

# Assessment of the ecological quality of the Minho estuary (Northwest Iberian Peninsula) based on metal concentrations in sediments and in *Corbicula fluminea*

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Received: 25/09/2013

Accepted: 21/03/2014

## ABSTRACT

### Assessment of the ecological quality of the Minho estuary (Northwest Iberian Peninsula) based on metal concentrations in sediments and in *Corbicula fluminea*

The concentrations of metals (Cd, Cr, Cu, Fe, Ni, Pb and Zn) in sediments and the soft tissues of *Corbicula fluminea* from the Minho estuary (Northwest Iberian Peninsula) were determined to assess the spatial variation of metal bioavailability and to classify the ecological quality of the estuary. Metal concentrations in sediments showed significant spatial variation ( $p < 0.0003$ ) and ranged in concentration (dry weight basis) from 0.025-0.36 mg Cd/kg, 7-16 mg Cr/kg, 2.7-24.6 mg Cu/kg, 7.3-45 mg Ni/kg, 4.1-15 mg Pb/kg and 37.3-110 mg Zn/kg. According to the Norwegian Pollution Control Authority metal concentration guidelines for sediments, this first ecological quality classification of the Minho estuary is “Class I/II-Background/Good.” Metal concentrations in the soft tissues of *C. fluminea* also showed significant spatial variation ( $p < 0.0001$ ) and ranged in concentration (dry weight basis) from 1.1-2.5 mg Cd/kg, 1.0-1.8 mg Cr/kg, 34-71 mg Cu/kg, 5.8-11 mg Ni/kg, 0.45-1.3 mg Pb/kg and 136-161 mg Zn/kg. The high Cu concentrations bioaccumulated by *C. fluminea* in the Minho estuary suggest that the estuary should be classified as “Class II/III-Moderately/Remarkably Polluted.” Thus, the primary contributions of this work are that *C. fluminea* proved to be a good and adequate biomonitor of metal contamination in the Minho estuary and that it could be used to assess the ecological quality of estuarine ecosystems worldwide.

**Key words:** Minho, sediments, soft tissues, spatial variations, *Corbicula fluminea*.

## RESUMEN

### Evaluación de la calidad ecológica del estuario del Miño (Península Ibérica Noroeste) basada en la concentración de metales en sedimentos y en *Corbicula fluminea*

La concentración de metales (Cd, Cr, Cu, Fe, Ni, Pb and Zn) en sedimentos y tejidos blandos de *Corbicula fluminea* procedentes del estuario del Miño (Noroeste Península Ibérica) fue determinada para evaluar variaciones espaciales en la biodisponibilidad de los metales y para comparar diferentes clasificaciones ecológicas de calidad. Los sedimentos retuvieron metales en rangos (peso seco) entre 0.025-0.36 mg Cd/kg, 7-16 mg Cr/kg, 2.7-24.6 mg Cu/kg, 7.3-45 mg Ni/kg, 4.1-15 mg Pb/kg y 37.3-110 mg Zn/kg, lo que revela una variación espacial significativa ( $p < 0.0003$ ). Las directrices sobre concentración de metales en sedimentos recomendadas por la Norwegian Pollution Control Authority permitieron obtener una primera clasificación de calidad ecológica del estuario del Miño designada como “Clase I/II-Basal/Buena”. En los tejidos blandos de *C. fluminea* también se encontraron variaciones espaciales significativas, ( $p < 0.0001$ ) y sus concentraciones variaron (peso seco) entre 1.1-2.5 mg Cd/kg, 1.0-1.8 mg Cr/kg; 34-71 mg Cu/kg, 5.8-11 mg Ni/kg, 0.45-1.3 mg Pb/kg y 136-161 mg Zn/kg. Sin embargo, teniendo en cuenta las altas concentraciones de Cu bioacumuladas por *C. fluminea* el estuario del

Miño debería estar clasificado como “Clase II/III-Moderadamente/Notablemente contaminado”. Así, la principal conclusión de este trabajo, es que *C. fluminea* ha demostrado ser un adecuado indicador de la contaminación en el estuario del Miño y podría ser utilizada para evaluar la calidad ecológica de los ecosistemas estuarinos a nivel mundial.

**Palabras clave:** Miño, sedimentos, tejidos blandos, variación espacial, *Corbicula fluminea*.

## INTRODUCTION

Anthropogenic contaminants are continually discharged into aquatic ecosystems, frequently without prior assessment of their potential effects on biota. Recently, growing concern over these problems has led to a large number of studies of such polluted ecosystems (Rodgers *et al.*, 1980; Abaychi & Mustafa, 1998; Bilos *et al.*, 1998; Baudrimont *et al.*, 2003; Marie *et al.*, 2006; Peltier *et al.*, 2008). Compared to freshwater and marine ecosystems, tidal freshwater wetlands have been less studied, and a deficit of ecological, ecotoxicological and biological knowledge still exists in these ecosystems, mainly due to their unique characteristics (Rundle *et al.*, 1998; Attrill & Rundle, 2002; Sousa *et al.*, 2008a). These transitional areas are subjected to several environmental threats, such as reservoir construction, eutrophication, chemical pollution, degradation of water quality, dredging and other hydrological modifications (Ysebaert *et al.*, 2003). Therefore, these ecosystems need special attention particularly because they are facing a considerable risk of biodiversity loss, primarily for those species particularly sensitive to chemical and abiotic stress.

