Universidade de Aveiro Departamento de Química
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Mónica Susana Gonçalves de Almeida Válega

Mobilidade, acumulação e transformação do mercúrio em sapais

Partition, accumulation and speciation of mercury in salt marshes

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Partition, accumulation and speciation of mercury in salt marshes

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Química, realizada sob a orientação científica da Doutora Maria Eduarda Pereira, Professora auxiliar do Departamento de Química da Universidade de Aveiro e do Doutor Miguel Ângelo Pardal, Professor associado com agregação do Departamento de Zoologia da Faculdade de Ciências e Tecnologia da Universidade de Coimbra

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(SFRH/BD/18682/2004)

… to my lovely daughter

o júri

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palavras-chave

Sapais, mercúrio, metilmercúrio, sedimentos, halófitas, Ria de Aveiro, Largo do Laranjo.

resumo

Com o trabalho desenvolvido nesta tese pretende-se contribuir para um melhor conhecimento do ciclo do mercúrio em sapais; como tal diversos aspectos relacionados com o ciclo biogeoquímico do mercúrio neste tipo de ecossistemas foram abordados, nomeadamente: o impacto que as descargas de mercúrio tiveram nos sapais da baía do Largo do Laranjo bem como o processo de recuperação do sapal (temporal e espacial) pela análise da diversidade do numero de espécies das plantas de sapal e das concentrações de mercúrio no sedimento ao longo do anos; a mobilidade do mercúrio em sedimentos de sapal colonizados pela espécie *Halimione portulacoides*, a sua distribuição nas camadas de sedimento e a sua incorporação na biomassa subterrânea da planta bem como a potencial exportação de mercúrio do sapal para as áreas adjacentes; o papel das plantas de sapal na conversão de espécies inorgânicas de mercúrio em espécies orgânicas; os processos bioquímicos por detrás do mecanismo de tolerância ao mercúrio da *H. portulacoides*; avaliação do potencial de uma planta de sapal muito difundida ao longo da costa Portuguesa como biomonitor da contaminação de mercúrio em sedimentos de sapal.

A análise dos resultados revelou que as descargas de mercúrio nos sapais da baía do Largo do Laranjo ocorridas no passado induziram uma diminuição na diversidade florística, conduzindo-o a um estado alternativo com a predominância de apenas uma espécie, nos anos de maiores descargas. Após o fim das descargas podemos concluir com base na diversidade do sapal que o sistema demonstra histerese na sua recuperação vindo a recuperar lentamente a sua diversidade florística ao longo dos anos.

Em ralação à mobilidade o mercúrio em sedimentos de sapal verificou-se que as taxas de *turnover* da biomassa subterrânea eram mais elevadas do que as que da biomassa aérea indicando uma maior mobilidade do mercúrio na rizosfera. Tendo em conta o *pool* de mercúrio encontrado na biomassa aéra verificou-se que a exportação de macro detritos da planta não é significativa para o balanço de mercúrio.

Os estudos de especiação de mercúrio realizados nos sedimentos e na biomassa demonstraram que nenhuma outra espécie de mercúrio orgânico foi encontrada para além do MeHg. As concentrações de MeHg nos sedimentos colonizados revelaram-se mais elevadas no entanto as percentagens relativamente aos valores de mercúrio total são baixas. Os resultados sugerem que as plantas de sapal contribuem para a metilação de mercúrio.

Os mecanismos de tolerância da *H. portulacoides* ao mercúrio envolvem essencialmente a sua imobilização nas paredes celulares, contudo o sequestro de mercúrio intracelularmente por fitoquelatinas foi demonstrado neste trabalho.

Finalmente demonstrou-se que para além da *H. portulacoides* ser um bom bioindicador pode também ser usada com um biomonitor da contaminação de mercúrio em sedimentos de sapal.

keywords

Salt marshes, mercury, methylmercury, sediments; halophytes, Ria de Aveiro, Laranjo bay.

abstract

This thesis intends to contribute to the better understand of mercury cycling in salt marsh ecosystems and thus several aspects regarding mercury biogeochemical cycle in salt marshes are discussed, namely the impact of mercury discharges and the recovery processes (temporally and spatially) by the examination of the richness of the species of salt marsh plants and mercury concentrations in sediments over the years; the mobility of mercury in a salt marsh colonised by the species *H.* and its redistribution in the sediment layers containing plants and subsequently incorporation into below ground biomass, as well the potential export of mercury from the salt marsh to the adjacent areas; the potential role of salt marsh plants on the conversion of inorganic mercury into organic mercury species; the biochemical processes behind mercury tolerance in a salt marsh plant species; the evaluation of the potential role of a well wide distribute salt marsh plant (*H. portulacoides*) along the Portuguese coast as biomonitor of mercury contamination.

