observed at ML contributed to the decrease of total abundance, abundance and diversity of amphipods, as well as polychaetes' abundance. Moreover, there was an increase of benthic equitability and the number of polychaetes' taxa (Table 2).

Discussion

According to the values proposed by Long et al. (1995) for the sediment quality guidelines, Cu and Ni in sediments of the three surveyed sites are above "Effects Range-Low" values (34 and 21 μg g^{-1}, respectively). Thus, potentially adverse biological effects could occur in the three sites of the lagoon. In general, metal levels in sediments of the Óbidos lagoon were higher than those recorded in a Mediterranean lagoon (D'Adamo et al. 2008) or fell within ranges found in other European systems (Accornero et al. 2008; Altun et al. 2009). Sediments of site BB presented slightly higher metal levels than the other sites and previous studies have also indicated that metal availability at BB is higher than at ML, due to the input from diffuse sources (Pereira et al. 2009b) and the pulse release of metals during summer nights associated with low oxygenated waters (Pereira et al. 2010a). Uniform levels of dissolved oxygen observed at BS site (unpublished data) suggest that such intense sediment–water exchanges did not occur in this branch. Total metal levels in sediments did not reflect their availability to aquatic organisms and cannot be used alone as an indicator of environmental quality. Environmental quality assessment based on macrobenthic communities alone leads also to unclear results. Despite some signs of poorer benthic quality in the BB area, significant differences on biological variables between sites were not detected and the biotic index M-AMBI pointed to a good environmental status within this area. Nevertheless, macrobenthic community structure was found to be significantly different between ML and BB, highlighting the differences between the two most environmentally contrasting areas. Previous works pointed to an impoverishment on benthic communities in the upper branch of Óbidos lagoon (Carvalho et al. 2005, 2006, 2011). The dominance of amphipods in the site BB, both in abundance and number of taxa, is in line with the low environmental quality. Likewise, the presence of amphipods together with oligochaetes in Ria Formosa (south of Portugal) was interpreted as showing the tolerance of

Table 2 Sorted rotated factor loadings of the 31 variables for the two principal factors resulting from the multivariate analysis

			Factor 1 (60.2%)	Factor 2 (39.8%)
Sediment characteristics	Al		−0.999	
	Fe		0.999	
	Mn			0.937
	Zn		1.000	
	Cu		0.919	
	Cr		0.784	
	Ni		0.832	
	Pb		0.985	
	Cd		0.962	
	OM			−0.855
Accumulated metal levels in *C. maenas*	Mn		0.951	
	Cu			−0.996
	Cr		0.988	
	Ni		0.736	
	Pb		0.972	
	Cd		−0.985	
Biological responses in *C. maenas*	CAT		−0.991	
	GPx		1.000	
	GST		0.981	
	GSH_t		−0.989	
	LPO		0.962	
Benthic community descriptors	S		−0.991	
	N			−0.978
	d		−0.925	
	J			1.000
	Polychaeta	N		−0.975
		S		0.998
	Mollusca	N		−0.717
		S		−0.985
	Amphipoda	N		−0.981
		S		−0.995

Sediment characteristics: loadings are related with the concentrations of metals in sediment and organic matter content (OM). Accumulated metal levels: loadings are related with the bioaccumulation of some metals in *Carcinus maenas*. Biological responses: loadings are related to the induction of several biological responses. Benthic community descriptors: loadings are related with alterations of benthic macrofauna, namely number of taxa (S), abundance (N), Margalef diversity (*d*) and Pielou's equitability (*J*). Only loadings equal or greater than 0.70 are shown

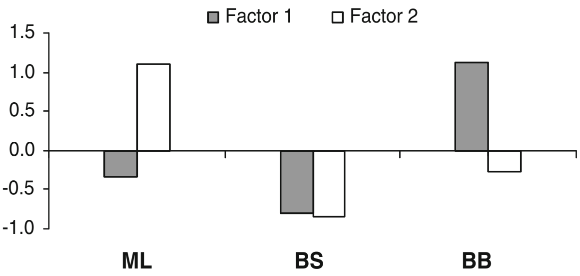

Fig. 3 Estimated factor scores for the three sampling sites. The factor scores quantify the prevalence of each factor for the sites and are used to confirm the factor description

some species of this group to deteriorated environmental conditions (Gamito 2008). Interestingly, the equitability of benthic communities as well as polychaetes' diversity was found to be enhanced in ML suggesting that the overall environmental conditions are suitable for the establishment and maintenance of macrobenthic communities typical of coastal lagoons. These results are consistent with the environmental features of this area, as it benefits from a much higher water renewal than both inner branches (BB and BS) and presents slightly lower metal levels in sediments. Therefore, depending on the methodology used on data analysis, different conclusions can be obtained, reinforcing the need for combined approaches of different disciplines as well as statistical methods.

