Aquatic Toxicology 102 (2011) 48–57

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Pb uptake by the marine mussel *Mytilus* sp. Interactions with dissolved organic matter

Paula Sánchez-Marín[a,*], Juan Bellas[b], Valentine K. Mubiana[c], J. Ignacio Lorenzo[a,c], Ronny Blust[c], Ricardo Beiras[a,d]

[a] Laboratorio de Ecoloxía Mariña (LEM), Universidade de Vigo, Campus Universitario, Crta. Colexio Universitario s/n, 36310 Vigo, Galicia, Spain
[b] Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Apdo. 1552, 36280 Vigo, Galicia, Spain
[c] Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium
[d] Estación de Ciencias Mariñas de Toralla (ECIMAT), Universidade de Vigo, Illa de Toralla, E-36331 Vigo, Galicia, Spain

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form
10 December 2010
Accepted 21 December 2010

Keywords:
Metal bioavailability
Lead
Mussel
Humic substances
DOM
Anodic stripping voltammetry

ABSTRACT

It is well known that dissolved organic matter binds metal ions and buffers them in natural waters. Although it is believed that a decrease in metal ion concentration should lead to a decrease in metal bioavailability, previous work has shown that Pb uptake by *Mytilus edulis* gills is greatly enhanced in the presence of humic acids. In the present work, the effect of more soluble organic matter (fulvic acids and DOM extracted from river) on Pb uptake by mussels and their gills is studied. Pb complexation by these organic substances was measured by anodic stripping voltammetry (ASV) and it is proven that Pb uptake by mussel gills in the presence of fulvic acids can be successfully predicted according to ASV-labile Pb concentrations. However, Pb uptake by whole mussels in the presence of river DOM is slightly higher than predicted on the basis of ASV measurements. The possible reasons leading to different effects of DOM on Pb uptake by mussels are discussed according to physicochemical properties of DOM.

1. Introduction

Filter-feeding bivalves, such as the mussels of the genus *Mytilus*, are exposed to large volumes of water for respiratory and feeding purposes. In this process, the gills and other exchange surfaces are exposed to the metals in the environment. Mussels accumulate these metals in the soft tissues and shells, which is one of the reasons why they are frequently used in environmental pollution monitoring programs (Sericano, 2000; Widdows and Donkin, 1992). It is well documented that the total or dissolved concentration of a metal is a poor predictor of its biological effects, because the chemical speciation of metals greatly affects their availability to aquatic organisms. A strong body of evidence supports the theory that the biological response elicited by dissolved metals is generally a function of their free metal ion activity (reviewed by Cambpell, 1995). This concept is usually referred to as the free-ion activity model (FIAM) (Morel, 1983). The FIAM considers the transport channels or surface-binding sites as ligands. If chemical equilibrium exists between metal ions and ligands in solution and the ligands on the cellular surfaces, it is expected that for a given situation, the bioavailability of the metal is directly related to the free-metal-ion activity under certain conditions (e.g., constant competing cations concentration) (Cambpell, 1995; Campbell et al., 2002).

Seawater is a buffered medium, with relatively small variations in pH, ionic strength, or ion composition. The inorganic speciation of metals can be determined rather accurately and remains almost constant in marine environments. However, organic complexation can vary considerably, and its effect on trace metal bioavailability has to be measured or modeled for each specific situation. Within the pool of dissolved organic matter (DOM) present in the marine environment, humic substances account for 5–25% in surface oceans (Benner, 2002). Humic substances have a relative high affinity for metal ions, especially the group of metals that naturally occur as carbonate and hydroxide complexes, such as Cu and Pb (Morgan and Stumm, 1991; Turner et al., 1981).

There is conflicting evidence regarding the role of DOM on the availability of dissolved metals to filter-feeding bivalves. Several studies have reported that humic substances enhanced Cd uptake by *Mytilus edulis* (George and Coombs, 1977; Kozuch and Pempkowiak, 1996; Pempkowiak et al., 1989). Cu uptake by whole mussels was higher than expected on the basis of free Cu, presumably due to the ingestion of metal–humic aggregates (Lorenzo et al., 2005). Using excised mussel gills, Lorenzo et al. (2005) tested Cu bioavailability to mussels excluding the digestive route of uptake, and showed that Cu uptake in the presence of humic acids (HA) followed the free ion concentration. In contrast, we have previously

* Corresponding author. Tel.: +34 986814087; fax: +34 986812556.
E-mail address: paulasanchez@uvigo.es (P. Sánchez-Marín).

0166-445X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2010.12.012

shown that Pb uptake by excised mussel gills was greatly enhanced in the presence of Aldrich HA (Sánchez-Marín et al., 2007). The reasons underlying the different effects that HA exert on Cu and Pb remain unknown, but it is thought that adsorption of HA to biological surfaces is involved and affects the uptake process in the case of Pb (Sánchez-Marín et al., 2007). The amphiphilic character of the humic acid molecules confers them surfactant properties and favours their aggregation in solution (Wershaw, 1993) and their accumulation on abiotic (Tipping, 1981) and biotic (Campbell et al., 1997) surfaces, which can modify membrane properties and affect metal uptake (Galvez et al., 2008; Glover and Wood, 2005; Knauer and Buffle, 2001; Lamelas et al., 2005; Myers et al., 1975; Parent et al., 1996; Slaveykova et al., 2003; Vigneault et al., 2000). In the present study we aim at testing the effect of other organic compounds (fulvic acids and natural organic matter extracted from river) that are more soluble than humic acids, on the bioavailability of Pb to the common mussel *Mytilus* sp.

In order to test if metal bioavailability follows variations in metal speciation according to complexation by DOM, speciation should be accurately measured or calculated with reliability. Among the analytical techniques available to determine the ambient chemical speciation of dissolved metals, electrochemical methods, as anodic stripping voltammetry (ASV), are frequently used because of its intrinsic capability in separating ionic and labile metal species from inert complexes (Florence, 1986). A fundamental problem underlying efforts to characterize metal complexation in the presence of natural DOM is the heterogeneity of the ligands. Several phenomena, such as the adsorption of DOM on the electrode, or the potential lability or semi-lability of the metal–DOM complexes, can occur in ASV measurements (Buffle et al., 1987; Buffle, 1990; Mota and Correia dos Santos, 1995). Despite these potential interferences, the effects of which should be evaluated, ASV has proven to be a useful tool to determine Cu bioavailability in seawater (Brooks et al., 2007; Lorenzo et al., 2002, 2006) and it has been equally useful for its application to Pb speciation measurements (Cobelo-García and Prego, 2004; Kozelka and Bruland, 1998; Sánchez-Marín et al., 2010a,b).

The aim of the present work is to study the influence of two types of dissolved organic matter, fulvic acids and river DOM, on the uptake of Pb by the mussel *M. edulis* or *Mytilus galloprovincialis* and their gills, using ASV to measure Pb speciation and test if Pb bioavailability behaves as it would be predicted according to FIAM. The results will be compared with those previously obtained with humic acids, more hydrophobic substances than fulvic acids and river DOM and which greatly enhanced Pb uptake by mussel gills.

2. Materials and methods

2.1. Reagents

All experimental solutions were prepared in chemically defined artificial seawater (ASW) according to the formulation described in Lorenzo et al. (2002). Distilled water purified by ion exchange (resistivity ≥ 18.2 MΩ/cm; MilliQ®; Millipore, Mosheim, France) and analytical grade salts were used. The seawater was aerated with 0.22 μm filtered air overnight to establish CO_2 equilibrium with the atmosphere. Before each experiment, pH and salinity were checked and found to be 8.14 ± 0.05 and 35 ± 0.5‰ respectively.

A 1.000 g Pb L^{-1} standard solution for spectrometry ($Pb(NO_3)_2$ in 0.5 N HNO_3; Panreac Química SA; Barcelona, Spain) was used for preparing Pb^{2+} dilutions. Fulvic acids (FA) were obtained from Fredriks research products (Amsterdam, Netherlands) and Suwannee River NOM (SRDOM) was purchased from the International Humic Substances Society (IHSS). Concentrated stocks of 1 g l^{-1} were prepared by dissolving FA or SRDOM in 7×10^{-3} M NaOH, and stock solutions were immersed for 30 min in an ultrasonic bath to dissolve DOM aggregates. FA and SRDOM solutions were stored at 4 °C in the dark to prevent photochemical ageing. DOC content of the stock solutions was analysed using a total carbon analyser (Shimadzu TOC-5000, model ASI-5000-S, Japan) and was found to be 0.34 and 0.38 g l^{-1} for FA and SRDOM respectively.

