Marine Pollution Bulletin xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Revisiting methods for the determination of bioavailable metals in coastal sediments

Mirella Peña-Icart [a,b], Carolina Mendiguchía [a], Margarita E. Villanueva-Tagle [c], Mario S. Pomares-Alfonso [b,*], Carlos Moreno [a]

[a] Department of Analytical Chemistry, Faculty of Marine and Environmental Sciences, University of Cadiz, República Saharaui, CP: 11510 Puerto Real, Cádiz, Spain
[b] Institute of Sciences and Technology of Materials, University of Havana, Zapata s/n, CP: 10400, La Habana, Cuba
[c] Department of General Chemistry, Faculty of Chemistry, University of Havana, Zapata s/n, CP: 10400, La Habana, Cuba

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Sediment metal contamination
Hydrochloric acid
Pepsin
Trypsin
Aquatic species digestive system
Metal bioavailability

ABSTRACT

A simple methodology for the determination of bioavailability of fourteen metals in coastal sediments has been developed by simulating the conditions of digestive process of marine fishes. With this aim, a representative sediment composite sample was treated with hydrochloric acid solutions at different pH values, temperatures and contact times, in the presence and absence of Pepsin and Trypsin. The addition of Pepsin and Trypsin did not affect the extraction of most elements. As a result of the present study, the digestion with a hydrochloric acid solution at pH 1, 40 °C and 12 h is proposed. Adjustments of the temperature and time reaction could be made according to the specific ecosystem under study. The amount of metal extracted by other methods based on acetic acid was lower than that extracted by HCl treatment proposed.

1. Introduction

It is well known that surface sediments are frequently employed to assess metal contamination in marine environments. Several methods have been proposed in order to achieve this purpose. One of the most used is the comparison of total concentration of metal in sediments with reference concentrations given in Sediment Quality Guidelines (Bartoli et al., 2012; Gao and Lia, 2012; Hahladakis et al., 2013; Hu et al., 2013; Louriño-Cabana et al., 2011; Rui et al., 2013; Varol, 2011; Xuelu and Chen-Tung, 2012). In the guidelines a relationship between the total concentration of metal in sediment and the probability of damage occurrence on biota is given. This way, the potential risk of contaminated sediment on biota is assessed. Undoubtedly, it is a very simple method. However, it is worth pointing out that it has a significant drawback: the level of predicted risk is true only if the relationship between the reference concentration of metal versus damage probability on biota is the same in the ecosystem where experimental data of guidelines was collected and also in the actual ecosystem where sediments are under study.

In another approach, the amount of metal transferred to the aquatic environment is considered to evaluate potential risk on biota of contaminated sediment. In many of the studies reviewed, the amount of metal transferred from the sediment to the water column is taken as the amount of metal extracted using some method of partial digestion of the sediment (Cappuyns and Swennen, 2008; Devesa-Rey et al., 2010; Ettler et al., 2007; Okuku and Peter, 2012; Quevedo et al., 2012; Peña-Icart et al., 2011; Pérez Santana et al., 2007; Sastre et al., 2002; Singare et al., 2012; Xiujuan et al., 2012). In others, it is taken as the amount transferred from the sediment ingested in the gastrointestinal tract of the aquatic species (Amiard et al., 1995, 2007; Ettanjani and Amiard, 1995; Griscom et al., 2000; Marisa et al., 2005; Ojo and Wood, 2007; Turner and Olsen, 2000; Turner et al., 2001). In both cases, the amount of metal transferred from the sediment to the environment is considered as the bioavailable part of the metal.

Probably, the 3-step BCR sequential digestion method is one of the most employed to estimate the amount of metal transferred from the sediment to the water (Devesa-Rey et al., 2010; Martínez et al., 2011; Mossop and Davidson, 2003; Pérez Santana et al., 2007; Queavauviller, 1998a,b; Rao et al., 2008; Rauret et al., 1999; Sahuquillo et al., 2002; Sahuquillo and Rauret, 2003; Yongmin et al., 2013; Zimmerman and Weindorf, 2010). For this method, reference concentrations of metal are given for the estimation of the potential risk caused by lixiviated metals on biota (Andrade Passos et al., 2011; Liu et al., 2009; Perin et al., 1985).

* Corresponding author. Tel.: +53 7 8781136.
E-mail address: mpomares@imre.oc.uh.cu (M.S. Pomares-Alfonso).

http://dx.doi.org/10.1016/j.marpolbul.2014.10.034
0025-326X/

Unfortunately, the 3-step BCR method is especially laborious and, consequently, it is not recommended for monitoring or routine analysis (Peña-Icart et al., 2011). Other more simple one-step extraction methods have also been investigated (Galí et al., 2011; Guang et al., 2011; Gupta et al., 2009; Quevedo et al., 2012; Peña-Icart et al., 2011; Rauret, 1998). Particularly, the extraction with 0.11 M acetic acid (HOAc) has been proposed to estimate highly mobile metals, mostly that fraction bound to carbonaceous phase of sediments (Sahuquillo and Rauret, 2003); while the 25% HOAc method has been, especially recommended for the evaluation of metal bioavailability in sediments (UNEP/IOC/IAEA, 1995).

