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Role of bacterial esterase on mercury dynamics in mangrove sediments

Paulo Roberto S. Lopes[1]
Maria das Graças S. Bispo[2]
Miriam A.C. Crapez[2]
Julio Cesar F. A. Wasserman[1,3]*

[1] Departamento de Geoquímica
Universidade Federal Fluminense
Outeiro de São João Batista s/n°
Centro, Niterói RJ Brazil,
CEP: 24220-007.

[2] Departamento de Biologia Marinha
Universidade Federal Fluminense
Outeiro de São João Batista s/n°
Centro, Niterói RJ Brazil
CEP: 24220-007

[3] Departamento de Análise Geo-Ambiental
Universidade Federal Fluminense
Av. Litorânea, s/n°
Boa Viagem Niterói RJ Brazil
CEP 24.230-340

Corresponding author
geowass@vm.uff.br

RESUMO

Áreas estuarinas tropicais apresentam condições microbiológicas e geoquímicas que mostraram ser importantes condições que controlam mecanismos de metilação de mercúrio, aumentando sua biodisponibilidade para níveis tróficos superiores. Apesar do potencial risco destes mecanismos para a saúde humana, poucos estudos foram realizados apresentando análises *in situ* em ecossistemas costeiros tropicais, relacionando a atividade bacteriana e concentrações de mercúrio. No presente trabalho, nós analisamos as relações entre alguns parâmetros ambientais relevantes que podem estimular a atividade bacteriana e controlam as concentrações de mercúrio em sedimentos de uma planície de mare de um mangue tropical. Quarenta e quatro amostras foram coletadas e analisadas para concentração de carbono orgânico total, carbono bacteriano, lipídeos, proteínas, atividade das esterase e concentração de mercúrio total e SEM/AVS. O potencial redox, temperatura e pH também foram medidos *in situ*. Os resultados mostraram uma intense atividade bacteriana e níveis de mercúrio crescentes, quando comparados aos valores naturais da região. A avaliação do SEM/AVS, recomentada pela USEPA como critério para checar a qualidade do sediment não mostra suficiente acurácia para permitir a previsão do risco de biodisponibilidade nos sedimentos, considerando que a quantidade de matéria orgânica é muito elevada. Esta metodologia parece necessitar novas abordagens para permitir a tomada de decisão
Palavras-chave Mercúrio, Sedimentos Estuarinos, Manguezal, Bacteria, Esterase

ABSTRACT

Tropical estuarine areas present microbial and geochemical conditions that have been pointed out as important conditions to trigger the mechanisms of mercury methylation improving its bioavailability to higher trophic levels. Despite the potential risk of these mechanisms to human health, few studies have reported *in situ* analysis in costal subtropical ecosystems, relating bacterial activity and mercury concentrations. In the present work we analyze the relationship between some relevant environmental parameters that may stimulate the bacterial activity and control mercury concentrations in the sediments of a tropical mangrove tidal flat. Forty-four samples were collected and analyzed for total organic carbon, bacterial organic carbon, lipids, proteins, esterase activity and mercury concentrations and SEM/AVS. The redox potential, temperature and pH were also measured *in situ*. The results showed intense bacterial activity and increasing mercury levels when compared with local backgrounds. The use of SEM/AVS indicator recommended by USEPA as a criterium to check the sediment quality, did not show enough accuracy to predict the ecological bioavailability risk in sediments with high organic matter content. This methodology requires new approach to help decision making.
Keywords: Mercury, Estuarine Sediments, Mangrove, Bacteria, Esterase

1. INTRODUCTION

The occurrence of mercury contamination in abiotic matrices (sediment, surface water and pore water) in costal and marine environments increase the exposure risk to higher trophic level consumers, including fishes, piscivorous birds and humans (Schaanning *et al.*, 1996; Kehrig *et al.*, 2001). In the coastal areas, the estuaries play an important role on the Hg cycle, acting as sites of inorganic Hg entrapment and biochemical reactions that result in MeHg production (Cossa *et al.*, 1997; Mason *et al.*, 2000). Once methylated, Hg complexes bioaccumulate and biomagnify up through the aquatic trophic web, exposing humans and other organisms to its toxic effects (Mergler *et al.*, 2007; Scheuhammer *et al.*, 2007). Although a number of works have assessed mercury chemistry in temperate environments (e.g.: Alberts *et al.*, 1974; Baldi *et al.*, 1995), a lot of gaps have been left for a precise understanding of its behavior. Under tropical conditions the scarce literature, shows that the behavior of mercury is very different from the reported in temperate areas (Quevauviller *et al.*, 1992; Marins *et al.*, 2000).