Lead and DOM solutions in ASW were allowed to equilibrate for 24 h before the complexation measurements or the uptake experiments. It was verified that neither HA nor lead additions altered the pH of the solutions.

All plastic labware was soaked for 24 h in 5% HNO_3 and rinsed several times with ultrapure water before use.

2.2. Pb complexation measurements

A series of increasing quantities of FA or SRDOM (13 additions ranging from 1 to 60 mg l^{-1}) were added to different sets of total Pb concentrations (0.25, 0.5, 1 and 2 μM) in ASW. Solutions were prepared in 20 ml polystyrene vials.

Electrochemically labile lead (Pb′) was measured by square wave anodic stripping voltammetry (SWASV) with a hanging mercury drop electrode, a Ag/AgCl reference electrode and a Pt-rod auxiliary electrode held in a Metrohm 663 VA polarographic stand coupled to an Eco-Chemie AutoLab PGSTAT10 pontenciostat (Metrohm; Herisau, Switzerland). Measurements were performed in a Teflon cell thermostated at 20 °C.

The cell was pre-conditioned with 10 ml of each sample for 5 min, then the solution was discarded and the remaining 10 ml of the sample were measured. After 250 s of purge with N_2, a deposition potential of −0.65 V was applied and Pb was accumulated on a mercury drop of 0.52 mm^2. Deposition time varied depending on Pb concentration (30 s for solutions with less than 1 μM of Pb and 10 s for higher Pb concentrations). Solutions were stirred at 3000 rpm during Pb accumulation and an equilibration time of 5 s was let before the voltage scan. For the voltage scanning (from −0.65 to −0.25 V) a square-wave with an amplitude of 25 mV, a frequency of 25 Hz and a scan increment of 2 mV was applied. Three voltammograms were recorded for each solution and labile Pb concentrations ([Pb′]) were calculated as $[Pb'] = i_p/S$; where i_p is the arithmetic mean of the peak current of the three voltammograms and S is the slope of the calibration curve.

Complexation curves were adjusted to a simple complexation model previously described (Lorenzo et al., 2002). Assuming one type of ligand and a stoichiometry of 1:1, a simple complexation model is obtained from the equation that describe the equilibrium and the mass balance equations,

$$[Pb'] = \frac{-a + \sqrt{a^2 + 4[Pb_T]/K'}}{2} \quad (1)$$

where $a = (-[Pb_T] + L + 1/K')$ and $L = [DOM] \times N$; $[Pb_T]$ is the total concentration of dissolved Pb; K' is the conditional stability constant and N (μmol Pb/g DOM) expresses the number of Pb binding sites present per gram of DOM.

In order to study the lability of Pb-DOM complexes, a series of samples with different [DOM] at a constant $[Pb_T]$ were measured at varying stirring speeds, from 1000 to 3000 rpm.

Additionally, to detect indications of lability or electroactivity of the complexes, pseudo-polarograms were constructed by measuring the Pb′ accumulated on the mercury drop at different deposition potentials. Four solutions with 1 μM of Pb and different FA or SRDOM additions were chosen for the pseudo-polarograms. For each of them, 30 voltammograms were recorded at 30 different deposition potentials (varied in small steps from −0.25 to −1 V).

2.3. Mussel collection and maintenance

M. galloprovincialis between 50 and 60 mm length collected at Canido (Ría de Vigo) were used for the whole mussel experiments and *M. edulis* collected from an intertidal site at Westkapelle (The Netherlands) were used for the mussel gills experiments.

In both cases, mussels were transported to the laboratory on ice in a cooling box. They were cleaned from epibionts and transferred to an acclimatization tank filled with artificial seawater or natural seawater of 35‰ at 15 °C. The mussels were fed with microalgae cultures until 2 days before the experiment.

2.4. Whole mussel experiments

Three experiments were carried out with whole mussels. Firstly, the effect of exposure time on Pb uptake by whole mussels was studied over a 92-h exposure period followed by 96-h of depuration. Secondly, the effect of exposure time on organ-specific Pb uptake by gills and rest of tissues was studied over a 24-h exposure period; Thirdly, the effect of SRDOM on Pb uptake was studied by exposing mussels for a fixed period of time (24 h) to different concentrations of Pb in the absence or presence of SRDOM.

To study the effect of exposure time on Pb uptake, 60 mussels were exposed to a fixed Pb concentration (0.34 μM) in 90 l aquariums made of poly(methyl-methacrylate). Samples of five individuals were taken after 0, 6, 12, 24, 48, 72 and 96 h of exposure, and after 24, 48, 72 and 96 h of depuration. Exposure was made in dark conditions and at constant temperature (15 °C). Exposure solutions were continuously aerated by 0.22-μm filtered air. Water was renewed once after the first 24 h of exposure and water samples were taken concurrently with mussels to monitor metal concentrations in the exposure solution. After exposure, the mussels were placed in clean artificial seawater for 10 min and washed with ultrapure water to remove the exposure solution from the mussel cavity and the weakly adsorbed metal. Subsequently, the soft tissues were transferred into 30-ml polypropylene tubes and dried for 72 h at 65 °C.

In the second experiment, mussels were exposed to 0.11 μM of Pb, and seven individuals were taken after 0, 1, 3, 6, 12 and 24 h. Experimental conditions and protocol were the same as described for the first experiment, except that in the second experiment the gills were dissected and transferred into 4-ml polypropylene vials to be analysed separately.

For the third experiment, experimental solutions were prepared in 15 l polypropylene aquariums, and 7 mussels were exposed in each aquarium for 24 h at 15 °C. Total lead concentrations in exposure solutions ranged from 0.1 to 0.5 μM, and [SRDOM] from 2.5 to 20 mg l^{-1}. The procedure was similar to the one explained for the exposure time experiments. Gills and rest of the tissues were analysed separately. Water samples were taken at the beginning and at the end of exposure for metal determinations and at several exposure times to control of changes in DOC concentrations.

2.5. Mussel gill experiments

The effect of FA on Pb uptake by excised mussel gills was studied over a 1-h exposure period. Experimental solutions for the mussel gill experiment were prepared in 2 l polypropylene bottles and distributed in six polypropylene containers (replicates) of 300 ml. The use of this volume of water assures that dissolved Pb concentrations after the exposure period are >96% of the initial [Pb] in experimental solution for the exposure time and metal concentrations used. Total lead concentrations in exposure solutions ranged from 0.1 to 0.4 μM, and [FA] ranged from 2.5 to 20 mg l^{-1}. Exposure solutions were left to equilibrate 24 h before gill exposure. Gills were cut from the mantle and incubated for an hour at 15 °C in the test solutions. After incubation, gills were washed twice for 15 s in ASW and once for 5 s in ultrapure water and then transferred into 4-ml polypropylene vials. Gill dry weight was determined after drying for 48 h at 65 °C. Water samples were taken before exposure to check metal concentrations.

2.6. Chemical analysis

Dried samples of mussel tissue were digested with a mixture of ultrapure 69% HNO_3 and 30% H_2O_2 following a microwave assisted procedure described in Sánchez-Marín and Beiras (2008). Blanks and reference material (Mussel tissue; CRM 278R; European Community Bureau of Reference Materials, Geel, Belgium) were also prepared and digested together with the samples. Pb concentration was measured by inductively coupled plasma atomic-emission spectroscopy (ICP-AES) using a Varian Liberty Series II ICP-AES (Varian, Mulgrave, Victoria, Australia). Samples with [Pb] below 20 μg l^{-1} were measured by inductively coupled plasma mass spectrometry using an X Series ICP-MS (Thermo Elemental, Cheshire, UK). Lead concentrations in gills were expressed on a dry-weight basis (μmol kg^{-1} dw). Measured Pb concentration in the reference material agreed well with the certified concentration, within ±6%.

Cu, Pb, Zn and Cd total concentrations in the water were measured by ASV after acidification of the sample to pH = 2 with HNO_3. [Pb′] was analysed by ASV as explained for the complexation measurements.

DOC samples were collected into 10 ml precombusted (450 °C, 12 h) glass ampoules. After acidification with H_3PO_4 to pH < 2, the ampoules were heat-sealed and stored in the dark at 4 °C until analysis. DOC was measured with a Shimadzu TOC V-CPH organic carbon analyser (Álvarez-Salgado and Miller, 1998).

2.7. Data treatment and statistics

Least squares regression analyses were performed by using GraphPad Prism version 4.00 for Windows (GraphPad software). Uptake rates obtained in different models were compared using the extra sum-of-squares *F* test by means of global fitting (Motulsky and Christopoulos, 2003). Goodness of fit of the models was reported by the coefficient of determination (R^2).