Estuaries from the northwest to south coast of Portugal, including the Douro estuary, “Ria de Aveiro”, Tagus, Sado and “Ria Formosa,” are contaminated by anthropogenic metals (Caetano *et al.*, 2002; Monterroso *et al.*, 2003; Mucha *et al.*, 2003; Caeiro *et al.*, 2005; França *et al.*, 2005). The Minho estuary (northwest Iberian Peninsula) was until recently considered “clean” and showed no indications of significant anthropogenic contamination (INAG, 1999; Paiva *et al.*, 2003). However, recent research by Moreno

*et al.* (2005) and Reis *et al.* (2009) has found metal enrichment in sediments from this estuary.

Mussels (e.g., *Mytilus* spp.), clams (e.g., *Mya arenaria* and *Spisula solida*), oysters (e.g., *Crassostrea gigas*) and other bivalve species have biological and ecological characteristics that make them useful biomonitors of contamination. These characteristics include their wide geographical distribution, ease of collection, abundance, ability to accumulate a vast range of contaminants and low mobility or sessile nature (Rodgers *et al.*, 1980; Araujo *et al.*, 1993; Abaychi & Mustafa, 1998; Bilos *et al.*, 1998; Baudrimont *et al.*, 2003; Marie *et al.*, 2006; Peltier *et al.*, 2008; Reis *et al.*, 2011). These species are likely to reflect changes in contamination levels in their habitat over time and have therefore been widely used in monitoring studies to assess the levels and effects of contamination (Marie *et al.*, 2006; Peltier *et al.*, 2008; Reis *et al.*, 2011).

The Asian clam *Corbicula fluminea* has invaded several aquatic ecosystems around the world, including the Minho estuary. Following invasion, *C. fluminea* are sometimes able to coexist with native species, but in several situations, they have caused the considerable decline of native bivalve species (Sousa *et al.*, 2008b). *C. fluminea* may be an ideal species in which to study contamination levels in aquatic ecosystems for several reasons: (i) it has become a major component of benthic communities, with a wide spatial distribution (Sousa *et al.*, 2008b); (ii) it may be found in both pristine and polluted environments; (iii) it may be very abundant (Phelps, 1994; Sousa *et al.*, 2008c, d, e); (iv) it has a great filtration capacity, allowing for the uptake of high amounts of contaminants; and (v) the size of adults facilitates easy dissection and

separation of the main organs, enabling specific analyses (Inza *et al.*, 1997).

The main objectives of this research were as follows:

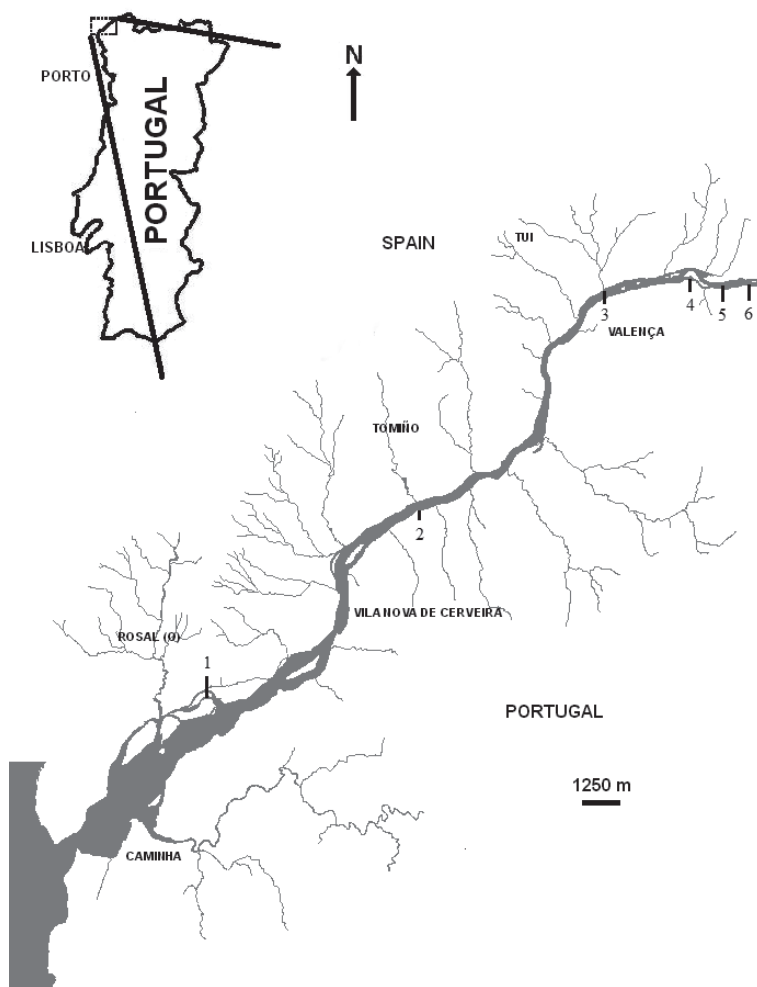
- (i) to assess metal (Cd, Cr, Cu, Fe, Ni, Pb and Zn) concentrations in sediments and in the soft tissues of *C. fluminea* in the Minho estuary, which will enable the construction of a database for comparison to future research;
- (ii) to assess the spatial variation of metal bioavailability in the Minho estuary;
- (iii) to assess the potential use of *C. fluminea* as a biomonitor of metal contamination in the Minho estuary; and

- (iv) to compare different ecological quality classifications of the Minho estuary.

