Available online at www.sciencedirect.com
SCIENCE DIRECT
Marine Pollution Bulletin 52 (2006) 674–680
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Can *Nassarius reticulatus* be used as a bioindicator for Hg contamination? Results from a longitudinal study of the Portuguese coastline

J.P. Coelho [a,*], J. Pimenta [b], R. Gomes [a], C.M. Barroso [b], M.E. Pereira [a], M.A. Pardal [c], A. Duarte [a]

[a] *Department of Chemistry, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal*
[b] *Department of Biology, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal*
[c] *IMAR (Institute of Marine Research), Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal*

Abstract

The focus of this work is to explore the use of the netted whelk, *Nassarius reticulatus* (L.), as an indicator of mercury (Hg) contamination, by assessing the concentration of Hg in the sediments and in the whelk along the entire Portuguese coast. Total Hg concentrations ranged from below the detection limit (0.01 ng absolute mercury) up to 0.87 mg kg^{-1} dry weight (dwt) in sediments and between 0.06 and 1.02 mg kg^{-1} (dwt) for organisms, with no significant differences between males and females. Although organic mercury was not detected in the sediments, it represented, on average, 52% of the total Hg in the whelk tissues, and as high as 88% in some cases, suggesting mercury accumulation from dietary intake. Significant negative correlations were found between the total Hg concentrations in the sediments and the $\log_{10}$ of Hg concentrations in whelk tissues males ($r = -0.64$; $P < 0.01$) and females ($r = -0.52$; $P < 0.01$) indicating that the species is a poor indicator of Hg contamination. Nevertheless, since the highest concentrations of organic mercury in the whelk tissues were found in the least contaminated areas, this species must be highly relevant in the trophic web, namely on the possible biomagnification of mercury. The high dietary mercury accumulation from feeding on carrion and the low bioavailability of mercury to whelks in estuarine sediments may be the basis of the mercury accumulation pattern in *N. reticulatus*.

Keywords: Mercury; Bioaccumulation; Biomagnification; *Nassarius reticulatus*; Bioindicator

1. Introduction

The contamination of coastal waters by metals and organometals derived from anthropogenic activities has long been a threat to the marine and coastal ecosystems. The accumulation processes of these contaminants in aquatic organisms will determine, in part, the enhancement of their adverse effects on the biota. Estuaries and coastal zones are surveyed frequently as part of the assessment of their environmental quality, since they are particularly impacted by anthropogenic inputs. Such assessments of the chemical and ecological status of the coastal zone 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 a 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 a complex set of studies. The trace metal content of organisms has been widely used in biomonitoring programmes of metal pollution in the marine environment, and provides a time-integrated measure of metal availability (Saiz-Salinas et al., 1996). Although most research in this area has been performed

* Corresponding author. Tel.: +351 234370737; fax: +351 234370084.
E-mail addresses: jcoelho@dq.ua.pt (J.P. Coelho), eduper@dq.ua.pt (M.E. Pereira).

0025-326X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2005.10.020

on species of genus *Mytilus* (Conti and Cecchetti, 2003; Liang et al., 2004; Roméo et al., 2005), it is important to use other organisms, with broader distributions and different biochemistries, for a more complete assessment of metal contamination in the environment. In the case of mercury, which is a highly deleterious environmental pollutant with recognized mutagenic and teratogenic effects, data on the mechanisms of such effects are very sparse and controversial in the literature (Calderón et al., 2003; Tchounwou et al., 2003). Problems derived from mercury contamination are enhanced when it enters the food chain, including human exposure to methylmercury, which is the most toxic form and is subject to biotic bioaccumulation and biomagnification (Mason et al., 1995; Tchounwou et al., 2003). However, studies specifically focused on mercury contamination are few, despite being one of the most hazardous elements. A few hotspots of mercury contamination are identified along the Portuguese coast, mainly in estuarine ecosystems such as the Tagus (Canário et al., 2003, 2005) and the Ria de Aveiro (Pereira et al., 1998; Ramalhosa et al., 2005a), whereas other systems retain nearly pristine conditions when referring to this metal, namely the Douro (Ramalhosa et al., 2005b) or the Mondego (Vale et al., 2002). Little is known, however, about the levels of mercury contamination in open coastal waters, and its effects on the associated benthic community.

Nassarius reticulatus is an abundant and ubiquitous marine prosobranch gastropod in European coastal waters, which is widely distributed in the Atlantic, the Mediterranean and Black Seas. The general biology and population dynamics of this species is provided by Tallmark (1980) and Fretter and Graham (1984) and the species was first proposed as a bioindicator of tributyltin (TBT) pollution by Stroben et al. (1992). Subsequently, it has been used in TBT monitoring programmes all over Europe (Oehlmann et al., 1993; Barreiro et al., 2001; Barroso et al., 2000, 2002). Thus, the question arises as to whether *N. reticulatus* is a suitable bioindicator for monitoring other contaminants, such as mercury, taking into account its wide ranging distribution and resistance to pollution.