3. Results and discussion

3.1. Quality control of experimental solutions

Total metal concentrations in the ASW were very low compared to the lead concentrations used in the experiments: [Zn] < 2 μg l^{-1} (0.03 μM), [Pb] and [Cu] < 1 μg l^{-1} (0.005 and 0.016 μM respectively) and Cd < 0.5 μg l^{-1} (4 nM). The pH was checked during the exposure period and it was verified that it did not depart from 7.9 to 8.2 values. Measured DOC concentrations in exposure solutions agreed well with nominal SRDOM concentrations.

3.2. Pb complexation by FA and SRDOM

Most speciation studies with ASV techniques consider that metal–DOM complexes are inert, and only the free metal ion and its inorganic complexes are reduced on the electrode surface (Srna et al., 1980; Coale and Bruland, 1988; Kozelka et al., 1997; Kozelka and Bruland, 1998). However, it has been suggested that Pb complexes with DOM may be kinetically labile (Buffle, 1990; Greter et al., 1979; Mota and Correia dos Santos, 1995). Kinetically labile complexes would contribute to the measured signal depending on the time they remain in the diffusion layer surrounding the electrode. To minimize the contribution of kinetically labile metal–DOM complexes to the measured signal, stirring rates

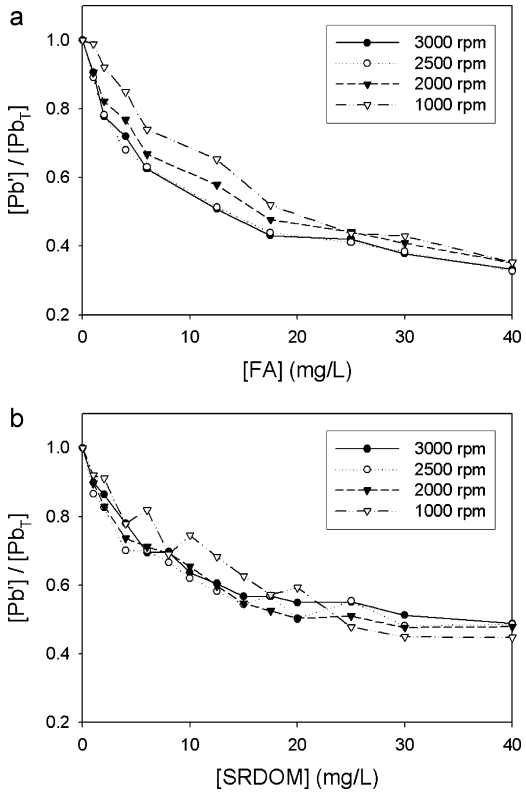

Fig. 1. Measured ASV-labile lead (Pb′) as a function of FA (a) or SRDOM (b) concentrations. Stirring speeds applied during deposition were varied from 1000 to 3000 rpm. Single measurements are represented.

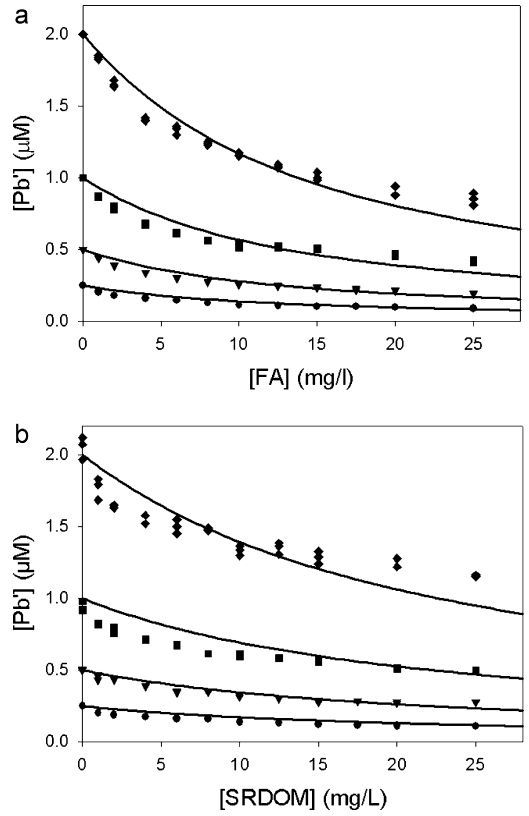

Fig. 2. Concentration of labile Pb, measured by ASV, in four series of solutions with 0.2 (●), 0.5 (▼), 1 (■) and 2 (♦) μM of Pb at increasing FA (a) or SRDOM (b) concentrations. Solid lines show the fit of the complexation model according to Eq. (1). Replicate measurements ($n = 3$) are represented.

are normally fixed to the maximum possible, therefore reducing diffusion layer thickness to a minimum (Capodaglio et al., 1995). To detect possible kinetic lability of the Pb–DOM complexes, Pb complexation with different concentrations of FA and SRDOM was measured at different stirring rates (Fig. 1). For FA (Fig. 1a), results showed that at the maximum stirring speed (3000 rpm) and at 2500 rpm, complexation curves are exactly the same, while at lower stirring speeds the fraction of Pb detected is slightly higher at [FA] < 20 mg l^{-1}, but remains unaltered with stirring speed at higher [FA]. Similar results were observed with SRDOM (Fig. 1b). It is therefore possible that FA–Pb and SRDOM–Pb complexes are labile and dissociate to a certain extent in the electrode diffusion layer at low stirring rates (<2000 rpm). However, contribution of Pb from kinetic dissociation is very small, and no significant differences were observed in the apparent conditional stability constants calculated according to Eq. (1) for the different rotation speeds. Similarly, Capodaglio et al. (1990) showed that Pb–organic ligand complexes from natural seawater samples were kinetically inert at rotation speeds of 4000, 3000 and 2000 rpm (rotating disk electrode). In the present work, to minimize the potential kinetic contribution of Pb–DOM complexes, the highest rotation speed of 3000 rpm was used for the complexation measurements.

Additionally, plots of peak intensity as a function of deposition potential (pseudo-polarograms) can be used to detect electroactivity or lability of complexes (Capodaglio et al., 1995; Town and Filella, 2000). Pseudo-polarograms showed a smooth sigmoidal shape both in the presence and absence of FA or SRDOM, not showing evidence of direct reduction of complexes, and the half-wave potential ($E_{1/2}$) was maintained at constant values with increasing ligand additions, which also indicates inert behaviour of complexes (Town and Filella, 2000).

Fig. 2 shows the ASV-[Pb′] measured at different total Pb concentrations in the presence of increasing FA or SRDOM concentrations. There is a clear decrease in [Pb′] with DOM additions. Data were fitted to Eq. (1), and complexation parameters ($\log K'$ and N) were obtained. The parameters N and $\log K'$ present a high correlation coefficient, meaning that their values are dependent, and similar fittings were obtained with higher $\log K'$ values together with lower N values. Based on the complexation of Cu and FA (Lorenzo et al., 2006), N was constrained to be 610 for comparison purposes. The parameters thus estimated with the standard errors from the fittings were: $\log K'_{\mathrm{PbFA'}} = 5.13 \pm 0.23$ and $N = 610 \pm 288$ μmol Pb/g FA. The model explains the 98.6% of the observed variation.

In the case of SRDOM, best fitting parameters values were: $\log K'_{\mathrm{PbSRDOM'}} = 4.55 \pm 1.12$ and $N = 1.3 \pm 2.6$ mmol Pb/g SRDOM. The model explains the 97.6% of the observed variation.

Both substances showed binding abilities for Pb in seawater, although higher complexation was observed in the presence of FA than for similar amounts of SRDOM. For instance, at 10 mg l^{-1} of FA, complexed Pb is around 45% of [Pb$_T$] while 10 mg l^{-1} of SRDOM bind 30% of [Pb$_T$].