The difference between the two most environmentally contrasting sites (BB and ML) was corroborated by biomarkers. Until the present, only few studies have used metal levels in *C. maenas* in an attempt to relate them with real field conditions making difficult the comparison of current data with previous. However, levels of Cu and Cd in *C. maenas* hepatopancreas from the Óbidos lagoon were lower than those recorded in *C. maenas* from Fal estuary (Pedersen and Lundebye 1996). Crabs from BB exhibited significantly higher levels of Ni and maximum levels of Mn, Cr and Pb. Moreover, oxidative stress parameters (particularly, GST and GSH_t) indicated that organisms were suffering from a pro-oxidant challenge at BB site. The impairment in the defence system by the chemical reactive species could reduce cell protection and the organism fitness becomes precarious (Escobar et al. 1996). Apparently, this is occurring in crabs of the Óbidos lagoon since, in general, a tendency for lower levels of CAT was observed at BB. This distinction was supported by factorial analysis for BB, as CAT activity was inversely related with accumulated levels of Cr, Mn, Ni and Pb and metals in sediments. Increased activity of antioxidant enzymes have also been described in several aquatic species from impacted sites (Guilherme et al. 2008; Martín-Díaz et al. 2008). A tendency to GPx enhancement was recorded at BB in line with its induction in a previous study (Pereira et al. 2009a). In the current work, GST activity significantly increased at BB in comparison with BS. GST induction was also previously observed in *C. maenas* exposed in situ to metal contaminated areas (Martín-Díaz et al. 2008), as well as at BB site in the Óbidos lagoon (Pereira et al. 2009a). In the present study, the induction of GST was associated by factor analysis with higher accumulation of Cr, Mn, Ni and Pb, particularly at BB. This sampling site was also put in evidence by factor analysis concerning the link of GST response with levels of metals in sediments. The depletion on GSH_t observed at BB indicates its increased use without a compensatory synthesis. The PCA linked GSH_t variation with accumulated levels of Cr, Mn, Ni and Pb. It is well-known that GSH_t may play a role in inducing resistance to metals by protecting macromolecules against attack by free radicals (Wang and Ballatori 1998). The absence of significant LPO increase in *C. maenas* from BB points to the effectiveness of the overall defence mechanism. Despite the pro-oxidant challenge in crabs at BB site, adaptive mechanisms were activated contributing eventually to their resistance to the adverse environmental conditions. However, other macrobenthic species with lower adaptive capability, namely regarding the antioxidant defence system, could have disappeared from BB site leading to community changes.

Environmental conditions observed at BS branch did not seem to be harmful to crab populations, since neither elevation of accumulated metals nor alterations in oxidative stress endpoints were recorded. Based on GST and GSH_t endpoints, BS branch exhibited a better environmental quality than the other lagoon inner branch (BB), where modulation of both parameters was recorded. These findings are in agreement with previous works that suggested BS branch as an intermediate hazard area compared with BB and ML (Pereira et al. 2009a). On the other hand, crabs at ML exhibited significantly lower Ni levels than those caught at BB where alterations regarding oxidative stress were detected. This was also observed with gills of *L. aurata* (Pereira et al. 2010a). Environmental conditions at ML did not seem to jeopardize organisms' fitness.

The factor analysis applied to all data set allowed the discrimination of BB from other sites, being BB the most impacted area of the Óbidos lagoon with a consequent risk for local populations. This is consistent with previous works using other sentinel species such as *Ulva* sp. (Pereira et al. 2009d) and *L. aurata* (Pereira et al. 2010a). The integrated approach, by proving the possibility to distinguish the environmental quality among areas of moderate degradation, appears to be particularly promising in monitoring programmes designed for specific descriptors used in the Water Framework Directive.