Taking the lack of knowledge about mercury contamination in the Portuguese coastal waters into account and the current interest in new bioindicators, the main objective of this work is to assess the level of Hg contamination in the sediments and in the netted whelk *N. reticulatus* (L.) along the Portuguese coast.

The specific objectives of the current work are: (i) to assess the general level of Hg contamination in sediments along the whole of the Portuguese coastline, which will provide a time referenced basis for monitoring a trend in temporal variation, (ii) to establish whether there is any relationship between the concentration of Hg in the sediments and its concentration in *N. reticulatus* tissues, (iii) to explore the possible use of *N. reticulatus* as a indicator of Hg contamination and (iv) to evaluate the potential risks of mercury contamination in *N. reticulatus* in terms of food web biomagnification.

2. Materials and methods

2.1. Sampling and pre-treatment

Sampling of *N. reticulatus* was performed from May to July 2000. Forty sampling stations were selected along the Western Portuguese coast (Fig. 1), including estuaries, covering a distance of approximately 700 km (Fig. 1). Geographical co-ordinates for each sampling site were determined with a mobile global positioning system (GPS). The specimens were collected by hand on the intertidal shore and with baited hoop nets at sublittoral sites. The animals were maintained in separate aquaria for 3 days to allow intestine and body surface depuration. In order to permit inter-site comparison, a common approach was adopted for selecting individual animals. They were only adult animals, i.e. those presenting white columellar callus and teeth on the outer lip, and were of similar ages and size. The shell height (distance from shell apex to lip of siphonal canal) was measured with vernier callipers to the nearest 0.1 mm. The shells were opened with a bench vice and the individuals were sexed under a stereomicroscope. About 20 females and 20 males were frozen separately at −20 °C, after removing the shell and the operculum. Samples were then homogenised, freeze-dried and preserved at −20 °C until the Hg analysis was performed.

Surface intertidal sediments (1–2 cm depth) were collected simultaneously from the same sites where *N. reticulatus* samples were obtained, whereas at sublittoral sites sediments were collected with a van Veen grab. Samples were placed in polyethylene bags and temporarily stored on ice during transport to the laboratory. Once in the laboratory the sediment was sieved through 100 μm polypropylene mesh with water taken from the same locality where the sediments were collected. The sediments were allowed to settle, the supernatant water decanted and the sediments were frozen at −20 °C. Samples were later homogenised, freeze-dried and preserved at −20 °C prior to Hg analysis.

The total concentration of mercury ($\sum$Hg) in sediments referred in this work was measured in the <100 μm sediment fraction, in order to provide inter-site comparisons. It is assumed that inorganic mercury (Hg_{inorg}) is equivalent to the difference between total ($\sum$Hg) and organic mercury (Hg_{org}). Total mercury determinations in the sediments and in the whelks, were carried out on samples of 200–500 mg, accurately weighed into a nickel boat. The samples were dried prior to combustion at 750 °C in an oxygen atmosphere. The mercury vapour evolved from the samples was trapped on the surface of a gold amalgamator, which was subsequently heated to 900 °C to quantitatively release the mercury. The vapour phase Hg was determined by atomic absorption spectrometry using a silicon detector at 253.6 nm (LECO, AMA-254) (Costley et al., 2000). Organic mercury determinations in biological tissues were determined on between 200 and 500 mg of dried sample. The samples were digested using a mixture of 18% KBr

