

- Godwin, L.S., Eldredge, L.G., 2001. South Oahu Marine Invasions Shipping Study. Bishop Museum Technical Report No. 20.
- Godwin, L.S., Eldredge, L.G., Gaut, K., 2004. The assessment of hull fouling as a mechanism for the introduction and dispersal of marine alien species in the Main Hawaiian Islands. Bishop Museum Technical Report No. 28.
- Grigg, R.W., 2006. The history of marine research in the Northwestern Hawaiian Islands: lessons from the past and hopes for the future. *Atoll Research Bulletin* 543, 13–22.
- Guinette, J.M., Orr, J., Cairns, S., Freiwald, A., Morgan, L., George, R., 2006. Will human-induced changes in seawater chemistry alter the distribution of deep-sea scleractinian corals? *Frontiers in Ecology and the Environment* 4 (3), 141–146.
- Hatcher, B.G., 1984. A maritime accident provides evidence for alternate stable states in benthic communities on coral reefs. *Coral Reefs* 3 (4), 199–204.
- Global Invasive Species Database (IUCN). <<http://www.issg.org/database/welcome>> (accessed 27.10.2006).
- International Comprehensive Ocean–Atmosphere Data Set (ICOADS), 2006. ICOADS home page. <<http://icoads.noaa.gov>> (accessed 13.02.2006).
- Jameson, S.C., Tupper, M.H., Ridley, J.M., 2002. The three screen doors: can marine “protected” areas be effective? *Marine Pollution Bulletin* 44, 1177–1183.
- Jenks, G.F., 1977. Optimal data classification for choropleth maps. Occasional Paper No. 2. University of Kansas, Department of Geography, Lawrence, Kansas.
- Keller, B.D., Jackson, J.B.C. (Eds.), 1993. Long-term Assessment of the Oil Spill at Bahia Las Minas, Panama, Synthesis Report, vol. II: Technical Report Part 1&2. OCS Study MMS 93-0048. US Dept. of Int., Minerals Management Service, Gulf of Mex. OCS Region. Off., New Orleans, LA, 1008 pp.
- Kim, K., Dobson, A.P., Gulland, F.M.D., Harvell, C.D., 2005. Diseases and the conservation of marine biodiversity. In: Norse, E.A., Crowder, L.B. (Eds.), *Marine Conservation Biology*. Island Press, 470 pp.
- McWilliams, J.P., Cote, I.M., Gill, J.A., Sutherland, W.J., Watkinson, A.R., 2005. Accelerating impacts of temperature-induced coral bleaching in the Caribbean. *Ecology* 86 (8), 2055–2060.
- National Oceanic and Atmospheric Administration (NOAA), 2006. Northwestern Hawaiian Islands Proposed National Marine Sanctuary Draft Environmental Impact Statement and Management Plan, vol. II. Honolulu, HI, 375 pp.
- Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.-K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.-F., Yamanaka, Y., Yool, A., 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437, 681–686.
- Pooley, S.G., Pan, M., 2006. Economic research on the NWHI – a historical perspective. *Atoll Research Bulletin* 543, 33–50.
- Rogers, C.S., Garrison, V.H., 2001. Ten years after the crime: lasting effects of damage from a cruise ship anchor on a coral reef in St. John, U.S. Virgin Islands. *Bulletin of Marine Science* 69 (2), 793–803.
- United States Coast Guard (USCG), 2006. United States Coast Guard Maritime Information Exchange Port State Information Exchange. <<http://cgmix.uscg.mil/psix/>> (accessed 08.08.2006).
- Western Pacific Fishery Management Council (WESPAC), 2006. Amendment to the Bottomfish and Seamount Groundfish Fishery Management Plan for the Hawaiian Archipelago. Honolulu, Hawaii, 221 pp. plus appendices.