The results showed that salt marshes of Laranjo bay shows how a considerable loading of mercury into a salt marsh for four decades has affected its resistance, inducing a change from salt marsh plants species richness into an alternative state dominated by one species. Ten years after the cessation of the loading of mercury and based on the salt marsh plants species richness, the system still shows an incomplete resilience due to the lag in recovery, named hysteresis.

With respect to the mercury mobility in the salt marsh sediments, the results shows that the turnover rates for below ground biomass were higher than those observed for above ground biomass, corresponding to higher mercury mobility within *H. portulacoides* rhizosphere. Taking into account the pool of mercury in above ground biomass, the export of mercury by macro-detritus is not significant for the mercury balance in the studied system.

Regarding the mercury speciation studies, no other organic mercury species rather than MeHg were found. MeHg concentrations in the vegetated sediments were higher than in non-vegetated sediments and although the percentages in the sediments were low, comparatively to the total mercury the results suggest that salt marsh plants contribute to methylation in sediments.

Mercury tolerance strategies of *H. portulacoides* seem to involve root cell wall immobilization as a major mechanism of metal resistance, rather than metal chelation in the cytosolic fraction. Intracellular mercury sequestration by PCs in the environment was also demonstrated in this work;however mercury chelation in environmental exposures seems to be a complexed, involving the formation of different types of complexes

Finally it was demonstrated that that besides *H. portulacoides* can be a suitable bioindicator, can also be used as a biomonitor for mercury pollution. Leaves responded following a positive linear model for a contamination range, while roots responded within the sigmoidal model. *H. portulacoides* may be considered an appealing tool for mercury pollution assessment in salt marshes.

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List of Acronyms

AAS - Atomic Absorption Spectroscopy

AVS - Acid Volatile Sulphide

CV-AFS - Cold Vapour Atomic Fluorescence Spectroscopy

LOD - Limit of Detection

LOI - Lost On Ignition

F-AAS - Flame Atomic Absorption Spectroscopy

GC-AFS- Gas Chromatography Atomic Fluorescence Spectroscopy

PCs - Phytochelatins

RP-HPLC - Reverse phase High Pressure Liquid Chromatography

SPM - Suspended Particulate Matter

1 Introduction

Pollution is a large concept used to define environmental damages usually caused by wastes discharges in the environment. Marine pollution results from the anthropogenic introduction, directly or not, of substances or energy into the marine environment. Some questions arises when pollution issues are discussed, namely what kind of pollutant, the type of sources and the deleterious effects caused in the ecosystems, especially in their respective organisms (plants and/or animals). Another issue not least important and that should must be taken into account is what is being done to reduce or remove the undesirable effects and what would be the consequences after sources elimination (Clark, 2001).

Due to their natural features, most of the large cities around the world are located nearby marine environments, namely estuaries, which made them vulnerable to anthropogenic discharges of all types of pollutants. The increase of anthropogenic emissions of metals in the environment has exposed populations to toxic levels of contaminants, especially through their diet and drinking water (Gailer, 2007). Metals can enter into the aquatic systems through atmospheric and land based effluent sources and can be classified as essential metals when are required for metabolic processes and non-essential metals or metalloids when none metabolic function is known. In general at high levels all metals are considered to be toxic, even essential metals; however mercury, cadmium, silver, nickel, selenium, lead, copper, chromium, arsenic and zinc (in order of toxicity) are among the most hazardous to marine life (Islam and Tanaka, 2004).

Mercury pollution is widely recognised as a growing risk to environment and public health. Mercury is a global pollutant and one which has been shown to have severe effects on organism health both as a neurotoxin and an endocrine disruptor. Occurring naturally in the environment, it is estimated that two thirds of the mercury in the biosphere is derived from anthropogenic sources (Mason et al., 1994). Due to its high industrial value, mercury was widely used in the past in several industrial processes which led to its effective dispersion in the environment. Adverse effects of mercury pollution are well recognised and still continue to prompt scientific investigations trough the years regarding important aspects like environmental sources, biogeochemistry, fate and effects, which made mercury issues a continuing scientific challenge (Wiener et al., 2003). Mercury fate and biogeochemistry in ecosystems is a topic of wide interest in the field of environmental toxicology and chemistry. Mercury chemistry and speciation is a diverse field and of interest to a wide range of researchers.

Restriction rules for anthropogenic loads of mercury during the last decades have resulted in the substantial decrease of its inputs into the aquatic systems. According to European community directives (82/176/EEC) the regulatory threshold value for mercury on chlor alkali plants effluents is 50 μg L^{-1}. Mercury pools in several environmental compartments remains a problem, especially in sediments where concentrations are usually higher. The potential release of mercury from sediments to the water column and to living organisms through bioaccumulation and/or trophic transfer processes can have a high impact in a local or a regional scale, particularly in areas highly dependent on fishery activities, endangering the system ecologically and economically with human health repercussions.

