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Pattern and annual rates of *Scrobicularia plana* mercury bioaccumulation in a human induced mercury gradient (Ria de Aveiro, Portugal)

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Abstract

Due to the lack of knowledge regarding annual bioaccumulation rates in estuarine and marine fauna, the main aim of this work was to study the annual mercury bioaccumulation in the well-documented bivalve species *Scrobicularia plana* along a human induced mercury gradient in the Ria de Aveiro coastal lagoon (Portugal) and in a nearby, non-polluted system (Mondego estuary), parallel to the risks associated with its consumption by humans.

Minimum total mercury concentration was as low as 0.019 mg kg^{-1} (wwt) in 4+ year old organisms in the reference site, where a significant negative correlation ($p < 0.05$) was found between total mercury concentrations and size, resulting in negative bioaccumulation rates (detoxification). On the other hand, values reached 1.8 mg kg^{-1} (wwt) in 3+ year old bivalves from the most contaminated area, where a strong positive correlation with size was found ($p < 0.01$) and annual bioaccumulation rates were as high as 0.25 mg kg^{-1} yr^{-1}. Annual bioaccumulation rates were highly correlated with suspended particulate matter mercury concentrations. Even though the levels of organic mercury contents increased parallel to the contamination gradient, at each sampling station, no increment was found with age, which corresponded to a decrease in organic mercury percentage with age.

In terms of ecological management and public health, the ratio of 0.01 consistently found between *Scrobicularia plana* annual mercury accumulation rates and SPM mercury levels for most sites may permit to roughly estimate *S. plana* contamination of commercial sized individuals (>2.5 cm) and, if verified and confirmed in other systems, be used as a simple management tool.
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Keywords: *Scrobicularia plana*; total mercury; organic mercury; bioaccumulation rates; lifespan bioaccumulation pattern; management

1. Introduction

The contamination of estuarine and coastal waters by metals and organometals derived from anthropogenic activities has long been a concern for researchers, managers and policy makers (Mikac et al., 1996; Usero et al., 2005). The accumulation processes of these contaminants in aquatic organisms will determine, in part, the enhancement of their adverse effects on the biota (Byrne and O'Halloran, 2001; Coelho et al., 2005, 2006; Usero et al., 2005). In the particular case of mercury, a highly deleterious environmental pollutant that has both natural and anthropogenic sources (Gustin, 2003; Nriagu and Becker, 2003; OSPAR Commission, 2004), contamination problems are enhanced when it enters the food chain. Food ingestion is the predominant pathway of human exposure to methylmercury, which is the most toxic form of mercury (Tchounwou et al., 2003). Through the processes of biomethylation and bioaccumulation through estuarine food webs, methylmercury finds its way to species usually consumed by humans (Clarkson et al., 2003; Coelho et al., 2005).

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Estuaries and coastal zones are therefore subject to frequent surveys as a part of the assessment of their environmental quality, which require exhaustive and complex studies on different aspects of the interactions between compartments and contaminants, including their chemical speciation. The use of bioindicators has been suggested as an effective methodology (Saiz-Salinas et al., 1996; Liang et al., 2004; Ugolini et al., 2004; Roméo et al., 2005) that could provide useful monitoring data without requiring such a complex set of studies. The trace metal content of organisms has been commonly used in biomonitoring programs of metal pollution in the marine environment, and provides a time-integrated measure of metal availability (Saiz-Salinas et al., 1996). Most research in this area has been performed on macroinvertebrate species, namely benthic bivalve and gastropod molluscs, known to be effective bioaccumulators (Mikac et al., 1996; Byrne and O'Halloran, 2001; Liang et al., 2004; Roméo et al., 2005; Coelho et al., 2006). There is, however, a lack of criteria homogeneity and comparison between different studies. This is true since each researcher often uses a single size class, or even size class is not referred. Since many of the biomonitored mollusc species are of commercial interest, and legislation on the commercial size of each species varies depending on location, it is important to have some knowledge on the lifespan and on the annual bioaccumulation patterns of each species. This will permit to extrapolate results from a specific size class to commercially available size classes, and hence assess the risk associated with the consumption by humans at any given location.