0025-326X/\$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2007.09.029

Assessment of methylmercury production in a temperate salt marsh (Ria de Aveiro Lagoon, Portugal)

M. Válega^{a,*}, A.I. Lillebø^a, M.E. Pereira^a, W.T. Corns^b, P.B. Stockwell^b,
A.C. Duarte^a, M.A. Pardal^c

^a CESAM and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

^b PSAnalytical Ltd, Arthur House, Crayfields Industrial Estate, Main Road, Orpington, Kent BR5 3HP, UK

^c IMAR–Institute of Marine Research, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal

During the last two decades, restriction rules for the anthropogenic load of mercury have resulted in a substantial decrease of the inputs of this metal into aquatic systems. However the pool of mercury presently in sediments remains a problem due to its potential release to other environmental compartments such as the atmosphere, the overlying water column, and to living organisms through bioaccumulation and/or trophic transfer processes. The potential release of methylmercury from sediments to the overlying water column can harm the environment on a

local or regional scale, particularly in areas highly dependent on fishery activities, endangering the system ecologically and economically as well as causing concern regarding human health.

The importance of salt marshes is recognised worldwide in providing essential ecological functions, such as hydrologic flux and storage, enhancement of metals accretion, high biological productivity, biogeochemical cycling and storage of contaminants, and habitat for fish and wildlife (Richardson, 1999; Mitsch and Gosselink, 2000). However, as transitional areas between land and sea, these ecotones can receive large inputs of pollutants. It is important to understand the mechanism of metals storage in salt marsh sediments and plants to avoid them becoming a metal

* Corresponding author. Tel.: +351 234 370 737; fax: +351 234 370 084.
E-mail address: mvalega@ua.pt (M. Válega).

source to the surrounding areas due to physicochemical and biological processes (e.g. erosion, dredging, early-diagenesis, bioturbation) which may remobilise metals from sediments to the water column (Lee and Cundy, 2001).

Metals that are accumulated in the root system are considered to be phytostabilized, but the potential conversion of inorganic mercury in the sediment due to root activity remains an open question. It is stated that plant roots can interact with the surrounding sediment, exuding oxygen and organic compounds influencing the distribution and availability of trace metals (Alloway, 1995; Mendelsohn et al., 1995).

Several reports have highlighted the potential role of vegetation on mercury speciation and availability, namely by enhancing mercury mobility in sediments: (a) through changes of the redox state in the vegetated sediment; (b) through mercury bio-accumulation into below ground and/or above ground plant tissue; (c) through the mineralisation of senescent plant material, and (d) by enhancing microbial methylmercury production in sediments associated with the root system. Salt marshes, where the redox chemistry of sulphate plays an important role, are among the environments with good conditions for mercury methylation (Hines et al., 1999).

Salt marshes have a high microbial activity, particularly during the warm season, and the redox transitional areas at plants roots appear to be ideal for methylation where organic carbon and sulphate for bacterial respiration are available. However, the presence of high total mercury concentrations is not indicative of high levels of methylmercury. Generally, methylmercury production in sediments is related to the factors that can control the bioavailability of mercury into the sediments and by the

factors that control the activity of bacteria responsible for mercury methylation (Hines et al., 1999). Methylmercury studies in salt marshes are scarce and very recent (Langer et al., 2001; Hung and Chmura, 2006; Kongchum et al., 2006).

The aim of this study was to evaluate the potential role of roots on the conversion of inorganic mercury into methylmercury. The work reports the first methylmercury values for a southern European Atlantic salt marsh and also provides the first insight into the potential role of two widely distributed estuarine salt marsh plant species (*Halimione portulacoides* (L.) Aellen. and *Arthrocnemum fruticosum* (L.) Moq.) in mercury speciation.

The study was conducted in Ria de Aveiro, a temperate, shallow coastal lagoon located along the Atlantic Ocean (45 km long; 10 km wide) on the northwest coast of Portugal (40°38'N, 8°44'W) (Fig. 1) with an wetland area of 83 km² at high tide and 66 km² at low tide. Ria de Aveiro is the most mercury-contaminated coastal system in Portugal. Between 1950 and 1994, a confined area of Ria de Aveiro (Laranjo Bay) received continuous mercury discharges from a chlor-alkali plant located in a industrial complex nearby Estarreja (Fig. 1). Although a fraction of this mercury has dispersed through the estuarine system, a considerable amount (25 tons) was estimated to be stored in the sediments of Laranjo Bay (Pereira et al., 1998). This area has extensive intertidal flats and salt marshes, with monotypic stands of *H. portulacoides* and *A. fruticosum*.

