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Sediment quality in the Douro river estuary based on trace metal contents, macrobenthic community and elutriate sediment toxicity test (ESTT)

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The aim of this study was to evaluate the sediment quality in the mesotidal Douro River estuarine environment, in order to identify areas where sediment contamination could cause ecosystem degradation. Samples were obtained in five locations and sediment characterised for grain size, total organic matter, total-recoverable metals (Al, Fe, Cu, Pb, Cr, Ni, Cd, Zn and Mn), as well as acid volatile sulfide (AVS) and simultaneously extracted metals (SEM). *In situ* effects were evaluated by examining the macrobenthic community structure. An elutriate sediment toxicity test (ESTT) was used to estimate the amount of metals and nutrients that could be exchanged with the water column through resuspension, and its positive or negative effects on the growth of the micro-alga *Emiliania huxleyi* in a 10 day test. Anthropogenic metal contamination was identified at the north bank of the Douro estuary, with deleterious effects on the macrobenthic community, namely decrease in number of species and diversity. This contamination could possibly also be toxic for water column organisms, in case of resuspension, as shown by the ESTT. Sediments from the salt marsh at the south bank showed an impoverished macrobenthic community and elutriate toxicity, which appeared to be due to anaerobic conditions. This study clearly shows the usefulness of the ESST approach to assess the biological effect of resuspension of estuarine sediments.

1. Introduction

The Douro estuary is the final region of the largest watershed of the Iberian Peninsula which covers over 98000 km^2. It drains into the Atlantic Ocean near Porto, Portugal's second largest city. Domestic sewage of over one million inhabitants, as well as industrial effluents, are still discharged, mostly without treatment, directly into the estuary and its tributaries. Sediments from the Douro estuary have been shown to receive metal contamination of anthropogenic origin that may have deleterious consequences for the macrobenthic communities.[1] A final stressor on the estuary is intense dredging from the sand mining industry.

The natural occurrence of metals in the environment complicates the assessment of potential sediment contamination. The grain size distribution is possibly the most important factor controlling sediment metal concentrations. Many sediment constituents have been used for normalization of this effect, including the grain size[2] itself and conservative elements such as aluminium (Al),[3,4] lithium (Li)[5] and iron (Fe).[6,7]

Several numerical sediment quality guidelines (SQGs) have been developed to provide interpretative tools for assessing the biological significance of individual chemicals.[8] One of these approaches established two guideline values, the ERL (effects range-low) and the ERM (effects range-median). They are derived from effect data sets composed of a compilation of results of sediment toxicity tests and benthic community surveys, thus defining the concentration ranges that are rarely, occasionally or frequently linked to adverse biological effects.[9–11]

Both the ERL and ERM used to be applied to total recoverable metal concentrations. Nevertheless, contaminants associated with sediments may not be available owing to their chemical and physical speciation. Bioavailability of sediment bound contaminants is determined by sediment composition, overlying and interstitial water chemistry, and the behaviour of organisms.[12] Partitioning models have been developed to account for the factors that are likely to influence metal bioavailability in sediments. For instance, the relative concentrations of extracted trace metals (SEM) and acid-volatile sulfides (AVS) have been proposed to account for the relative bioavailability of five trace metals (Cd, Cu, Ni, Pb and Zn) in sediments,[13,14] since they form insoluble sulfides and their toxicity is limited by the amount of sulfide in the sediment.

On the other hand, the elutriate sediment toxicity test (ESTT) provides information about the expected toxicity associated with resuspension processes such as floods or dredging operations, which frequently occur in estuaries. The ESTT is considered the best method to estimate the amount of contaminants that are exchanged between the sediment and the aqueous phase during dredging and disposal.[15] Several authors have used micro-algae as test organisms in the ESTT.[16–18]

The purpose of this study was to evaluate the sediment quality at five sampling stations in the lower Douro estuary, in order to identify areas where sediment contamination could be responsible for ecosystem degradation. Sediment characterisation included grain size, total organic matter and total-recoverable metals, as well as AVS and SEM. *In situ* effects were evaluated by examining macrobenthic community structure. ESTT was used to estimate the amount of metals and nutrients that could be exchanged to the water column during sediment resuspension, and its effects on the growth of the micro-alga *Emiliania huxleyi* in a 10 day test.

2. Materials and methods

All materials were previously acid cleaned (1 M HCl for at least 24 h), rinsed with deionised water (conductivity <0.1 μS cm^{-1})

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and sterilized by microwave (700 W for 10 min) only for organism manipulation. All sample manipulations were carried out using gloves, in a Class 100 laminar flow hood in a clean room with high efficiency particulate air filtered air.

2.1. Sampling strategy

Based on a previous study,[1] five sampling stations (Fig. 1), one on the northern bank and four on the southern bank, were selected as being representative of the different intertidal sediments of the lower Douro estuary. Samples were collected (in November 1998) at low spring tide, down to a depth of 2 dm. Three replicate samples for macrobenthic organisms assessment were collected with a 0.35 dm^2 core sampler, sieved on site through 0.5 mm mesh size and fixed in 5% buffered estuarine water–formalin stained with rose bengal. Two replicate samples of sediment were collected with a plastic corer, and analysed individually for grain size distribution, in duplicate for organic matter (OM) content, AVS and SEM, and in triplicate for total-recoverable metals. For ESTT, composed samples of sediment were collected. All samples were collected synoptically. For ESTT and chemical analysis, sediments were stored at 4 °C in plastic bags, and analysed as soon as possible, within one month after collection.

