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**Different mercury bioaccumulation kinetics by two macrobenthic species: the bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor***

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**ABSTRACT**

Mercury is a global priority pollutant and given its huge relevance in terms of environmental damage and a threat to human health, it is important to study the bioaccumulation processes at the level of macrobenthic organisms and evaluate possible consequences for the trophic chain. In this work we evaluate the mercury accumulation rates of two distinct and economically important macrobenthic species, the polychaete *Hediste diversicolor* and the bivalve *Scrobicularia plana*, through a mesocosms laboratory experiment.

The present experiment demonstrated different bioaccumulation kinetics for each species: while *S. plana* has a rapid accumulation after 48 hours of exposure to contaminated sediments and then reaches a steady state, *H. diversicolor* has a linear mercury accumulation throughout the experiment time (31 days). The different patterns observed can be related to different feeding strategies and assimilation efficiencies. In addition, we can conclude that independently of the mercury concentration in the sediments, the two studied species in both contaminated sediments reached the same percentage (25%) of mercury accumulated at the end of the experiment when compared to the maximum concentrations recorded in the field.

This experiment highlights that the mercury bioaccumulation rate by these two economically and ecologically important macrobenthic species is a reasonably rapid process which can promote serious consequences for the higher trophic levels, constituting a severe risk to the natural environment and ultimately to human health.

**KEYWORDS:** Mercury; bioaccumulation; kinetics; *Hediste diversicolor*; *Scrobicularia plana*

## 1. INTRODUCTION

Sediments represent, quantitatively, the major compartment for metal storage in aquatic environments. Although the total metal concentrations in sediments give a convenient measure of their metal contamination, such measures do not necessarily predict the toxicity of these contaminants to aquatic organisms. The ecotoxicological risk posed by contaminated sediments will depend on metal availability as well as of the ability of living organisms to assimilate metals (Amiard et al., 2007). Bioaccumulation at organism level is the result of uptake mechanisms, which control the entry of metals through the biological barriers separating individuals from their surrounding environment, and excretion mechanisms, which give rise to transfers into the external environment (Inza et al., 1998).

Over the past 10 years, a considerable interest has been generated regarding the kinetic of metal bioaccumulation in aquatic invertebrates, mainly bivalves. However, much information is lacking, specifically about the bioaccumulation rates of an important trace metal, mercury, by different economically important macrobenthic species.

In the specific case of mercury, a highly deleterious chemical element that has both natural and anthropogenic sources, contamination problems are enhanced when it enters the food web via biomethylation and bioaccumulation processes. The sources, fate and bioaccumulation of mercury have received increasing attention (US EPA 1997, Regulation EC 466/2001) due to the impact of fish consumption on human health. Knowledge of the concentration of heavy metals in biological tissues is required, particularly in benthic fauna, in order to understand the bioconcentration in the food web.

The relationship between metal accumulation and the feeding behaviour of the benthic organisms is an important factor in terms of bioavailability of different metals in the aquatic environment (Monperrus et al., 2005). The common ragworm, *Hediste diversicolor*, is one of the few polychaete species to have a commercial interest because of its use as bait in recreational fishing and as food in aquaculture (Scaps, 2002). *H. diversicolor* is omnivorous but is also an active predator. It may behave as a deposit-feeder collecting food near its burrow opening (Nielsen et al., 1995, Scaps, 2002). As a burrowing species, this worm lives in close contact with sediments and, as a consequence, may be appointed as an appropriate indicator of contamination. *Scrobicularia plana* is a long-lived deposit feeding bivalve species living in muddy-sandy sediments. It has a wide geographical distribution (Verdelhos et al., 2005) and has been previously used in biomonitoring programs and toxicity tests (Coelho et al., 2006).

Considering that the above mentioned macrofaunal species have high economic relevance and represent an important position in the food web, constituting the food source for higher trophic levels, the main goals of the present study were (1) to determine the kinetics of mercury

bioaccumulation in those two distinct macrobenthic species (*Hediste diversicolor* and *Scrobicularia plana*) and (2) to evaluate the influence of mercury sediment concentration on the mercury accumulation rate of these two species.