## MATERIALS AND METHODS

### Study Area

The Minho estuary is located in northwest (NW) Iberian Peninsula, draining North-Northeast-South-Southwest (NNE-SSW) into the Atlantic Ocean (Fig. 1). This estuary is partially mixed and, during the period of high floods, tends to evolve toward a salt wedge estuary (Antunes &



**Figure 1.** Location of sampling sites (sites 1 to 6) in the Minho estuary (Northwest Iberian Peninsula). *Localización de los sitios de muestreo (sitios 1 a 6) en el estuario del Miño (Noroeste de la Península Ibérica).*

**Table 1.** Metal concentrations (mg/kg, dry wt. basis) obtained in SRM 2702 and SRM 2976, certified for inorganic elements in marine sediments and mussel tissue of *Mytilus galloprovincialis*, respectively. *Concentraciones de metales (mg/kg, peso seco) obtenidos en SRM 2702 y SRM 2976 certificados para elementos inorgánicos en sedimentos marinos y tejidos del mejillón Mytilus galloprovincialis respectivamente.*

Element (mg/kg)	SRM 2702		SRM 2976	
	(Certified)	(Obtained)	(Certified)	(Obtained)
Cd	0.817 ± 0.011	0.956 ± 0.049	0.82 ± 0.16	0.82 ± 0.02
Cr	352 ± 22	359 ± 63	0.50 ± 0.16	0.55 ± 0.02
Cu	117.7 ± 5.6	102.4 ± 2.4	4.02 ± 0.33	3.98 ± 0.20
Ni	75.4 ± 1.5	83.7 ± 1.1	0.93 ± 0.12	0.93 ± 0.11
Pb	132.8 ± 1.1	114.2 ± 1.5	1.19 ± 0.18	1.07 ± 0.12
Zn	485.3 ± 4.2	485 ± 20	137 ± 13	148 ± 18

Dias, 2005; Sousa *et al.*, 2005). The Minho River drains a hydrological basin of 17,080 km<sup>2</sup>, 95 % of which is in Spain and 5 % in Portugal. This river is 300 km long (the last 70 km mark the Portuguese/Spanish border) and has a mean annual freshwater discharge of 300 m<sup>3</sup>/s (Sousa *et al.*, 2005). The influence of spring tides extends approximately 40 km upstream, comprising a total estuarine area of 23 km<sup>2</sup> (Sousa *et al.*, 2005).

In the last decade, several studies have been conducted on the macroinvertebrates and fish species in this estuary to characterise the major environmental and ecological processes (Antunes & Dias, 2005; Sousa *et al.*, 2005; 2006a, b; 2007a, b; 2008a, e; Costa-Dias *et al.*, 2010a, b).

## Reagents and Materials

All reagents used in this work were at least of pro analysis or equivalent grade, without further purification: HNO<sub>3</sub> (Fluka, pro analysis). Solutions were prepared with high-purity water from a Milli-Q system (conductivity: 0.054 µS/cm at 25 °C). The metal standard solutions used in

the analysis were prepared daily by weight from a stock solution of 1000 mg/L (Fluka, pro analysis). All sampling and treatment materials had been decontaminated in a nitric acid solution (20 %, v/v) for at least for 24 h, washed with deionised water (conductivity < 0.066 µS/cm at 25 °C) and dried in an oven

## Collection and treatment of samples

Sediments and adult Asian clams (*C. fluminea*) were collected at six sites in the Minho estuary in April 2007 (Fig. 1). One sediment sample per site was prepared and analysed for granulometry and organic matter (OM) content, as previously described in Sousa *et al.* (2005, 2006a). To test for metal contamination, three sediment replicates per site were collected and transported to the laboratory within two hours. Samples were dried at room temperature until constant weight and were sieved through 2-mm nylon nets to remove large stones and gravel (Reis *et al.*, 2009). Afterwards, 300 mg of each sediment replicate was digested with 5 ml of concentrated HNO<sub>3</sub> in a domestic

**Table 2.** Metal concentrations (mg/kg, dry wt. basis) obtained in sediments from the Minho estuary. *Concentraciones de metales (mg/kg, peso seco) obtenidos en sedimentos procedentes del río Miño.*

Element (mg/kg)	Limit of detection	Sampling Site					
		1	2	3	4	5	6
Cd	0.011	0.025 ± 0.006	0.03 ± 0.01	0.113 ± 0.002	0.20 ± 0.02	0.36 ± 0.02	0.21 ± 0.02
Cr	0.055	10 ± 2	7 ± 1	12 ± 1	15 ± 1	16 ± 2	11.7 ± 0.6
Cu	2.62	2.7 ± 0.1	3 ± 1	12.7 ± 0.4	17.4 ± 0.9	24.6 ± 0.7	14.8 ± 0.7
Ni	2.53	11 ± 2	7.3 ± 0.6	20 ± 2	26 ± 1	45 ± 2	31.1 ± 0.8
Pb	0.24	4.8 ± 0.5	4.1 ± 0.6	11.3 ± 0.2	8.2 ± 0.5	15 ± 2	7.5 ± 0.3
Zn	6.7	37.3 ± 0.9	38 ± 4	78.2 ± 0.9	96 ± 5	110 ± 5	108 ± 3

microwave system (model NE-1037, Panasonic) using Parr teflon reactors (model 4782, Parr) at high pressure following the method of Reis *et al.* (2009).