Scrobicularia plana, a long-lived deposit feeding bivalve species present in muddy to sandy sediments (Verdelhos et al., 2005), has a wide geographical distribution and has been previously used both in biomonitoring programs (Cheggour et al., 2005) and toxicity tests (Byrne and O'Halloran, 2001; García-Luque et al., 2004; Pérez et al., 2004; Riba et al., 2004). Its life cycle in southern Europe is well studied, where it has a lifespan of 5 years, with one annual recruitment period and growth rates of roughly 1 cm yr^{-1} (Verdelhos et al., 2005). Nevertheless, little is known on the mercury accumulation patterns during the lifespan in this species. Taking that in mind, the main objectives of this work were: (1) to study mercury accumulation in both total and organic forms in all annual size classes of the clam *S. plana*; (2) to assess the annual mercury bioaccumulation pattern during the lifespan; and (3) to calculate annual mercury accumulation rates eligible to be used for extrapolations in biomonitoring studies elsewhere.

2. Materials and methods

2.1. Study site

The study was conducted in the Ria de Aveiro coastal lagoon, northwestern coast of Portugal (Fig. 1), which since the 1950s has continuously received mercury effluents from a chlor-alkali industry, inducing an environmental contamination gradient inside the lagoon (Pereira et al., 1998; Coelho et al., 2005).

Sampling consisted of two campaigns, the first carried out in summer 2003. This sampling campaign was conducted in nine locations on the Ria de Aveiro coastal lagoon (Fig. 1, sites A2, A5–A7 and A10–A14) and one location in the Mondego estuary (A15), 60 km south from the Ria, which was considered to have pristine conditions referring to heavy metals (Vale et al., 2002), used as a reference site for comparison purposes. This campaign was intended to evaluate the population structure of *Scrobicularia plana* on both systems and also to provide basic information on the overall mercury contamination, essential for the selection of the sampling points for the second sampling effort, in autumn 2003. The second field campaign focused on the annual mercury bioaccumulation rates of *S. plana* during its lifespan along the contamination gradient in the Aveiro lagoon (A1, A2, A4, A5 and A11) and also on the reference system (non-contaminated), the Mondego estuary (A15).

At each site, a composite sediment sample was obtained from the top 30 cm, consisting of five replicate cores (15 cm diameter) pooled together in order to account for within-site variability. On arrival at the laboratory, sediments were oven-dried to constant weight at 60 °C, homogenized and sieved through a 1-mm sieve before storage until analysis. Water samples were collected from intertidal water pools at each site in acid-washed plastic bottles and kept on ice during transportation to the laboratory, where they were filtered through 0.45-μm Millipore cellulose acetate membrane filters, acidified to $pH < 2$ and stored at 4 °C until analysis.

All biological samples were collected on the mudflats during low water conditions. For population structure analysis, 30 sediment cores (0.429 m^2) were collected to a depth of 30 cm, at locations A2 and A5 (15 at each station) and washed through 1-mm mesh bags. On arrival to the laboratory, the cores were sorted and all individuals of *Scrobicularia plana* were counted and measured. For total mercury analyses, larger individuals were collected by hand, while the smaller classes were collected by sediment coring as explained above. Based on the 30 cores of the two stations, a frequency distribution analysis was performed by ANAMOD software package (Nogueira, 1992), as used in previous literature (Pardal et al., 2000; Verdelhos et al., 2005) to analyze the population structure. Based on the present results, which are in agreement with those for a nearby system (Verdelhos et al., 2005), five size classes were defined (0–1, 1–2, 2–3, 3–4 and >4 cm, corresponding to 0+, 1+, 2+, 3+ and 4+ year old individuals, respectively) (Verdelhos et al., 2005), and six individuals per size class were collected at each site. For organic mercury quantifications, being a higher sample mass demanding procedure, composite samples (10–15 individuals) of the three largest size classes were collected at sites with different mercury contamination (A2, A5, A11 and A15). All samples were transported to the laboratory in a cool box, left to depurate overnight, dissected, freeze-dried at −50 °C and 0.06 bars, and homogenized for analysis.