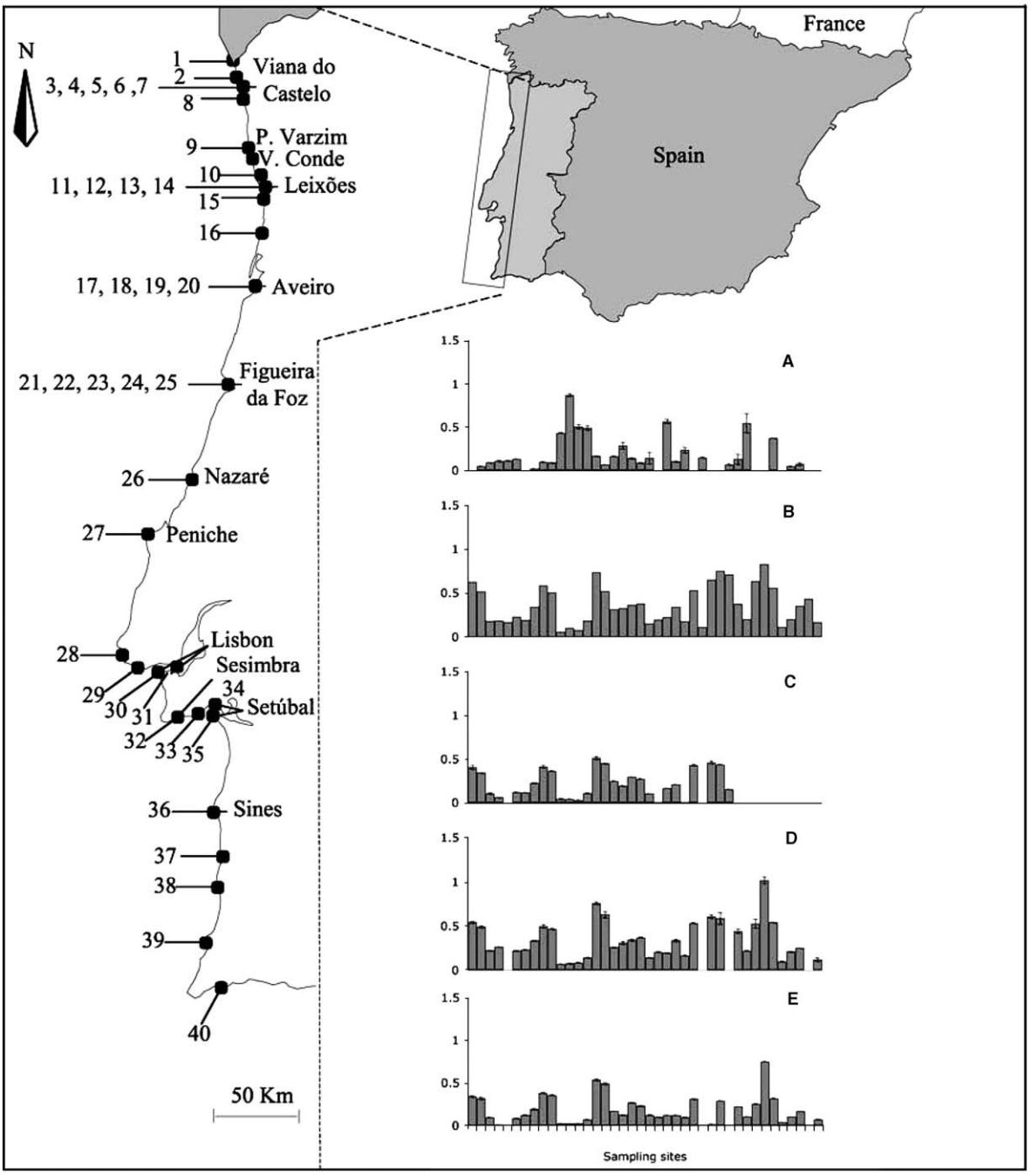

Fig. 1. Map of the sampling stations, total mercury concentrations in sediments (A), female (B) and male *N. reticulatus* (D) tissues, plus organic mercury concentrations in female (C) and male *N. reticulatus* (E). (Error bars represent standard deviation of samples, all values in mg kg^{-1}.)

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 a $Na_2S_2O_3$ solution, was then analysed for Hg by thermal decomposition atomic absorption spectrometry with gold amalgamation. The analytical quality of the total and organic mercury determinations was assessed by applying the analytical procedure to standard reference materials TORT-2 (for biological samples), MESS-2 (for total mercury in sediments) and CRM-580 (for organic mercury in sediments). Results for this assessment are summarized in Table 1.

3. Results

3.1. Mercury concentration in the sediments

The $\sum$Hg in the sediments along the Portuguese coast varied from below the detection limit (0.01 ng absolute mercury) up to 0.87 mg kg^{-1} (dwt) (Fig. 1). The lowest

Table 1
Obtained and certified mercury concentrations and extraction efficiency for analyses of CRM

	Reference material	[Hg] ± Std dev (mg kg^{-1})	n	[Hg] cert ± Std dev (mg kg^{-1})	Efficiency (%)
Total Mercury	TORT-2	0.28 ± 0.021	43	0.27 ± 0.03	102.6
	MESS-2	0.089 ± 0.001	21	0.092 ± 0.005	96.7
Organic Mercury	TORT-2	0.152 ± 0.004	27	0.152 ± 0.006	100.0
	CRM-580	0.074 ± 0.003	14	0.076 ± 0.002	97.7

Std dev = standard deviation; n = number of analyses.

concentrations of Hg in sediments was observed at sites located along the open coast (Stations 2, 8, 9, 10, 16, 30, 37, and 38) where concentrations did not exceed 0.10 mg kg^{-1} and showed a mean value of 0.07 mg kg^{-1}. The remainder of the sampling stations were located inside estuaries and generally gave higher values, which varied between 0.09 and 0.87 mg kg^{-1}, with a mean value of 0.28 mg kg^{-1}. The highest concentrations were observed at Leixões, an estuarine system that has a major port and receives industrial effluents. At this location it was possible to depict a clear increasing $\sum$Hg gradient from the adjacent coastal zone towards the estuarine interior (Stations 9–16). The values of organic mercury in sediments were always below the detection limit, accounting for less than 0.5% of total mercury (calculated from the detection limit of the instrument and the total mercury levels in the sediments).