## 2. MATERIALS AND METHODS

### 2.1 Study sites description

Sediments were collected in October 2007 in two different temperate estuarine systems, the Mondego Estuary (40° 08' N, 8° 50' W) (Figure 1A) and the Ria de Aveiro (40° 38' N, 8° 44' W) (Figure 1B) located both on the Atlantic coast of Portugal. The Mondego Estuary is located 60 km south from the Ria de Aveiro. Station 1 at the Mondego Estuary has been used as a reference condition since no local source of mercury contamination is known (Coelho et al., 2006 and references therein). Ria de Aveiro was contaminated by effluents coming from a mercury cell chlor-alkali plant from the 1950s until 1994. The discharges resulted in an accumulation of about 33 ton of mercury in the lagoon, much of which (about 27 ton) are known to be trapped in the sediments of the basin named Laranjo (Pereira et al., 2008). In the last decade, the industrial mercury discharge decreased considerably, being nowadays within the regulatory levels ( $50 \mu\text{g L}^{-1}$ , the limit value for discharges from chlor-alkali electrolysis industry, in accordance with Directive 82/176/EEC). In this Laranjo basin two sampling sites were chosen: one characterized by strongly contaminated sediments (Laranjo - station 3), collected approximately 450 m away from the mercury point source, and the other one corresponding to a moderately contaminated sediments (Chegado - station 2), collected at the opposite end of the basin, approximately 1250 m away from the mercury point source towards Chegado Quay (referred along the text as Chegado) (Figure 1B). The three sediment environments can be considered as similar in terms of physical characteristics (muddy sediments)

and representing a gradient of mercury contamination. Furthermore, only the superficial layer of the sediments (10 cm depth) was considered relevant in this study.

## 2.2 Invertebrates

The bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor* were collected from the Mondego station (reference condition). In the laboratory, the polychaete organisms were measured (first setiger width) and all the organisms used in the experiment belonged to the class < 1 mm, corresponding to an average burrow surface area of 1 cm<sup>2</sup> (Mortimer et al., 1999). *Scrobicularia plana* individuals were, also, measured in terms of total length. All the individuals used in the experiment belonged to the class 2-3 cm, corresponding to 2+ year old individuals (Verdelhos et al., 2005). The organisms used in the experiment were left in clean seawater at 20°C during four days to acclimate and depurate. Oxic conditions were ensured by air-bubbling the water.

## 2.3 Experimental set-up

The experiments were performed in acid-washed glass containers of 3 litres (Ø: 14 cm). To reduce the natural heterogeneity and to achieve similar starting conditions, all the sediments were homogenised before use and their macrofauna was removed. The water used in the experiment was collected in the Mondego Estuary (reference condition) and filtered through 0.45 µm pore size Millipore filters, in order to retain particles present in the water. Sediment mesocosms were established by transferring 2000 g (wet weight-wwt) of homogenised and fauna-free sediment to each glass container. Then, 1.0 L of filtered (0.45µm) estuarine water (salinity - 30) from the Mondego Estuary was added to each container, always maintained under oxic conditions. The glass containers were randomly distributed in a dark room to avoid primary production and kept at 20°C.

The containers were maintained in these conditions during 48 hours to stabilize before introducing the organisms. In each of the glass containers 5 individuals of *Hediste diversicolor* and 3 individuals of *Scrobicularia plana* were introduced, according to the mean densities observed in the field (Cardoso et al., 2007, Verdelhos et al., 2005). At defined times, the organisms (*Hediste diversicolor* and *Scrobicularia plana*) were removed from the glass containers, rinsed with seawater and then placed in constantly aerated clean seawater at 20°C for 48 hours to allow depuration, being afterwards dissected in the case of *S. plana*, and then freeze-dried for later mercury analysis.

For each of the three conditions (reference, low and high contamination) four different sampling times were chosen (t1 – 2 days; t2 – 5 days; t3 – 9 days; t4 – 31 days). At each sampling condition and occasion three replicates were carried out. Therefore, the experimental set-up included thirty six (3x4x3) sub-experiments.

To evaluate the total mercury accumulation ability of the studied organisms in the field, the two species under study were collected at each sampling site. In the laboratory, the individuals were subjected to depuration, following the same procedure as described above.

## **2.4 Analytical methodologies**

### **2.4.1 Sediment samples**

Sediment samples of each site were analysed for water and organic matter contents, grain size, total carbon, mercury, iron and manganese. The physico-chemical properties of the sediments were analysed to evaluate if there were any differences that could influence the availability and the accumulation of mercury and to achieve the degree of metal contamination in each site.

The sediments were previously homogenised, freeze-dried and manually sieved through a 1 mm mesh to remove coarser material and debris.



The water content of the sediments was determined by subtracting the value of dry weight from the wet weight of a sediment sample. Organic matter content of the sediments was evaluated as weight loss on ignition (% LOI) at 450°C during 8 hours. Fine particles (less than 63 µm) were determined by wet sieving of a dried sample using a 63 µm mesh size sieve.