Thirty-five adult individuals of *C. fluminea* (20-25-mm shell length) were collected at each site and transported to the laboratory in plastic bags within two hours after their collection. In the laboratory, soft tissues were isolated and pooled samples of ten clams were lyophilised (Labconco's lyophiliser, 48 hours). As described above, 300 mg of each tissue replicate was digested with diluted HNO<sub>3</sub> solution in a microwave following the method of Reis & Almeida (2008).

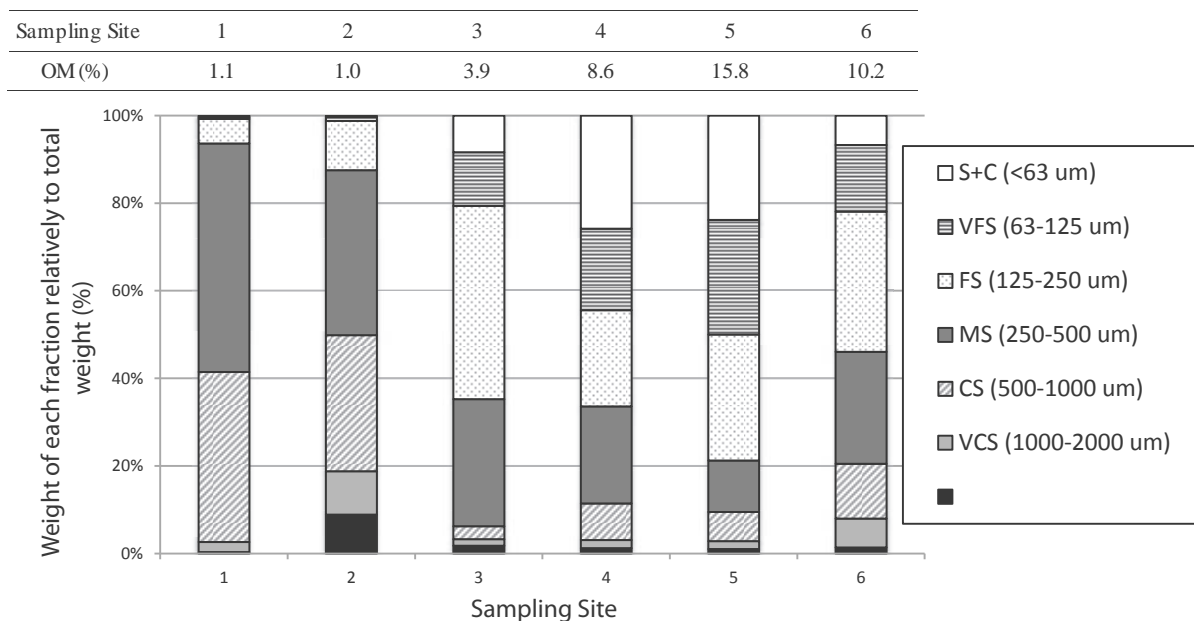
### Determination of metals in samples

Metals (Cd, Cr, Cu, Fe, Ni, Pb and Zn) were assessed using Atomic Absorption Spectrometry (SpectrAA 220 FS, Varian) with flame (Marck 7, Varian) and electrothermal atomisation (Autosampler GTA 110, Varian), depending on the

metal concentrations, with a deuterium background correction (D<sub>2</sub>-BG) system using APHA recommendations (APHA, 1998b, c).

For each solution analysed, Varian software was programmed to give values with precisions below 10 % between readings, in a maximum of four readings per replicate, to assure reproducibility of the measurements (Varian, 1988). External calibrations for each metal were carried out with aqueous standards using the operating conditions recommended by Varian manuals (Varian, 1988, 1989). Blank solutions were prepared following the appropriate sample treatment and metal concentrations in blank solutions were always below the limit of detection of the analytical procedure for all elements (APHA 1998d). The limits of detection were calculated using APHA recommendations (APHA, 1998d).

The Standard Reference Material, SRM 2702, certified for "inorganics in marine sediments" was used to check the suitability of the analytical procedure for estuarine sediments (Reis *et al.*,



**Figure 2.** Percentage of organic matter (OM) content and granulometry in sediments from the Minho estuary. Fractions: Silt and Clay (S+C, < 63 μm); Very Fine Sand (VFS, 63-125 μm); Fine Sand (FS, 125-250 μm); Medium Sand (MS, 250-500 μm); Coarse Sand (CS, 500-1000 μm); Very Coarse Sand (VCS, 1000-2000 μm); and Gravel (G, > 2000 μm). *Porcentaje de materia orgánica (OM) y granulometría en sedimentos procedentes del estuario del Miño. Leyendas de cada granulometría: limo y arcilla (S+C, < 63 μm); arenas muy finas (VFS, 63-125 μm); arenas finas (FS, 125-250 μm); arenas medias (MS, 250-500 μm); arenas gruesas (CS, 500-1000 μm); arenas muy gruesas (VCS, 1000-2000 μm) y grava (G, > 2000 μm).*

2009). The analyses of SRM 2702 revealed satisfactory recoveries for all elements that were higher than 86 % (Table 1). Recovery values below 100 % were expected for some metals because the HNO<sub>3</sub> digestion of the sediments only allows for the determination of total-recoverable metal contents (US-EPA, 1994). This method has the advantage of being a strong acid digestion that dissolves all elements that could become “environmentally available” and that have ecotoxicological potential (US-EPA, 1994).