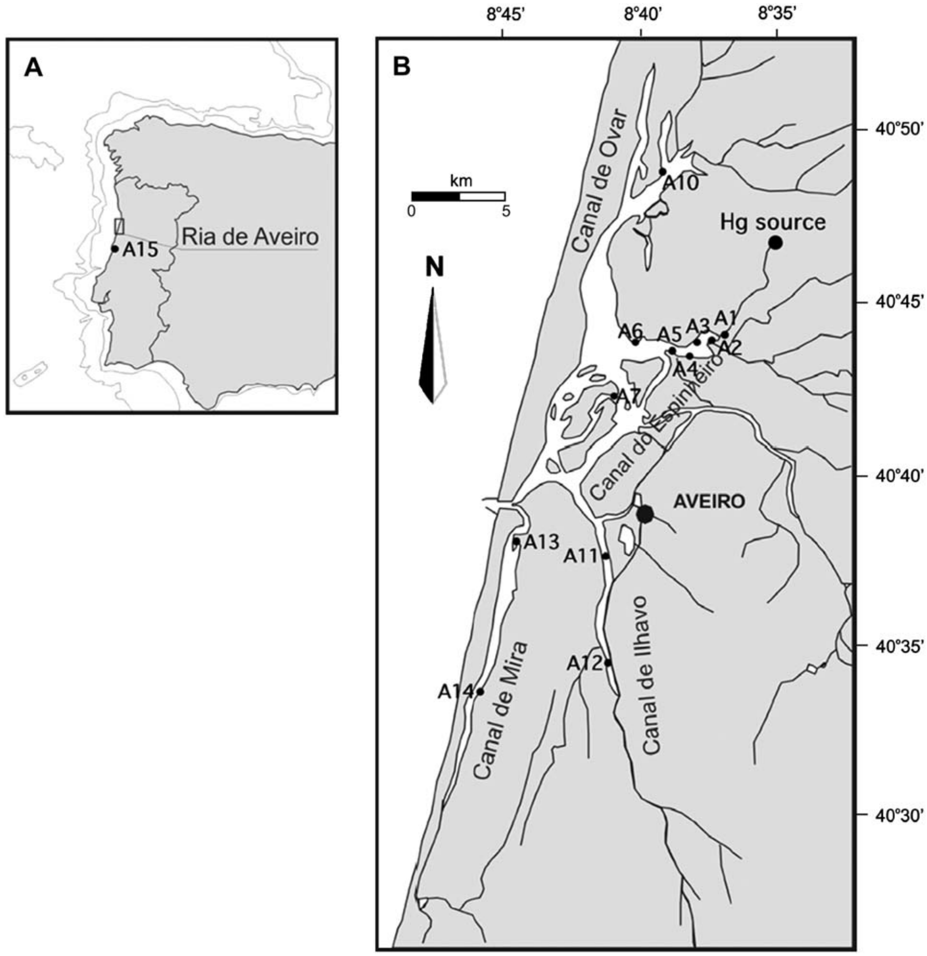

Fig. 1. The Ria de Aveiro coastal lagoon with the sampling locations indicated.

2.2. Laboratory procedures

Sediment and biological samples were analyzed for total mercury by thermal decomposition atomic absorption spectrometry with gold amalgamation, using a LECO AMA-254 (Advanced Mercury Analyzer).