Sediment samples were analysed for total mercury by thermal decomposition coupled to atomic absorption spectrometry after gold amalgamation, using a LECO AMA-254 (Advanced Mercury Analyser). Analytical quality control was ensured by using certified reference material (CRM). The CRM used were MESS-3 (marine sediments) and PACS-2 (polluted marine sediments). Measured and certified values for total mercury in the CRMs used were in agreement, with recoveries between 88 and 109% and standard deviations always less than 5%.

Total iron and manganese concentrations were determined on approximately 100 mg of freeze-dried sediment added into an acid-washed Teflon reactor with further addition of 1 mL of aqua regia and 6 mL of concentrated hydrofluoric acid. The Teflon reactors were sealed and placed in an oven for 1 hour at 100°C. After cooling, the solution was quantitatively transferred into acid cleaned 100 mL volumetric flasks that contained 5.6 g of boric acid (Rantala and Loring, 1977). The volume was completed with Milli-Q water and mixed to promote the dissolution of the boric acid, and the solution was transferred to plastic bottles and stored at 4°C. Afterwards, iron and manganese were determined by Flame Atomic Absorption Spectrophotometry (Perkin Elmer, model AAnalyst 100).

A partial digestion using a hydroxylamine hydrochloride-acetic acid mixture (HAA) was used to evaluate the content of iron and manganese hydrous oxides of the sediments. This extraction was performed using approximately 1g of freeze-dried sediment, to which 20 mL of 0.04 mol L<sup>-1</sup> hydroxylamine hydrochloride in 25% glacial acetic acid was added. The selective extractions with HAA were conducted in centrifuge tubes, which were subjected to continuous agitation during 6

hours (Canário et al., 2003). After extraction, solid-solution separation was obtained by centrifuging (B. Braun, model Sigma 4-10) at 6000 rpm for 30 minutes. The supernatant was filtered through 0.45  $\mu\text{m}$  pore size Millipore filters and analysed by using Flame Atomic Absorption Spectrophotometry (Perkin Elmer, model AAnalyst 100).

#### **2.4.2 Biological samples**

Biological samples were analysed for total mercury also by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254. Analytical quality control was performed by using TORT-2 as certified reference material. The recovery values obtained for TORT-2 analysis ranged from 102-118% and standard deviations always less than 3%.

#### **2.5 Data analysis**

The model based on a Michaelis-Menten equation applied to the bivalve *Scrobicularia plana* was performed using the GraphPad Prism software package, version 5.

### **3. RESULTS**

#### **3.1 Sediment characterization and mercury concentrations**

The percentage of organic matter (% LOI) of the sediments of the three studied sites was similar, with Laranjo showing slightly higher organic matter content than Chegado and Mondego Estuary sites (Table 1). The percentage of water content of the sediments of the three areas was also similar, ranging between 42% for the Mondego Estuary and 50% for Chegado and Laranjo (Table 1). In terms of percentage grain size < 63  $\mu\text{m}$ , the sediment samples were quite similar in the three sites (around 50%).

Mercury concentrations in the sediments from the three sites clearly show the existence of a strong contamination gradient. The sediments from the Laranjo contain more mercury than those

from the Chegado and both sites have high levels when compared to the reference station, the Mondego Estuary (Table 1).

Concentrations of total iron were higher in Mondego Estuary ( $27133 \pm 30 \mu\text{g g}^{-1}$ ) and Laranjo ( $29156 \pm 909 \mu\text{g g}^{-1}$ ) than in Chegado ( $23378 \pm 270 \mu\text{g g}^{-1}$ ) site (Table 1). Concentrations of total manganese were higher in the Mondego Estuary ( $236 \pm 1.3 \mu\text{g g}^{-1}$ ) than in Chegado ( $177 \pm 4.7 \mu\text{g g}^{-1}$ ) and Laranjo ( $126 \pm 2.3 \mu\text{g g}^{-1}$ ) sites (Table 1).

The highest concentrations of iron hydrous oxides occurred in Chegado followed by Mondego Estuary and Laranjo. In Chegado, concentrations of iron hydrous oxides were  $2918 \pm 101 \mu\text{g g}^{-1}$  (12%), while Mondego Estuary and Laranjo presented similar concentrations, around  $2770 \pm 325 \mu\text{g g}^{-1}$  (10%) for the first one while the latter recorded  $2564 \pm 135 \mu\text{g g}^{-1}$  (9%) (Table 1). Regarding the concentrations of manganese hydrous oxides, this metal presented a different pattern compared to iron, since it was more abundant in the Mondego Estuary,  $36.2 \pm 0.8 \mu\text{g g}^{-1}$  (15%) than in the Chegado,  $18.8 \pm 0.7 \mu\text{g g}^{-1}$  (11%). Laranjo presented the lowest concentrations of manganese hydrous oxides, around  $10.3 \pm 0.5 \mu\text{g g}^{-1}$  (8%) (Table 1).