3.2. Mercury concentrations in N. reticulatus

As shown in Table 2, 75–93% of the $\sum$Hg was found in the soft tissues of *N. reticulatus*, whilst the shell contributed no more than 3% and the operculum up to 22% (only in one location, the others varied from 5% to 9%). The body burden of $\sum$Hg in *N. reticulatus* varied between 0.07 and 1.02 mg kg^{-1} (dwt) in males and 0.06 and 0.83 mg kg^{-1} (dwt) in females along the Portuguese coast (Fig. 1). It was lower in estuarine systems (from 0.06 to 0.38 mg kg^{-1} dwt) than in the open coast (from 0.11 to 1.02 mg kg^{-1} dwt). The body burdens in the two genders were similar across sites (regression equation: males = 0.97 * females; $r = 0.96$; slope's 95% interval of confidence 0.97 ± 0.1, not statistically different from 1). Significant inverse correlations were obtained between the $\sum$Hg sediment concentration and the $\log_{10}\sum$Hg body burden of females ($r = -0.52$; $P < 0.01$) (Fig. 2A) and males ($r = -0.64$; $P < 0.01$) (Fig. 2B).

Table 2
Mercury concentrations (mg kg^{-1}) in the soft tissues, operculum and shell of *N. reticulatus* in locations 8, 9, 16 and 25

Site	$\sum$[Hg] (mg kg^{-1})		
	Soft tissues	Operculum	Shell
8	0.303 (88.5%)	0.031 (9.1%)	0.0082 (2.4%)
9	0.468 (90.5%)	0.036 (7.0%)	0.013 (2.5%)
16	0.657 (93.0%)	0.037 (5.2%)	0.013 (1.9%)
25	0.121 (74.9%)	0.036 (22.3%)	0.0045 (2.8%)

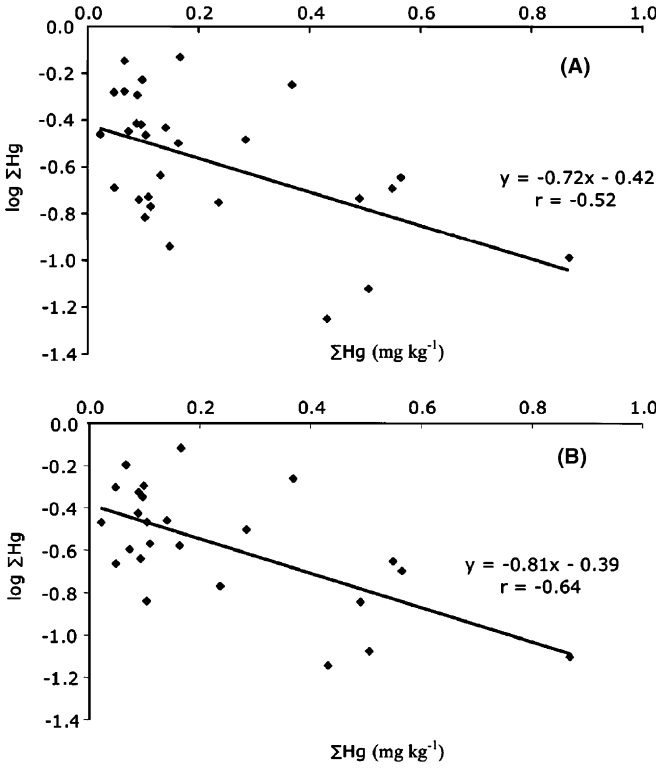

Fig. 2. Log$\sum$Hg body burden to sediment $\sum$Hg (mg kg^{-1}) concentrations for female (A) and male *N. reticulatus* (B).

About 32–88% (mean 52%) of the $\sum$Hg was found to be in the Hg_{org} form. Both the $\log_{10}Hg_{org}$ and the $\log_{10}Hg_{inorg}$ body burdens were negatively correlated with the $\sum$Hg sediment concentration, in females, respectively, $r = -0.64$, $P < 0.01$ (Fig. 3A) and $r = -0.48$, $P < 0.02$, and for males $r = -0.66$, $P < 0.001$ (Fig. 3B) and $r = -0.55$, $P < 0.01$. A high similarity of mercury body burden between genders was observed for organic mercury (regression equation: males = 1.10 * females; $r = 0.96$; slope's 95% interval of confidence 1.10 ± 0.12, not statistically different from 1).