The studies on mercury chemistry show that under favorable chemical or biological conditions within the sediment, inorganic mercury (Hg(II) and Hg(0)) can be alkylated to very harmful complexes (mainly methyl-mercury and dimethyl-mercury) that will reach humans through the food chain (Ross, 1996). In many cases, human contamination can reach serious proportions attaining adults (Haraguchi *et al.*, 2000) and children (Marsh *et al.*, 1995). In the sedimentary, coastal environmental bacteria are recognized as organisms that play a major role in mercury biogeochemical transformations.

Heterotrophic bacteria have a major role in organic matter cycling due to its use as a source of carbon and energy. Any type of organic substrate can be used by specialized bacteria, which is metabolized through extracellular reactions, into less complex molecules, becoming available for membrane transportation. As part of these metabolic systems, esterases (EST) are an assemblage of extracellular enzymes that play a relevant role on the organic matter digestion. In this process, esterases break down ester bonds from proteins, lipids and carbohydrates, yielding monomers and olygomers that constitute dissolved organic matter that is easily absorbed by the organisms. Therefore, the activity of the esterases can be used as an indicator of the microorganisms metabolic potential to transform metals into alkyl-metallic forms. Early works reported tin (Guard *et al.*, 1981) and mercury (Jensen & Jernelov, 1969) methylation by bacteria. However more recent studies (*e.g.*: Choi & Bartha, 1993; Costa & Liss, 2000) indicate that the chemistry of mercury may be affect by physical and chemical processes, bacterial activity is still shown to be very significant (Hintelmann & Wilken, 1995; Duran *et al.*, 2008). Furthermore, the bacterial breakdown of organic macromolecules by the esterases activity may release metals trapped into these complexes, making them available for the food chain and increasing exposure.

Although strongly contaminated with cadmium and zinc, the Sepetiba bay (Rio de Janeiro, Brazil) historically has been subjected to small Hg loads. However, the recent increase in urbanization and industrialization of the drainage basin, have improved the contamination of this metal in the water column (Marins *et al.*, 2000) and in the sediments (Veeck, 1999; Wasserman *et al.*, 2001). Furthermore, measurable inputs of atmospheric mercury, probably of anthropogenic origin have already been recorded in the bay (Marins *et al.*, 1996). These non-point mercury inputs have already yielded very high concentrations in the sediment of the region (Barrocas & Wasserman, 1998).

The sedimentary environment in Sepetiba Bay is anoxic (Wasserman *et al.*, 2002). The hydrodynamics due to the tidal driven current are not strong enough to produce oxidation of sediments, mainly in the large mangrove forests that grow alongside its margin. Under such environmental conditions, sulphate that is largely supplied by seawater tends to be reduced to sulfide, forming refractory complexes like meta-cinnabar (Kim *et al.*, 2000). These complex of mercury-sulfides yields insoluble compounds (solubility constant = 5.0×10^{-58}), unavailable for bacterial methylation (Craig & Moreton, 1986; Benoit *et al.*, 1999). Nonetheless, this behavior has not been observed in tropical coastal environments. In the neighboring Guanabara Bay (Rio de Janeiro), results from sequential extractions of the sediments showed that, although redox conditions and sulfide availability favor sulfide complexation, mercury is rather associated with organic matter (Barrocas & Wasserman, 1998). Speciation assays in tropical sediments show that its relationship with organic matter is still more complex, since the volatile dimethyl-mercury could be present in the sediments (Quevauviller *et al.*, 1992). These authors proposed that volatile forms of mercury (like dimethylmercury) would be associated with organic matter, probably humic substances, avoiding mercury to be degassed from the sediment. Although there was a suspect of spurious dimethylmercury formation during the analytical procedure (Tseng *et al.*, 1999), a recent research confirmed the presence of dimethylmercury in the sediment (Wasserman *et al.*, 2002).

Due to the high temperatures observed in mangrove sediments, bacterial activities as established by esterase measurements showed high levels (Crapez *et al.*, 2003). Although a large amount of sulphide is supplied by seawater and the redox conditions could induce to mercury-sulfide complex formation, this metal seems to be in available forms, particularly Hg(II) (Wasserman *et al.*, 2002).

The present work evaluates concentrations and the relationship between mercury and bacterial activity through esterase measurement in tidal flat sediments from a mangrove area (Garças Cove) in the Sepetiba Bay, Rio de Janeiro, Brazil. Total organic carbon, bacterial organic carbon, proteins, and lipids were also measured in order to establish the factors that might be relevant for the bacterial activities.