The Standard Reference Material, SRM 2976, certified for “trace elements and methylmercury in mussel tissues” was used to check the suitability of the entire analytical procedure for clam tissues (Reis & Almeida 2008; Reis *et al.* 2012a,b). The analyses of SRM 2976 also revealed satisfactory recoveries for all elements that were higher than 90 % (Table 1).

For each sediment and tissue sample, three replicates were analysed, and the respective average and standard deviation were calculated (APHA, 1998d; Reis & Almeida, 2008; Reis *et al.*, 2009, 2012a, b). To compare contaminant concentrations among sites, ANOVA and multivariate analyses were applied using the Classification CLUSTER, based on the Euclidean distance matrix, available in the PRIMER package (Clarke & Warwick, 2001).

## RESULTS AND DISCUSSION

### Abiotic characterisation of the sediments

The OM content and granulometry of the sediments from the different sites showed spatial variation (Fig. 2). The OM contents and silt and clay fractions (S+C, < 63 µm) were significantly higher in sites 3-6 (upstream sites) than in sites 1 and 2 (downstream sites), in which the fractions of median (MS) and coarse sand (CS) were dominant. The OM contents measured in this study (1.0-15.8 %) were higher than those previously reported by Maze *et al.* (1993) in the Minho estuary (< 0.250-0.624 %) and by Reis *et al.* (2009) in the Minho saltmarsh areas (1.3-10.7 %). The percentage of OM in the sediments was significantly positively correlated with their fine frac-

tions (S+C, < 63 µm), which can be explained by smooth hydrodynamic conditions, promoting the retention of particles, and by the presence of plants (Reis *et al.*, 2009). The Minho estuary followed this tendency with high accumulations of detritus from the margins after the winter period, mainly in upstream sites. Indeed, site 5 showed high percentages of fine sediments and OM due to the fact that it is located in a sheltered area. In contrast, sites 1 and 2, located in downstream areas, showed coarser sediments and lower percentages of OM.

### Metal concentrations of the sediments

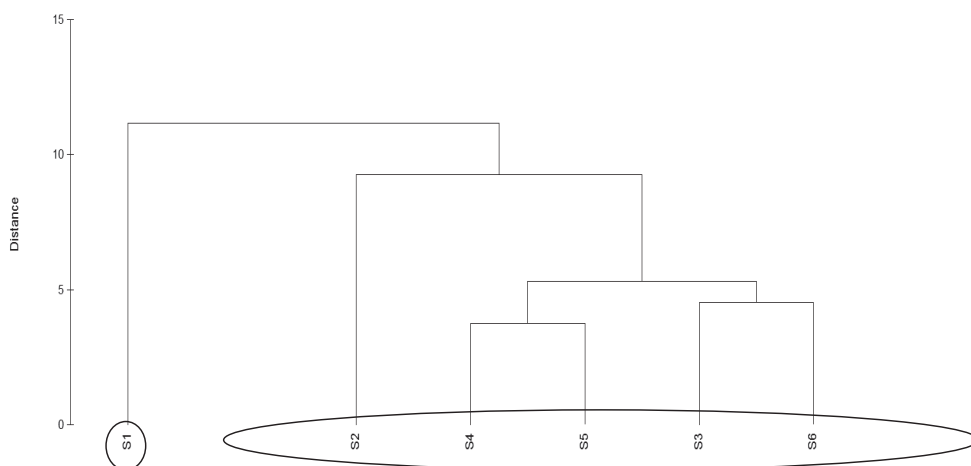
The metal (Cd, Cr, Cu, Fe, Ni, Pb and Zn) concentrations of the sediments from the Minho estuary are shown in Table 2. Metals in sediment samples varied spatially ( $p < 0.05$ ), and the concentrations (dry wt. basis) ranged from 0.025-0.36 mg Cd/kg, 7-16 mg Cr/kg, 2.7-24.6 mg Cu/kg, 7.3-45 mg Ni/kg, 4.1-15 mg Pb/kg and 37.3-110 mg Zn/kg.

The CLUSTER analysis based on the Euclidean distance index, using data of granulometry, OM contents and metal concentrations, identified two distinct groups of sites (Fig. 3): Group A and Group B. Group A was composed of only site 1 (downstream site), which had sediments with the lowest metal concentrations, low OM contents and mainly median, coarse and very coarse sand fractions (i.e., site 1 had concentrations of Cd, Cu and Ni approximately 5-10 times lower than sites 4, 5 and 6). Group B was composed of sites 2-6 (upstream sites), which had high metal concentrations, high OM contents and sediments composed mainly of silt and clay, very fine and fine sand fractions. These finer granulometry and higher OM contents allow sediments to retain more metals by adsorption and complexation, although the more sensitive redox elements can be remobilised during the degradation of OM (Monterroso *et al.*, 2003). This CLUSTER analysis showed that the natural characteristics of sediments highly influence the concentrations of metals they retain. Indeed, the natural characteristics (granulometry and OM contents) of sediments from both groups can be considered the primary

reason for the higher metal concentrations observed in sites 2-6 relative to site 1.