3.3. Pb uptake by mussels and their gills

3.3.1. Pb uptake and depuration kinetics

Uptake of Pb by whole mussels as function of time was evaluated in two different experiments. In the first one, mussels were exposed to 0.38 μM of Pb and uptake was followed over a 96-h period followed by depuration another 96-h in clean seawater. In the second one, mussels were exposed to 0.11 μM of Pb during a 24-h period and Pb content in gills and rest of tissues were analysed independently. Analysis of water samples revealed that dissolved

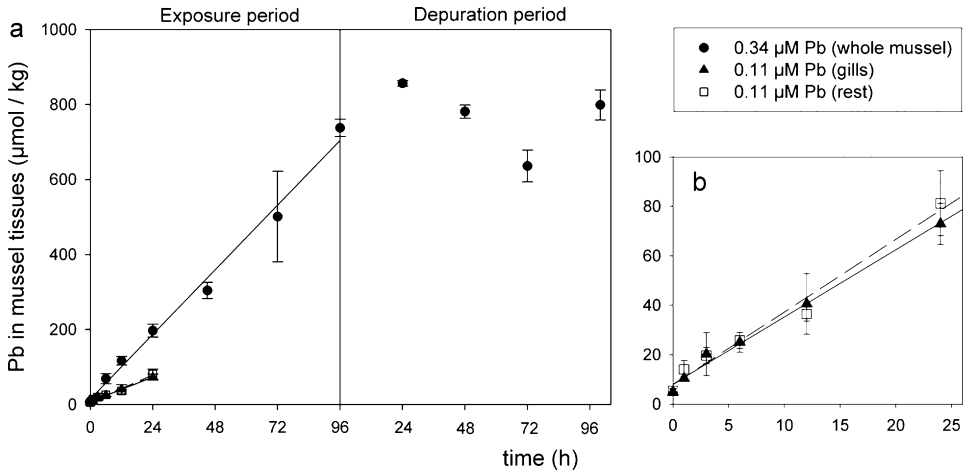

Fig. 3. Pb content in mussels (●), gills (▲), and rest of the tissues (□) as a function of exposure time. Mussels were exposed to 0.34 μM (●) or 0.11 μM of waterborne Pb (▲, □). Depuration period in clean seawater was left after the 96 exposure to 0.34 μM of Pb. (a) shows the whole data set and (b) is a detail of the first 24 h showing Pb uptake in gills and rest of tissues separately. Mean ± standard deviation (n = 5–7) are represented.

Pb concentrations were maintained above 80% of the initial concentrations during all the exposure period.

Pb uptake was linear with time and no saturation was observed for the 96-h exposure (Fig. 3). Pb was not eliminated from the tissues after 96 h of depuration in clean seawater. Separate analysis of gills and rest of tissues revealed that Pb concentrations were the same in the both types of tissues, even at the shortest exposure time (1 h).

Linear uptake of Pb with time has been reported to continue even at longer exposure times (40 days) (Schulz-Baldes, 1974). Although Pb elimination was not observed in our experiments (96-h depuration), it has been shown that mussels can eliminate Pb from the tissues, but at very low rates (Schulz-Baldes, 1974). In that work, significant decrease of Pb concentration in mussel tissues was only observed after 20 days of depuration in clean seawater. Mussels transplanted from a polluted site to a clean one revealed that Pb depuration is not complete, and about a 50% of the Pb originally present in the mussel cannot be excreted even after 5 years of depuration (Riget et al., 1997).

3.3.2. *Pb uptake as a function of Pb dissolved concentrations*

Whole mussels were exposed to different Pb concentrations during 24 h and gills and the rest of the tissues were analysed separately. As observed in the kinetic experiments where uptake of Pb was followed in time (Fig. 3), Pb concentrations in gills and in the rest of the tissues were similar (Fig. 4) for all concentrations tested. Therefore, only Pb concentration in the gills will be presented for the whole mussels experiments.

Pb uptake was linear with Pb concentration in water (Fig. 5, black dots) within the range of Pb concentrations tested (0–0.5 μM of Pb). Uptake data was fitted to the following equation:

$$Pb_{gills} = Pb_0 + k_u \cdot [Pb] \cdot t \quad (2)$$

where Pb_{gills} is the concentration in the gills in $\mu mol\,kg^{-1}$, expressed on a dry weight basis; Pb_0 is the initial Pb concentration in the gills; [Pb] is the Pb concentration in the water expressed in $\mu mol\,L^{-1}$ and t is the exposure time in hours. The uptake rate (k_u) obtained from the fitting was 8.93 (±0.28) $l\,h^{-1}\,kg^{-1}$.

Short term experiments using excised gills also showed a linear relationship of Pb uptake with exposure concentrations (Fig. 6, black dots). Fitting of uptake data to Eq. (2) gave an uptake rate constant of 102 ± 4 $l\,h^{-1}\,kg^{-1}$, which is about 10 times higher than the uptake rate observed in gills of exposed whole mussels.

The observed difference in uptake rate can be explained according to the size of the organism or tissue that accumulates Pb in relation with the uptake and elimination flux of the metal. Gills are known to be the main gate for uptake of dissolved metals in mussels. In a whole mussel, Pb is taken up by the gills, but rapidly redistributed to the rest of the organs, as observed by the equal Pb concentrations achieved in gills and the rest of tissues for all exposure times and Pb concentrations tested (Fig. 4). For the excised gills, however, the Pb taken up by the gills remains in the gills; as a result, concentration in isolated gills is higher than in gills of exposed mussels. If we convert uptake rates (normalized per dry weight) to net uptake rates, by multiplying k_u by the size of the organism or tissue (0.34 g dw for a whole mussel and 0.038 g dw for a excised gill), we obtain that net uptake rates for the gills of exposed whole mussels and for excised gills are respectively 3.04 ± 0.10 and 3.88 ± 0.15 $ml\,h^{-1}$, which is a close match given the differences in exposure conditions and mussels origin.

3.4. *Pb uptake by excised mussel gills in the presence of FA*

The use of excised gills in metal uptake experiments provides a well-defined model system to study the uptake of metals by the gill epithelium. Pb uptake by excised mussel gills decreased in the presence of FA (Fig. 6a, white dots) and the decrease was higher

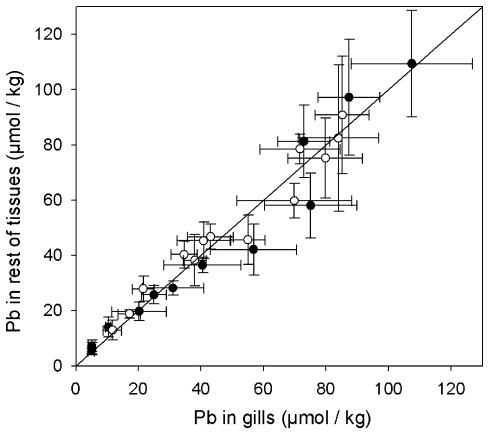

Fig. 4. Comparison of Pb uptake by the gills and the rest of mussel tissues after whole mussel exposure to different concentrations Pb (black dots) or Pb + SRDOM (white dots) at different exposure times (from 1 to 24 h) and different dissolved Pb concentrations (from 0.1 to 0.5 μM). Straight line represents a 1:1 relationship.

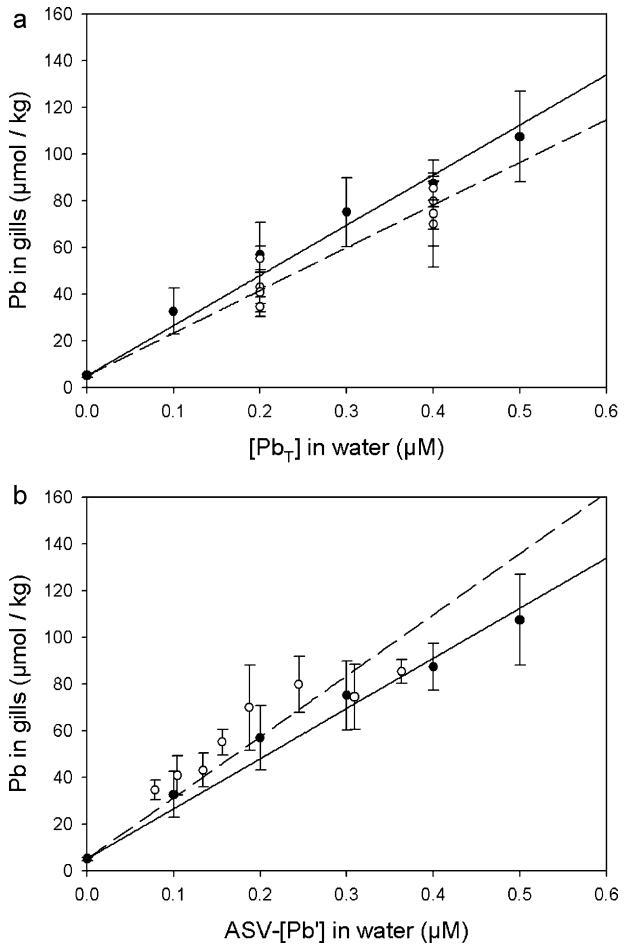

Fig. 5. Pb concentration in gills after 24-h whole mussel exposure to Pb in the absence (●) or presence (○) of different concentrations of SRDOM (from 2.5 to 20 mg l^{-1}) as a function of total (a) or labile (b) Pb concentration in water. Means ± standard deviation (n = 7) are represented. Solid and dashed line represent fitted uptake model considering the exposure in the absence and presence of SRDOM respectively.