### 3.2 Mercury bioaccumulation rates

Field mercury concentrations of the studied macrobenthic species (*S. plana* and *H. diversicolor*), as expected, presented lower mercury concentrations in the Mondego Estuary, followed by the Chegado and finally the Laranjo (Table 1). In the case of *S. plana*, this bivalve exceeded the maximum mercury levels allowed for human consumption ( $0.5 \mu\text{g g}^{-1}$  wet weight, Regulation EC 466/2001). This species reached higher mercury concentrations than the polychaete *H. diversicolor*, which may be due in part to its longer life span (Coelho et al. 2007) (Table 1).

Concerning the total mercury concentration accumulated by the studied species during the experimental period, it can be observed that the two macrobenthic species showed different kinetics. In the reference condition (Mondego Estuary), *S. plana* and *H. diversicolor* did not show

significant differences in terms of mercury concentration between the beginning and the end of the experiment (*t*-test, *S. plana*:  $t_4=0.22$ ,  $p>0.05$ ; *H. diversicolor*:  $t_4=-0.7$ ,  $p>0.05$ ).

On the other hand, in Chegado and Laranjo, the bivalve presented a model based on a Michaelis-Menten equation (Chegado,  $R^2=0.897$ ; Laranjo,  $R^2=0.786$ ). In both contaminated areas, *S. plana* reached higher mercury concentrations throughout the first 48 hours, stabilizing during the rest of the experimental period. After 48 hours of exposition to contaminated sediments, the bivalves attained 25% of mercury accumulated in relation to the mercury concentrations recorded in the field (Figures 2A-C).

For the polychaete *H. diversicolor*, its body burdens of total mercury increased all over the experimental period, for both contaminated sediments, Chegado and Laranjo (Chegado  $R^2=0.994$ ; Laranjo  $R^2=0.980$ ) (Figures 2D-F). At the end of the experiment, the ragworm reached the same percentage of mercury accumulated (25%) in relation to the values recorded in the field. A steady state did not appear to occur in *H. diversicolor* during the 31-day accumulation period for total mercury. According to the regression equations (Fig. 2E, F), the time to achieve the steady state for total mercury in *H. diversicolor* was estimated to be approximately 120-180 days, respectively, in Laranjo and Chegado.

A comparison of mercury body burdens for both macrobenthic species in the high and intermediate contaminated sediments showed that accumulation was higher in the Laranjo (high contamination) than in the Chegado (intermediate contamination) sediments.

Analysing the mercury accumulation rate per day for the two studied species, we can observe that for *S. plana* there was a huge accumulation of mercury after the first 2 days (equivalent to  $0.026 \mu\text{g g}^{-1}$  per day). However, after 5 days of exposure, the increment on mercury concentrations in *S. plana* declined abruptly ( $0.0009 \mu\text{g g}^{-1}$  per day, approximately two orders of

magnitude). Since then and until the end of the experiment, the mercury accumulation rate was even lower. On the other hand, for *H. diversicolor*, the mercury accumulation was linear, with a regular increment ( $0.0006 \mu\text{g g}^{-1}$  per day) throughout the experiment (Figures 3A, B).

#### 4. DISCUSSION

This study showed that total mercury concentrations in *Scrobicularia plana* and *Hediste diversicolor* correlate well with total mercury levels in sediments, which is in accordance with other works (Coelho et al., 2007, Zizek et al., 2007). On the other hand, the bivalve *S. plana* recorded higher levels of total mercury than the polychaete *H. diversicolor*, which can be related in part to their life spans and their bioaccumulation rates. *S. plana* is a long lived bivalve species with a lifespan of 5 years (Verdelhos et al., 2005), while for the polychaete *H. diversicolor* its lifespan ranges between 12 and 15 months (Abrantes et al., 1999). Therefore, a possible explanation for the higher levels of mercury measured in the bivalve *Scrobicularia plana* could be related to the fact that during its longer life period it accumulates more mercury than the polychaete *H. diversicolor*, even though bioaccumulation rates could be different for the two species.

The mesocosms experiment also demonstrated that these two different macrobenthic species revealed distinct behaviours concerning the mercury accumulation kinetics. *S. plana* exhibits a model similar to a Michaelis-Menten like kinetics, reaching a maximum accumulation rate after 48 hours of exposure and then accumulation stabilises until the end of the experiment. The polychaete *H. diversicolor* shows a linear accumulation rate throughout the experiment, which means that the steady state is reached later relative to the bivalve *S. plana*. According to the observed model, we can estimate that *H. diversicolor* will take approximately 120 to 180 days to reach the field mercury concentrations, respectively in Laranjo and Chegado.