2. MATERIALS AND METHODS

Various biogeochemical measurements were made in surface sediments of forty-four sites in the Garças Cove (Figure 1), Sepetiba bay, Rio de Janeiro, Brazil. Sediment samples were collected during two campaigns: February 2001 and February 2002. The study focused on surface sediments, because previous research suggests that this is typically the most active zone for microbial activity (Benoit *et al.*, 2002). Measurements were made during the summer since temperature is related to microbial activity (Benoit *et al.*, 2002; Fitzgerald *et al.*, 2007). For each campaign, multiple samples were collected at the tidal flat zone, along transects that were perpendicularly projected from the mangrove vegetation to the low tide line (4 transects in the first campaign, 3 transects in the second one; Figure 2). These samples were stored during 2 h in sealed polythene bags, conditioned in ice and taken to the laboratory. The analyses were performed in sediment samples in triplicates. Measurements were made for abiotic and biotic parameters, including microbial activity (Esterase activity - EST and electron transport system activity – ETSA). Also, we verified the total mercury concentration (THg) and many ancillary chemical and physical parameters. The variables chosen for this study were based on our understanding of the controls on Hg in other ecosystems.

2.1 SITE DESCRIPTION

The Garças Cove is located in the geographic co-ordinates 43°38' W and 22°59' S (Figure 1). It is a typical tropical coastal area with large mangrove stands covering an extensive tidal range region. Annual average temperatures vary from 21° up to 24° C and rainfall can be as high as 1500 mm year^{-1}. In the early 1970's this area became target of an industrial development with the construction of a large harbor and a significant population inflow. Nowadays, there are around 400 industries that can be grouped into two main groups: pyrometallurgic with two large steel plants, aluminum and electricity power plants. This industrial park is responsible for the input of large amounts of heavy metals (mainly zinc and cadmium) in the surrounding environments, reaching the coastal area either through the rivers (Lacerda *et al.*, 1987; Rodrigues, 1990; Wasserman *et al.*, 1991; Barcellos, 1995; Barcellos *et al.*, 1998) or through atmospheric deposition (Pedlowsky *et al.*, 1991; Silva-Filho *et al.*, 1998). No distinct point source of mercury is reported in the drainage basin, so far.

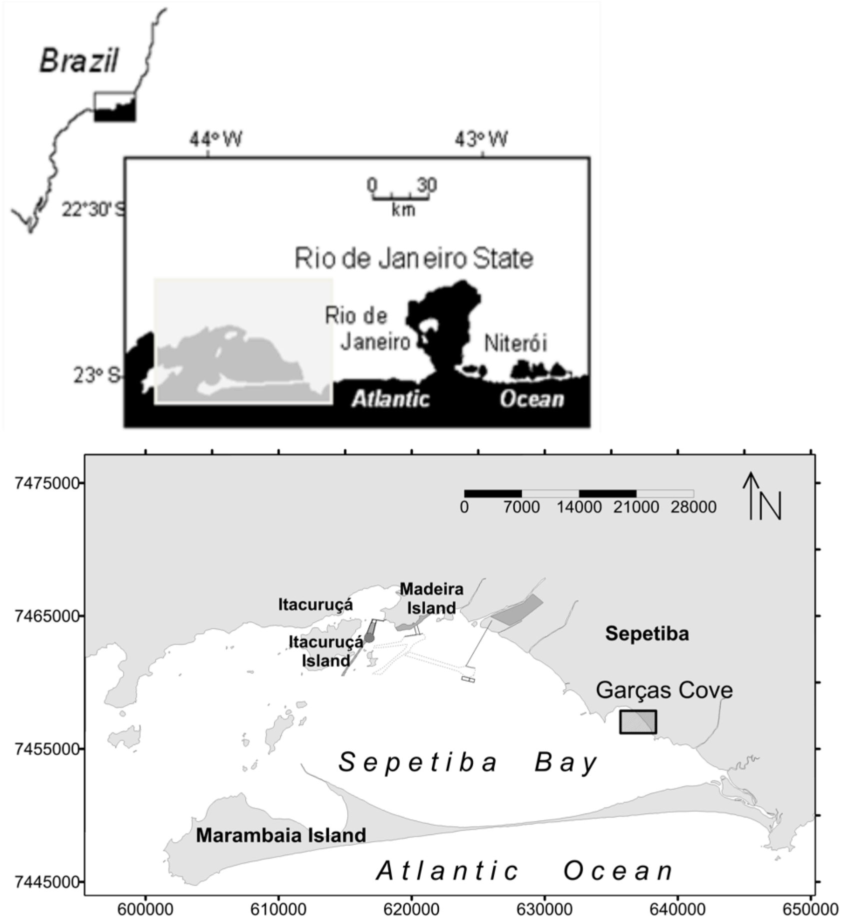

Figure 1
The study area with location of the sampled mangrove tidal flat.