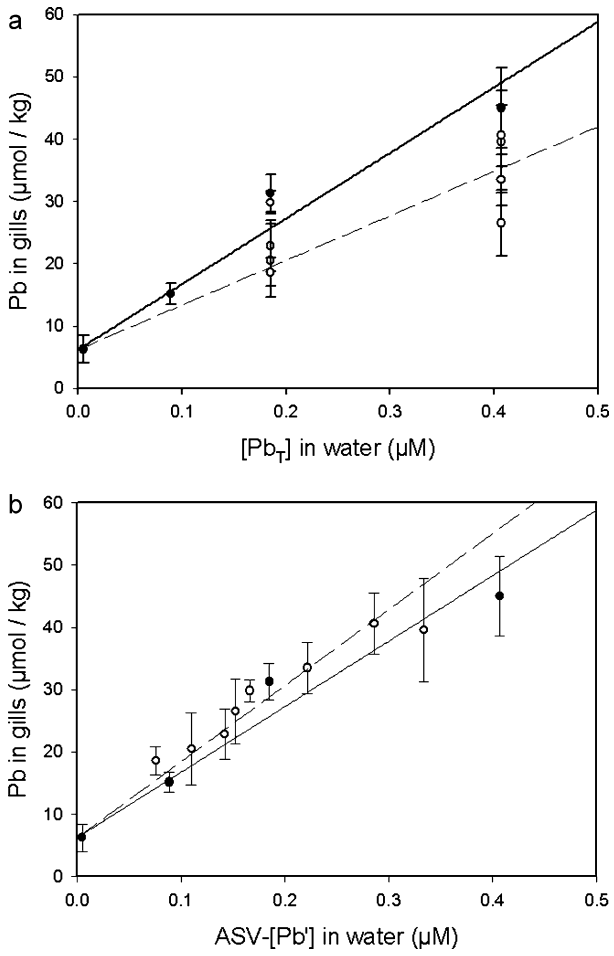

Fig. 6. Pb concentration in excised gills after 1-h exposure to Pb in the absence (●) or presence (○) of different concentrations of FA (from 2.5 to 20 mg l^{-1}) as a function of total (a) or labile (b) Pb concentration in water. Means ± standard deviation (n = 6) are represented. Solid and dashed line represent fitted uptake model considering the exposure in the absence and presence of FA respectively.

at higher FA concentrations (Fig. 7a). Regression analysis showed that, at constant [Pb$_T$], [FA] had a significant protecting effect on Pb$_{gills}$, with slopes of -0.63 ± 0.10 ($p < 0.001$) at 0.2 µM of Pb$_T$ and -0.89 ± 0.14 ($p < 0.001$) at 0.4 µM of Pb$_T$. The linear uptake model (Eq. (2)) was fitted to the uptake data obtained from the exposure to Pb and FA (white dots and dashed line, Fig. 6a) and the k_u obtained (74 ± 9 l h^{-1} kg^{-1}) was significantly lower ($p < 0.001$) than the k_u obtained in the Pb only exposure (102 ± 6 l h^{-1} kg^{-1}; Table 1).

To quantify if these protecting effect was related to the decrease in [Pb′] caused by Pb complexation with FA, Pb uptake was plotted versus ASV-[Pb′] (Fig. 6b). No differences were found between the obtained uptake rate (113 ± 41 l h^{-1} kg^{-1}) and the uptake rate obtained in the Pb only exposure (102 ± 41 l h^{-1} kg^{-1}), and therefore it can be concluded that ASV-Pb′ measurements can adequately predict Pb uptake by mussel gills in the presence of FA, in agreement with the free ion model.

3.5. Pb uptake by mussels in the presence of SRDOM

DOC concentrations in the ASW were around 0.2 mg l^{-1} and did not change during the 24-h exposure period. Also monitored DOC concentrations in the SRDOM exposure water did not change during the 24-h exposure period.

Table 1
Fitted parameters ± standard error of regression analysis to the excised gills or mussel gills uptake data according to the model shown.

	Model	Excised gills			Whole mussel gills		
		k_u (l h^{-1} kg^{-1})[a]	c	r^2, df	k_u (l h^{-1} kg^{-1})[b]	c	r^2, df
Only Pb	$Pb_{gills} = Pb_0 + k_u$ [Pb] t	102 ± 6	–	0.908, 30	8.93 ± 0.53	–	0.865, 39
Pb + DOM[c]	$Pb_{gills} = Pb_0 + k_u$ [Pb$_T$] t	$74 \pm 9^{***}$	–	0.379, 55	$7.50 \pm 0.70^{***}$	–	0.676, 50
Pb + DOM[c]	$Pb_{gills} = Pb_0 + k_u$ [Pb′] t	113 ± 11^{ns}	–	0.532, 55	$10.95 \pm 0.95^{***}$	–	0.480, 50
Pb + DOM[c]	$Pb_{gills} = Pb_0 + k_u$ [Pb′] $t + c$·[PbDOM[c]]·t	102	21 ± 15^{ns}	0.540, 55	8.93	$4.95 \pm 1.22^{***}$	0.712, 50

Comparison of parameters (k_u and c) obtained in the different models with those of the Pb only exposure (0 in the case of c) is indicated by: ***(significantly different at the $p < 0.001$ level) and ^{ns}(not significantly different at the $p = 0.05$ level).

[a] Uptake rate corresponding to 1-h exposure of excised mussel gills to Pb or Pb + FA.
[b] Uptake rate corresponding to 24-h exposure of whole mussels to Pb or Pb + SRDOM.
[c] DOM represent either FA or SRDOM.

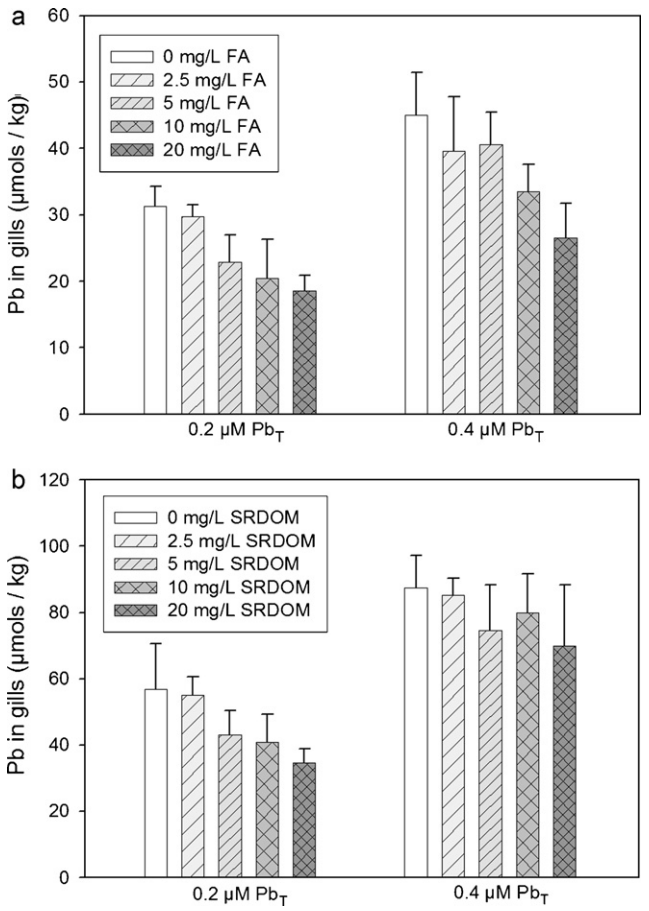

Fig. 7. Pb concentrations in gills exposed for 1 h to different Pb_T concentrations with increasing additions of FA (a) or in gills of mussels exposed for 24-h to different Pb_T concentrations with increasing additions of SRDOM (b). Error bars represent standard deviations ($n = 6–7$).