Filters were oven-dried at 60 °C and digested with HNO_3 (4 mol L^{-1}) for determination of the total mercury concentration in suspended particulate matter (for detailed information on the method see Pereira et al., 1998). Dissolved reactive mercury and suspended particulate matter (SPM) mercury analyses were performed by cold-vapor atomic fluorescence spectrometry (CV-AFS) using a PSA model Merlin 10.023 equipped with a detector PSA model 10.003, with tin chloride as reducing agent (2% in 10% HCl). Organic mercury determinations in biological tissues were determined through digestion with a mixture of 18% KBr in 5% H_2SO_4, followed by extraction of organic mercury into toluene (Cai et al., 1997). The extractions were always performed in triplicate. The aqueous fraction, resulting from the addition of an $Na_2S_2O_3$ solution, was then analyzed for mercury by thermal decomposition atomic absorption spectrometry with gold amalgamation.

Analytical quality control was performed by using certified reference material (CRM). The CRM used were IAEA-356 and MESS-2 (for sediments), while for biota NIST-2976 and TORT-2 were used. The results were corrected according to the daily recovery percentage of the CRM analyses. The values obtained for the whole of the CRM analysis ranged from 81 to 102% (MESS-2: 81 ± 3%; IAEA-356: 98 ± 1%; TORT-2: 102 ± 2%; NIST-2976: 101 ± 3%). Analyses were always performed in triplicate. Reference material NIST-2976 was used to validate organic mercury analyses, and an extraction efficiency of roughly 80% was achieved.

Bioconcentration factors (BCF) were calculated, defined as $[Hg]_{biota}/[Hg]_{environment}$ for sediments, dissolved reactive fraction and SPM.

3. Results

3.1. Population structure

Five cohorts were identifiable (Table 1), results that concur with the findings of Verdelhos et al. (2005), who found a similar population structure in the Mondego estuary, 60 km south from our study site (sampling site A15). They identified an annual recruitment period of a spring cohort, which seems also to be the case in our study which was expected since latitudinal variations are never found in such close systems (60 km apart)

Table 1
Population structure, cohorts and size classes

Size class	Size (mm)	Std. dev.	n	Age
1	4.97	2.54	95	5–6 months
2	19.72	3.34	7	1.5 years
3	28.84	2.90	44	2.5 years
4	34.76	3.00	13	3.5 years
5	42.00	2.32	3	4.5 years
G-test = 24.898	D.F. = 22	$P = 0.301957$	Not significant ($P > 0.05$)	

(Pardal et al., 2000). Size class 2 (1–2 cm) unexpectedly showed very low abundance, probably due to adverse conditions during the recruitment period (corresponded to a season of strong floods during all the winter and early spring of 2001).

3.2. Scrobicularia plana contamination in the Ria de Aveiro

The results from the first sampling campaign show that mercury contamination is mainly restricted to the area in the vicinity of the contamination source (A2 and A5–A7), while in the remainder of the system mercury levels in water, SPM and sediments are close to baseline levels found in the non-contaminated site (A15) (Fig. 2). Mercury in *Scrobicularia plana* (based only on adult individuals) followed a similar trend, with higher levels found in sites A2 and A5, where some individuals exceeded the 0.5 mg kg^{-1} threshold permitted for human consumption (EPA, 2001). Very high positive linear regressions were found between *S. plana* mercury contamination and environmental mercury levels ($r^2 = 0.73$, $p < 0.01$ for sediments, $r^2 = 0.91$, $p < 0.01$ for dissolved reactive mercury and $r^2 = 0.96$, $p < 0.01$ for SPM). The observed regressions are strong indicators of a possible correlation between the feeding tactics of *S. plana*, a surface deposit feeder, and the intake of mercury mostly from the water and suspended particulate matter. Bioconcentration factors were rather variable, but a common trend of higher bioconcentration farther from the contamination source was discernible (Fig. 3), and suggest the use of decontamination strategies by *S. plana* in the most contaminated area.

These results were the basis for the selection of sampling sites for the annual and lifespan bioaccumulation study, conducted in the most contaminated area (A1, A2, A4 and A5, two more sites were selected to increase resolution), with a local, less contaminated control site (A11) and another site in the reference system, the Mondego estuary (A15), assumed as an Hg-free ecosystem.