A possible explanation for the distinct bioaccumulation kinetics of mercury by the two studied species may be due to a combination of factors, such as different feeding strategies, ingestion rates and assimilation efficiencies. *H. diversicolor* is omnivorous but it is also an active predator. However, it can also behave as a deposit-feeder collecting food near its burrow opening (Scaps, 2002). On the other hand, *S. plana* is a facultative surface deposit-feeder. It is able to take its food from the surface of the sediment and out of the water column (as filter feeder) (Kamermans, 1994, Chícharo et al., 2002). Considering that deposit/filter feeder species, like *S. plana* feeds essentially on sediment particles to which mercury is associated (Monperrus et al., 2005) it is possible that this species accumulates more mercury than *H. diversicolor*. Besides feeding behaviour, species-specific physiological properties of organisms, such as efficiency of assimilation, metal excretory rates and biochemical properties, including the number of metal binding ligands may also influence the accumulation of metals in organisms' tissues (Ernst and Frey, 2007). Therefore, all these processes together may explain the higher mercury accumulation by the bivalve *S. plana*.

From this experiment we conclude that independently of the mercury concentration in the sediments, the two studied species in both contaminated sites, Chegado and Laranjo, reached the same percentage (25%) of mercury accumulated at the end of the experiment compared to the concentrations recorded in organisms collected in the field and living in each site. However, the mercury accumulation rate for both species at the two sites was different. For *S. plana*, the accumulation rate was 3 times higher in Laranjo than in Chegado, while for *H. diversicolor* it was 2 times higher. This can be explained by the higher mercury concentrations of the Laranjo sediments and also the feeding strategy and assimilation efficiency of *Scrobicularia plana*.

It would be expected that *S. plana* as a filter feeder, could also accumulate mercury from the water column (dissolved fraction); however, according to a previous study by Cardoso et al. (2008), it was possible to conclude that the mercury concentration in the water caused by the bioturbation

activity of *H. diversicolor* was very low (10-15 ng.L<sup>-1</sup>). So, the water column should not be considered as a mercury source. This phenomenon can be related to the sediments' characteristics from the Ria de Aveiro, since these sediments are rich in organic matter and in iron/manganese hydrous oxides compared to other worldwide systems (see Cardoso et al., 2008). These physico-chemical characteristics of the sediments may explain, in part, the high retention of mercury in sediments of this system.

To our knowledge there are only a few studies (e.g. Inza et al., 1998, Burton et al., 2005) concerning the kinetics of mercury bioaccumulation by different macroinvertebrate species, despite the enormous importance of this trace metal to the biota. Most of the published studies (e.g. Pan and Wang, 2004, Baudrimont et al., 2005) used different species of bivalves as biomonitor species, such as mussels, cockles and other species of clams. Very few studies examined the bioaccumulation rates of mercury in worms. Despite the different habitat occupied by earthworms, our results are in agreement with Burton et al. (2005): the earthworm *Eisenia fetida* had the same behaviour as *H. diversicolor* in our study, showing a linear accumulation rate of mercury over the experimental period and also the accumulation increased with increasing concentrations of mercury in the sediments.

Regarding other studies using bivalves as biomonitor species, they revealed a different behaviour from the one observed with *S. plana* in the present study. According to Inza et al. (1998), the bivalve *Corbicula fluminea* demonstrated a linear trend for mercury accumulation.

In conclusion, the bioaccumulation rate of mercury by these two economically and ecologically important macrobenthic species is a quite rapid process that can promote serious consequences for the higher trophic levels (e.g. fishes and birds) which use these species as food resources. Therefore, the mercury biomagnification process can be very fast, constituting a severe risk to the human health.

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Figure 1 – Sediment sampling sites: A – Station 1 - Mondego Estuary; B – Ria de Aveiro (Station 2 – Chegado and Station 3 – Laranjo).

Figure 2 – Mercury concentrations ( $\mu\text{g g}^{-1}$ ) accumulated by the two species during the experimental period. A – *S. plana* in the Mondego estuary; B – *S. plana* in the Chegado; C – *S. plana* in the Laranjo; D – *H. diversicolor* in the Mondego estuary; E - *H. diversicolor* in the Chegado; E - *H. diversicolor* in the Laranjo (error bars represent standard error).

Figure 3 – Mercury accumulation per day ( $\mu\text{g g}^{-1}/\text{day}$ ) in the two species, during the experimental period. A – *S. plana*; B – *H. diversicolor*.