Pb uptake in the presence of SRDOM was lower than in its absence for constant dissolved lead concentrations in exposure solutions (Fig. 5a), and the decrease in uptake was proportional to the increase in SRDOM concentrations (Fig. 7b). Regression analysis showed that, at constant [Pb_T], [SRDOM] had a significant protecting effect on Pb_{gills}, with slopes of -1.07 ± 0.22 ($p < 0.001$) at 0.2 µM of Pb_T and -0.76 ± 0.31 ($p = 0.02$) at 0.4 µM of Pb_T. Pb uptake rate in the presence of SRDOM calculated according to [Pb_T] was $7.5 \pm 0.7\ l\ h^{-1}\ kg^{-1}$, significantly lower ($p < 0.001$) than the uptake rate obtained in the Pb only exposure, i.e. $8.93 \pm 0.53\ l\ h^{-1}\ kg^{-1}$ (Table 1 and Fig. 5a).

To test if Pb uptake by mussel gills could be explained on the basis of [Pb′], uptake data was fitted to the linear uptake model as a function of [Pb′] (Fig. 5b and Table 1). The uptake in the presence of SRDOM was slightly higher than predicted from the ASV measurements. Uptake rate resulting from the linear equation according to [Pb′] was $10.95 \pm 0.95\ l\ h^{-1}\ kg^{-1}$, a value significantly higher ($p < 0.001$) than the uptake rate obtained from the Pb only exposure. Therefore, ASV measurements of [Pb′] in the presence of SRDOM slightly underestimate Pb bioavailability for the mussels. The concentration of Pb in the form of Pb–SRDOM complexes in the bulk solution was calculated as the difference between total Pb and labile Pb, given that $Pb_T = Pb' + Pb–SRDOM$. Considering that both Pb′ and Pb–SRDOM would contribute to Pb uptake, contribution of Pb–SRDOM was estimated from the multiple regression analysis to be $4.95 \pm 1.22\ \mu mol\ kg^{-1}/\mu mol\ L^{-1}$ and per h (Table 1), around half the contribution of Pb′.

It is clear that SRDOM decreases Pb uptake, even though less than predicted from ASV-measurements, while in the presence of FA the decrease in Pb uptake was accurately predicted by the ASV measurements of [Pb′]. Within this context we should consider the possibility of underestimation of Pb′ by ASV measurements in the case of SRDOM. As previously discussed, uncertainties in ASV measurements can occur due to DOM adsorption on the electrode or to the partial lability of complexes. Based on the complexation measurements at different stirring speeds, it was concluded that the possibility of labile Pb–SRDOM complexes contributing to the measured signal is improbable under the applied measurement conditions. Similarly, pseudo-polarograms have not shown the typical double curvature observed in case of electroactive complexes (Town and Filella, 2000). Nevertheless, in case these phenomena would affect the results of speciation measurements, the effect would be the opposite, leading to an overestimation of [Pb′], by either measuring labile or electroactive complexes, and could never explain the apparent underestimation of Pb bioavailability based on ASV-Pb′ measurements.

The slightly different effect of both types of DOM can be due either to the organic matter type or to the different models used for the exposure, i.e. excised gills or whole mussels. As occurred for Cu–HA complexes (Lorenzo et al., 2005), whole mussels could be able to take up colloidal forms of Pb–SRDOM complexes via the digestive system. The digestive route of uptake for mussels may be important for uptake of colloidal or particulate metal (Guo et al., 2001; Lorenzo et al., 2005; Mubiana, 2006). Lorenzo et al. (2005) observed production of faecal materials in tanks with starved mussels after exposure to HA, indicating that mussels are able to filtrate and ingest particulate forms of HA formed due to the aggregative behaviour of HA in seawater. Similarly, Mubiana (2006) monitored HA concentration with time during exposure of mussels to HA and metals, and observed that [HA] decreased about a 20% after 24-h exposure. Additionally, more faecal materials were produced in the experimental tanks with HA. In the present work, a significant decrease was not observed in DOC concentrations in the water measured at different times during the 24-h exposure period and faeces production was not observed in the tanks with mussels exposed to SRDOM. Humic acids are not truly dissolved species but, rather, suspensions susceptible to forming colloidal particles with aggregation properties. FA and SRDOM showed a much lower tendency to aggregate in seawater, as compared with HA, as demonstrated by centrifugation of solutions of these substances in artificial seawater. After centrifugation at 4000 rpm, a precipitated pellet was observed in the case of HA, while this pellet was absent in the case of SRDOM or FA solutions (unpublished data). Therefore, the contribution of the digestive route of uptake does not seem to be so important for these substances. On the other hand, it could be that mussels may be taking directly dissolved organic matter from the water, not in a particulate/ingested form, but as direct absorption of dissolved DOM, as has been shown for the freshwater mussel *Dreissena polymorpha* (Roditi et al., 2000; Voets et al., 2004); and that the quantity of absorbed DOM is not high enough to be detected by changes in DOC in exposure water.

If we assume that whole mussels take up Pb by the gill surfaces in a dissolved form, and that the ingestion of Pb–DOM complexes is not involved, the slightly enhanced Pb uptake in the presence of SRDOM should be due to the different properties of these substances compared to fulvic acids. Different possibilities were evaluated.

One possibility considers the possible lability of the Pb–SRDOM complexes at the time scale of the uptake process. If Pb uptake fluxes are higher than the flux of Pb′ to the gill surface, Pb–SRDOM complexes may dissociate within the diffusion layer surrounding the gill surface. The limiting diffusional flux of Pb to the gill surface of the mussel was calculated according to the following equation

(Jansen et al., 2002):

$$J_{dif} = \frac{D_M \cdot (c_M - c_0)}{\delta_M} \quad (3)$$

where D_M is the diffusion coefficient of Pb (8×10^{-6} cm^2 s^{-1} (Kariuki and Dewald, 1996)); c_M is the bulk metal concentration; c_0 is the metal concentration at the cell surface (0); and δ is the diffusion layer thickness (estimated to be ~20 μm (Jansen et al., 2002)). For the lowest [Pb′], 0.08 μM, $J_{dif} = 3.2 \times 10^{-9}$ mol m^{-2} s^{-1}. The biouptake flux at 0.08 μM, J_u, calculated from the uptake model (Table 1) and assuming that the total gill surface area for a mussel of 55 mm length is 38 cm^2 (Jones et al., 1992), is 1.8×10^{-11} mol m^{-2} s^{-1}. Therefore J_{dif} is two orders of magnitude higher than J_u ($J_{dif}/J_u = 181 \gg 1$) and therefore diffusion limited uptake is not probable under the present conditions. Moreover considering that J_{dif} was calculated for a static system, which is not the case for a filtering mussel.

Another explanation would be related to DOM adsorption to biological surfaces. Dissolved organic matter has been shown to adsorb to the surface of living cells (Campbell et al., 1997; Knauer and Buffle, 2001; Slaveykova et al., 2003). DOM adsorbed to cell surfaces can modify cell membrane characteristics, as surface charge (Galvez et al., 2008; Knauer and Buffle, 2001; Lamelas et al., 2005; Myers et al., 1975; Slaveykova et al., 2003) and membrane permeability (Parent et al., 1996; Vigneault et al., 2000). DOM adsorbed to cell surfaces has been shown to enhance sodium uptake by *Daphnia magna* (Glover and Wood, 2005) and to enhance Pb bioavailability to freshwater microalgae (Knauer and Buffle, 2001; Slaveykova et al., 2003). HA adsorption to surfaces has been considered to explain the observed increase in Pb uptake and toxicity to marine invertebrates in the presence of these substances (Sánchez-Marín et al., 2007). However, it remains unclear how this effects would affect Pb bioavailability but not that of Cu, which has been shown to depend on [Cu′] in the presence of HA (Kozuch and Pempkowiak, 1996; Lorenzo et al., 2002) or FA (Lorenzo et al., 2006). An additional effect of HA adsorption to surfaces has been recently shown by Bourgeault et al. (2009). They have shown that HA caused an increase in filtration rates by zebra mussels, presumably in response to the low oxygenation produced by clogging of gill surfaces due to HA adsorption. The increase in filtration rate was used to explain the observed increased Cd uptake rates in the presence of HA also observed in this organism. The conventional rate coefficient models relate the concentration in the organism with that in the water, independently of filtration rate. Metal uptake dependence on filtration rate will only apply in the case of diffusion limited uptake. It could be that in case Pb uptake is diffusion limited but Cu uptake is not, because of its smaller hydrodynamic radio and higher diffusion coefficient, this would explain why Pb uptake increases in the presence of HA while Cu uptake does not. Diffusion limited uptake has been reported for Cd in clams. Tran et al. (2001) have shown increased Cd accumulation by clams as ambient O_2 decreased, and filtration rates increased, using Cd dissolved concentrations < 0.02 μM. Calculated J_{dif}/J_u in their system is 3.3, but calculated J_{dif}/J_u in our system Pb–SRDOM-mussels was much higher (181). Therefore, diffusion limited uptake in the present study is unlikely as previously discussed. Moreover, this would not explain the higher uptake observed for excised gills in the presence of HA (Sánchez-Marín et al., 2007), given that to increase filtration rates due to low oxygenation is a whole organism response.