Three sediment core samples of 12 cm depth were collected in August 2005 during ebb tide in monotypic stands of *H. portulacoides* and *A. fruticosum* and in the adjacent sediment without vegetation (at a distance of approximately 20 m). Above ground plant materials were

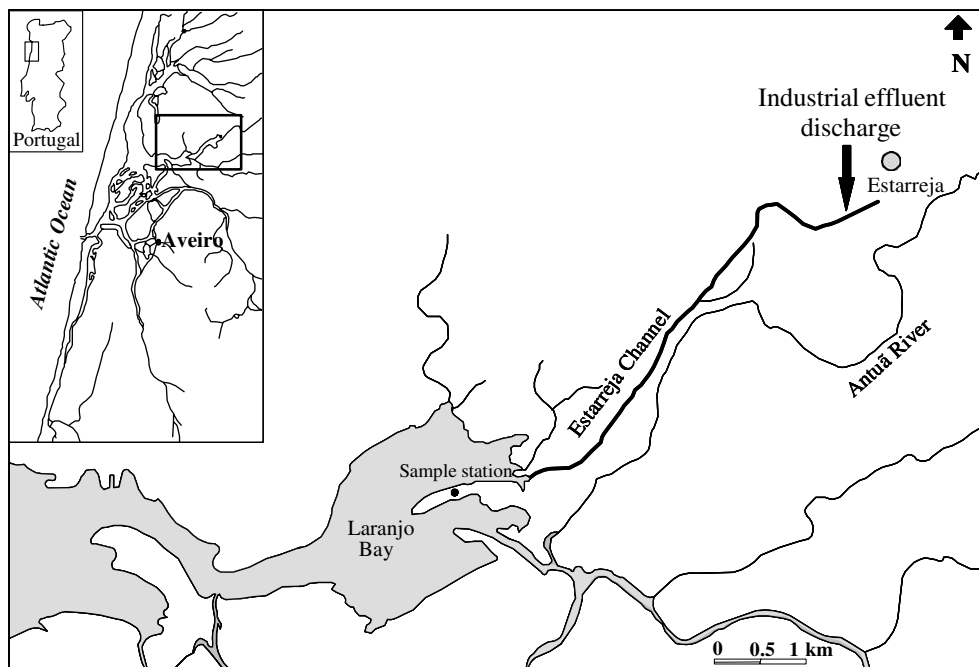


Fig. 1. Location of the Ria de Aveiro and the sampling site.

also sampled. This sampling design allowed the assessment of the effect of plants on mercury speciation. August was the selected month due to known temperature dependency of bacterial activity; i.e., maximum bacterial activity is achieved with higher temperatures (Ullrich et al., 2001). Thus, the first 12 cm was chosen for the evaluation since the root system is more active in this region, and the oxic/anoxic interface has been recognised as the most important area for mercury methylation (Ullrich et al., 2001). Above ground biomasses of the two studied plants were also estimated. Sediment cores were sliced in the field into 1 cm sections, transported to the laboratory under refrigerated conditions, and immediately frozen (-20°C). Prior to sediment segmentation, *in situ* redox potential (Eh) and pH were measured (six independent measurements) in each layer. In the laboratory, sediment samples were homogenised, freeze-dried and sieved (1 mm) in order to eliminate roots and other debris. Roots were separated from the sediment and washed with distilled water to assure that no sediment particles remained. Plant material (roots and above ground biomass) were also freeze-dried. After this process all samples were kept in the refrigerator under dark conditions until analysis.