The importance of salt marshes is worldwide recognised and very well described in bibliography for providing several vital ecological functions; however, as transitional areas between the land and sea these ecotones are subject to large inputs of pollutants in consequence of all urban and industrial development. From an ecological point of view it is vital to understand the mechanisms of pollutants storage, namely metals, in salt marsh sediments and plants to avoid that they may become a metal source to the surrounding areas due to physicochemical and/or biological processes (e.g. erosion, dredging, early-diagenesis, bioturvation) which may remobilise metals from sediments to the water column (Lee and Cundy, 2001).

The research work of this thesis is focused essentially in the Ria the Aveiro Lagoon which is as an important wetland classified as “ZPE-Ria de Aveiro” (special protection area for wild birds, Decreto-Lei nº 384-B/99 de 23 de Setembro) since 1999.

Ria de Aveiro is a costal lagoon located in the northwest coast of Portugal (40°38’N; 8°44W). With an extensive area of approximately 45 Km length and 8.5 Km wide (maximum value), it is comprised by four main branches (Ovar, Murtosa, Vagos and Mira) and connects with the sea only by a very narrow artificial channel (approximately 1.3 Km long, 350m wide and 20 m depth). An irregular and complex geometry with narrow channels and an extensive area of mudflats and salt marshes (wetlands) (83 km^2-high tide and 66 km^2-low tide) are the its main features. It is a mesotidal system, where tides are semi-diurnal and propagate from the mouth to the inner lagoon areas. The tides are semidiurnal with a minimum tidal range of 0.6 m (neap tides) and a maximum tidal range of about 3.2 m (spring tides) (Dias et al., 2000). In general the depth of the lagoon is lower than 3 m, with the exception of the navigation channel were the

depths can reach 7 m dues to the efforts of dredging operations, in the upper areas of the lagoon the depth is about 1m. Ria de Aveiro is supplied with freshwater by two major rivers (Antuã and Vouga rivers) and the influence of sea water is more significantly than the freshwater. The mean river discharge during a tidal cycle is about 1.8 x 10^6 having the Vouga River a higher contribution (approximately 60%) (Dias et al., 2000).

1.1 Ria de Aveiro and mercury pollution

Like the major estuaries around the world, Ria de Aveiro is subject to pressures caused by the industrial and urban development in its vicinities. The major source of pollution of Ria de Aveiro was identified as being an industrial chemical-complex located nearby Estarreja where it was installed a chlor-alkali plant that used mercury cells in the past in its industrial processes. Ria de Aveiro is known for its problematic with mercury contamination which is largely documented since the middle of the 80s until nowadays (e.g. Hall et al., 1985; Pereira et al., 2009).

Ria de Aveiro (Figure 1.1) is one of the most mercury-contaminated aquatic systems in Europe. Between 1950 and 1994, Ria de Aveiro received continuous discharges of untreated mercury-rich effluents, mainly from a chlor-alkali plant located in the chemical complex of Estarreja. Subsequently, mercury-rich effluents were dispersed into the system, mainly in the Estarreja Channel and in the Laranjo Bay (Figure 1.2) and its salt marshes. In 1998 Pereira et al. estimated that 33 tonnes of mercury were buried in the sediments of the Lagoon being approximately 25 tons of mercury stored in the Laranjo Bay.

Figure 1.1- Map of Ria de Aveiro with the identification of the principal channels.

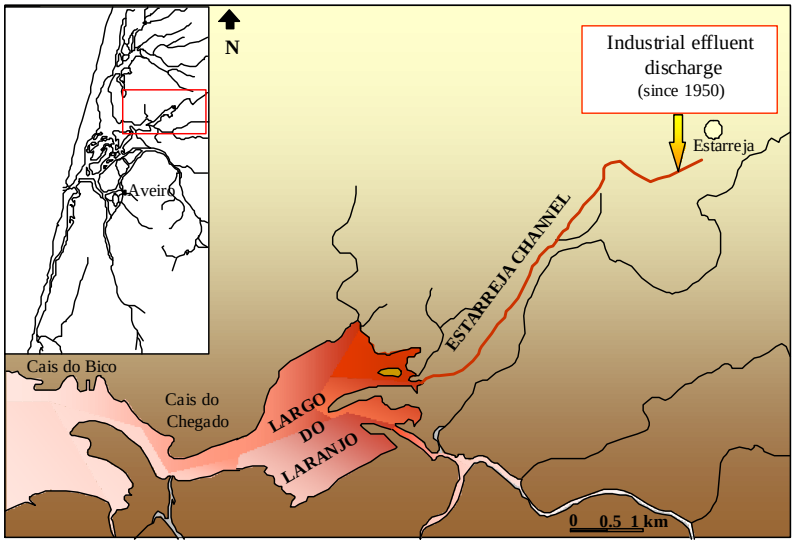


Figure 1.2- Mercury rich effluent discharges in the Ria de Aveiro.