2.2. Sediment characteristics

Sediments were dried at 60 °C. Contents of OM in sediment were determined by loss on ignition (4 h at 500 °C). Grain size fractionation was performed by wet ($<$0.063 mm) and dry (0.063–0.125 mm; 0.125–0.25 mm; 0.25–0.5 mm; 0.5–1 mm; 1–2 mm; $>$2 mm) sieving of samples previously treated with hydrogen peroxide.

For metal analysis, about 0.5 g of dry sediment was digested in high-pressure Teflon vessels, using a microwave (MLS-1200 Mega, Millestone), with 6 ml of suprapure concentrated nitric acid. The metal contents in the obtained solution were assayed by atomic absorption spectrophotometry with flame atomisation (AAS-F, Philips PU 9200 X) for Al, Fe, Mn, Zn, Cu, Cr, Pb and Ni and with electrothermal atomisation (AAS-EA, Perkin-Elmer 4100 ZL) for Cd. Reference standard sediment with certified total metal concentration (BCR 277 estuarine sediment n° 068) was used to check the accuracy of the digestion and analysis procedures for the determination of Zn, Cu, Pb, Cr, Ni and Cd. For the other metals, the total concentrations were not certified in the reference standard, and only indicative values were available. The analysis of the reference standard sediment revealed satisfactory recoveries (Zn (94%), Cu (94%), Pb (93%), Cr (85%), Cd (85%), Ni (84%) and Fe (82%)) except for Mn (74%) and Al (40%). Some lower values were expected as the total-recoverable metals instead of total contents were measured.

Concentrations of AVS and SEM were determined in 5 g of wet sediment according to the method described by Allen *et al.*[19] The sulfide concentration was determined by spectrophotometry at 670 nm (UNICAM 5675) and the metal contents by AAS.

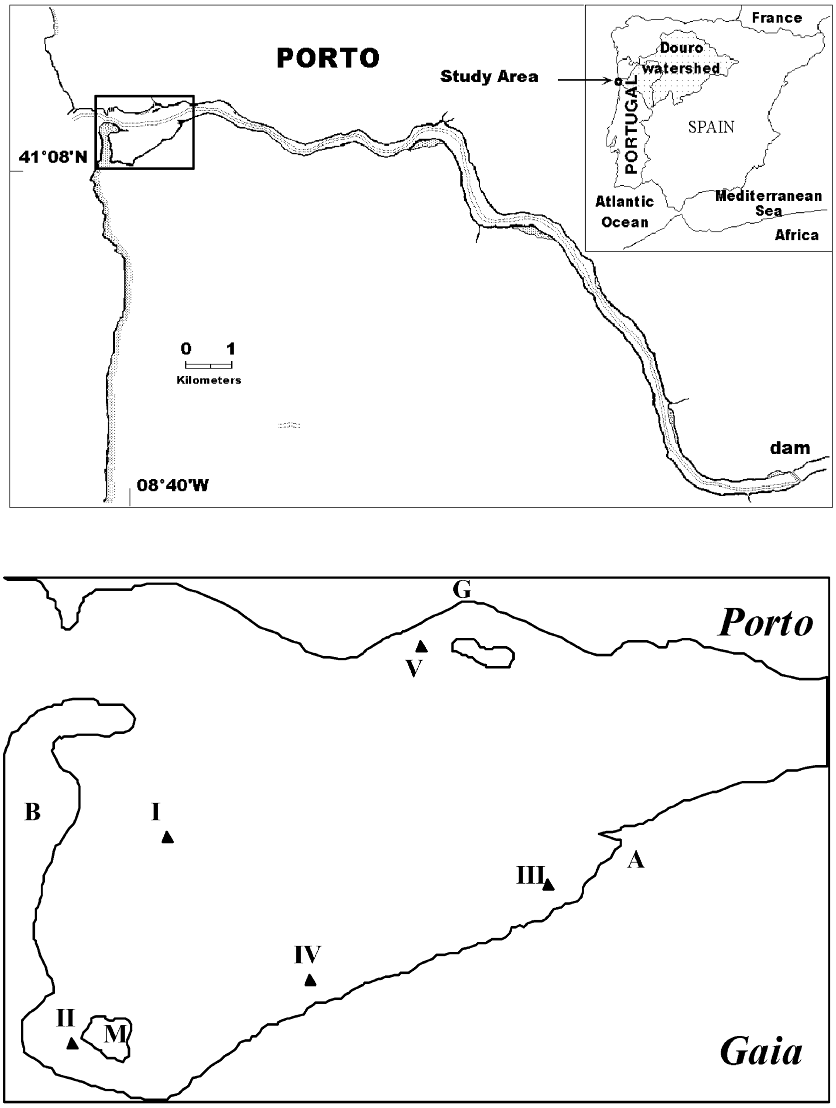

Fig. 1 Area of study with the location of the different sampling stations. A – Afurada village, B – sand bar, M – marsh area, G – Granja tributary.