The metal concentrations in sediments from the main Portuguese estuaries reported over the years are shown in Table 3. Although our study measured total-recoverable metal concentrations, comparisons with total-total metal concentrations from other studies can be considered acceptable because our recovery percentages in SRM 2702 were higher than 86 % (Table 1). To reflect the variation, we observed in metal concentrations across sediment types; comparisons of our data to other studies of the Minho estuary should also take into account the spatial and seasonal variation in sediment characteristics. Our metal concentrations were similar to those observed by Paiva *et al.* (1993), INAG (1999) and Reis *et al.* (2009) and lower than the concentrations reported by Moreno *et al.* (2005) (Table 3). Moreno *et al.* (2005) found that the most sheltered areas of the Minho estuary, which coincided with our sites 5 and 6, retained the highest concentrations of Cr, Cu, Ni and Zn and were associated with anthropogenic contamination. Additionally, our metal concentrations were lower than those reported in the other main Portuguese estuaries, suggesting relatively low metal contamination of the Minho estuary (Table 3).

In 2004, the Norwegian Pollution Control Authorities developed a guideline for environmental risk assessment of marine sediments to be used as a supportive tool in the decision making processes on remediation of polluted sediments (Bakke *et al.*, 2010). In 2007, the Norwegian Pollution Control Authorities modified the approach to categorise environments as one of five environmental quality classes based on the concentration of contaminants (Bakke *et al.*, 2010). These environmental quality classes were based on ecotoxicology and followed the principles of contaminant risk assessment of the European Community (Bakke *et al.*, 2010). These guidelines included 50 inorganic and organic compounds, which were focused on toxicity to aquatic organisms as well as impact on human health (Bakke *et al.*, 2010). In 2010, the Norwegian classification system changed from one based on the distribution of contaminant concentrations to one based on environmental effects and closely linked to the risk assessment principles of the European Union. This final ecological classification system is consistent with the Norwegian guidelines for risk assessment of marine sediments and represents a useful tool for marine environmental management (Bakke *et al.*, 2010). This Norwegian approach



**Figure 3.** Groups of sampling sites from the Minho estuary based on Euclidean distance, considering granulometry, OM contents and metal concentrations of sediments. *Agrupaciones de estaciones en el estuario del Miño basados en distancias euclideas, considerando la granulometría, contenido en materia orgánica y concentración de metales del sedimento.*

is preferable to the United States Environmental Protection Act and Canadian sediment quality guidelines (SQGs) because it represents a final agreement established and ratified during the Oslo-Paris (OSPAR) Convention involving 14 European countries (Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland and the United Kingdom) and has been approved by the European Community management (Bakke *et al.*, 2010). Thus, these guidelines for sediments can be used in ecological quality classifications in different coastal and estuarine areas in Europe, including Portugal (Region IV) (Bakke *et al.*, 2010). Using the Norwegian Pollution Control

Authority metal concentrations guidelines for sediments (Table 3), we determine the first ecological quality classification of the Minho estuary as a coastal ecosystem of “Class I/II-Background/Good” quality (Bakke *et al.*, 2010).

This preliminary ecological quality classification of the Minho estuary, based on metal concentrations in sediments, would be strengthened by confirmation using a metal biomonitor species, such as *C. fluminea*, *Chthamalus montagui* or *Pollicipes* (Reis *et al.*, 2012a,b). Because biomonitor species vary in their metal bioaccumulation factors (BAFs), it is important to assess the suitability of a given biomonitor species in the particular ecosystem of interest prior to a contamination assessment. Reis *et al.* (2012a,b)

**Table 3.** A. Range of metal concentrations ( $\mu\text{g}/\text{kg}$  or  $\text{mg}/\text{kg}$ , dry wt. basis) in sediments from the Minho estuary. B. Range of metal concentrations ( $\mu\text{g}/\text{kg}$  or  $\text{mg}/\text{kg}$ , dry wt. basis) in sediments from other Portuguese wetlands. C. Ecological quality classification of coastal ecosystems based on sediment metal concentrations according to the Norwegian Pollution Control Authority (Bakke *et al.*, 2010). A. *Intervalos de concentración de metales ( $\mu\text{g}/\text{kg}$  o  $\text{mg}/\text{kg}$ , peso seco) en sedimentos procedentes del estuario del Miño.* B. *Intervalos de concentración de metales ( $\mu\text{g}/\text{kg}$  o  $\text{mg}/\text{kg}$ , peso seco) en sedimentos procedentes de otros humedales portugueses.* C. *Clasificación ecológica de los ecosistemas costeros basada en las concentraciones de metales en sedimentos de acuerdo a la Norwegian Pollution Control Authority (Bakke *et al.*, 2010).*