Sediment samples were analysed for water content (wet weight minus dry weight; 120°C for 24 h), organic matter content as percentage of loss on ignition (%LOI) (dry weight minus ash free dry weight; 500°C for 4 h), and for percentage of fine particles after wet sieving of dried sample through a $63\ \mu\text{m}$ mesh size sieve.

Total mercury concentrations in sediments and roots were directly analysed by atomic absorption spectrometry (AAS) with thermal decomposition, using an advanced mercury analyser (AMA) LECO 254, according Costley et al. (2000). The accuracy and precision of the analytical methodology for mercury determinations was assessed by the analysis of certified materials, BCR 60 (trace elements in an aquatic plant), NRC PACS 2 and NRC Mess 3 (trace elements in estuarine sediment) every day. Certified and measured values were in general agreement, varying the recovery efficiency between 94–110%, 97–107%, 90–95% for PACS 2, Mess 3 and BCR 60, respectively.

Methylmercury was extracted from the sediments according to Cai et al. (1997) and quantified by capillary GC-AFS. This integrated gas chromatography – mercury atomic fluorescence spectrometer (P.S. Analytical Ltd., UK) comprised an Agilent Technologies gas chromatography, model (6890) – equipped with an autosampler coupled to a PSA Merlin detector via a pyrolysis unit (PSA 10.750). A fused silica analytical column ($15\ \text{m} \times 0.53\ \text{mm}$ ID-Megabore) coated with a $1.5\ \mu\text{m}$ film thickness of DB-1 (J&W Scientific) was used. The accuracy and precision of the analytical methodology for methylmercury determinations was assessed by replicate analysis of certified materials, CRM-580 (estuarine sediment) and methylmercury spikes. Recovery of methylmercury spikes varied between 73–91% for sediments and 100–105% for plant biomass.

All Laranjo Bay sediments consisted of a mixture of sand and mud. Considering the percentage of fine particles ($53.9 \pm 10.6\%$), there were no statistically significant differences between vegetated and adjacent non-vegetated sediments ($P > 0.05$). Nevertheless, organic matter content (%LOI) was significantly higher in vegetated sediments (mean value for the whole core, $22.5 \pm 3.2\%$) than in non-vegetated (mean value for the whole core, $8.7 \pm 0.5\%$) ($P < 0.05$). Vegetated sediments were generally more acidic ($P \leq 0.001$, Mann-Whitney rank sum test), and presented a significantly higher redox potential (Eh) ($P = 0.003$, Mann-Whitney rank sum test) than the adjacent non-vegetated sediments (Fig. 2).

Salt marsh plants are able to aggregate sediment by acting as sediment traps, and this may constitute the main source of mercury through the settlement of suspended particulate matter. In the present study, vegetated sediments were 1.4 times more contaminated than adjacent non-vegetated sediments which is an indication that this salt marsh contributes to the retention of mercury even although the mercury discharges in the lagoon have ceased. Concentrations of total mercury in the roots (Table 1) were approximately two times higher than the concentrations found in vegetated sediments (Fig. 3), which indicates bioaccumulation of mercury by both studied plants.

Total mercury concentrations in the above ground biomass (Table 1) were comparatively low, suggesting that in both plants mercury is mainly cycled between sediment and roots and only a small fraction is translocated to the above ground portions of the plant.

No other organic mercury species rather than methylmercury were found in the sediments and plants. Methylmercury concentrations of $16.2\text{--}56.4\ \text{ng g}^{-1}$ were found in sediments of *H. portulacoides* and $10.2\text{--}75.8\ \text{ng g}^{-1}$ for *A. fruticosum*, while in non-vegetated sediments the values ranged between 1.2 and $14.7\ \text{ng g}^{-1}$ (Fig. 3). According to Langer et al. (2001), during daylight the first 5 cm are potential zones for methylmercury production in estuarine sediments because of high sulphate reducing rates and relatively low sulphide concentrations. In fact, this is observed in the non-vegetated sediments as opposed to the vegetated sediments, probably because of the activity of the root system. Although values found in the vegetated sediments can be considered high according to Ebinghaus and Wilken (1996), they correspond to low percentages of methylmercury (Fig. 4). Heyes et al. (2006) showed for several ecosystems (Chesapeake Bay, Guanabara Bay, Gulf of Trieste, Lavaca Bay, Hudson River, Tampa Bay and Florida Bay) a lack of direct dependence between methylmercury and total mercury concentrations in sediments. In fact, in that study, the highest percentages of methylmercury were found in sediments with lower concentrations of total mercury. The difference in the mean values of the sediments colonised by the two studied plants is not significantly different ($P = 0.480$, Mann-Whitney rank sum test), while the difference in the mean values of the vegetated sediments and non-vegetated sediments is significantly