2.3. Macrobenthic community

Macrobenthic organisms were sorted, enumerated and identified to the lowest possible taxon (usually to the species level). Data on individuals per species were used to calculate biological parameters such as abundance (A) (individuals dm^{-2}), number of species (S), Margalef's species richness index (R),[20] Shannon diversity index (H'),[21] Pielou's evenness index (J')[22] and Simpson dominance index (D).[23]

The biological environmental gradients procedure (BIO-ENV), from the PRIMER software package,[24,25] was used to establish a link between the macrobenthic community structure and the sediment characteristics. Such a procedure enabled selection of the environmental variables subset that maximises the rank correlation between biotic and abiotic (dis)similarity matrices, as measured by the Spearman rank coefficient (ρs).

2.4. Elutriate sediment toxicity test

Elutriates were prepared with natural seawater enriched only with 176 µM N ($NaNO_3$) and 7.26 µM P (NaH_2PO_4). The seawater had been collected 150 m from the seashore, filtered (0.1 µm polycarbonate membrane, Millipore) and stored in HDPE containers in the dark. The elutriation was carried out by mixing sediment to seawater in a 1 : 4 solid to liquid volumetric ratio, stirring vigorously for 30 min with a magnetic stirrer (stirring manually every 10 min to ensure complete mixing), and filtering the supernatant (0.45 µm cellulose nitrate membrane, Millipore) after 1 h settling.[26] Elutriates were prepared less than 24 h before the beginning of the experiments, and were used without dilution (100%), and with dilutions 1 : 1 (50%) and 1 : 10 (10%).

Sixteen different experiments (natural seawater used as control plus three concentrations of the five elutriates) were conducted in 25 ml STERILIN flasks. *E. huxleyi* from cultures on day 7 of growth (exponential phase) prepared as before[27] were inoculated in the media providing an initial concentration of 0.75×10^6 cells l^{-1}. The cultures were incubated under continuous (24 h) illumination at 18 °C for 10 days. Bottles were shaken every day and cells numbers were counted every 2 days. All experiments were carried out in triplicate. Details on algae culture are described elsewhere.[28] Statistical differences between algae growth in elutriates and control were tested with ANOVA and results presented refer to a level of 95% significance ($p < 0.05$).

Initial concentrations of total dissolved Zn, Cu, Pb, Cd, Fe, Mn, Ni and Cr were assessed in the natural seawater and elutriates (undiluted). Aliquots of the initial culture media were acidified to pH 2.2 with 10 M HCl and digested by ultraviolet (UV) radiation for 1 h. Total dissolved metals ($[M]_d$) were determined by cathodic stripping voltammetry (Fe, Mn, Ni and Cr) and anodic stripping voltammetry (Zn, Cu, Pb and Cd). The procedures and conditions adopted for the voltammetric determinations for all metals are described elsewhere.[27,28] The $[M]_d$ was also determined, using the same procedures, in a seawater reference material for trace metals (NASS-5 from National Research Council of Canada, Ontario, Canada), and the experimental and certified values were found to be statistically identical (t-test, $p = 0.05$).

The initial concentrations of dissolved nutrients in both natural seawater and elutriates were determined before N and P additions. The concentration of orthophosphate (PO_4^{3-}), nitrite (NO_2^-), ammonium (NH_4^+) ions and dissolved silicate (DSi) were determined using methods described in Grasshoff *et al.*[29] The nitrate (NO_3^-) anion was assayed using an adaptation of the spongy cadmium reduction technique.[30]

2.5. Scaled values

Trace metal concentration in the sediment, macrobenthic community and ESTT data were used together, in order to rank the stations for a particular parameter and to calculate a mean rank for each station. The ranks for each parameter have been scaled in order to maximize the relative nature of the data among stations. All values, for a particular parameter, were scaled to a 0–99 range using the following formula:[31]

$$\text{Scaled value} = [(\text{value} - \text{minimum})/(\text{maximum} - \text{minimum})] \times 99$$

The mean of the scaled values for metals of toxicological significance (Cd, Cr, Cu, Pb, Ni and Zn), according to the SQGs,[9–11] was used to estimate the potential impact of the trace metal contamination. The mean of the scaled values for the several biological parameters (A, S, R, H', J', D), was used in the ranking procedure to estimate the impact on the macrobenthic community. The mean of the scaled values for the results of the ESTT in the three concentrations of elutriates (100%, 50% and 10%) was used as a measure of toxicity. ESTT results and biological parameters, except dominance (D), were transformed in their inverse to calculate the scaled value, since they are known to decrease when sediment quality decreases. As with any ranking procedure, there is an element of subjectivity. Even so, the ranking procedure will provide a valid means of making comparisons among stations.