Table 1 – Physico-chemical characterization of the sediments and mercury content of *S. plana* and *H. diversicolor* in the field conditions for the three sampling sites (\* – Standard deviation; \*\* – Standard error).

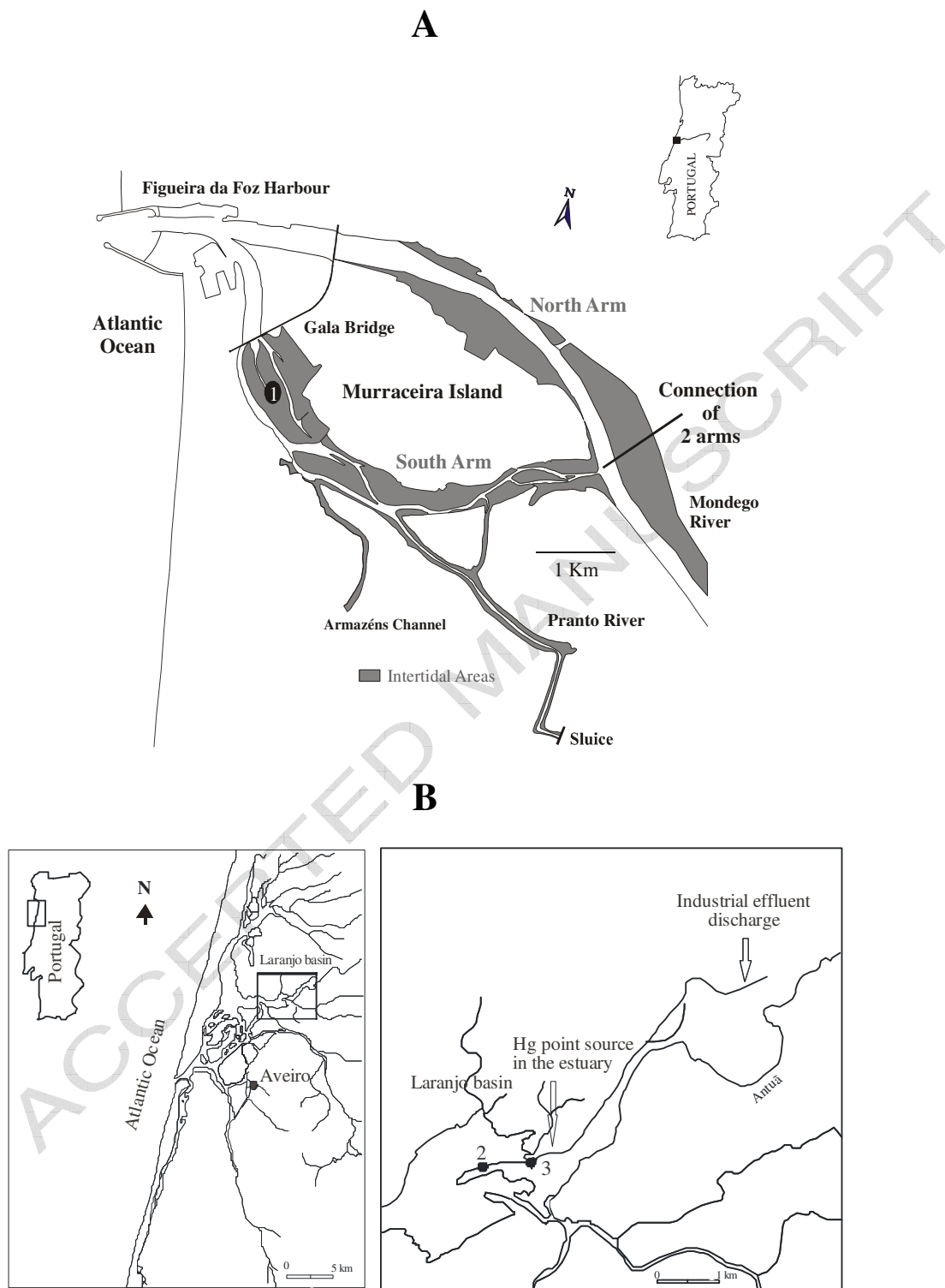


Figure 1

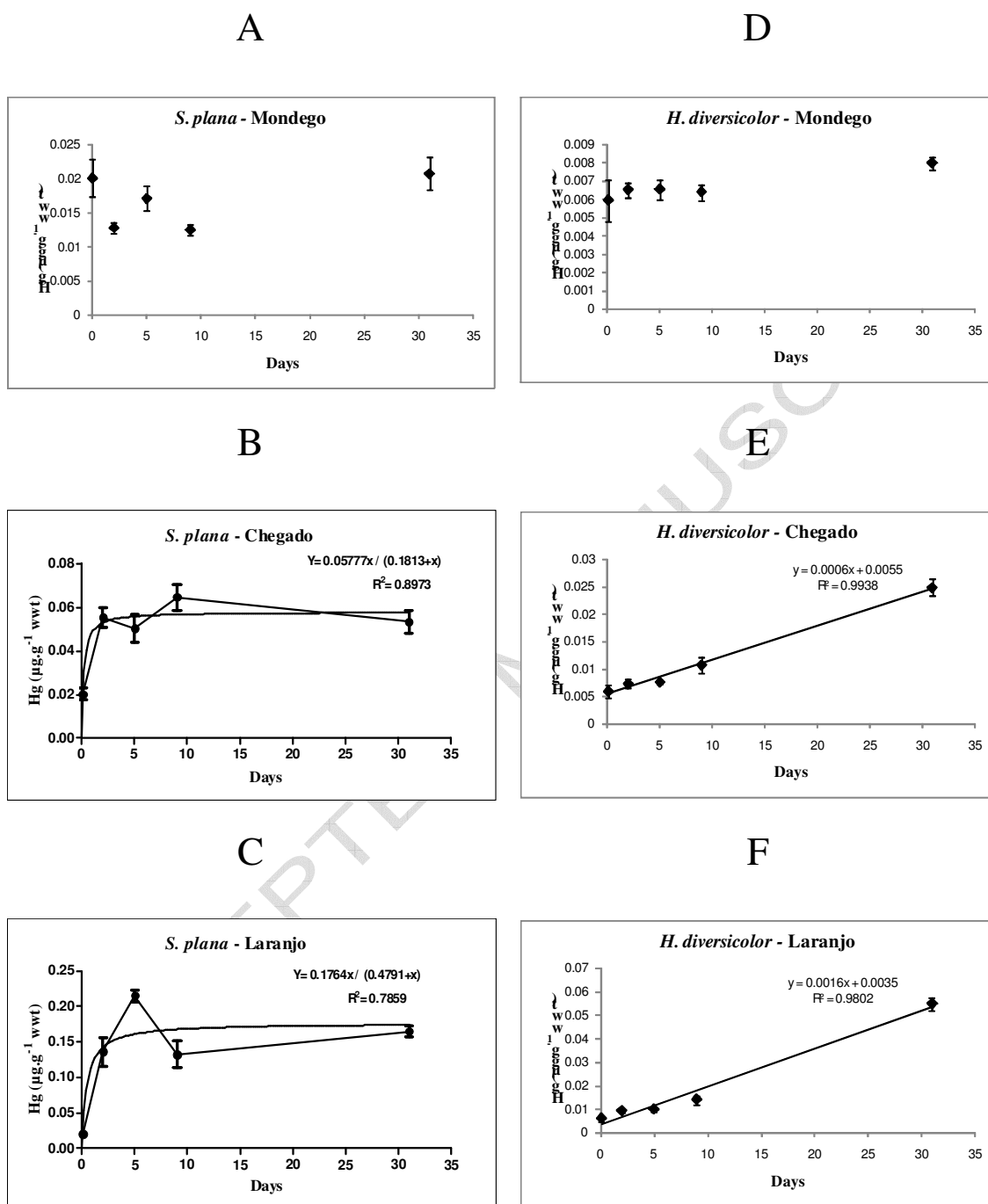
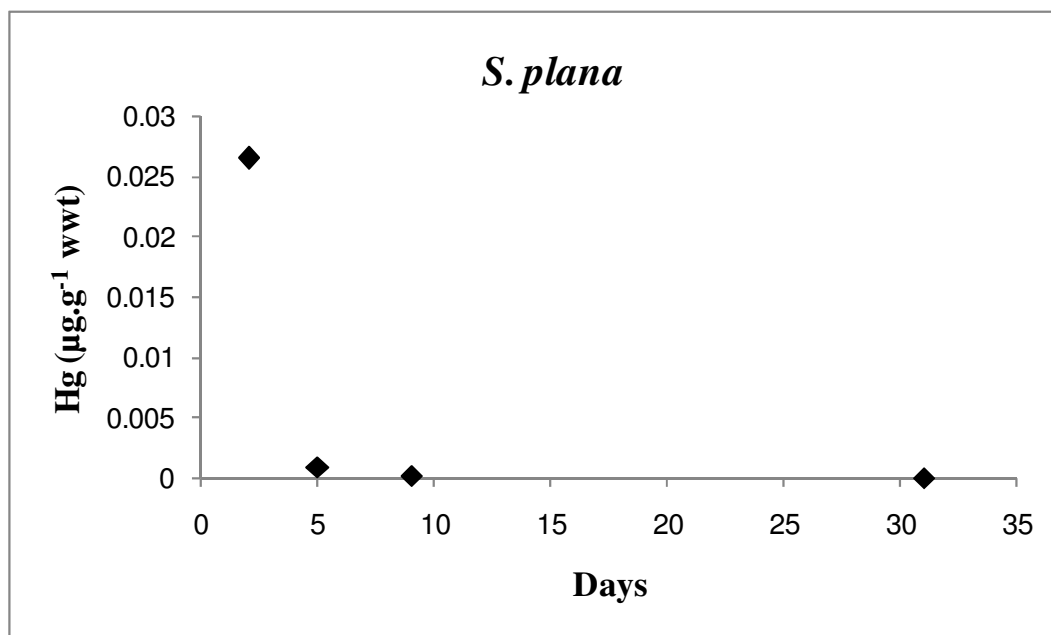