Since the middle of 80s the impact of mercury contamination has been reported in different compartments, biotic and abiotic, of Ria de Aveiro. The first studies regarding mercury contamination in Ria de Aveiro were performed by Hall and others (1985) and Lucas and others (1986) and reported extremely high values of mercury concentrations in the superficial sediments especially in the area comprised between the Estarreja channel (15 e 435 µg g^{-1}) and the Laranjo bay (10 a 100 µg g^{-1}). Significant lower values were found outside this area, e.g. in Cais do

Chegado were concentrations reached 5 μg g^{-1}. More recent studies (e.g. Ramalhosa et al., 2001; Micaelo et al., 2003) concluded that the mercury hotspots remained, although mercury concentrations in the superficial sediments have been reduced and the highest concentrations were found in deepest sediments layers of Estarreja channel and Laranjo bay.

With respect to mercury studies in biota, the studies concluded that organisms collected in Laranjo bay have higher values of mercury in their tissues compared to other collected in other areas. According to Abreu et al. (2000), sea bass presented higher values in Laranjo bay (0.2-1.7 μg g^{-1}) from those captured in other zones of Ria de Aveiro (0.03-0.7 μg g^{-1}). The same pattern was observed for the bivalve *Scrobicularia plana* (0.7±0.2 μg g^{-1} in Laranjo bay and 48±9 ng g^{-1} in other areas (Coelho et al., 2006) and for *Carcinus maenas* where values of Laranjo bay ranged between 0.03-0.63±0.2 μg g^{-1} and 0.04-0.18±0.2 μg g^{-1} outside Laranjo bay (Pereira et al., 2006). Attending to mercury contamination levels within the Laranjo bay, Human consumption of *Scrobicularia plana*, *Carcinus mae*nas and *Dicentrarchus labrax* captured within Laranjo bay represents a risk for human health (Pereira et al., 2009).

The exportation of mercury from Laranjo bay to the entire lagoon (Pereira et al., 1998) and more recently between the lagoon and the adjacent costal waters (Pato et al., 2008) has also been assessed. Pereira *et al*. (1998) estimated that approximately 69 Kg of mercury associated to suspended particulate matter were exported from the Laranjo bay to the entire lagoon and more recently, according to Pato et al., 2008 the exportation of dissolved and particulate mercury to the Atlantic Ocean was estimated in the range of 42-77 Kg per year, having little impact in the coastal waters. Based on the actual knowledge of mercury contamination in Ria de Aveiro, all attentions are focused on Laranjo bay, where mercury seems to be strongly associated to the solid fraction of the sediments. Therefore, as long as sediments of the most contaminated areas remain undisturbed by dredging activities, it does not present a risk to the near shore environment. The main concern for mercury cycling in the Ria is the re-suspension of contaminated sediments by storm events and high-energy phenomena (Pereira et al., 2009).

1.2 Objectives and aims of the study

The main objective of the research was to increase the knowledge on mercury cycling in salt marshes and its redistribution in the different environmental compartments. Several aspects regarding mercury biogeochemical cycle and its potential effects in these ecotones were

therefore questioned and scientific explanations are purposed. The most important objectives of the study were:

- To investigate the resilience of the salt marsh vegetation, here defined as ‘‘the ability of an ecosystem to return to its original state after being disturbed”, through a passive recovery.
- To establish the dynamic of mercury within a temperate salt marsh contaminated by mercury
- To evaluate of the potential role of roots on the conversion of inorganic mercury into methylmercury.
- To investigate the biochemical processes behind mercury tolerance in salt marsh plants.
- To investigate the potential use of salt marsh plants as biomonitors tools for mercury pollution assessment.

To a better understand, this thesis is organised in eight chapters. **Chapter I** and **Chapter II** are introductory chapters where it is given general concepts involved in the entire work. Analytical methodologies adopted in this work as well samples treatment and quality control are described in detail in **Chapter III**.

Chapter IV investigates the impact of mercury discharges and the recovery processes (temporally and spatially) by the examination of the richness of the species of salt marsh plants and mercury concentrations in sediments over the years. To accomplish these objectives two approaches were used: a) a spatial/horizontal assessment following the contamination gradient and the change in the diversity of salt marsh plants (salt marsh species richness) as a function of distance from the mercury source and b) a temporal/vertical assessment to track the temporal changes in the richness of the salt marsh species as a function of mercury loading.

The study described in **Chapter V** study intends to increase the knowledge on the mobility of mercury in a salt marsh colonised by *Halimione portulacoides* (L.) Aellen (Caryophyllales: Chenopodiaceae), its redistribution in the sediment layers containing plants and its incorporation into below ground biomass. For these purposes, mercury pools in *Halimione portulacoides*

biomass and in sediments were assessed, as well the potential export of mercury from the contaminated salt marsh to the adjacent areas. Thus, this study provides important data about the dynamic of mercury inside the salt marsh, comprising the below ground and the above ground system.