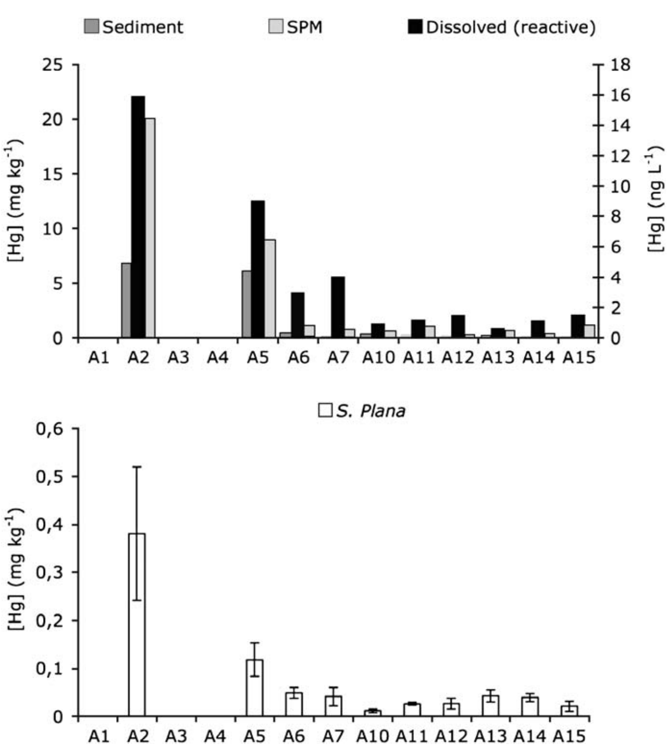

Fig. 2. Average ($n = 10$, error bars represent standard deviation) total mercury concentrations in *S. plana* (mg kg^{-1}), sediment (mg kg^{-1}), SPM (mg kg^{-1}) and dissolved reactive mercury (ng L^{-1}).

3.3. Total mercury annual bioaccumulation patterns in Scrobicularia plana

As expected, and following a similar pattern to the preliminary sampling, mercury contamination was higher in the vicinity of the mercury source (Fig. 4). Despite not always statistically significant due to high standard deviation values, a trend of increasing mercury accumulation with age was apparent from the results (Fig. 4), except in the reference site (Mondego estuary, A15) where the estimated values were always very low. In the most contaminated site (A1) no class 5 individuals were found.

Two different scenarios emerged in terms of annual mercury accumulation in *Scrobicularia plana* during lifespan, depending on environmental mercury levels. In low contamination sites (A11 and A15), absence of or negligible accumulation occurred, with accumulation rates (calculated from the slope of the regression lines) of 0.009 mg kg^{-1} yr^{-1} in site A11 and no net accumulation in the reference site A15 (Fig. 5, Table 2) (one-way ANOVA, $p = 0.53$), where an apparent growth dilution effect was observed, similar to previous studies on other bivalve species (Joiris et al., 1998). In the other locations, bioaccumulation followed a linear trend,

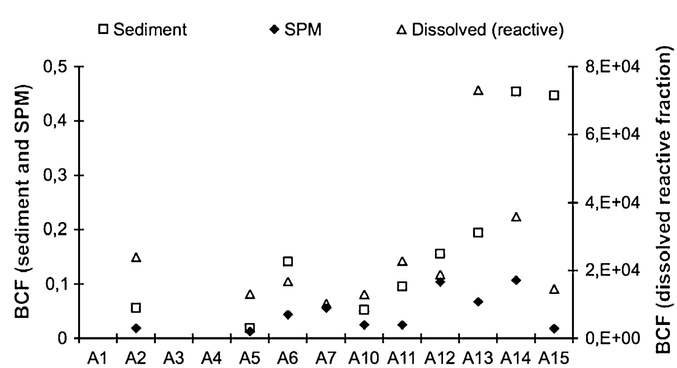

Fig. 3. Bioconcentration factors ($[Hg]_{biota}/[Hg]_{environment}$) for sediments, suspended particulate matter (SPM) and dissolved reactive fraction, in the first sampling campaign.