The bioconcentration factor (BCF) was estimated as the ratio of $\sum$Hg body burden (dwt) to $\sum$Hg sediment concentration. It varied from 0.03 to 4.4 in males and from 0.04 to 4.5 in females and the BCFs for the two genders were not correlated. The BCF was inversely correlated to $\sum$Hg sediment concentration on a $\log_{10}$-to-$\log_{10}$ basis for females ($r = -0.90$, $P < 0.01$) (Fig. 4A) and males ($r = -0.92$, $P < 0.001$) (Fig. 4B).

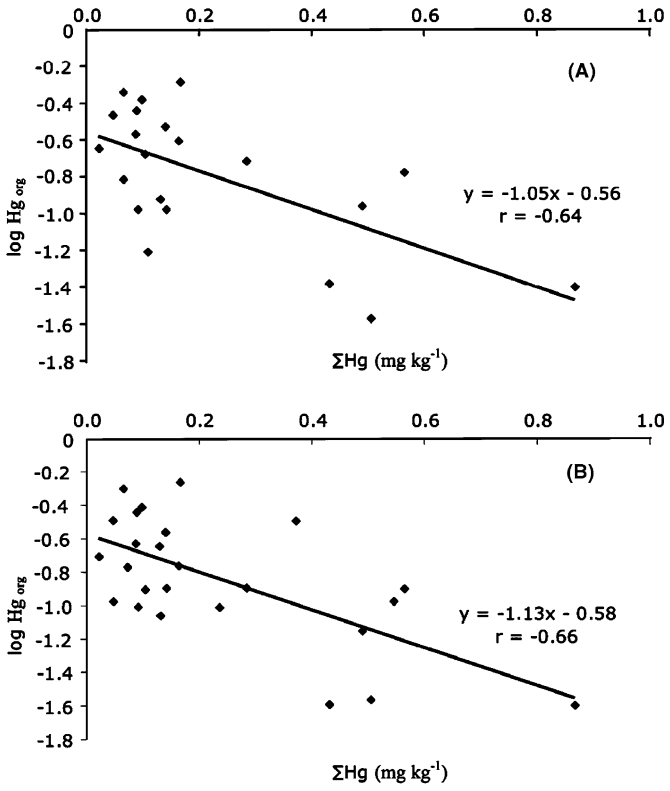

Fig. 3. Log Hg_{org} concentrations in female (A) and male (B) *N. reticulatus* to sediment $\sum$Hg concentrations (mg kg^{-1}).

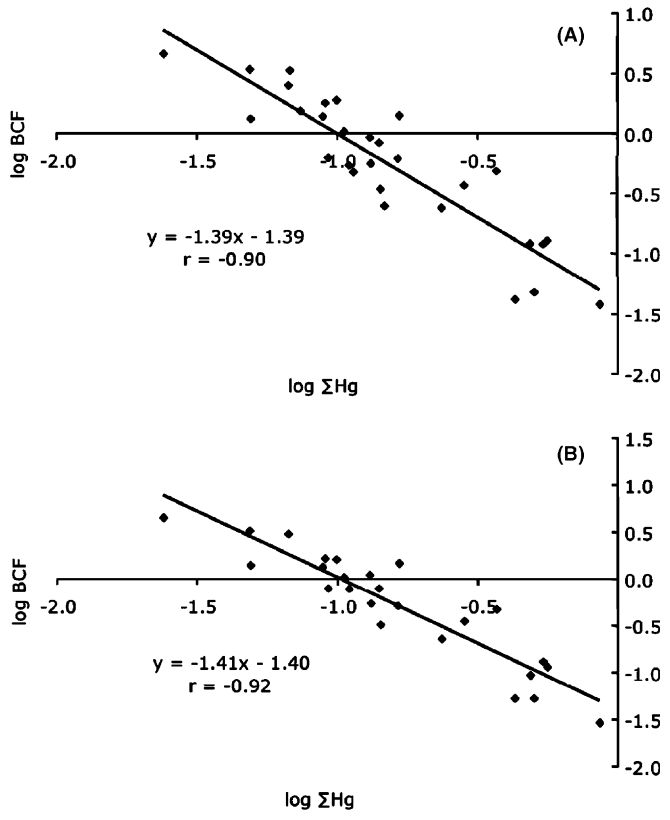

Fig. 4. BCF to sediment $\sum$Hg concentrations (log to log basis) in female (A) and male (B) *N. reticulatus*.