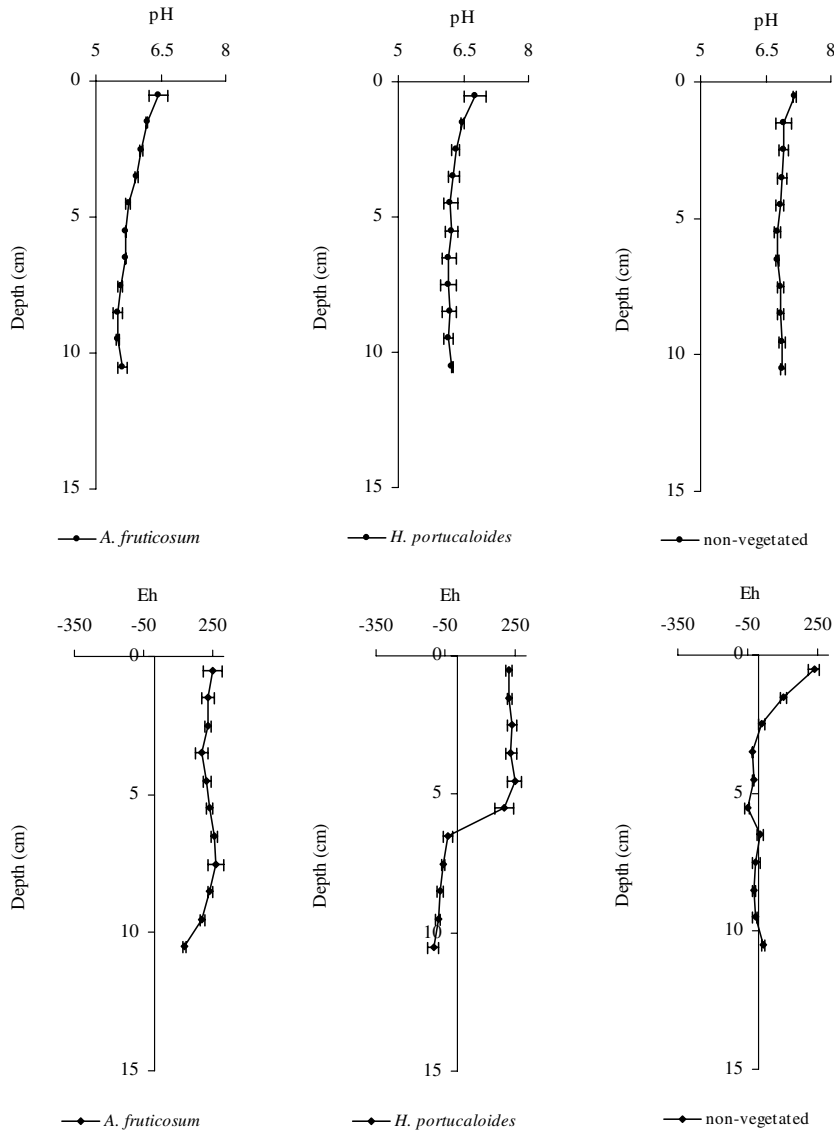


Fig. 2. Eh (mV) and pH values (mean \pm standard deviation) of Laranjo Bay salt marsh sediments colonised by *H. portulacoides* and *A. fruticosum* and in non-vegetated sediments.