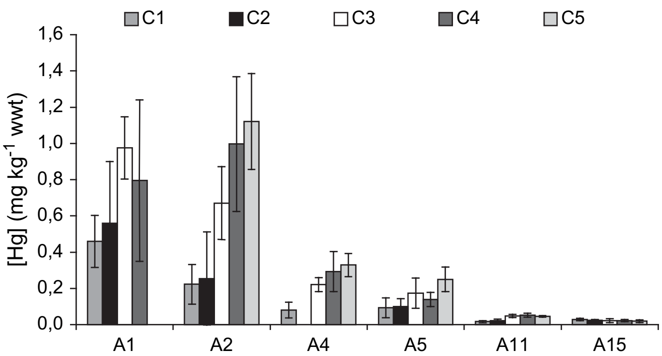

Fig. 4. Average mercury contamination (error bars represent standard deviation) in all size classes of *S. plana* (mg kg^{-1}).

with annual accumulation rates increasing parallel to environmental contamination (Table 2). In site A1, however, despite a 10-fold increase in sediment and reactive dissolved mercury levels, the annual bioaccumulation rate (not considering the highest size class) was similar to that of site A2.

A highly significant linear regression ($r^2 = 0.93$, $p < 0.01$) was found between annual bioaccumulation rates and SPM concentrations, suggesting this to be the primordial pathway for mercury incorporation in *Scrobicularia plana*.

3.4. Organic mercury annual bioaccumulation patterns in Scrobicularia plana

Despite the levels of organic mercury contents increasing parallel to the contamination gradient, at each sampling station, no increment was found with age (Fig. 6A). This feature resulted in a discernible reduction in the organic mercury fraction with increasing size (Fig. 6B), consisting of what can be considered as a dilution effect of organic mercury with age, more evident in sites A2 and A15.

4. Discussion

Scrobicularia plana is a commonly used bioindicator for metal associated environmental monitoring (Byrne and O'Halloran, 2001; García-Luque et al., 2004; Pérez et al., 2004; Riba et al., 2004; Cheggour et al., 2005), but some background is needed to integrate the different and fragmentary literature available, since not always the same size classes are used and therefore intercomparison becomes very complex.

Our data on the population structure, together with previous studies in nearby systems (Verdelhos et al., 2005) suggest that this species has a single annual recruitment period, producing a single cohort per year. The existence of five different cohorts allows to estimate the lifespan in more than 4 years. Verdelhos et al. (2005) also calculated the annual growth rate to be approximately 1 cm yr^{-1}. This information is invaluable, since such detailed data on the species may permit to study the lifespan bioaccumulation patterns in the field, unlike most studies on bioaccumulation that consist of (short time) laboratory studies (Anandraj et al., 2002; García-Luque et al., 2004; Riba et al., 2004). The Ria de Aveiro functions as a natural laboratory due to its human induced mercury gradient, and *Scrobicularia plana* was found to respond to this gradient, with increasing accumulation parallel to environmental mercury levels. Bioconcentration factors were found to increase farther from the contamination source and in lower environmental contamination sites, similar to what was found for various mollusc species (McGeer et al., 2003), and various macroalgae (Coelho et al., 2005). According to McGeer et al. (2003), this trend contrasts with the accumulation of other metals by molluscs, which is generally higher for increasing metal levels.