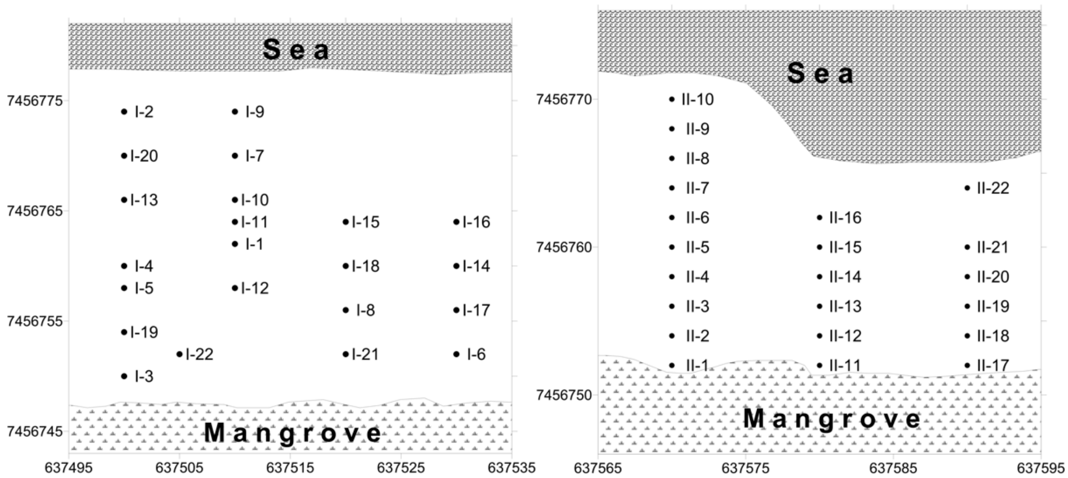

Figure 2
Scheme of the transects where sediment samples were collected

2.2. ABIOTIC PARAMETERS

Temperatures, pH and Eh were measured *in situ*. The granulometry was determined by passing wet sediment through a 63 µm sieves. The measurement was done by the difference of weigh obtained between the sieved and the retained fraction.

Samples were freeze dried and grounded with an agate mortar. To evaluate the total mercury (THg) concentrations, dried samples were submitted to a 50° C extraction in "cold finger" Erlenmeyers, using the procedure described by Malm *et al.* (1989) and performed by cold vapor atomic absorption spectrometry (CVASS). Stannous chloride was used as reducing/derivatizing agent. The procedure accuracy was tested using a standard reference material (BCR reference material 320, river sediment) that give results within the standard deviation established by the European Bureau of References.

2.3. BIOTIC PARAMETERS

The viable cells (EPI) were counted by epifluorescence (Kepner Jr. & Pratt, 1994) in a Axovert optical microscope using a 1000 times magnification. Biomass calculations also permitted the determination of the bacterial carbon (BC) (Carlucci *et al.*, 1986). The sediment total organic carbon (TOC) was measured by titration with ferrous sulphate (MERK-PA) after wet oxidation with sulfuric acid/dichromate solution (MERK-PA; Strickland & Parsons, 1972). Esterase enzyme activity (EST) was analyzed according to Stubberfield and Shaw (1990). This analysis is based on enzymatic transformation of fluorogenic compounds, into fluorescent products that can be quantified using a spectrophotometer (490 nm). These enzymes act on biopolymers (carbohydrates, proteins and lipids) and transform them into low-molecular-weight products, assimilable organic carbon fraction which is taken up by the viable bacteria. The results are given in µg of fluorescein h^{-1} g^{-1} (wet weight of sediment). Determination of the electron transport system activity (ETSA) was made according to Trevors (1984) and Houri-Davignon and Relexans (1989). The reagent 2-(*p*-Iodophenyl)-3(*p*-nitrophenyl)-5-phenyl tetrazolium chloride accepts electrons from the dehydrogenase enzymes and is reduced to a red-colored formazan (INTF). The reaction was quantified by colorimetric analysis (475 nm). The authors modified the assay to observe microbial enzymatic activity, in real-time, and established the relation between O_2 consumption and INTF production in both bacterial cultures and sediment samples. Results are thus expressed as electron transportation system activity to wet weight of sediment (µL O_2 h^{-1} g^{-1}). The total proteins were measured by the method of Lowry (Lowry *et al.*, 1951) and the total lipids were detected following the method described by Folch *et al.* (1957).