4. Discussion

Most mercury is released into the environment as inorganic mercury, and it is mainly bound to particulate and organic matter (Beckvar et al., 1996). Sediments are thus of primary importance in mercury accumulation in aquatic environments and represent a major potential reservoir for this element, where it may persist for many decades (Ullrich et al., 2001). The current work has shown that the highest levels of $\sum$Hg sediment contamination along the Portuguese coast are found in estuaries, probably due to the concentrated local discharges of urban and industrial wastes. The values of mean $\sum$Hg in the sediment in these areas was about 4 times higher than those observed at the open coast, where mean concentrations are similar to the reported for pristine, uncontaminated sediments (Ullrich et al., 2001). As Hg preferentially binds to silt/clay particles and organic matter, which were much more abundant in estuarine than in open coast sediments, the mercury concentrations are potentially higher in estuaries than that reported here for the <100 μm fraction. The determinations of organic mercury in samples in this work were always below the detection limit, confirming the widely accepted idea that inorganic species are the predominant form of mercury in sediments. Methylmercury concentrations in sediments are usually 1–1.5% of total mercury concentrations in freshwater systems and tend to be lower (0.5%) in estuarine and marine environments (Ullrich et al., 2001).

The recent OSPAR evaluation of Ecotoxicological Assessment Criteria (EACs—which are expressions of biological and/or ecotoxicological risk) (Bignert et al., 2004), gives the provisional EAC for Hg in sediments in the range 0.05–0.5 mg kg^{-1}. Most of our results fall in this range, signalling possible biological effects, such as impaired growth and reproduction. Some of the Hg concentrations in the surface sediments are above the upper EAC threshold and long-term biological effects, such as mortality, are indicated.

The concentration of $\sum$Hg in *N. reticulatus* tissues was based in similar proportions of inorganic and organic forms. Of major relevance is the fact that the $\sum$Hg concentration of *N. reticulatus* was higher outside the estuaries and both inorganic and organic mercury body burdens in the animal were inversely correlated with $\sum$Hg sediment concentration. No differences were found between males and females, suggesting that mercury uptake and bioaccumulation is independent of differential physiological responses between sexes and reproductory condition, and that bioaccumulation follows a similar pattern irrespective of gender.

The whelk lives on the sediment and frequently burrows in it, which favours the accumulation of Hg_{inorg} from this source. However, it is known that the inorganic mercury is easily taken up by the biota from the sediments but it is also depurated quickly, resulting in a net low accumulation (Beckvar et al., 1996), which may explain that only half of the mercury body burden is in an inorganic form

in the whelk. The negative correlation found between sediments and tissues could eventually be due to the lower bioavailability of Hg to the whelk, particularly inside estuaries. For instance, the presence of sulfides in the sediments may strongly bound the Hg[II] and make it unavailable to the biota (Beckvar et al., 1996). On the other hand, McGeer et al. (2003) made an extensive literature review regarding the relationship between bioconcentration/bioaccumulation factors and the exposure concentration for metals in aquatic organisms. They reported an accumulation of mercury in molluscs which contrasts with that observed in other phyla, specifically fish and arthropods, since molluscs show a reduction, instead of an increase, in the tissue concentration of Hg as inorganic Hg exposure increases. According to the same authors, this trend also contrasts with the accumulation of other metals by molluscs, which is generally higher for increasing exposure to metals. If this applies to the whelk, it could also explain the negative relationship found between the Hg_{inorg} body burden and the $\sum$Hg in the sediments along the Portuguese coast, and hence the negative correlation of bioconcentration factor to sediment contamination.

There is little evidence that molluscs can convert inorganic mercury to organic mercury (Fowler et al., 1978), thus the Hg_{org} measured in the whelk tissues is primarily accumulated from external sources. The most common form of Hg_{org} in the environment is methylmercury, which is produced by microorganisms in both sediment and water through the methylation of the bivalent mercury (Hg[II]) (Mason et al., 1995; Beckvar et al., 1996; Clark, 2001). Methylmercury accumulates quickly and depurates very slowly, so that it biomagnifies in higher trophic levels (Mason et al., 1995; Beckvar et al., 1996; Lawson and Mason, 1998). The whelk is a scavenger, with a preference for detritus among juveniles to carrion among adult snails (Tallmark, 1980; Cheung and Wong, 2001; Cheung et al., 2002) and as a carrion feeder the species may accumulate large quantities of methylmercury from its feeding habit. However, the Hg_{org} concentrations are less than in most adult fish where methylmercury generally represents more than 90% of the Hg body burden (EPA, 2001). One possible reason for the observed negative correlation between Hg_{org} whelk body burden and the $\sum$Hg in the sediments could be the lower bioavailability of Hg in estuaries as reported above. If it is strongly bound to the sediments it will be less available for methylation by biota (Beckvar et al., 1996). Parks et al. (1989) reported that methylmercury in a freshwater system was inversely related to the inorganic mercury concentrations in the sediments and that methylmercury concentrations in surface water and in fish was highest about 80–100 km downstream from the source, which could be due to a downstream decrease in the sulphide concentration of the sediments. This caused the methylmercury concentration to be inversely correlated with Hg_{inorg} in the sediments, which seems also to occur in the present study. This negative correlation arises important methodological implications for monitoring programmes, since most environmental monitoring occurs in the vicinity of contamination sources, when in fact biotic contamination hotspots may arise hundreds of kilometres away, in more pristine areas, and implicate risks in case of human consumption. Moreover, the adverse effects of episodes of acute mercury discharges will probably emerge with considerable delay in those distant contamination hotspots.