On the other hand, in most studies that focus the desorption of metals from sedimentary particles in the gastrointestinal tract of aquatic species, sediments are treated using gastric juice previously extracted from a target marine species, the results being of limited application (Fan and Wang, 2003; Griscom and Fisher, 2004; Mayer et al., 1996; Turner and Olsen, 2000; Wang et al., 2002; Zhong and Wang, 2008). Only in very few works, treatment of the sediment was simulated in the lab by using the main chemical (hydrochloric acid) and enzymatic (pepsin and trypsin) agents that principally act on the sediment in the gastrointestinal tract of aquatic organisms (Amiard et al., 1995; Bignasca et al., 2011; Turner and Olsen, 2000; Turner et al., 2001). Particularly, the lixiviation effect of hydrochloric acid on a limited amount of elements (Ag, As, Cd, Cu, Pb, and Zn) was described as predominant if compared to enzymatic digestion (Amiard et al., 1995). However, it should be noted that experiments were performed in limited laboratory conditions. For example, studied ranges of contact time and temperature of 2–6 h and 19–21 °C, respectively, are relatively short in comparison to the whole range of conditions that can be found. Besides, results for some elements of ecotoxicological interest (As, Cd, Cr, Cu, Ni, Pb and V) are very scarce. Recently, Bignasca et al. (2011) have employed pepsin and trypsin, to evaluate bio-accessibility of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, V and Zn from marine sediments. The amounts of metal extracted by the pepsin and trypsin enzymes, by proteinasa K and by 0.11 M HOAc were compared. In this context, it should be noted that 25% HOAc (Turner and Olsen, 2000) was also employed in order to simulate acidic conditions of sediment digestion in the stomach of aquatic organisms.

At this point, it is convenient to remark that the lack of international consensus regarding the concept of metal bioavailability; which has been defined in different forms, such as: amount of metal transferred from sediment to aquatic environment, in terms of plant uptake, amount absorbed by microorganisms or by superior organisms, and in terms of biodegradation, among others, as described in the review work of Naidu et al. (2008). The concept of metal bioavailability defined in (Turner and Olsen, 2000) will be used in the present study, i. e., the maximum amount of a contaminant that is available or dissolved in the gastro-intestinal tract of an organism.

For the living aquatic organisms considered in the present work, sediment digestion takes place, basically, in two consecutive steps in the gastrointestinal tract (Edward and Hume, 1995). The first step occurs in the stomach in the presence of pepsin, a gastric enzyme, which is activated under acidic conditions generated by the secretion of HCl. The second step occurs in the intestines, where trypsin, segregated by the pancreas, is the principal acting enzyme. Acidity in the intestine varies from pH 7 at the beginning of this organ, to a slightly basic pH at the end.

The aim of the present work was to study the desorption of Al, As, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, Pb, Ti, V, and Zn from marine sediments by simulating the digestive process in the gastrointestinal tract of aquatic organisms by using both chemical and enzymatic reagents in different conditions. With this purpose, the metal extraction with HCl at different values of pH, temperature and contact times was studied. The action of the two principal enzymes, pepsin and trypsin, involved in the digestion of aquatic organism, was also considered. As a result of this study, a simple method is proposed for the estimation of the bioavailability of metals present in sediments. In addition, the results obtained by the proposed method are compared with those reported by the HOAc-based digestion methods, mostly used by environmentalists, to evaluate potential over- or underestimation of metal uptake by fishes.

2. Material and methods

2.1. Sediment samples

The studies were done with a low contaminated representative composite sample, taken from an aquaculture farm dedicated to the culture of *Sparus Aurata* in earthen ponds, described elsewhere (Tovar et al., 2000). Although the study of moderately to highly contaminated sediments would be simpler, the chosen option is more representative of most ecosystems.

The sample was prepared as follows: at the end of a culture cycle, eight portions of surface sediments were collected in sites distributed homogenously at a pond. At laboratory, the samples were separately sieved through 500 μm metal sieve in order to eliminate mollusc shells and other particles of a considerable size. Further, samples were dried at 40 °C and then, an appropriate equal amount of each one was put together and mixed by milling in agate mortar. Finally, the composite sample was properly labelled and stored in plastic bottle until analysis.

2.2. Treatment of sediments with the principal chemical (HCl) and enzymatic (Pepsin and Trypsin) components acting in the digestive process of aquatic organisms

HCl treatment of sediment was carried out at pH of 1, 2, 3, 4, 5, and 6 in separated experiments; while temperature and reaction time were fixed at constant values. These six treatments were run for all nine different combinations of temperature of 10 °C, 20 °C, and 40 °C and reaction time of 1 h, 12 h, and 24 h. Then, a total of fifty-four treatments were tested; in which the entire range of temperatures that can be found in different geographical zones and the whole range of time that can stay sedimentary particles in the gastrointestinal tract of different aquatic specimens were studied. For each treatment, 25 ml of HCl solution at a fixed pH was added to 0.25 g of sediment in a polytetrafluoroethylene reactor. Next, sample was mechanically shaken. At the end, samples were filtered through 0.45 μm cellulose acetate filters and solutions were reserved in polyethylene bottles until analysis. Each one of the fifty-four treatments was repeated three times with three portions of sediments.

Hydrochloric acid plus Pepsin treatment of sediment was made with 25 ml HCl solutions prepared at pH of 1, 2, 3 and 4 that contained also 0.15% w/v of Pepsin A (activity = 600 units/mg from Scharlab, Spain). The four prepared HCl + Pepsin solutions at different pHs were added, correspondingly, to 0.25 g of sediment, previously put in polytetrafluoroethylene reactors. Then, samples were mechanically shaken for 12 h at 40 °C, filtered through a 0.45 μm cellulose acetate filter and reserved in polyethylene bottle until analysis.

Trypsin treatment of sediment was made by adding 25 mL of a 0.15% w/v Trypsin solution to 0.25 g of sediment in polytetrafluoroethylene reactors. pH of solution was adjusted to 7.6 by adding an appropriate amount of NaOH. The enzyme used was Trypsin II-S with activity 1300 BAEE units/mg (Scharlab, Spain). The rest of procedure was similar to that described for Pepsin.

Please cite this article in press as: Peña-Icart, M., et al. Revisiting methods for the determination of bioavailable metals in coastal sediments. Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.10.034