3. Results

3.1. Sediment characteristics

Sediment characterisation is summarised in Table 1. Metal normalization to the silt and clay fraction (<0.063 mm)

Table 1 Values[a] of organic matter (OM), grain size <63 µm, metals, simultaneously extracted metals (SEM), acid volatile sulfide (AVS) and SEM-AVS measured in the sediments from the 5 sampling stations

		Station				
Parameter		I	II	III	IV	V
OM	%[b]	0.43 ± 0.02	4 ± 1	1.8 ± 0.2	2.0 ± 0.4	0.7 ± 0.1
<63 µm	%[b]	1.5 ± 0.2	34 ± 7	14 ± 5	22 ± 3	2.2 ± 0.4
Al	%[b]	0.34 ± 0.06	1.9 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	0.45 ± 0.03
Fe	%[b]	0.5 ± 0.1	1.5 ± 0.1	1.11 ± 0.05	1.2 ± 0.2	0.48 ± 0.04
Mn	$\mu g\ g^{-1}$	58 ± 10	93 ± 7	46 ± 4	47 ± 6	32 ± 12
Cd	$\mu g\ g^{-1}$	0.07 ± 0.07	0.27 ± 0.05	0.28 ± 0.04	0.27 ± 0.03	0.08 ± 0.01
Cr	$\mu g\ g^{-1}$	5.4 ± 0.9	26 ± 2	25 ± 4	25 ± 3	57 ± 18
Cu	$\mu g\ g^{-1}$	2.6 ± 0.8	19 ± 2	18 ± 1	19 ± 4	74 ± 69
Ni	$\mu g\ g^{-1}$	11 ± 4	26 ± 3	22 ± 3	24 ± 3	20 ± 2
Pb	$\mu g\ g^{-1}$	0.4 ± 0.4	37 ± 8	34 ± 3	24 ± 2	20 ± 5
Zn	$\mu g\ g^{-1}$	22 ± 4	106 ± 11	83 ± 7	78 ± 9	203 ± 59
SEM	$\mu mol\ g^{-1}$	0.12 ± 0.04	0.8 ± 0.2	0.31 ± 0.05	0.4 ± 0.2	1.7 ± 0.6
AVS	$\mu mol\ g^{-1}$	n.d.	1.3 ± 0.2	1.04 ± 0.17	0.8 ± 0.6	0.4 ± 0.2
SEM-AVS	$\mu mol\ g^{-1}$	+0.12 ± 0.04	−0.46 ± 0.08	−0.7 ± 0.2	−0.4 ± 0.6	+1.3 ± 0.5

[a] Arithmetic mean ± SD. [b] Percentage of the total dry sediment weight. n.d. - not detected value.

(Fig. 2) indicated the presence of anthropogenic contamination in terms of Cu, Cr and Zn only at station V, shown by the outliers in the graphics. Metal normalization to Fe provided similar conclusions (results not shown). Fig. 2 also permits a comparison of the metals concentration with the respective ERLs (sediment quality guidelines). Values above ERMs were not found in any case.

Station I was dominated by very coarse sand (44%) and displayed the lowest content of OM, silt and clay fraction and all metals but Fe and Mn. AVS was not detected.

Station II was dominated by the silt and clay fraction (34%) and had the highest contents of OM, Ni (above its ERL), Pb, Fe, Mn and Al. It displayed the highest AVS levels and negative values of SEM-AVS, denoting very low potential for metal bioavailability.

Stations III and IV were dominated, respectively, by gravel (36%) and very coarse sand (32%), with some contribution of the silt and clay fraction (14% and 22%, respectively). These sediments exhibited intermediate values for almost all the measured sediment parameters and negative values of SEM-AVS and, therefore, also low metal bioavailability. Ni was above its ERL in both sediments.

Station V was dominated by gravel (46%) and displayed the lowest levels of Fe and Mn but the highest levels of Cu, Cr and Zn. Concentrations of Ni, Cu and Zn were above their respective ERLs. In addition, positive values of SEM-AVS were found, indicating a potential for metal bioavailability.

3.2. Macrobenthic community

Abundance of the different species, as well as the calculated biological parameters of the macrobenthic community, are summarised in Table 2. A total of 12 species were found in the studied area. All stations were dominated by oligochaetes with the exception of station IV which was dominated by a polychaete (*Streblospio benedicti*). Station I registered the highest abundance (*A*), number of species (*S*) and species richness (*R*) while station II presented the lowest abundance (*A*). Station III showed the lowest dominance (*D*) and station IV the highest diversity (*H*′) and evenness (*J*′), while station V registered the lowest number of species (*S*), species richness (*R*), diversity (*H*′) and evenness (*J*′) but the highest dominance (*D*).

BIO-ENV procedure was used to select the combinations of sediment characteristics that best associate the samples in a consistent manner with the macrobenthic community structure. The single variable that best matched species abundances was Cd (ρs = 0.70), followed by SEM (ρs = 0.58), Cr (ρs = 0.53) and Ni (ρs = 0.53). The combination of Cd and SEM with grain size fraction <0.063 mm constituted the overall optimum (ρs = 0.84). Although the BIO-ENV procedure does not give the direction of such correlation, it indicates that these