Location	Cd ( $\mu\text{g}/\text{kg}$ )	Cr ( $\text{mg}/\text{kg}$ )	Cu ( $\text{mg}/\text{kg}$ )	Ni ( $\text{mg}/\text{kg}$ )	Pb ( $\text{mg}/\text{kg}$ )	Zn ( $\text{mg}/\text{kg}$ )
Minho estuary (our study)	25-360	7.0-16	2.7-24.6	7.3-45	4.1-15	37-110
<b>A</b>						
<b>Range of metal concentrations observed in sediments from Minho estuary</b>						
Minho Estuary [Paiva <i>et al.</i> , 1993]	—	5-56	5-29	9-30	18-50	22-94
Minho Estuary [Reis <i>et al.</i> , 2009]	< 100	6-15	3.2-7.5	0.9-9.3	9-16	33-44
Minho Estuary [Moreno <i>et al.</i> , 2005]	—	20-62	9-36	9-47	12-42	32-157
Minho Saltmarsh [Moreno <i>et al.</i> , 2005]	—	26-116	13-91	9-76	21-80	53-160
Minho Saltmarsh [Reis <i>et al.</i> , 2009]	21-265	7.0-20	2.8-22	3.6-22	4.8-15	38-92
<b>B</b>						
<b>Range of metal concentrations observed in sediments from several Portuguese wetlands</b>						
Douro Estuary [Mucha <i>et al.</i> , 2003, 2004]	70-280	1-94	1-229	11-26	0.3-192	6-457
Ria de Aveiro [Monterroso <i>et al.</i> , 2003]	40-1550	—	3-46	—	6-26	51-589
Tagus Estuary [Caçador <i>et al.</i> , 1996; França <i>et al.</i> , 2005]	1700-5900	35-55	28-89	25-35	65-199	168-427
Sado Estuary [Caeiro <i>et al.</i> , 2005]	200-8000	0.6-63	1-191	—	2-69	2-507
Ria Formosa [Padinha <i>et al.</i> , 2000; Caetano <i>et al.</i> , 2002]	40-6300	11-64	1.1-59	9-24	11-46	7-167
<b>C</b>						
<b>Ecological quality of the ecosystem [Bakke <i>et al.</i>, 2010]</b>						
Class I-Background	< 250	< 70	< 35	< 30	< 30	< 150
Class II-Good	250-2,600	70-560	35-51	30-46	30-83	150-360
Class III-Moderate	2,600-15,000	560-5,900	51-55	46-120	83-100	360-590
Class IV-Bad	15,000-140,000	5,900-59,000	55-220	120-840	100-720	590-4,500
Class V-Very Bad	> 140,000	> 59,000	> 220	> 840	> 720	> 4,500



have previously shown that *C. montagui* and *P. pollicipes* can be used as biomonitors of metal contamination along the NW coast of Portugal, including in the Minho estuary. They obtained significant linear correlations ( $p < 0.05$ ) between metal concentrations in water samples and those accumulated in biomonitor tissues. However, they did not assess the suitability of *C. fluminea*, which may also be an appropriate biomonitor species of metal contamination in the Minho estuary and aquatic ecosystems worldwide.

### Metal concentrations of *C. fluminea*

The metal concentrations measured in the soft tissues of the Asian clam *C. fluminea* from the Minho estuary are shown in Table 4. Total-total metal concentrations were considered acceptable, as the analytical methodology obtained recovery percentages in SRM 2976 higher than 90 % (Table 1). Metal concentrations (dry wt. basis) in *C. fluminea* ranged from 1.1-2.5 mg Cd/kg, 1.0-1.8 mg Cr/kg, 34-71 mg Cu/kg, 5.8-11 mg Ni/kg, 0.45-1.3 mg Pb/kg and 136-161 mg Zn/kg.

Metal concentrations in *C. fluminea* also showed significant spatial variation ( $p < 0.05$ ). The lowest metal concentrations were found in site 5 (Cd, Cr and Zn) and in sites 1, 4 and 6 (Cu, Ni and Pb, respectively). The greatest metal concentrations were found in site 3 (Cr, Cu, Ni and Zn) and in sites 1 and 4 (Pb and Cd, respectively). Thus, *C. fluminea* from downstream areas (sites 1-3) bioaccumulated greater concentrations of metals than those from upstream areas (sites 4-6), most likely as a result of higher anthropogenic influences in the lower estuarine areas, which were not revealed by our

sediment analysis. Downstream areas of the Minho estuary are located near the cities of Caminha and Vila Nova de Cerveira, which may be discharging untreated domestic and industrial effluents into the waters of the Minho estuary. Overall, it appears that there is greater metal availability to organisms in downstream areas than upstream areas of the Minho estuary, and similar significant spatial variations should also be expected for metal bioavailability.

Because this study only included six sampling sites, we cannot extrapolate our findings to the whole ecosystem. From our data, it appears that the high metal concentrations found in sediments, particularly in sites 5 and 6, had no significant effects on *C. fluminea*; therefore, the Minho estuary should be considered "clean" of metals. However, previous studies have found evidence of metal contamination in *C. fluminea* from the Minho estuary, particularly by Cd and Cu (Table 5). For example, *C. fluminea* from the Minho estuary were found to have 10 times greater Cd and Cu concentrations than *C. fluminea* from Cazaux-Sanguinet Lake in France (Baudrimont *et al.*, 1997). At the present time, the source of this potential metal contamination remains unidentified.

The Norwegian Pollution Control Authority has also developed guidelines for classifying the ecological quality of coastal ecosystems using biomonitor species (Molvaer *et al.*, 1997). There are currently not guidelines specific to *C. fluminea*, but using the guidelines (SFT TA-1467/1997) for blue mussels *Mytilus edulis* (Table 5), metal concentrations in *C. fluminea* suggest that the Minho estuary be classified as "Class II/III – Moderately/Remarkably Polluted" because of their high Cu concentrations

**Table 4.** Metal concentrations (mg/kg, dry wt. basis) obtained in soft tissues of *C. fluminea* from the Minho estuary. *Concentraciones de metales (mg/kg, peso seco) obtenidas en tejidos blandos de C. fluminea procedentes del estuario del Miño.*