Final remarks

The results obtained in Óbidos lagoon, a coastal ecosystem characterised by moderate metal contamination associated with eutrophication symptoms (Pereira et al. 2009b), pointed to a slight impoverishment of benthic assemblages at the BB site, where pulse release of metals from the sediments occurs repeatedly during summer (Pereira et al. 2009b, 2010b). Despite the presence of eutrophication symptoms and increase of metal availability, BB site was classified as presenting a good environmental status, after the calculation of M-AMBI. A high score was also obtained for the other two sites. The search of alterations at a lower level of the biological organisation, namely using *C. maenas* hepatopancreas as a model for assessing metal availability, indicated a higher metal accumulation in crabs and associated biochemical responses at the site BB. Therefore, the sensitivity of crab hepatopancreas to the metal availability suggests the advantage of including a bioindicator organism/tissue in conjugation with benthic community and sediment chemistry in order to discriminate environmental status in moderately contaminated ecosystems. On the basis of this integrated analysis, areas with potential risk to local organisms could be identified and adequate measures adopted.

Acknowledgements Patrícia Pereira (SFRH/BD/17616/2004) and Susana Carvalho (SFRH/BPD/26986/2006) benefit from Ph.D. and post-doctoral grants, respectively, awarded by Fundação para a Ciência e a Tecnologia" (FCT). Authors would like to thank to Dulce Subida for help with PERMANOVA analysis. This work was financially supported by the company "Águas do Oeste" within the project "Monitoring and modelling the Óbidos lagoon and the Foz do Arelho submarine outlet". The manuscript was greatly improved by the comments of two anonymous reviewers.

References

Accornero, A., Gnerre, R., & Manfra, L. (2008). Sediment concentrations of trace metals in the Berre Lagoon (France): an assessment of contamination. *Archives of Environmental Contamination and Toxicology, 54*, 372–385.

Allan, I. J., Vrana, B., Greenwood, R., Mills, G. A., Roig, B., & Gonzalez, C. (2006). A “toolbox” for biological and chemical monitoring of the European Union’s Water Framework Directive. *Talanta, 69*, 302–322.

Altun, O., Saçan, M. T., & Erdem, A. K. (2009). Water quality and heavy metal monitoring in water and sediment samples of the Küçükçekmece Lagoon, Turkey (2002–2003). *Environmental Monitoring and Assessment, 151*, 345–362.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+for PRIMER: guide to statistical methods* (p. 214). Plymouth: PRIMER-E.

Blanchet, H., Lavesque, N., Ruellet, T., Dauvin, J. C., Sauriau, P. G., Desroy, N., Desclaux, C., Leconte, M., Bachelet, G., Janson, A.-L., Bessineton, C., Duhamel, S., Jourde, J., Mayot, S., Simon, S., & de Montaudouin, X. (2008). Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats—implications for the implementation of the European Water Framework Directive. *Ecological Indicators, 8*, 360–372.

Borja, A., Franco, J., & Pérez, V. (2000). A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin, 40*, 1100–1114.

Carvalho, S., Moura, A., Gaspar, M. B., Pereira, P., Cancela da Fonseca, L., Falcão, M., Drago, T., Leitão, F., & Regala, J. (2005). Spatial and inter-annual variability of the macrobenthic communities within a coastal lagoon (Óbidos lagoon) and its relationship with environmental parameters. *Acta Oecologica, 27*, 143–159.

Carvalho, S., Gaspar, M. B., Moura, A., Vale, C., Antunes, P., Gil, O., Cancela da Fonseca, L., & Falcão, M. (2006). The use of the marine biotic index AMBI in the assessment of the ecological status of the Óbidos lagoon (Portugal). *Marine Pollution Bulletin, 52*, 1414–1424.

Carvalho, S., Pereira, P., Pereira, F., de Pablo, H., Vale, C., & Gaspar, M. B. (2011). Factors structuring temporal and spatial dynamics of macrobenthic communities in a eutrophic coastal lagoon (Óbidos lagoon, Portugal). *Marine Environmental Research, 71*, 97–110.

Choueri, R. B., Cesar, A., Torres, R. J., Abessa, D. M. S., Morais, R. D., Pereira, C. D. S., Nascimento, M. R. L., Mozeto, A. A., Riba, I., & DelValls, T. A. (2009). Integrated sediment quality assessment in Paranaguá Estuarine System, Southern Brazil. *Ecotoxicology and Environmental Safety, 72*, 1824–1831.