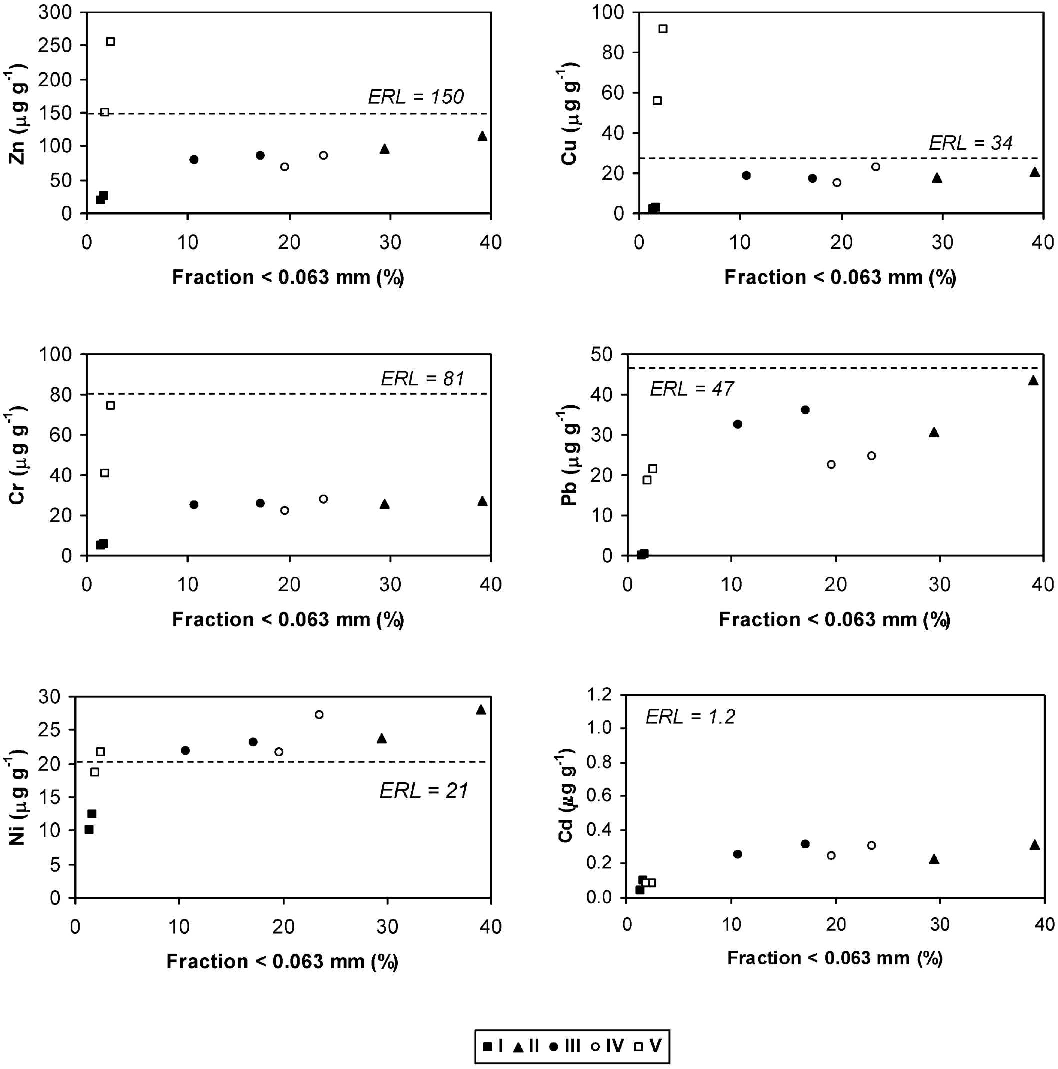

Fig. 2 Normalized plots of trace metal concentrations to the fraction <0.063 mm (silt and clay) in the 5 sampling stations (2 replicates each). The presence of outliers indicates anthropogenic contamination. Dashed lines represent the Effect Range Low (ERL) value for each metal.

Table 2 Abundance (ind. dm^{-2}) of the different species and values of total abundance (A) (ind. dm^{-2}), number of species (S), Margalef's species richness index (R), Shannon diversity index (H'), Pielou's evenness index (J') and Simpson dominance (D), measured at the five sampling stations

	Stations				
	I	II	III	IV	V
Hediste diversicolor	0	0	89	0	0
Streblospio benedicti	0	17	80	226	0
Enchytraeidae	1149	0	0	0	11
Tubificidae	754	326	866	83	651
Tubifex costatus	34	9	609	154	0
Nematoda	20	0	0	0	0
Cyathura carinata	6	0	49	14	0
Corophium volutator	29	0	0	0	0
Sphaeroma serratum	17	0	0	0	0
Scrobicularia plana	0	3	0	6	0
Bivalvia	3	0	0	0	0
Bryozoa	3	0	0	0	0
A	2014	354	1691	483	663
S	9	4	5	5	2
R	1.05	0.51	0.54	0.65	0.15
H'	0.94	0.36	1.11	1.18	0.08
J'	0.43	0.26	0.69	0.73	0.12
D	0.47	0.85	0.40	0.35	0.97

variables possibly influence the differences in community structure found among samples.

3.3. Elutriate sediment toxicity test

Data on total dissolved metals and macronutrients that were found in control and elutriates (undiluted) are presented in Table 3. Elutriate sediment from station I displayed the lowest levels of Pb, Cr, Mn and Fe, but the highest levels of all the macronutrients. Elutriate sediment from station II contained the highest level of Mn but the lowest levels of PO_4^{3-} and NH_4^+. Elutriate sediment from station III had the lowest levels of Cd, Ni, Cu and Zn but the highest level of Cr. Elutriate sediment from station IV showed the lowest level of NO_2^-. Finally, elutriate sediment from station V, presented the highest levels of Cd, Pb, Ni, Cu, Zn and Fe and the lowest level of NO_3^-.