different ($P < 0.001$, Mann-Whitney rank sum test). Kongchum et al. (2006) reported for freshwater marshes percentages of 3% for methylmercury while lower values were found in salt marshes (1.7%). Lower methylmercury ratios may be related to the higher concentrations of sulphate and consequently higher concentration of sulphides resulting from sulphate reduction, which can restrict the formation of methylmercury due to the reduction of available mercury substrate (Gilmour and Henry, 1991). According to Gilmour et al. (1998) sulphide, as a result of sulphate reduction, is considered to be most effective in reducing mercury bioavailability for methylation processes.

Of the comparatively small amounts of mercury that reach the above ground biomass, a significant fraction is transformed to methylmercury since the percentage values of methylmercury were considerable high: 17% was found in the stems of *H. portulacoides* and 14% in the shoots of *A. fruticosum* (Table 1). These values are in agreement with

the studies performed by Heller and Weber (1998) in the Great Bay estuary (NH–USA) where high methylmercury percentages were found in aerial portions of *Spartina alterniflora* (6.23–48.1%). Methylmercury concentrations found in the roots were high, but corresponded only to 0.7% and

Table 1

Total mercury concentrations (ng g^{-1}) and methylmercury (MeHg, ng g^{-1}) in above ground biomass and roots of *H. portulacoides* and *A. fruticosum* collected at Laranjo Bay salt marsh

	[Hg] (ng g^{-1})	[MeHg] (ng g^{-1})
<i>A. Fruticosum</i>		
Shoots	95.9	13.3
Roots	11.35	80.6
<i>H. portulacoides</i>		
Stem	26.5	4.4
Leaves	54.1	<LOD
Roots	14.77	73.1