Element (mg/kg)	Limit of detection	Sampling Site					
		1	2	3	4	5	6
Cd	0.003	2.3 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	2.5 ± 0.3	1.1 ± 0.2	2.1 ± 0.3
Cr	0.033	1.6 ± 0.6	1.6 ± 0.1	1.8 ± 0.1	1.4 ± 0.2	1.0 ± 0.2	1.3 ± 0.3
Cu	3.31	34 ± 2	40 ± 1	71 ± 3	61 ± 1	44 ± 8	65 ± 9
Ni	0.067	8 ± 2	7.3 ± 0.3	11 ± 2	5.8 ± 0.5	8 ± 2	9 ± 2
Pb	0.064	1.3 ± 0.2	1.03 ± 0.06	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.2	0.45 ± 0.09
Zn	6.7	141 ± 4	159 ± 4	161 ± 5	141 ± 6	136 ± 5	152 ± 10

(Molvaer *et al.*, 1997). In conclusion, *C. fluminea* proved to be a useful biomonitor species of metal contamination in the Minho estuary.

The distribution of *C. fluminea* continues to grow, and this species can now be found in several countries, including Argentina, the United States, France and Iraq, where it also has the potential of being used as a biomonitor species of metal contamination (Abaychi & Mustafa, 1998; Bilos *et al.*, 1998; Marie *et al.*, 2006; Peltier *et al.*, 2008). Compared to *C. fluminea* from these other countries, Portuguese *C. fluminea* were found to have similar or higher metal concentrations in their tissues, confirming that the Minho estuary has experienced metal contamination (Table 5).

In summary, this study showed that metal concentrations in sediments and in soft tissues of *C. fluminea* from the Minho estuary (North-west Iberian Peninsula) were spatially variable. Generally, *C. fluminea* from upstream estuarine areas had bioaccumulated lower metal concentrations than those from downstream areas,

revealing different levels of metal bioavailability along the Minho estuary. Metals in the Minho estuary were associated not only with natural sources but also with anthropogenic sources. Metal concentrations measured in sediments allowed us to determine the first ecological quality classification of Minho estuary as “Class I/II-Background/Good.” However, high Cu concentrations accumulated by *C. fluminea* suggest that the Minho estuary should instead be classified as “Class II/III-Moderately/Remarkably Polluted.” The primary contributions of this research are that *C. fluminea* can be used as a biomonitor of metal contamination in the Minho estuary and can be used to classify the ecological quality of estuarine ecosystems worldwide.

The metal concentrations that we have reported in both sediments and *C. fluminea* from the Minho estuary can be used as a baseline and will be incorporated into a database for comparison to future research. Future research should investigate regulation mechanisms and physiological parameters, such as uptake rate constants

**Table 5.** A. Metal concentrations (mg/kg, dry wt. basis) obtained in soft tissues of *C. fluminea* in other similar studies. B. Ecological quality classification of coastal ecosystems based on blue mussel *Mytilus edulis* soft tissues metal concentrations (SFT TA-1467/1997) according to the Norwegian Pollution Control Authority (Molvaer *et al.*, 1997). Cr and Ni guideline values are not available. A. *Concentraciones de metales (mg/kg, peso seco) obtenidos en tejidos blandos de C. fluminea reportados en otros estudios similares.* B. *Clasificación de la calidad ecológica de ecosistemas acuáticos basados en la concentración de metales en tejidos blandos del mejillón azul Mytilus edulis (SFT TA-1467/1997) de acuerdo con la Norwegian Pollution Control Authority (Molvaer et al., 1997). Los valores umbrales para el Cr y Ni no están disponibles.*

Location	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Minho estuary (our study)	1.1-2.5	1.0-1.8	34-71	5.8-11.0	0.45-1.30	136-161
<b>A.</b>						
<b>Range of metal concentrations observed in soft tissues of <i>C. fluminea</i> from several international wetlands</b>						
Shatt al-Arab River, Iraq [Abaychi & Mustafa, 1998]	2.2-70	—	40-1065	4.7	—	31-83
Rio de la Plata, Argentina [Bilos <i>et al.</i> , 1998]	0.5-1.9	1.3-11	28-89	1.3-6.4	—	118-316
Cazaux-Sanguinet Lake, France [Marie <i>et al.</i> , 2006]	0.15-4.2	—	—	—	—	20-29
Georgia Piedmont Basin, USA [Peltier <i>et al.</i> , 2008]	0.8-4.1	—	32.0-87.7	—	—	189.4-544.0
New River, Virginia, USA [Rodgers <i>et al.</i> , 1980]	5.9-6.9	8.2-55	6.3-12	—	—	500-564
Cazaux-Sanguinet Lake, France [Baudrimont <i>et al.</i> , 2003]	0.165-0.231	—	5.5-7.7	—	—	23.5-28.5
<b>B.</b>						
<b>Ecological quality of the ecosystem (SFT TA-1467/1997) [Molvaer <i>et al.</i>, 1997]</b>						
Class I-Unpolluted / Slightly Polluted	< 2	—	< 10	—	< 3	< 200
Class II-Moderately Polluted	2-5	—	10-30	—	3-15	200-400
Class III-Remarkably Polluted	5-20	—	30-100	—	15-40	400-1000
Class IV-Highly Polluted	20-40	—	100-200	—	40-100	1000-2500
Class V-Very Highly Polluted	> 40	—	> 200	—	> 100	> 2500

from water, efficiencies of assimilation from diets and efflux rate constants, involved in the bioaccumulation of each metal by *C. fluminea*.

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