Growth curves of *E. huxleyi* in the different media are disclosed in Fig. 3. Relative to the control, sediment elutriate from station I stimulated the growth of the algae. In fact, the culture media with undiluted elutriate (100%), displayed an algae growth significantly higher ($p < 0.05$) than that of the control, at days 4, 6 and 10. For 50% elutriate dilution, algae growth was still significantly higher than that of the control ($p < 0.05$) at days 6 and 10. For the 10% dilution, although the growth curve shows average values slightly higher than the control, no statistically significant differences could be found.

Growth curves representing algae exposure to sediment elutriate from station II exhibited an initial inhibition on day 2, for all the elutriate concentrations, since the cells numbers were significantly lower than those for the control ($p < 0.05$). Further incubation showed that the inhibition effect was registered only in day 8 (values significantly lower than those for the control ($p < 0.05$)) in the undiluted elutriate (100%).

Where sediment III is concerned, the undiluted elutriate (100%) stimulated algae growth on days 4 and 6 (values significantly higher than those for the control ($p < 0.05$)). The 50% elutriate concentration also stimulated the growth. However statistically significant differences ($p < 0.05$) were registered only on day 4. For the 10% elutriate, the results were not significantly different from the control.

Growth curves in sediment elutriate from station IV were quite similar to those for the control. Nevertheless, for the diluted elutriates (50% and 10%), significant growth stimulation ($p < 0.05$) was registered at day 4, but inhibition was found at day 8 in the 100% experiment.

Algae exposed to undiluted elutriate (100%) from sediment V exhibited a statistically significant inhibition ($p < 0.05$) of growth, on days 8 and 10. For the 50% elutriate, values significantly lower than the control ($p < 0.05$) were registered only on day 8. No statistically significant differences to the control were observed in the growth curve for 10% of elutriate.

3.4. Scaled values

Scaled values for data on trace metals in sediments, macrobenthic community and ESTT are presented in Table 4. Station V exhibited the lowest relative ranking, resulting from a high trace metal contamination, namely in terms of Cu, Cr and Zn, the highest impact in macrobenthic community and the highest inhibition of algae growth in the ESTT, after 10 days of growth. Station II had the second lowest relative ranking followed by station III and station IV. Station I presented the highest relative ranking which is compatible with the lowest trace metal concentrations, the least impacted macrobenthic community and the highest stimulation of the algae growth in the ESTT.

4. Discussion

Based on the obtained data, the different stations can be classified as follows: station I, located in a high hydrodynamic zone, appeared as the least degraded with the highest relative ranking. This sandy station had the lowest metals concentration and a macrobenthic community with relatively high diversity and the highest abundance, number of species and

Table 3 Concentration of total dissolved metals[a] (nM) and total dissolved macronutrients[b] (μM) in natural seawater (control) and elutriates (undiluted) from sediments from the five locations in the Douro estuary

		Elutriates				
	Seawater (Control)	I	II	III	IV	V
Fe	75 ± 7	110 ± 2	900 ± 9	362 ± 23	284 ± 3	(210 ± 1) × 10
Mn	15.7 ± 0.3	85 ± 3	(140 ± 9) × 10^2	(121 ± 0) × 10	(185 ± 9) × 10	(211 ± 1) × 10
Cd	0.20 ± 0.05	0.65 ± 0.07	0.69 ± 0.06	0.63 ± 0.04	0.88 ± 0.08	2.5 ± 0.1
Cr	9.0 ± 0.2	9.4 ± 0.1	12 ± 2	45.1 ± 0.1	34 ± 2	32.4 ± 0.7
Cu	10 ± 2	82 ± 1	11.5 ± 0.2	11 ± 1	13 ± 1	176 ± 8
Ni	9 ± 3	65.8 ± 0.4	149 ± 12	66 ± 4	72 ± 2	(139 ± 2) × 10
Pb	0.83 ± 0.01	0.48 ± 0.01	1.1 ± 0.2	3.7 ± 0.1	13.8 ± 0.2	22 + 1
Zn	23 ± 4	44 ± 3	152 ± 8	33.3 ± 0.2	150 ± 9	(350 ± 8) × 10^2
NO_3^-	71.2	585	112	112	99.8	75.0
NO_2^-	0.069	1.9	1.3	1.3	0.38	0.68
NH_4^+	5.01	108	18.8	19.6	68.5	90.2
PO_4^{3-}	0.60	11	0.12	0.17	0.33	0.21

[a] Arithmetic mean ± SD (three replicates). [b] Before addition of N and P.