The negative correlation feature seems to be a general trend for mercury, and it has been previously reported for other molluscs (McGeer et al., 2003) and fish (Parks et al., 1989). Through bioaccumulation and biomagnification processes (mainly of organic forms of mercury) throughout food webs involving molluscs and economically important fish species may retain an excessive mercury load in regions with a low Hg load and highly exploited coastal areas, representing therefore an unexpected risk to human health.

The inverse relationship observed between the Hg contamination in the sediments and in *N. reticulatus* leads to the conclusion that this species is an unsuitable bioindicator for Hg contamination. Further research is needed to clarify the mechanisms involved in the accumulation of mercury in molluscs and more clearly explain the inverse relationship to environmental concentrations. Monitoring of biotic mercury contamination in coastal waters (mainly in fishing areas) should also be seriously considered, and may prove to be more important than monitoring close to contamination sources, since our results suggest that in fact, dilution of the pollutant concentration is not the solution to biological contamination, at least in the case of mercury.

References

Barreiro, R., González, R., Quintela, M., Ruiz, J.M., 2001. Imposex, organotin bioaccumulation and sterile females in *Nassarius reticulatus* from polluted areas of NW Spain. Marine Ecology Progress Series 218, 203–212.

Barroso, C.M., Moreira, M.H., Gibbs, P.E., 2000. Comparison of imposex and intersex development in four prosobranch species for TBT monitoring of a Southern Europe estuarine system (Ria de Aveiro, NW Portugal). Marine Ecology Progress Series 201, 221–232.

Barroso, C.M., Moreira, M.H., Bebianno, M., 2002. Imposex, female sterility and organotin contamination of the prosobranch *Nassarius reticulatus* (L.) from the Portuguese coast. Marine Ecology Progress Series 230, 127–135.

Beckvar, N., Field, J., Salazar, S., Hoff, R., 1996. NOAA Technical Memorandum NOS ORCA 100. Hazardous Materials Response and Assessment Division, National Oceanic and Atmospheric Administration, Seattle, 74 pp.

Bignert, A., Cossa, D., Emmerson, R., Fryer, R., Füll, C., Fumega, J., Laane, R., Martinez Calls, H., McHugh, B., MillerR, B., Millward, G.E., Moffat, C., Pijnenberg, J., Roose, P., Ruus, A., Schmolke, S., Smedes, F., Strand, J., Stronkhorst, J., Thain, J., Tissier, C., Traas, T., Tronczynski, J., 2004. OSPAR/ICES Workshop on the Evaluation and Update of Background Reference Concentrations (B/RCs) and Ecotoxicological Assessment Criteria (EACs) and How These Assessment Tools Should be Used in Assessing Contaminants in Water, Sediments and Biota. Final Report to OSPAR Commission, The Hague, 116 pp.

Cai, Y., Tang, G., Jaffé, R., Jones, R., 1997. Evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary gas chromatography–atomic fluorescence spectrometry. International Journal of Environmental Analytical Chemistry 68, 331–345.

Calderón, J., Ortiz-Pérez, D., Yáñez, L., Díaz-Barriga, F., 2003. Human exposure to metals. Pathways of exposure, biomarkers of effect, and host factors. Ecotoxicology and Environmental Safety 56, 93–103.

Canário, J., Vale, C., Caetano, M., Madureira, M.J., 2003. Mercury in contaminated sediments and porewaters enriched in sulphate (Tagus Estuary, Portugal). Environmental Pollution 126, 425–433.

Canário, J., Vale, C., Caetano, M., 2005. Distribution of monomethylmercury and mercury in surface sediments of the Tagus Estuary (Portugal). Marine Pollution Bulletin 50, 1142–1145.

Cheung, S.G., Wong, W.H., 2001. Effect of food availability on the energetics of the intertidal scaveging gastropod *Nassarius festivus*. Vie Milieu 51, 181–188.

Cheung, S.G., Tai, K.K., Leung, C.K., Siu, Y.M., 2002. Effects of heavy metals on the survival and feeding behaviour of the sandy shore scavenging gastropod *Nassarius festivus* (Powys). Marine Pollution Bulletin 45, 107–113.

Clark, R.B., 2001. Marine Pollution, fifth ed. Oxford University Press Inc., New York, 237 p.