Claiborne, A. (1985). Catalase activity. In R. A. Greenwall (Ed.), *CRC handbook of methods in oxygen radical research* (pp. 283–284). Boca Raton, FL: CRC Press.

Cohen, A. N., Carlton, J. T., & Fountain, M. C. (1995). Introduction, dispersal and potential impacts of the green crab Carcinus maenas in San Francisco Bay, California. *Marine Biology, 122*, 225–237.

D’Adamo, R., Di Stasio, M., Fabbrocini, A., Petitto, F., Roselli, L., & Volpe, M. G. (2008). Migratory crustaceans as biomonitors of metal pollution in their nursery areas. The Lesina lagoon (SE Italy) as a case study. *Environmental Monitoring and Assessment, 143*, 15–24.

Depledge, M., & Galloway, T. S. (2005). Healthy animals, healthy ecosystems. *Frontiers in Ecology and the Environment, 3*, 251–258.

Elliott, M., & Quintino, V. (2007). The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin, 54*, 640–645.

Escobar, J. A., Rubio, M. A., & Lissi, E. A. (1996). SOD and catalase inactivation by singlet oxygen and peroxyl radicals. *Free Radical Biology & Medicine, 20*(3), 285–290.

Fernandes, C., Fontaínhas-Fernandes, A., Ferreira, M., & Salgado, M. A. (2008). Oxidative stress response in gill and liver of *Liza saliens*, from the Esmoriz-Paramos Coastal Lagoon, Portugal. *Archives of Environmental Contamination and Toxicology, 55* (2), 262–269.

Filho, D. W., Tribess, T., Gáspari, C., Cláudio, F. D., Torres, M. A., & Magalhães, A. R. M. (2001). Seasonal changes in antioxidant defences of the digestive gland of the brown mussel (*Perna perna*). *Aquaculture, 203*, 149–158.

Gamito, S. (2008). Three main stressors acting on the Ria Formosa lagoonal system (Southern Portugal): physical stress, organic matter pollution and the land-ocean gradient. *Estuarine, Coastal and Shelf Science, 77*, 710–720.

Gornall, A. C., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry, 177*, 751–766.

Guilherme, S., Válega, M., Pereira, M. E., Santos, M. A., & Pacheco, M. (2008). Antioxidant and biotransformation responses in *Liza aurata* under environmental mercury exposure—relationship with mercury accumulation and implications for public health. *Marine Pollution Bulletin, 56*, 845–859.

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry, 249*, 7130–7139.

Long, E. R., MacDonald, D. D., Smith, S. L., & Calder, F. D. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management, 19*, 81–97.

Malhadas, M. S., Leitão, P. C., Silva, A., & Neves, R. (2009). Effect of coastal waves on sea level in Óbidos lagoon, Portugal. *Continental Shelf Research, 29*, 1240–1250.

Martín-Díaz, M. L., Blasco, J., Sales, D., & DelValls, T. A. (2007). Biomarkers study for sediment quality assessment in Spanish ports using the crab Carcinus maenas and the clam Ruditapes philippinarum. *Archives of Environmental Contamination and Toxicology, 53*, 66–76.

Martín-Díaz, M. L., Blasco, J., Sales, D., & DelValls, T. A. (2008). Field validation of a battery of biomarkers to assess sediment quality in Spanish ports. *Environmental Pollution, 151*, 631–640.

Mohandas, J., Marshall, J. J., Duggins, G. G., Horvath, J. S., & Tiller, D. (1984). Differential distribution of glutathione and glutathione related enzymes in rabbit kidney. Possible implications in analgesic neuropathy. *Cancer Research, 44*, 5086–5091.

Morales-Caselles, C., Riba, I., Sarasquete, C., & DelValls, T. A. (2008). The application of a weight of evidence approach to compare the quality of coastal sediments affected by acute (Prestige 2002) and chronic (Bay of Algeciras) oil spills. *Environmental Pollution, 156*, 394–402.

Morales-Caselles, C., Riba, I., & DelValls, T. A. (2009). A weight of evidence approach for quality assessment of sediments impacted by an oil spill: the role of a set of biomarkers as a line of evidence. *Marine Environmental Research, 67*, 31–37.