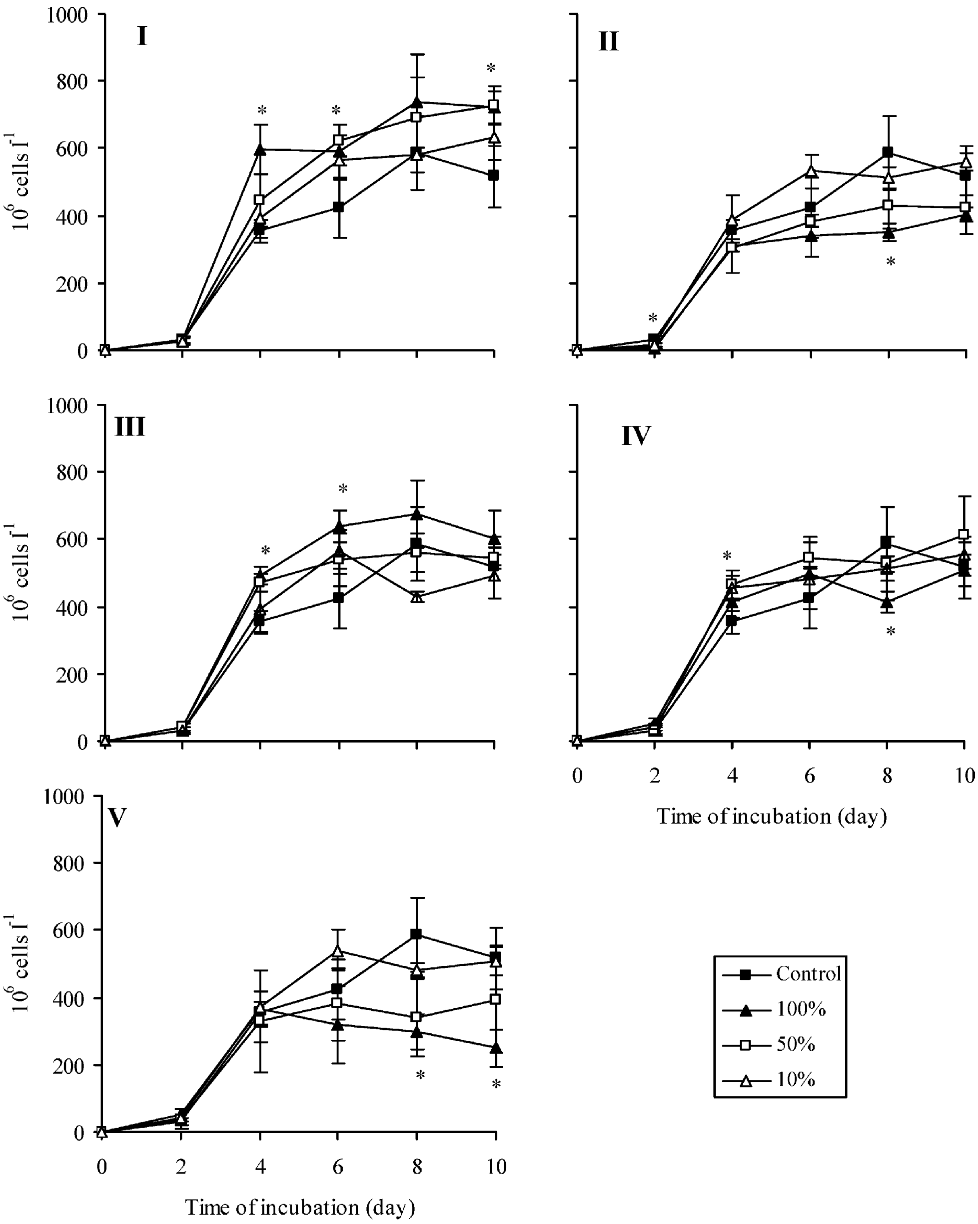

Fig. 3 *E. huxleyi* growth in the control medium and in the three concentrations (100%, 50% and 10%) of the sediment elutriates from stations I to V. Bars correspond to standard deviation ($n = 3$). Asterisks indicate statistically significant differences relative to the control ($p < 0.05$) in at least one of the treatments.

species richness. Elutriates from this sediment fostered the highest algae growth. This stimulation could be related to the high macronutrient concentrations and also to the absence of a metal inhibitory effect (which may reflect the absence of anthropogenic contamination) since this elutriate presented the lowest dissolved metal contents of all the locations. Actually, in a separate study the high sink rates of water column nutrients to the sediment were demonstrated.[32]

Station II, located within the salt marsh in a low energetic high sedimentation zone, dominated by silt and clay, had the second lowest relative ranking. The relatively high trace metal contents found at this station could reflect natural enrichment rather than anthropogenic contamination, as stated by the metal normalization to the silt and clay fraction. In addition, the availability of the metals to organisms is assumed to be very low, as negative SEM-AVS values were observed. Nevertheless, this station had relatively low diversity and the lowest abundance of the macrobenthic community, which could arise from the anaerobic conditions of sediments. For instance, the presence of free sulfide (associated to relatively high levels of AVS) is known to be toxic to some organisms.[33] Elutriates from this sediment induced inhibition of algae growth in the initial phase. The initial concentrations of Mn and Fe in elutriates were rather high. As Mn and Fe are nutrient metals and not toxicants,[34] inhibitory effect may be related to the presence of reduced sulfur species that, despite not being measured in the elutriates, were found in the sediment. However, factors not measured in this work, including organic and/or organo-metallic contaminants, could also condition the observed results.

Stations III and IV, located also along the south bank, had intermediate relative rankings. Again, trace metal contents reflected natural enrichment rather than anthropogenic contamination and with reduced availability to organisms, as SEM-AVS values were negatives. Biological effects were not detected since these stations had the highest diversities of macrobenthic community. In terms of ESTT, stimulation of algae growth was registered in sediment elutriate from station III while that from station IV displayed small differences relative to the control.