Conti, M.E., Cecchetti, G., 2003. A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. Environmental Research 93, 99–112.

Costley, C.T., Mossop, K.F., Dean, J.R., Garden, L.M., Marshall, J., Carroll, J., 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. Analytica Chimica Acta 405, 179–183.

EPA (United States), 2001. Mercury Update: Impact on Fish Advisories. EPA-823-F-01-011, EPA, Washington, 10 p.

Fowler, S.W., Heyraud, M., La Rosa, J., 1978. Factors affecting methyl and inorganic mercury dynamics in mussels and shrimp. Marine Biology 46, 267–276.

Fretter, V., Graham, A., 1984. The prosobranch molluscs of Britain and Denmark. Part 8—Neogastropoda. Journal of Molluscan Studies (supp. 15).

Lawson, N.M., Mason, R.P., 1998. Accumulation of mercury in estuarine food chains. Biogeochemistry 40, 235–247.

Liang, L.N., He, B., Jiang, G.B., Chen, D.Y., Yao, Z.W., 2004. Evaluation of molluscs as biomonitors to investigate heavy metal contaminations along the Chinese Bohai Sea. Science of the Total Environment 324, 105–113.

Mason, R.P., Reinfelder, J.R., Morel, F.M.M., 1995. Bioaccumulation of mercury and methylmercury. Water, Air and Soil Pollution 80, 915–921.

McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., Green, A., 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. Environmental Toxicology and Chemistry 22, 1017–1037.

Oehlmann, J., Stroben, E., Fioroni, P., 1993. Fréquence et degré d'expression du pseudohermaphrodisme chez quelques prosobranches sténoglosses des côtes françaises (surtout de la baie de Morlaix et de la Manche). 2. Situation jusqu'au printemps de 1992. Cahiers de Biologie Marine 34, 343–362.

Parks, J.W., Lutz, A., Sutton, J.A., 1989. Water column methylmercury in the Wabigoon/English River–Lake System: factors controlling concentration, speciation, and net production. Canadian Journal of Fisheries and Aquatic Sciences 46, 2184–2202.

Pereira, M.E., Duarte, A.C., Millward, G.E., Abreu, S.N., Vale, C., 1998. An estimation of industrial mercury stored in sediments of a confined area of the Lagoon of Aveiro (Portugal). Water Science and Technology 37, 125–130.

Ramalhosa, E., Pereira, E., Vale, C., Válega, M., Duarrte, A.C., 2005a. Distribution of mercury in the upper sediments from a polluted area (Ria de Aveiro, Portugal). Marine Pollution Bulletin 50, 682–686.

Ramalhosa, E., Pereira, E., Vale, C., Válega, M., Monterroso, P., Duarrte, A.C., 2005b. Mercury distribution in Douro Estuary (Portugal). Marine Pollution Bulletin 50, 1218–1222.

Roméo, M., Frasila, C., Gnassia-Barelli, M., Damiens, G., Micu, D., Mustata, G., 2005. Biomonitoring of trace metals in the Black Sea (Romania) using mussels *Mytilus galloprovincialis*. Water Research 39, 596–604.

Saiz-Salinas, J.I., Ruiz, J.M., Frances-Zubillaga, G., 1996. Heavy metal levels in intertidal sediments and biota from the Bidasoa estuary. Marine Pollution Bulletin 32, 69–71.

Stroben, E., Oehlmann, J., Fioroni, P., 1992. The morphological expression of imposex in *Hinia reticulata* (Gastropoda: Buccinidae): a potential indicator of tributyltin pollution. Marine Biology 113, 625–636.

Tallmark, B., 1980. Population dynamics of *Nassarius reticulatus* (Gastropoda, Prosobranchia) in Gullmar Fjord, Sweden. Marine Ecology Progress Series 3, 51–62.

Tchounwou, P.B., Ayensu, W.K., Ninashvili, N., Sutton, D., 2003. Environmental exposure to mercury and its toxicopathologic implications for public health. Environmental Toxicology 18, 149–175.

Ugolini, A., Borghini, F., Calosi, P., Bazzicalupo, M., Chelazzi, G., Focardi, S., 2004. Mediterranean *Talitrus saltator* (Crustacea, Amphipoda) as a biomonitor of heavy metals contamination. Marine Pollution Bulletin 48, 526–532.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Critical Reviews in Environmental Science and Technology 31, 241–293.

Vale, C., Ferreira, A., Caetano, M., Brito, P., 2002. Elemental composition and contaminants in surface sediments of the Mondego river estuary. In: Pardal, M.A., Marques, J.C., Graça, M.A. (Eds.), Aquatic Ecology of the Mondego River Basin. Global Importance of Local Experience. Imprensa da Universidade de Coimbra, Coimbra, pp. 243–256.