In **Chapter VI** the problematic of methylmercury in the environment is explored and it is evaluated the potential role of salt marsh plants on the conversion of inorganic mercury into organic mercury species, namely methylmercury. For this purposes two different salt marsh plants were studied, *Halimione portulacoides* and *Sarcocornia perennis* and methylmercury concentration were assessed in the sediments, belowground biomass and above ground biomass.

After mercury accumulation in plants, a new issue arises that has to do with biochemical processes behind mercury tolerance. **Chapter VII** discusses the role of phytochelatins in these processes. With this purpose, two fractions of mercury were separated: buffer-soluble (mainly cytosolic) and insoluble mercury (mainly associated with membranes and cell walls). The amount of both fractions of metal was compared and related to metal distribution within plant organs. It was assessed if the tolerance of this species was associated with the induction of metal chelation by phytochelatins. With this purpose, protein-mercury complexes were isolated and analysed for their thiol content.

In **Chapter VIII** it is discussed the potential role of *H. portulacoides* as biomonitor tool for mercury contamination assessment of salt marsh sediments. This study covers the entire Portuguese coast where the *Halimione portulacoides* species can be found, comprising eight estuaries with different degrees of mercury contamination.

This research resulted in the following scientific publications, which will be the basis of this dissertation:

Válega, M., Lillebø, A.I., Pereira, M.E., Duarte, A.C. Pardal, M.A., 2008. Long-term effects of mercury in a salt marsh: hysteresis in the distribution of vegetation following recovery from contamination. Chemosphere, 71, 765-772.

Link: http://dx.doi.org/10.1016/j.chemosphere.2007.10.013

Válega, M., Lillebø, A.I., Pereira, M.E., Corns, W.T., Stockwell, P.B., Duarte, A.C., Pardal, M.A., 2008. Assessment of methylmercury production in a temperate salt marsh (Ria de Aveiro Lagoon, Portugal). Marine Pollution Bulletin/Baseline, 56, 136-162.

Link: http://dx.doi.org/10.1016/j.marpolbul.2007.09.033

Válega, M., Lillebø, A.I., Caçador, I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Mercury mobility in a salt marsh colonised by *Halimione portulacoides*. Chemosphere, 72, 1607-1613.

Link: http://dx.doi.org/10.1016/j.chemosphere.2008.04.007

Válega, M., Lillebø, A.I., Pereira, M.E., Caçador, I., Duarte, A.C., Pardal, M.A., 2008. Mercury in salt marshes ecosystems: *Halimione portulacoides* as biomonitor. Chemosphere 73, 1224-1229.

Link: http://dx.doi.org/10.1016/j.chemosphere.2008.07.053

Válega, M., Lima, A.I.G., Figueira, E.M.A.P., Pereira, E., Pardal, M.A., Duarte, A.C., 2009. Mercury intracellular partitioning and chelation in a salt marsh plant, *Halimione portulacoides* (L.) Aellen: strategies underlying tolerance in environmental exposure. Chemosphere, 74, 530-536.

Link: http://dx.doi.org/10.1016/j.chemosphere.2008.09.076

1.3 References

Abreu, S., Pereira, E., Vale, C., Duarte, C., 2000. Accumulation of mercury in sea bass from a contaminated lagoon (Ria de Aveiro, Portugal). Mar Pollut Bull. 40, 293-297.

Clark, R.B., 2001. Marine Pollution fifth edition, Oxford university press

Coelho, J.P., Rosa, M., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2006. Pattern and annual rates of *Scrobicularia plana* mercury bioaccumulation in a human induced mercury gradient (Ria de Aveiro, Portugal). Estuar Coast Shelf S. 69, 629 - 635.

Coelho, J.P., Nunes, M., Dolbeth, M., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. The role of two sediment dwelling invertebrates on the mercury transfer from sediments to the estuarine trophic web. Estuar Coast Shelf S. 78, 3, 516 - 523.

Dias, J.M., Lopes, J.F., Dekeyser, I., 2000. Tidal propagation in Ria de Aveiro lagoon, Portugal. Phys Chem Earth PT B 25, 369-374.

Gailer, J., 2007. Arsenic-selenium and mercury-selenium bonds in biology. Coordin Chem Rev. 251, 234-254.

Hall, A., Lucas, M.F., Caldeira, M.T., Duarte, A.C., 1985. Presença de mercúrio nos sedimentos da Ria de Aveiro. In: Jornadas da Ria de Aveiro, Vol. I- Poluição da Ria de Aveiro, Câmara Municipal de Aveiro.

Islam, S., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Mar Pollut Bull. 48, 624-649

Lee, S.V., Cundy, A.B., 2001. Heavy metal contamination and mixing processes in sediments from the Humber Estuary, Eastern England. Estuar Coast Shelf S. 53, 619-636.

Lucas, M.F., Caldeira, M. T., Hall, A., Duarte, A. C., Lima, C., 1986. Distribution of mercury in the sediments and fishes of the lagoon of Aveiro, Portugal. Water Sci Technol. 18, 141-148.