2.4. SIMULTANEOUSLY EXTRACTABLE METALS/ACID VOLATILE SULFIDES

The SEM/AVS (Simultaneously Extracted Metals – Acid Volatile Sulfides) was measured in the first campaign to estimate the mercury bioavailability. The AVS measurements were based on the procedure described by Allen *et al.* (1993) and Casas and Crecelius (1994). For sediment samples, sulfide is first volatilized after the addition of acid by converting it to gaseous hydrogen sulfide (H_2S) at room temperature. The H_2S is purged from the sample by an inert gas (nitrogen) and trapped in a sodium hydroxide (NaOH) solution. The abundance of reduced sulfur is quantified using a colorimetric method with the reagent MDR (mixed-diamine reagent). The sulfide binds with the reagent forming a methylene blue color. The intensity of the colored complex is determined using a spectrophotometer. The acid treatment to recover reduced sulfur also releases metals associated with the sulfide minerals into the acid solution. The metals released into the acid are then analyzed for SEM using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). Common metals usually determined include: cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), silver (Ag) and zinc (Zn). Mercury (Hg) was determined by cold vapor atomic spectrometry (CVAAS) Perkin Elmer.

2.5. SPATIAL VALUE DISTRIBUTION

In order to observe the existence of a spatial pattern among the measured parameters we used the software package Surfer® 8 that interpolate XYZ data in contour maps by a deterministic interpolator program.

2.6. DATA TREATMENT

All analyses were conducted using the statistical software program, Statsoft – Statistic 10. The relationship between EPI values and the other variables across the sample sites were determined using analysis of Spearman and were considered significant for $p<0.05$.

3. RESULTS AND DISCUSSION

Regardless the fact that sampling positions were very close one to the other, the results indicate a significant variability for almost all parameter during each campaign, except for temperature, pH and viable cells. As expected, in these marine estuarine sediments, the values were typical from mangrove environments with favorable characteristics for bacterial development (table 1). The sediment samples were fine grained (fraction smaller than 63µm ranged from 20.8 to 99.3%) and composed with high contents of organic matter - ranged from <0.1 to 25.6%. The values obtained for redox potential were highly negative favoring the organic matter preservation (ranging from -92 to -357 mV). Moreover, the pH levels (varied between 6.1 and 9.0) indicate shifts between autotrophic and heterotrophic (chemotrophic) condition of the surface anoxic sediments which are compatible with the biochemical reactions that might promote mercury changes in bioavailability. This process seems to be reinforced by the biological parameters that indicated intense microbial activity (Table 1), which could promote biochemical modifications of mercury. If these processes are relevant, the bioavailability of mercury should be severely influenced, triggering Mercury biomagnification of fish, a relevant source of incomes for large populations in Sepetiba Bay. It is interesting to note the difference of two orders of magnitude between the viable cell densities from the both campaigns. Probably these discrepancies were related with the lower environmental temperatures observed in the second campaign (Table 1).

Table 1 - Environmental parameters - Average and standard deviation values obtained from 44. samples.

1st Campaign	**Total Hg** (µg kg^{-1})	**TOC** (%)	**< 63 µm** (%)	**pH**	**Eh** (mv)	**EST** (µg Fluor. h^{-1} g^{-1})	**EPI** (cells g-1)
average	292	18.9	84.8	8.3	-235.3	6.67	3.45E+07
SD	130	4.6	19.5	0.4	55.8	1.56	3.80E+06
(% of average)	44.5	24.2	23	4.4	23.7	23.4	11.0
2nd Campaign							
average	173.6	13.8	62.9	6.9	-210	4.73	3.86E+05
SD	80.5	5.6	21.9	0.4	67.3	0.62	3.16E+05
(% of average)	46.4	40.6	34.8	5.8	32.0	13.1	81.9