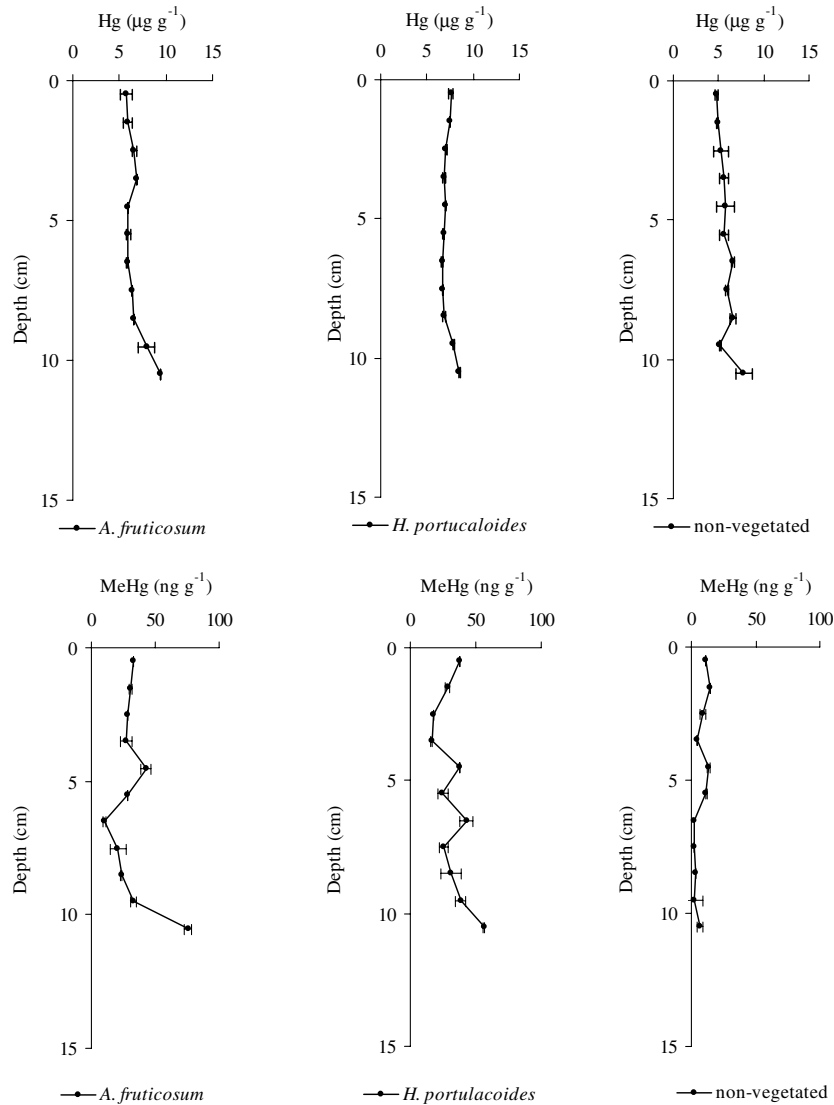


Fig. 3. Total mercury concentrations ($\mu\text{g g}^{-1} \pm$ standard deviation) and methylmercury (MeHg, $\text{ng g}^{-1} \pm$ standard deviation) in Laranjo Bay salt marsh sediments colonised by *H. portulacoides* and *A. fruticosum* and in non-vegetated sediments.

0.5% of the total mercury for *A. fruticosum* and *H. portulacoides*, respectively. Since the salt marsh vegetation areas is extensively used for agricultural purposes and grazing areas for cattle, its contamination is an environmental and human health concern.

As methylmercury is the most toxic mercury compound to living organisms, future research should evaluate the ecological and toxicological implications of methylmercury fluxes from pore water to the water column, and its exposure to living organisms. Because the salt marsh areas are vegetated sediments with lower pH values compared to non-vegetated sediments, desorption processes of methylmercury could possibly take place with subsequent increases of methylmercury in pore water, as discussed by Ullrich et al. (2001). Holmes and Lean (2006) describe sediment-water fluxes of methylmercury between $1.6\text{--}10.02 \text{ ng m}^{-2} \text{ day}^{-1}$ for a freshwater marsh. Although *H. portulacoides* and *A. fruticosum* marshes in Ria the Aveiro presented similar methylation ratios at the root system,

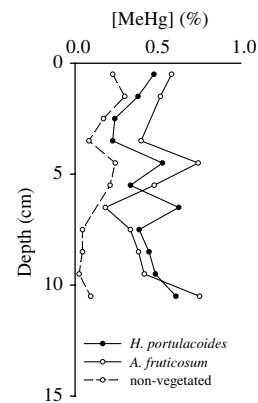


Fig. 4. Values of methylmercury (MeHg, %) in Laranjo Bay salt marsh sediments colonised by *H. portulacoides* and *A. fruticosum* and in non-vegetated sediments.

other salt marsh plants with more distinct physiology, depth of active roots, life cycle, and tolerance to heavy

metals contamination (e.g. *Phragmites australis*, *Scirpus maritimus* and *Juncus maritimus*), may provide evidence of the distinct interactions between plant, bacteria and mercury and on mercury methylation and availability. Thus possible plant species specific processes should also be assessed.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) through a Ph.D grant number SFRH/BD/18682/2004 (M. Válega). The authors would like to acknowledge PS Analytical Ltd (UK) for the kindness in offering all the laboratory facilities and conditions to perform the methylmercury analysis.