Mason, R.P., Fitzgerald, W.F., Morel, F.M.M., 1994. The biogeochemical cycling of elemental mercury: anthropogenic influences. Geochim Cosmochim Ac. 58, 3191-3198.

Pato, P., Lopes, C., Válega, M., Lillebø, A.I., Dias, J.M., Pereira, E., Duarte, A.C., 2008. Mercury fluxes between an impacted coastal lagoon and the Atlantic Ocean. Estuar Coast Shelf S. 76, 4, 787-796.

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. Sci Total Environ. 213, 157-163.

Pereira, E., Abreu, S.N., Coelho, J.P., Lopes, C.B., Pardal, M.A., Vale, C., Duarte, A.C., 2006. Seasonal fluctuations of tissue mercury contents in the European shore crab *Carcinus maenas* from low and high contamination areas (Ria de Aveiro, Portugal). Mar Pollut Bull. 52, 1450-1457.

Pereira, M.E., Lillebø, A.I., Pato, P., Válega, M., Coelho, J.P., Lopes, C., Rodrigues, S., Cachada, A., Otero, M., Pardal, M.A., Duarte, A.C., 2009 Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. Environ Monit Assess (doi: 10.1007/s10661-008-0416-1) in press).

Wiener, J.G., Krabbenhoft, D.P., Heinza, G.H., Scheuhammer, A.M., 2003. Ecotoxicology of mercury. In: Hoffman, D.J. et al. (Eds). Handbook of Ecotoxicology. CRC Press, Boca Raton-Florida, pp. 409-463.

2 Mercury in the environment

2.1 Introduction

Pollution is caused when an input from human activities, increases the concentration of a certain substance in the water, sediments and/or organisms, above the natural background levels for that area (Clark, 2001).

The history of aquatic pollution starts with the beginning of human civilization; however it was only after the first visible adverse consequences in the environment and in the organisms that aquatic pollution started to receive special attention. Industrial revolution represents the starting point of the intense remobilisation of metals from the geosphere to the hydrosphere, biosphere and atmosphere. After this period the increase of the anthropogenic activities, namely fossil consumption, metals mining, smelting, fertilisation, waste incineration, leaching of toxic metalloids from coal and ash and the use of growth promoters became important sources of metals (Gailer, 2007).

Heavy metals are defined as metallic elements with specific gravity higher than 5 gcm^{-3}, and are distinguish by a tendency to form stable complexes with partly covalent metal-ligand bonds (e.g. chelates, sulphides and hydroxylated species) (Jackson, 1998). Mercury belongs to the non-transitional heavy metals (Group IIB), produced from sulphide ore (cinnabar) which dispersion in the environment is considered to be a global concerning problem. Mercury is considered the most toxic metal and none essential biochemical function of is known, presenting a high toxic degree to biota in all its forms. Mercury is known by its unique physico-chemical characteristics, especially due to the fact that it is the only metal liquid at room temperature (melting point -38.89 ^{o}C; boiling point 357.25^{o}C). Its main characteristics are its low electrical resistivity, high thermal neutron capture cross section (360 barnes), high surface tension, high thermal conductivity, uniform volume expansion over its entire liquid range and probably the most known, that it is the amalgamation ability with a high number of metals, namely gold. Mercury vapour pressure (14 mg cm^{-3} at 20 ^{o}C; 72 mg m^{-3}) and density (13.54 g cm^{-3} at 20 ^{o}C; 43.95 g cm^{-3} at 0 ^{o}C) are dependent on room temperature (Andren and Nriagu, 1979; Yu, 2001).

Due to the fact that mercury is a conservative pollutant, once released in the environment, it is permanent and difficult to remove. It can occurs naturally in the environment due to natural processes which include degassing of geologic materials, volcanic emissions and volatilisation from vegetation and marine/aquatic environments, (USEPA, 1997a; Farago, 2000) but it is the anthropogenic sources that are the major contributors for mercury pollution.

According to Mason et al. (1994) it was estimated that approximately that two thirds of the mercury in modern global fluxes is from anthropogenic sources, while one third is form natural sources. Its ability to conduct electricity, very good responses to temperature and pressure changes and amalgamation with other metals are the main features to its high industrial value in the past, with a wide range of applications for more than 2000 years in several areas. Thus it was frequently used in electrical industry (e.g. wiring devices and switches and batteries), navigation devices, generally in instruments that measure temperature and pressure, production of chlorine and caustic soda, wood processing (as anti fungal) and solvent for reactive and precious metals (e.g. gold mining). The biggest consumers of mercury were the chlor-alkali plants, which made them also the biggest polluters (Wiener et al., 2003).