3.1 SPATIAL DISTRIBUTION

To highlight any spatial trend among the measured parameters, each variable was plotted in contour maps with the software Surfer® 8.0. The distribution maps are presented in Figures 3 and 4, first campaign (February 2001) and second campaign (February 2002) respectively. Despite, the expected distribution trend would be related to tidal oscillation, all parameters did not show any particular tendency. The obtained maps did not show overlap coherence among the measured variables, even between those that presented a significant correlation as Epifluorescence (EPI) – Esterase (EST) and EST – Total Organic Carbon (TOC). However, it is possible to observe a mild correspondence between the spatial distribution obtained during the first campaign for TOC, granulometry and pH. The graphics (Figure 3) show two points (I.7 and I.13), which presented the higher bacterial density and pH levels, but they were situated in areas with different levels of TOC and THg. Dissociated patterns were also obtained with samples in the second campaign, when the higher bacterial densities were found in samples with low pH. In general, the contour maps (Figures 3 and 4) showed great variabilities of measured values, probably as consequence of significant spatial variation. This may occur by organic particles aggregation where attached bacteria could inhabit in rich microzones (Azam *et al.*, 1994). These particles create a spatial heterogeneity in the distribution of organic matter, in the remineralized nutrients, and in the species composing the bacterial populations, which could influence the biochemical reactions on the sediment components.

3.2 BIOLOGICAL PARAMETERS

The results found for the biological parameters, shown in Figures 3 and 4 were similar to those obtained in an earlier study which was realized on sewage samples from the Guanabara Bay (Crapez *et al.*, 2001). This behavior is expected in environmental profiles from mangrove sediments, very rich in organic matter and, probably, under domestic and industrial effluent contamination. Our measurements presented a significant positive correlation ($p<0.05$, $n=44$) between EST and almost all the other measured variables. However, some correlations as those between EST, granulometry, temperature and THg were not significant with values above 0.01. Moreover, the average values for EST, bacterial organic carbon (BOC), lipids and proteins suggested an intense bacterial activity (Table 1). EST activity showed an average of 6.67 µg fluorescein h^{-1} g^{-1}, in the first campaign (Figure 3), when the counts of viable cells were around 3.5E7. In the second campaign (Figure 4), the EST average was 4.75 µg h^{-1} g^{-1} and the EPI was around 3.9E5 (Table 1). A previous report in similar ecosystem suggested seasonal variation for ETSA and EST levels, pointing the organic matter quality as a relevant factor to control the organic carbon turnover (Crapez *et al.*, 2003). Samples from Boa Viagem beach at Guanabara bay presented ETSA levels maximum of 0.18 in winter; 7.48 µg h^{-1} g^{-1} in summer; EST levels maximum were 0.14 in winter and 0.17 µg h^{-1} g^{-1} in summer. In contrast, our results showed maximum levels for ETSA and EST, in summer, at 3.24 and 8.6 µg h^{-1}. g^{-1} respectively. Considering that Garças cove sediments suffered less anthropogenic impact than those from Boa Viagem, our data seems to confirm the importance of organic source to drive the enzyme reactions and the electron membrane transport activity. These variable levels might have considerable implications on the mercury dynamics and its concentrations. According to Baldi *et al.* (1991), under anaerobic conditions bacteria would alkylate mercury and methyl-mercury to the volatile dimethyl-mercury, constituting a detoxification mechanism for sediments. Previous reports detected high dimethyl-mercury production at the same place of the present study (Quevauviller *et al.*, 1992). Apparently, this process was not confirmed by our results. Despite of the negative redox potential with prevalence values below -200mV, that characterise a highly anoxic environment, no correlation was obtained between Eh and THg. Moreover, the sulfides measurements showed averages higher than 200 mg kg^{-1}. As reported in the current literature, such sulfides levels could induce the mercury trapping into insoluble complex that inhibits the bacterial methylation mechanisms (Morel *et al.*, 1998; Moreau *et al.*, 2015). However, in a recent survey on mercury speciation, Wasserman *et al.* (2002) suggested that mercury seems to be available. After treating the sediment with 6 N hydrochloric acid digestion, mercury was recovered in the same levels of the total concentrations reported in a previous work (Veeck, 1999). Also, the authors detected, in the same region, production of dimethyl-mercury triggered by microbial metabolism related with biogeochemical process in the recycle of this metal.

Although the biological parameters levels indicate a strong bacterial activity, the presence of an extensive contamination with metals, mainly zinc (up to 2500 µg g^{-1}) and cadmium (up to 5 µg g^{-1}) (Barcellos *et al.*, 1998; Pellegatti *et al.*, 2001), could inhibit this metabolic process. Earlier data from this region, obtained in 1994 and 1996, showed levels of total Hg rising from 50 to 200 µg g^{-1} (Barrocas & Wasserman, 1998; Veeck, 1999). Seven years later, our data registered THg concentration until three times higher (601µg g^{-1}), suggesting a trend of increasing Hg contamination that could represent a dangerous risk to living organisms including humans.