It is possible that other direct effects caused by DOM adsorption to the gill surface can account for the observed differences between FA and SRDOM. The different effects of FA and SRDOM on Pb uptake would probably be caused by the different properties of these substances, such as their hydrophobicity and surfactant activity.

3.6. Differences in SRDOM and FA effects on Pb uptake, comparison with HA

Even though the differences between SRDOM and FA effects on Pb uptake by whole mussels and mussel gills respectively are difficult to explain, the main finding of the present study is that both FA and SRDOM are capable to complex Pb in seawater, and as a result of this complexation Pb bioavailability decreases. These results are in contrast with the effect caused by HA, which increased Pb bioavailability despite of their Pb complexation properties (Sánchez-Marín et al., 2007). To explain these differences, one should consider the physicochemical properties of these substances. For instance, the average molecular weight of Aldrich HA is two times higher than that of the FA and SRDOM used in this study (Chin et al., 1994; Karanfil et al., 1996; Ritchie, 2005), also the aromatic carbon content is similar for FA and SRDOM (23–28%) (IHSS, 2008a; Karanfil et al., 1996) while it is 41–45% for HA (Ashley, 1996; Zhou et al., 1994). Also, the O/C ratio is lower for HA (0.47) than for FA (0.83) or SRDOM (0.81) (IHSS, 2008b; Karanfil et al., 1996). All these characteristics show that HA differ in their physicochemical properties as compared with FA and SRDOM. Due to these properties, HA are more hydrophobic and tend to adsorb more to organic (and inorganic) surfaces (Zhou et al., 1994). Other experiments performed by the present authors (unpublished results) have shown that adsorption of HA to phytoplankton cells is around three times higher than adsorption of FA and SRDOM. So, adsorption of HA due to its more hydrophobic behaviour, lower solubility and other properties may cause effects on cell membranes leading to increased Pb uptake.

Different characteristics of commercial HA as compared with soil and aquatic DOM have already been reported (Glover and Wood, 2005; Malcolm and MacCarthy, 1986), leading to consider that they are not representative of aquatic DOM. Aldrich HA are extracted from soil and are more hydrophobic and of higher molecular weight than aquatic humic and fulvic acids. They may represent an extreme (in terms of hydrophobicity and aromatic carbon content) within the range of variation of the heterogeneous mixture of compounds present in natural DOM. Galvez et al. (2008) used Aldrich HA as a positive control in a study of the effects of DOM extracted from different freshwater sources on fish gills, and very high correlations were found between specific absorption coefficients of DOM and changes in transepithelial potentials in fish gills exposed to these substances. Aldrich HA fitted very well in the regression line (even though it was in the highest effect extreme). Also, other unpublished results by the present authors have shown that FA increase Pb toxicity to *Paracentrotus lividus* larvae over what would be expected according to [Pb′], although the enhancing effect of FA is lower than that of HA.

4. Conclusion

In summary, dissolved organic matter has a double and contrary effect on Pb bioavailability. On the one hand, Pb complexation by DOM decreases Pb bioavailability and on the other hand, other effects of DOM probably caused by their adsorption on cell membranes may enhance Pb bioavailability. Which effect will prevail in natural conditions will depend on physico-chemical characteristics of the natural DOM and the structural and functional organisation of the epithelial interface for different biological species and exposure conditions.

Acknowledgements

P.S.-M. was granted with a FPU fellowship from the Spanish Ministry of Education and Science. Rocío Rendo is gratefully acknowledged for technical assistance. Part of this work was per-

formed in the marine facilities of the ECIMAT (University of Vigo). ICP analysis was performed in the CACTI (University of Vigo) or by Marcel Selens (University of Antwerp). X.A. Álvarez-Salgado and M. Nieto-Cid are acknowledged for the DOC measurements. This study was partly financed by the Galician Government (Research Project PGIDIT05RMA31201PR).

References

Álvarez-Salgado, X.A., Miller, A.E.J., 1998. Simultaneous determination of dissolved organic carbon and total dissolved nitrogen in seawater by high temperature catalytic oxidation: conditions for precise shipboard measurements. Mar. Chem. 62, 325–333.

Ashley, J.T.F., 1996. Adsorption of Cu (II) and Zn (II) by estuarine, riverine and terrestrial humic acids. Chemosphere 33, 2175–2187.

Benner, R., 2002. Chemical composition and reactivity. In: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter. Elsevier, USA, pp. 59–90.

Bourgeault, A., Gourlay-Francé, C., Tusseau-Vuillemin, M.-H., 2009. Bioaccumulation of dissolved metals in zebra mussels: influence of water composition. In: SETAC Europe 19th Annual Meeting , Goteborg, Sweden.

Brooks, M.L., Meyer, J.S., McKnight, D.M., 2007. Photooxidation of wetland and riverine dissolved organic matter: altered copper complexation and organic composition. Hydrobiologia 579, 95–113.

Buffle, J., 1990. Complexation Reactions in Aquatic Systems: An Analytical Approach. Ellis Horwood Limited, Coll House, UK.

Buffle, J., Vuilleumier, J.J., Tercier, M.L., Parthasarathy, N., 1987. Voltammetric study of humic and fulvic substances. V. Interpretation of metal ion complexation measured by anodic stripping voltammetric methods. Sci. Total Environ. 60, 75–96.

Cambpell, P.G.C., 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley and Sons Ltd., NY, USA, pp. 45–102.

Campbell, P.G.C., Twiss, M.R., Wilkinson, K.J., 1997. Accumulation of natural organic matter on the surfaces of living cells: implications for the interaction of toxic solutes with aquatic biota. Can. J. Fish. Aquat. Sci. 54, 2543–2554.

Campbell, P.G.C., Errécalde, O., Fortin, C., Hiriart-Baer, V.P., Vigneault, B., 2002. Metal bioavailability to phytoplankton-applicability to the biotic ligand model. Comp. Biochem. Physiol. C 133, 189–206.

Capodaglio, G., Coale, K.H., Bruland, K.W., 1990. Lead speciation in surface waters of the Eastern North Pacific. Mar. Chem. 29, 221–233.

Capodaglio, G., Scarponi, G., Toscano, G., Barbante, C., Cescon, P., 1995. Speciation of trace metals in seawater by anodic stripping voltammetry: critical analytical steps. Fresenius J. Anal. Chem. 351, 386–392.

Chin, Y.-P., Aiken, G., O'Loughlin, E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ. Sci. Technol. 28, 1853–1858.

Coale, K.H., Bruland, K.W., 1988. Copper complexation in the Northeast Pacific. Limnol. Oceanogr. 33, 1084–1101.

Cobelo-García, A., Prego, R., 2004. Chemical speciation of dissolved copper, lead and zinc in a ría coastal system: the role of resuspended sediments. Anal. Chim. Acta 524, 109–114.

Florence, T.M., 1986. Electrochemical approaches to trace element speciation in waters. A review. Analyst 111, 489–505.

Galvez, F., Donini, A., Playle, R.C., Smith, D.S., O'Donnell, M.J., Wood, C.M., 2008. A matter of potential concern: natural organic matter alters the electrical properties of fish gills. Environ. Sci. Technol. 42, 9385–9390.

George, S.G., Coombs, T.L., 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Mar. Biol. 39, 261–268.

Glover, C.N., Wood, C.M., 2005. The disruption of *Daphnia magna* sodium metabolism by humic substances: mechanism of action and effect of humic substance source. Physiol. Biochem. Zool. 78, 1005–1016.

Greter, F.L., Buffle, J., Haerdi, W., 1979. Voltammetric study of humic and fulvic substances. Part I. Study of their complexing properties with lead. J. Electroanal. Chem. 101, 211–229.

Guo, L., Hunt, B.J., Santschi, P.H., Ray, S.M., 2001. Effect of dissolved organic matter on the uptake of trace metals by American oysters. Environ. Sci. Technol. 35, 885–893.

IHSS, 2008. 13C NMR estimates of carbon distribution in IHSS samples. http://www.ihss.gatech.edu/thornnmr.html (last accessed July 2010).

IHSS, 2008. Elemental compositions and stable isotopic ratios of IHSS samples. http://www.ihss.gatech.edu/elements.html (last accessed July 2010).