Mucha, A. P., Bordalo, A. A., & Vasconcelos, M. T. S. D. (2004). Sediment quality in the Douro river estuary based on trace metal contents, macrobenthic community and elutriate sediment toxicity test (ESTT). *Journal of Environmental Monitoring, 6*, 585–592.

Muxika, I., Borja, A., & Bald, J. (2007). Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. *Marine Pollution Bulletin, 55*, 16–29.

Nunes, M., Coelho, J. P., Cardoso, P. G., Pereira, M. E., Duarte, A. C., & Pardal, M. A. (2008). The macrobenthic community along a mercury contamination in a temperate estuarine system (Ria de Aveiro, Portugal). *Science of the Total Environment, 405*, 186–194.

Pedersen, S. N., & Lundebye, A.-K. (1996). Metallothionein and Stress Protein Levels in Shore Crabs (*Carcinus maenas*) along a Trace Metal Gradient in the Fal Estuary (UK). *Marine Environmental Research, 42*(1–4), 241–246.

Pereira, E., Abreu, S. N., Coelho, J. P., Lopes, C. B., Pardal, M. A., Vale, C., & Duarte, A. C. (2006). Seasonal fluctuations of tissue mercury contents in the European shore crab *Carcinus maenas* from low and high contamination areas (Ria de Aveiro, Portugal). *Marine Pollution Bulletin, 52*, 1450–1457.

Pereira, P., de Pablo, H., Subida, M. D., Vale, C., & Pacheco, M. (2009a). Biochemical responses of the shore crab (*Carcinus maenas*) in a eutrophic and metal-contaminated coastal system (Óbidos lagoon, Portugal). *Ecotoxicology and Environmental Safety, 72*, 1471–1480.

Pereira, P., de Pablo, H., Vale, C., Franco, V., & Nogueira, M. (2009b). Spatial and seasonal variation of water quality in an impacted coastal lagoon (Óbidos Lagoon, Portugal). *Environmental Monitoring and Assessment, 153*, 281–292.

Pereira, P., de Pablo, H., Vale, C., Rosa-Santos, F., & Cesário, R. (2009c). Metal and nutrient dynamics in a eutrophic coastal lagoon (Óbidos, Portugal): the importance of observations at different time scales. *Environmental Monitoring and Assessment, 158*, 405–418.

Pereira, P., de Pablo, H., Rosa-Santos, F., Vale, C., & Pacheco, M. (2009d). Metal accumulation and oxidative stress in *Ulva* sp. substantiated by responses integration into a general stress index. *Aquatic Toxicology, 91*, 336–345.

Pereira, P., de Pablo, H., Vale, C., & Pacheco, M. (2010a). Combined use of environmental data and biomarkers in fish (*Liza aurata*) inhabiting a eutrophic and metal-contaminated coastal system - gills reflect environmental contamination. *Marine Environmental Research, 69*, 53–62.

Pereira, P., de Pablo, H., Carvalho, S., Vale, C., & Pacheco, M. (2010b). Daily availability of nutrients and metals in a eutrophic meso-tidal coastal lagoon (Óbidos lagoon, Portugal). *Marine Pollution Bulletin, 60*, 1868–1872.

Pereira, P., de Pablo, H., Subida, M. D., Vale, C., & Pacheco, M. (2011). Bioaccumulation and biochemical markers in feral crab (*Carcinus maenas*) exposed to moderate environmental contamination—the impact of non-contamination-related variables. *Environmental Toxicology, 26*, 524–540.

Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., & Dimming, A. (2004). Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine Pollution Bulletin, 49*, 728–739.

Salas, F., Neto, J. M., Borja, A., & Marques, J. C. (2004). Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). *Ecological Indicators, 4*, 215–225.

Specchiulli, A., Renzi, M., Scirocco, T., Cilenti, L., Florio, M., Breber, P., Focardi, S., & Bastianoni, S. (2010). Comparative study based on sediment characteristics and macrobenthic communities in two Italian lagoons. *Environmental Monitoring and Assessment, 160*, 237–256.

Wang, W., & Ballatori, N. (1998). Endogenous glutathione conjugates: occurrence and biological functions. *Pharmacological Reviews, 50*, 335–352.