3.3. SEM/AVS

The SEM/AVS ratio used in this work to evaluate metals (Zn, Cu, Ni, Cd and Pb) bioavailability in the sediments, presented values < 1.0 for all stations (Figure 3), indicating that other toxic metals seem not to affect bacterial activities. However, the use of this negative indicator for sediment toxicity has to be considered with caution when applied to mercury bioavailability. In a previous study, Chen *et al.* (2009) found that excess AVS did not prevent Hg bioaccumulation in benthic fauna. In fact, the higher biotic THg and MeHg concentrations in sites with the excess AVS suggests that the SEM/AVS is not a good predictor of bioavailability for mercury. This report reinforces our hypothesis that in marine sediments, rich in organic matter, the bioavailabiliy of Hg appears to be strongly influenced by organic carbon, that could act as an intermediate factor to the bacterial methylation process. Thus, despite the Hg concentration and SEM/AVS levels, it is important to realize the metals risk analysis for the sediment toxicity including a sequential extraction to determinate the mercury bioavailability.

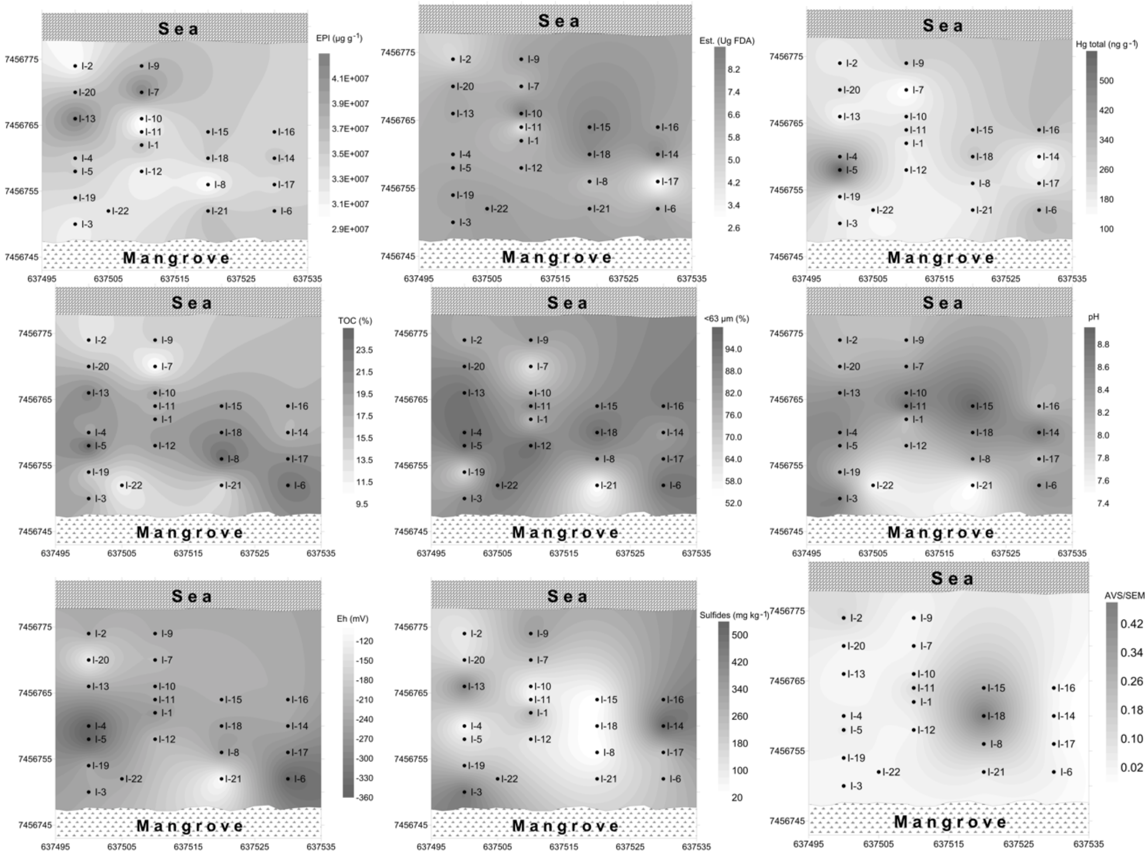

Figure 3

Contour maps of measured variables during the first campaign (February 2001). EPI -bacterial biomass with epifluorescence; Est ~esterase; Hg Total; TOC – total organic carbon; %<63μm – granulometric fraction smaller than 63 μm; pH of the interstitial water; Eh – redox potential of the interstitial water; Sulfides; SEM/AVS – ratio simultaneously extracted metals, acid volatile sulfides.