Scrobicularia plana was found to accumulate mercury throughout its lifespan, in a linear pattern. Accumulation rates showed a significant positive correlation to SPM mercury levels, explainable by its feeding tactics, deposit feeding (Byrne and O'Halloran, 2001; Verdelhos et al., 2005). Location A1 was considered a highly stressful environment, reflected by the 10-fold increase in sediment mercury levels and the fact that no 5-year-old individuals could be found there. Mercury daily uptake rates were calculated for mussels *Mytilus galloprovincialis* (Mikac et al., 1996), ranging from 0.02 to 1.44 ng Hg_{tot} d^{-1}, but typically below 0.75 ng Hg_{tot} d^{-1}. Calculations for an average 4+ year old individual (with a wet weight of 2.5 g) in site A1 or A2 result in a mercury intake of 1.71 ng Hg_{tot} d^{-1}, considerably higher. Much higher daily uptake rates (200 ng Hg_{tot} d^{-1}) were observed for chronic exposure experiments in the closely related species *Macoma balthica* (Boisson et al., 1998), but these results were obtained in laboratory experiments and are not compatible with long-term survival of organisms.

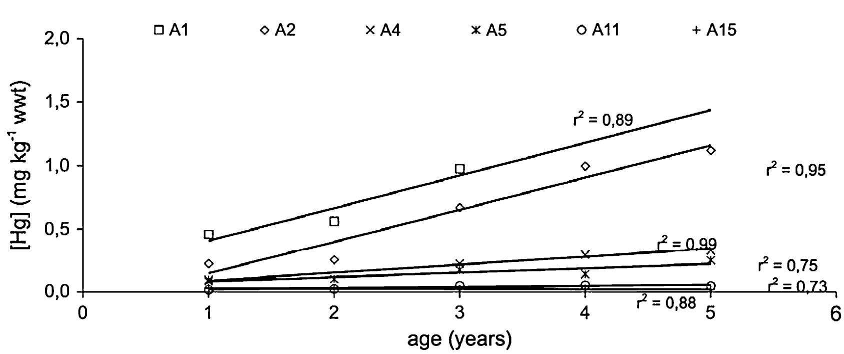

Fig. 5. Lifespan mercury accumulation in *S. plana*. The trendlines calculated from average levels in each year represent the annual net accumulation of mercury by the bivalves.

Table 2
Mercury concentrations in sediment (mg kg^{-1}), dissolved reactive fraction (ng L^{-1}) and suspended particulate matter (mg kg^{-1}) in the second sampling moment, annual bioaccumulation rates in *S. plana* (calculated from the slope of regression lines, mg kg^{-1} yr^{-1}) and the ratio between annual accumulation rates and SPM Hg concentrations (mean values ± Std. dev.)

Station	Sediment [Hg] (mg kg^{-1})	Reactive dissolved [Hg] (ng L^{-1})	Suspended particulate matter [Hg] (mg kg^{-1})	Annual bioaccumulation rates	Acc. rate/SPM
A1	51.7 ± 4.8	60.5 ± 9.6	25.8 ± 0.4	0.258	0.01
A2	6.8 ± 0.3	15.8 ± 1.0	20.1 ± 2.6	0.254	0.013
A4	2.5 ± 0.1	28.3 ± 1.8	6.5 ± 0.2	0.064	0.01
A5	6.2 ± 0.1	9.0 ± 1.7	8.9 ± 0.5	0.035	0.004
A11	0.3 ± 0.1	1.1 ± 0.5	1.0 ± 0.1	0.009	0.009
A15	0.1 ± 0	1.5 ± 0.4	1.2 ± 0.5	−0.002	−0.002

Our results suggest the existence of a threshold accumulation rate for *Scrobicularia plana* (around 0.25 mg kg^{-1} yr^{-1}), above which stress may result in physiologic alterations and elevated mortality (4+ year old individuals in site A1 presented abnormal mercury levels). *Scrobicularia plana* was found to synthesize metallothioneins as a response to heavy metal contamination and the salinity of the medium found to influence this physiological response (García-Luque et al., 2004). The rupture of this detoxifying mechanism may be the basis for the results found in the top classes of the most contaminated site. Another possible explanation was the inhibition of enzymatic activity, described earlier by Mazorra et al. (2002), but this study employed very high levels of mercury (1–20 mg L^{-1}, several orders of magnitude higher than the dissolved concentrations found, up to 20 ng L^{-1}) and no evidence on whether the levels observed in the field may induce such an effect was found.