References

- Alloway, B.J., 1995. Trace Metals in Soils. Chapman and Hall, Great Britain, pp. 11–35.
- Cai, Y., Jaffe, R., Jones, R., 1997. Ethylmercury in the soils and sediments of the Florida Everglades. *Environmental Science & Technology* 31 (1), 302–305.
- Costley, C., Mossop, K., Dean, J., Garden, L., Marshall, J., Carroll, J., 2000. Determination of mercury in environmental and biological samples using pyrolysis atomic absorption spectrometry with gold amalgamation. *Analytica Chimica Acta* 405, 179–183.
- Ebinghaus, R., Wilken, R.-D., 1996. Mercury distribution and speciation in a polluted fluvial system. In: Calmano, W., Förstner, U. (Eds.), *Sediments and Toxic Substances: Environmental Effects and Ecotoxicity*. Springer Verlag, Berlin Heidelberg, pp. 215–244.
- Gilmour, C.C., Henry, E.A., 1991. Mercury methylation in aquatic systems affected by acid deposition. *Environmental Pollution* 71, 131–169.
- Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A., Stordal, M.C., 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry* 40, 327–345.
- Heyes, A., Mason, R.P., Kim, E.-H., Sunderland, E., 2006. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. *Marine Chemistry* 102 (1–2), 134–147.
- Hines, M., Evans, R.S., Genthner, B.R.S., Willis, S.G., Friedman, S., Rooney-Varga, J., Devereux, R., 1999. Molecular phylogenetic and biogeochemical studies of sulfate-reducing bacteria in the rhizosphere of *Spartina alterniflora*. *Applied Environmental Microbiology* 65 (5), 2209–2216.
- Holmes, J., Lean, D., 2006. Factors that influence methylmercury flux rates from wetland sediments. *Science of the Total Environment* 368, 306–319.
- Hung, G.A., Chmura, G.L., 2006. Mercury accumulation in surface sediments of salt marshes of the Bay of Fundy. *Environmental Pollution* 142 (3), 418–431.
- Kongchum, M., Devai, I., DeLaune, R.D., Jugsujinda, A., 2006. Total mercury and methylmercury in freshwater and salt marsh soils of the Mississippi river deltaic plain. *Chemosphere* 63 (8), 1300–1303.
- Langer, C.S., Fitzgerald, W.F., Visscher, P.T., Vandal, G.M., 2001. Biogeochemical cycling of methylmercury at Barn Island Salt Marsh, Stonington, CT, USA. *Wetlands Ecology and Management* 9, 295–310.
- Lee, S.V., Cundy, A.B., 2001. Heavy metal contamination and mixing processes in sediments from the Humber Estuary, eastern England. *Estuarine, Coastal and Shelf Science* 53, 619–636.
- Mendelsohn, I., Kleiss, B., Wakeley, J., 1995. Factors controlling the formation of oxidized root channels in wetland plants: a review and annotated bibliography. *Wetlands* 15, 37–47.
- Mitsch, W.J., Gosselink, J.G., 2000. *Wetlands*, third ed. John Wiley & Sons, Inc., USA.
- Pereira, M.E., Duarte, A.C., Millward, G.E., Vale, C., Abreu, S.N., 1998. Tidal export of particulate mercury from the most contaminated area of Aveiro's lagoon, Portugal. *Science of the Total Environment* 213, 157–163.
- Richardson, C.J., 1999. Plenary session presentation: ecological functions of wetlands in the landscape. In: Lewis et al. (Eds.), *Ecotoxicology and Risk Assessment for Wetlands*. SETAC Press, Florida-USA, pp. 9–25.
- Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. *Critical Reviews in Environmental Science and Technology* 31 (3), 241–293.

0025-326X/\$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2007.09.033

Tracing sewage pollution in the Pearl River Delta and its adjacent coastal area of South China Sea using linear alkylbenzenes (LABs)

Xiao-Jun Luo^{a,*}, She-Jun Chen^{a,b}, Hong-Gang Ni^{a,b}, Mei Yu^{a,b}, Bi-Xian Mai^{a,b}

^a State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

^b Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

LABs with a C₁₀–C₁₄ normal alkyl chain are the raw material for the synthesis of linear alkylbenzene sulfonates.

Residue LABs in the linear alkylbenzene sulfonates have been used as markers of municipal wastewater to monitor sewage impacts on fluvial and coastal environments in many regions (Eganhouse et al., 1983; Eganhouse and Sherblom, 2001; Hartmann et al., 2000; Heim et al., 2004; Isobe et al., 2004; Macias-Zamora and Ramirez-

* Corresponding author. Tel.: +86 20 85290146; fax: +86 20 85290706.
E-mail address: luoxiaoj@gig.ac.cn (X.-J. Luo).