Nowadays the use of mercury it is scarce, due to existence of several economically viable mercury-free alternatives for almost all applications of mercury involved processes; however it is still used in some pharmaceutical products as preservative agent (e.g. thimerosal), lightning (fluorescence lamps) and dental amalgams. The adverse effects caused by mercury led to its reduction in uses and consequently in its production; in fact after 1970 the production of mercury has been reduced by almost one order of magnitude (Hylander and Meili, 2003). During the last decades, the restrictive rules for anthropogenic emissions of mercury resulted in a substantial decrease of its release; however the historical local contaminations stills a concerning problem (Mason, 2003). Over recent decades, the anthropogenic sources of mercury in aquatic systems have been reduced, yet, mercury is still one of the most hazardous contaminants present in the aquatic environment and it is included in the list of high priority environmental pollutants within the scope of the European Water Framework Directive (WFD). The initial efforts to identify sources of mercury to the environment and consequently the efforts to reduce industrial discharges started around the 70s in America, Canada and in many other industrialised countries.

Mercury dispersion into the environment through decades have resulted in its accumulation and biomagnification through the trophic chain being the humans the last target and subjected to high concentrations of mercury, especially in areas where fish and seafood are the main components of the diet (Farago, 2000; Wiener et al., 2003). Once released in the environment, even in small amounts, mercury bioaccumulates through the food chain reaching dangerous levels in fish for human consumption (Wiener et al., 2003). The biomagnification of mercury in marine ecosystems is very well established (Sadiq, 1992; Wiener et al., 2003).

2.2 Biogeochemical cycle of mercury

The circulation and interaction of the elements between the different environmental compartments (biotic and abiotic) that result in the transfer or transformation of matter and energy is called the biogeochemical cycle (Catallo et al., 1999). After the release in the environment, the elements may undergo for several biotic (e.g. methylation, oxidation, reduction) and abiotic reactions (e.g. dissolution, precipitation, hydrolysis), generating new chemical species affecting their degree of bioavailability for the organisms (Clark, 2001; Gailer, 2007).

The environmental mercury cycle has four strongly interconnected compartments: atmospheric, terrestrial, aquatic and biotic (Wiener et al., 2003). Mercury cycle comprises a network of physical, chemical and biological interactions between the abiotic (sediments and water) and biotic (organisms) compartments (Sadiq, 1992).

In Figure 2.1 it is represented the simplified biogeochemical cycle of mercury in the environment. Mercury can occur in three stable oxidation states, Hg^0, Hg^+ and $Hg^{2+,}$ thus its properties and chemical behavior is highly dependent on its oxidation state. Elemental mercury is a volatile liquid at most earth-surface temperatures and its oxidation to HgO is thermodynamically low. The species Hg^+ it is not thermodynamically stable and can be found in the environment mainly as Hg_2^{2+}; however it rapidly transforms in Hg^0 and Hg^{2+} in the presence of ligands that bind Hg(II) being Hg^{2+}, the mainly oxidation state that can be found in the environment (Jackson, 1998). Due to the fact that is a weak Lewis acid, this species (Hg^{2+}) has a high affinity for ligands highly polarized (weak Lewis bases) like sulphides. Mercury can occur in the form of inorganic complexes or associated to the organic mater by covalent and ionic bonds (EPA, 1997). Mercury can establish more stable complexes with organic matter than other metals (Ravichandran, 2004) deserving special attention to mercury compounds with covalent bonds to the organic matter. These compounds are denominated organic mercury species and play an important role in mercury contamination studies due to its high toxicity effects.