Station V, located on the north bank, appeared as the most degraded, with the lowest relative ranking. This station had

Table 4 Scaled values of trace metal concentration in sediments, impact on macrobenthic community and toxicity in the ESTT

	Stations				
Scaled values[a]	I	II	III	IV	V
Based on total-recoverable trace metal concentration					
Cu	0	23	21	23	99
Pb	0	99	87	42	28
Cr	0	40	38	38	99
Ni	0	99	76	89	60
Cd	0	94	99	94	8
Zn	0	46	33	30	99
Mean	0	67	59	53	65
Based on macrobenthic community					
A^{-1}	0	99	4	67	43
S^{-1}	0	35	23	23	99
R^{-1}	0	18	16	11	99
H'^{-1}	2	18	0	0	99
J'^{-1}	14	36	1	0	99
D	18	80	7	0	99
Mean	6	48	9	17	90
Based on ESTT (10 day growth^{-1})					
100%	0	41	10	22	99
50%	0	82	39	22	99
10%	0	44	99	49	82
Mean	0	56	50	31	93
Mean of scaled values	2	57	39	34	83
Relative ranking[b]	5	2	3	4	1

[a] All values were scaled to 0–99 range using the following formula : scaled value = [(value − minimum)/(maximum − minimum)] × 99. [b] The higher the ranking the less degraded the station relative to the other stations.

clear anthropogenic contamination in terms of Zn, Cr and Cu, and SEM-AVS positive values, suggesting a potential for metal bioavailability. A major source of metals is a nearby tributary, a stream that crosses the city of Porto. Indeed, several industries (plating, soap, textiles and tannery) still discharge their effluents into the stream without treatment despite the existence of an urban sewage system. Sediment contamination seems to affect the macrobenthic community, as shown by the lowest number of species, species richness, diversity and evenness, and the highest dominance registered at this station. If resuspension occurs, sediment contamination could pass to the water column and possibly affect planktonic communities, as shown by the inhibition of algae growth in the ESTT. In fact, this elutriate presented the highest levels of almost all the dissolved metals. It should be emphasised that Ni and Zn concentrations were 1 and 2 orders of magnitude, respectively, above those of the other elutriates. Metal contaminations will be a major cause of the observed toxicity, despite the fact that other unmeasured compounds (namely organic and/or organometallic) could also condition growth inhibition. In a separate study, with elutriates of sediments from the same estuary,[28] organic ligands (measured in equivalents of Cu) up to 800 nM were found. These concentrations, despite being relatively high (and higher than the Cu concentration in the elutriate) were quite significantly below the Ni and Zn concentrations found in the present work, suggesting high bioavailability of these two elements in the elutriate.

In the Douro estuary, salinity fluctuations ranging from 0 to 35 PSU occur not only seasonally but also within a tidal cycle,[35] due to the discharge regime of the dams along its watershed. The strong salinity fluctuations that occur within estuarine boundaries could be one explanation for the low diversity of the macrobenthic community, which is dominated by a few opportunistic species. Only twelve macrobenthic species were found in the five stations sampled, which is in agreement with a total of nineteen species registered in a previous study carried out in thirty five stations spread along the entire lower Douro estuary.[1] The BIO-ENV procedure selected, among the measured parameters, the trace metal content, in combination with the grain size distribution, as the major factors that correlate with the community structure. Nevertheless, other physical, chemical and biological factors, both natural and anthropogenic, will contribute to the observed situation. For instance, it is foreseeable that the physical instability of the sediments affects the macrobenthic community. Nevertheless, despite recognizing that strong resuspension processes, such as floods and dredging operations, are known to occur regularly in the Douro estuary, specific data about its impact in the sediments structure are not yet available, therefore deserving investigation.

Comparing values of metal contents in sediments with those in elutriates, it was found that amounts of metal exchanged to the liquid phase during the elutriation process varied markedly among the sediment samples, as illustrated next for some remarkable cases. The levels of total-recoverable Cd in sediments were similar at stations II, III and IV and about four fold as large as those at station I and V. Notwithstanding, the undiluted elutriate of station V sediment exhibited about six fold more dissolved Cd than those of the sediments from the other stations. Much higher solubility of Ni and Pb from station V sediment was also observed. The contrary was observed, for instance, for Cr, for which the dissolved fraction in the undiluted elutriate of station V was lower than those in the elutriates of stations III and IV, despite the fact that the Cr concentration in sediment from station V was significantly higher. These results show differences in the nature of binding metal/sediment among the several stations. They emphasise the impossibility of estimating effects to water column organisms by just analysing sediments. Approaches such as the ESTT provide an estimation of the amount of chemical contaminants that can be exchanged between sediment and solution, and its biological effects through exposure to the water column. However, it must be stressed that, even when present in the water column, metals bioavailability is strongly dependent on the presence of organic ligands, which could transform metals into stable complexes.[27,36]

In conclusion, the present study disclosed anthropogenic metal contamination in the north bank of the Douro estuary, with probable deleterious effects on the macrobenthic community. Such contamination could possibly be toxic also for water column organisms, in case of resuspension, as shown by the ESTT. Sediments from the salt marsh at the south bank showed an impoverished macrobenthic community and elutriate toxicity, related to its anaerobic characteristics rather than to metal contamination. Finally, this study clearly shows the usefulness of the ESST approach to assess the biological effect of the resuspension of estuarine sediments, due to the potential impact in promoting phytoplankton growth (eutrophication) or inhibition (reduction of primary production, toxicity) when submitted to floods or dredging operations, as occurs in the Douro estuary.

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