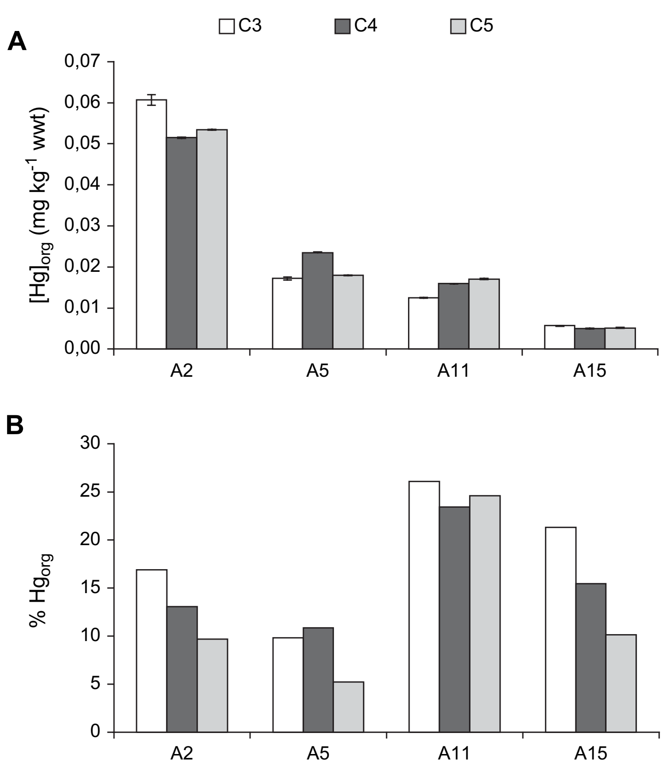

Fig. 6. Organic mercury accumulation study in *S. plana*: organic mercury (A) in composite samples of *S. Plana* (mg kg^{-1}); and organic mercury percentage of total mercury (B) in composite samples of *S. plana*.

Along the contamination gradient, the organic mercury accumulation followed a pattern similar to that of total mercury. Nevertheless, at each site no consistent accumulation with age was observed, and the contribution of the organic mercury fraction to the total mercury load tended to decrease with age, regardless of environmental contamination. These results imply that the rate of inorganic mercury uptake is higher than that for organic mercury, causing a "dilution effect" observable with increasing age, and also with increasing contamination, since higher contamination sites had a lower percentage of organic mercury. Mercury was released in inorganic forms, resulting in low organic mercury fraction in sediments (below 1%) and therefore higher exposure to inorganic mercury. Also, inorganic mercury is more strongly bound to particulate organic matter than organic mercury species, hence being more bioavailable to deposit feeding organisms.

The tolerable daily intake for organic mercury oscillates depending on the selected source, but Ipolyi et al. (2004) calculated from the most conservative sources and average mollusc consumption a tolerable average residue level of 0.0435 mg kg^{-1} (wwt). In the present study, organic mercury concentrations ranged from 0.005 in site A15 to 0.061 mg kg^{-1} (wwt) in site A2, therefore exceeding the tolerable average residue level.

Our findings may have important implications for management policies and biomonitoring programs, by giving a better insight into the process of mercury bioaccumulation in the clam *Scrobicularia plana*. For all but two sampling sites, the ratio between annual bioaccumulation rates and suspended particulate matter mercury concentrations was very close to 0.01, which if observed and confirmed elsewhere, may reveal to be a good predictor of accumulation rates for *S. plana*. Since bioaccumulation appears to follow a linear model, predicting annual accumulation rates will allow to estimate mercury levels at any given size class, and take necessary management actions accordingly in order to avoid deleterious effects on humans through direct consumption of bivalves.

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