Figure 2  
20

A



B

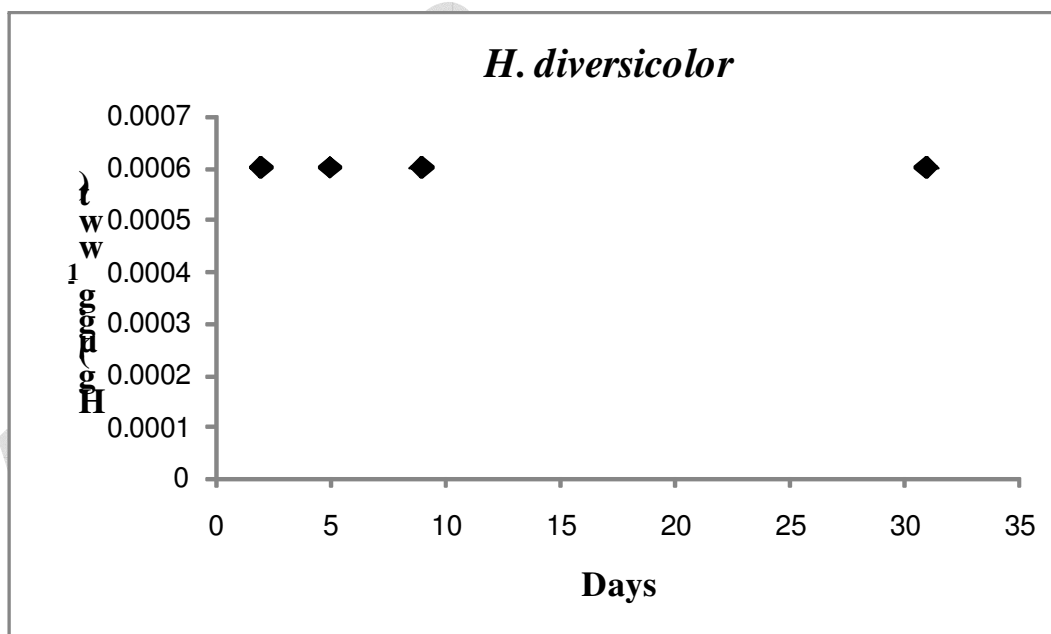


Figure 3

Table 1

	SEDIMENTS							FAUNA (field conditions)	
	LOI (%)	Water content (%)	Hg ( $\mu\text{g}\cdot\text{g}^{-1}$ dwt)	Fe ( $\mu\text{g}\cdot\text{g}^{-1}$ dwt)	Mn ( $\mu\text{g}\cdot\text{g}^{-1}$ dwt)	Fe hydrous oxides ( $\mu\text{g}\cdot\text{g}^{-1}$ dwt)	Mn hydrous oxides ( $\mu\text{g}\cdot\text{g}^{-1}$ dwt)	<i>S. plana</i> Hg ( $\mu\text{g}\cdot\text{g}^{-1}$ wwt)	<i>H. diversicolor</i> Hg ( $\mu\text{g}\cdot\text{g}^{-1}$ wwt)
<b>Mondego Estuary</b>	4.2±0.2*	42.2±0.8*	0.07±0.004*	27134±30.5**	236±1.3**	2771±325**	36.1±0.8**	0.02±0.002**	0.006±0.001**
<b>Chegado</b>	6.3±0.2*	50.3±0.4*	5.3±0.2*	23379±270**	177±4.7**	2918±101**	18.8±0.7**	0.2±0.03**	0.1±0.02**
<b>Laranjo</b>	7.5±0.1*	51.8±0.3*	75±0.6*	29157±909**	126±2.3**	2563±135**	10.3±0.5**	0.6±0.1**	0.2±0.03**