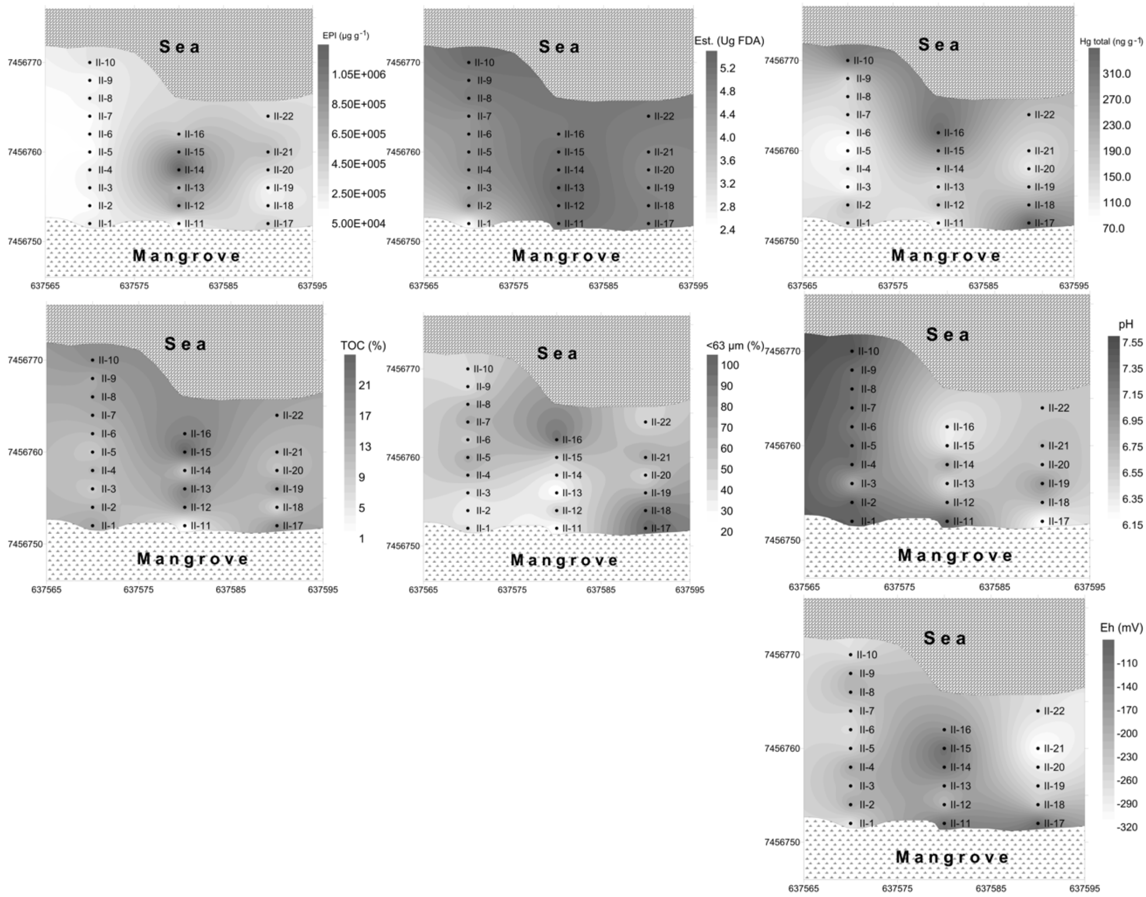

Figure 4
Contour maps of measured variables during the second campaign (February 2002). EPI -bacterial biomass with epifluorescence; Est ~esterase; Hg Total; TOC – total organic carbon; %<63µm – granulometric fraction smaller than 63 µm; pH of the interstitial water; Eh – redox potential of the interstitial water.

4. CONCLUSIONS

In the present study it was shown that, although the mechanisms of transformation of mercury are still unclear, biotic and abiotic elements considered as inductors of the organo-mercury compounds synthesis, are present in the Garças cove sediment. Although this potential conditions for mercury alkylation was present, methyl-mercury formation observed was limited (Wasserman *et al.*, 2002), suggesting the need to expand the studies on biochemical controls for the mercury transformations. The results of this study strengthen the hypothesis that heterogenic spatial patterns were consequence from sampling design, area dimension and high organic matter content that could aggregate the particles in microzones which support different microbial community and metabolic activities. The contour maps confirm these heterogenic patterns. Further, it should be verified if these patterns occur in deeper layers, analyses from sediment cores should be done.

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