Jansen, S., Blust, R., Van Leeuwen, H.P., 2002. Metal speciation dynamics and bioavailability: Zn(II) and Cd(II) uptake by mussel (*Mytilus edulis*) and carp (*Cyrpinus carpio*). Environ. Sci. Technol. 36, 2164–2170.

Jones, H.D., Richards, O.G., Southern, T.A., 1992. Gill dimensions, water pumping rate and body size in mussel *Mytilus edulis* L. J. Exp. Mar. Biol. Ecol. 155, 213–237.

Karanfil, T., Kilduff, J., Schlautman, M.A., Weber Jr., W.J., 1996. Adsorption of organic macromolecules by granular activated carbon. 1. Influence of molecular properties under anoxic solution conditions. Environ. Sci. Technol. 30, 2187–2194.

Kariuki, S., Dewald, H.D., 1996. Evaluation of diffusion coefficients of metallic ions in aqueous solutions. Electroanalysis 8, 307–313.

Knauer, K., Buffle, J., 2001. Adsorption of fulvic acid on algal surfaces and its effect on carbon uptake. J. Phycol. 37, 47–51.

Kozelka, P.B., Bruland, K.W., 1998. Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. Mar. Chem. 60, 267–282.

Kozelka, P.B., Sañudo-Wilhelmy, S., Flegal, A.R., Bruland, K.W., 1997. Physicochemical speciation of lead in South San Francisco Bay. Estuar. Coast. Shelf Sci. 44, 649–658.

Kozuch, J., Pempkowiak, J., 1996. Molecular weight of humic acids as major property of these substances influencing the accumulation rate of cadmium by a blue mussel (*Mytilus edulis*). Environ. Int. 22, 585–589.

Lamelas, C., Wilkinson, K.J., Slaveykova, V.I., 2005. Influence of the composition of natural organic matter on Pb bioavailability to microalgae. Environ. Sci. Technol. 39, 6019–6116.

Lorenzo, J.I., Nieto, O., Beiras, R., 2002. Effect of humic acids on speciation and toxicity of copper to *Paracentrotus lividus* larvae in seawater. Aquat. Toxicol. 58, 27–41.

Lorenzo, J.I., Beiras, R., Mubiana, V.K., Blust, R., 2005. Copper uptake by *Mytilus edulis* in the presence of humic acids. Environ. Toxicol. Chem. 24, 973–980.

Lorenzo, J.I., Nieto, O., Beiras, R., 2006. Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. Environ. Toxicol. Chem. 25, 36–44.

Malcolm, R.L., MacCarthy, P., 1986. Limitations in the use of commercial humic acids in water and soil research. Environ. Sci. Technol. 20, 904–911.

Morel, F.M.M., 1983. Principles of Aquatic Chemistry. John Wiley, New York, NY, USA.

Morgan, J.J., Stumm, W., 1991. Chemical processes in the environment, relevance of chemical speciation. In: Merian, E. (Ed.), Metals and their Compounds in the Environment. John Wiley, NY, USA, pp. 67–103.

Mota, A.M., Correia dos Santos, M.M., 1995. Trace metal speciation of labile chemical species in natural waters: electrochemical methods. In: Tessier, A., Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. John Wiley and Sons Ltd., Chichester, UK, pp. 205–257.

Motulsky, H., Christopoulos, A., 2003. Fitting Models to Biological Data using Linear and Nonlinear Regression. A Practical Guide to Curve Fitting. GraphPad Software, Inc., San Diego CA.

Mubiana, V.K., 2006. Delineating factors influencing the uptake and accumulation of heavy metals in the intertidal mussel *Mytilus edulis*. Ph. D Thesis. University of Antwerp.

Myers, V.B., Iverson, R.L., Harriss, R.C., 1975. The effect of salinity and dissolved organic matter on surface charge characteristics of some euryhaline phytoplankton. J. Exp. Mar. Biol. Ecol. 17, 59–68.

Parent, L., Twiss, M.R., Campbell, P.G.C., 1996. Influences of natural dissolved organic matter on the interaction of aluminum with the microalga Chlorella: a test of the free-ion model of trace metal toxicity. Environ. Sci. Technol. 30, 1713–1720.

Pempkowiak, J., Bancer, B., Legezynska, E., Kulinsky, W., 1989. The accumulation and uptake of cadmium by four selected Baltic species in the presence of marine humic substances. In: Proceedings, 21st European Marine Biology Symposia , Gdansk, Poland, September 14–19, 1986, pp. 599–607.

Riget, F., Johansen, P., Asmund, G., 1997. Uptake and release of lead and zinc by blue mussels. Experience from transplantation experiments in Greenland. Mar. Pollut. Bull. 34, 805–815.

Ritchie, J.D., 2005. The distribution of charge and acidic functional groups in natural organic matter: the dependence on molecular weight and pH. Ph.D. Thesis. Georgia Institute of Technology.

Roditi, H.A., Fisher, N.S., Sañudo-Wilhelmy, S.A., 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature 407, 78–80.

Sánchez-Marín, P., Beiras, R., 2008. Lead concentrations and size dependence of lead accumulation in the clam Dosinia exoleta from shellfish extraction areas in the Galician Rías (NW Spain). Aquat. Living Resour. 21, 57–61.

Sánchez-Marín, P., Lorenzo, J.I., Blust, R., Beiras, R., 2007. Humic acids increase dissolved lead bioavailability for marine invertebrates. Environ. Sci. Technol. 41, 5679–5684.

Sánchez-Marín, P., Santos-Echeandía, J., Nieto-Cid, M., Álvarez-Salgado, X.A., Beiras, R., 2010a. Effect of dissolved organic matter (DOM) of contrasting origins on Cu and Pb speciation and toxicity to *Paracentrotus lividus* larvae. Aquat. Toxicol. 96, 90–102.

Sánchez-Marín, P., Slaveykova, V.I., Beiras, R., 2010b. Cu and Pb accumulation by the marine diatom Thalassiosira weissflogii in the presence of humic acids. Environ. Chem. 7, 309–317.

Schulz-Baldes, M., 1974. Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edulis*. Mar. Biol. 25, 177–193.

Sericano, J., 2000. The Mussel Watch approach and its applicability to global chemical contamination monitoring programs. Int. J. Environ. Pollut. 13, 340–350.

Slaveykova, V.I., Wilkinson, K.J., Ceresa, A., Pretsch, E., 2003. Role of fulvic acid on lead bioaccumulation by *Chlorella kesslerii*. Environ. Sci. Technol. 37, 1114–1121.

Srna, R.F., Garret, K.S., Miller, S.M., Thum, A.B., 1980. Copper complexation capacity of marine water samples from southern California. Environ. Sci. Technol. 14, 1482–1486.

Tipping, E., 1981. The adsorption of aquatic humic substances by iron oxides. Geochim. Cosmochim. Acta 45, 191–199.

Town, R.M., Filella, M., 2000. Determination of metal ion binding parameters for humic substances. Part 2. Utility of ASV pseudo-polarography. J. Electroanal. Chem. 488, 1–16.

Tran, D., Boudou, A., Massabuau, J.-C., 2001. How water oxygenation level influences cadmium accumulation pattern in the Asiatic clam *Corbicula fluminea*: a laboratory and field study. Environ. Toxicol. Chem. 20, 2073–2080.

Turner, D.R., Whitfield, M., Dickson, A.G., 1981. The equilibrium speciation of dissolved components in freshwater and seawater at 25 °C and 1 atm pressure. Geochim. Cosmochim. Acta 45, 855–881.
Vigneault, B., Percot, A., Lafleur, M., Campbell, P.G.C., 2000. Permeability changes in model and phytoplankton membranes in the presence of aquatic humic substances. Environ. Sci. Technol. 34, 3907–3913.
Voets, J., Bervoets, L., Blust, R., 2004. Cadmium bioavailability and accumulation in the presence of humic acid to the zebra mussel, *Dreissena polymorpha*. Environ. Sci. Technol. 38, 1003–1008.
Wershaw, R.L., 1993. Model for humus in soils and sediments. Environ. Sci. Technol. 27, 814–816.
Widdows, J., Donkin, P., 1992. Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: Gosling, E. (Ed.), The Mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Elsevier, Amsterdam, The Netherlands, pp. 383–424.
Zhou, J.L., Rowland, S., Fauzi, R., Mantoura, R.F.C., Braven, J., 1994. The formation of humic coatings on mineral particles under simulated estuarine conditions. A mechanistic study. Water Res. 28, 571–579.