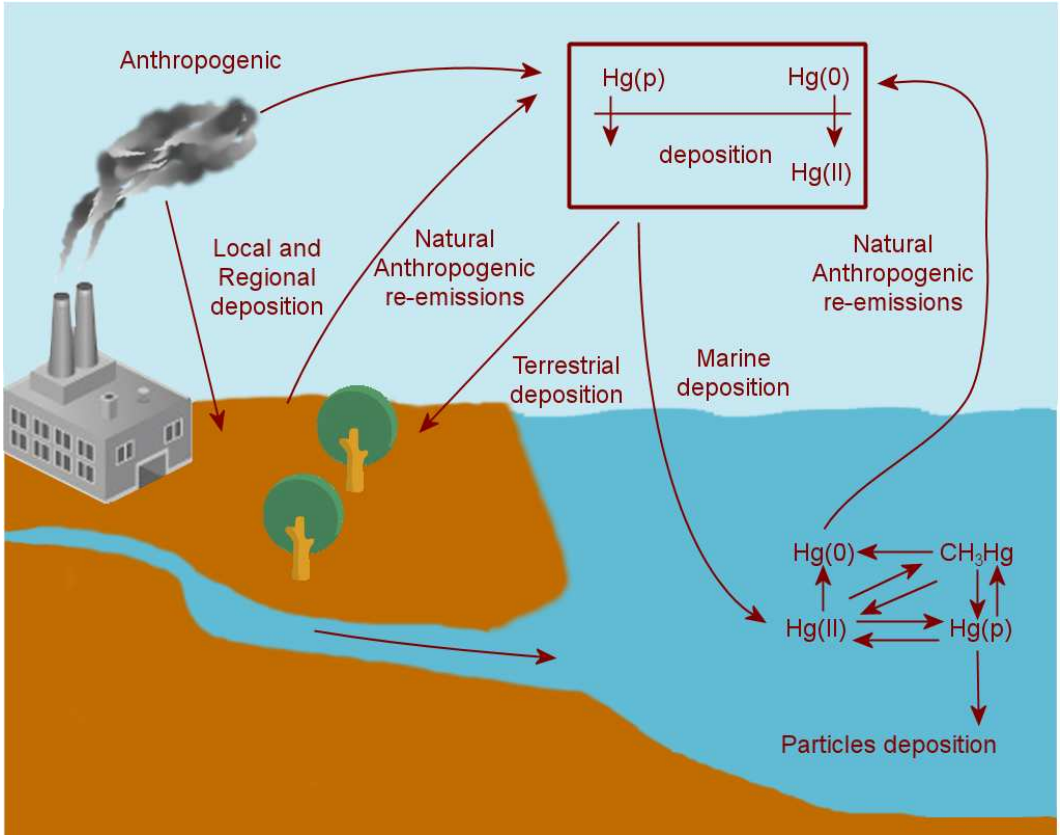


Figure 2.1- Schematic representation of the global cycle of mercury in the environment (adapted from Environmental Protection Agency- EPA)

The most common forms of mercury in the environmental circulation are: $HgCl_2$, $Hg(OH)_2$, HgS, CH_3HgCl, CH_3HgOH (methylmercury compounds), dimethylmercury and phenylmercury, being the last two the more rarely forms (USEPA, 1997b). As referred by Farago (2000), mercury species can be classified as volatile species (Hg^0, $(CH3)_2Hg$), water soluble species-borne reactive species: Hg^{2+}, HgX_2; HgX^{3-}, HgX_4^{2-}, where X= OH^-, Cl^- or Br^-, HgO in aerosol particles and Hg^{2+} complexed with organic acids and non-reactive species: CH_3Hg^{2+}, CH_3HgCl, CH_3OH, $Hg(CN)_2$, HgS and Hg^{2+} associated to sulphur of humic mater.

In general the most predominant form of mercury in the atmosphere is the elemental mercury Hg(0) in the terrestrial and aquatic environment is dominated by Hg(II), while in the biotic compartment methylmercury is the most predominant species (Wiener et al., 2003).

2.3 Mercury in the atmosphere

The atmosphere plays an important role in the global dispersion of mercury. In the atmosphere, mercury can be widely dispersed and transported thousands of miles from likely emission sources, depending on the chemical and physical form of the mercury emitted (USEPA, 1997b). Mercury can be found in the atmosphere in the form of elemental mercury (Hg^0) which has a high residence time in the atmosphere (approx. 1year), although it can be removed after oxidation in the aqueous phase (cloud droplets) to water soluble species that can be deposited (wet deposition) in the hydrosphere and lithosphere (USEPA, 1997a; Farago, 2000). Hg(II) can also associate to particulates forms due to the presence of soot in the atmosphere, which posses S atoms in theirs matrix. The resulting particulate forms of mercury can be also removed by wet deposition if the particles are associated with cloud droplets or dry deposition (USEPA, 1997a). On the contrary to elemental mercury, the residence time of oxidised mercury compounds in the atmosphere is uncertain, but is generally believed to be on the order of a few days or less (USEPA, 1997b). The oxidation occurs by the reaction with de ozone (USEPA, 1997a). As referred by Wiener et al., (2003) more than 95% of the total mercury in the atmosphere is predominantly elemental mercury and in less quantities, less than 5%, can also be found particulate ionic mercury Hg(II), gaseous divalent mercury and methylmercury. Natural waters are usually statured with Hg(0) (aq) when compared to the above atmosphere, promoting a flux from the water to the atmosphere (Morel et al., 1998).

2.4 Mercury in the aquatic environment

It was estimated that between 6000-7500 tonnes were introduced annually in the sea, where 50-75% are the result of anthropogenic activities (Clark, 2001). The contamination of mercury in estuaries is a concern as most estuarine and coastal environments are in close proximity to urban centres; however, currently there is no legislation concerning its concentrations in brackish or coastal waters used as fishery resource. In the aquatic environment, mercury can be found in the water column, associated to suspended particulate matter (SPM) and in the sediments (Jackson, 1998; De Marco 2006; Covelli et al., 2007); in fact sediments are considered to be the principal depository for mercury in aquatic environments (Sadiq, 1992).

Mercury can be introduced in the aquatic environment by atmospheric deposition and be retained in the sediments; however it can return to the water column through resuspension and diagenetic processes and be incorporated into the trophic chain or be released to the

atmosphere. Once in the aquatic environment (Figure 2.2) mercury can enter in a complex cycle and be subjected to conversion processes between different mercury species.

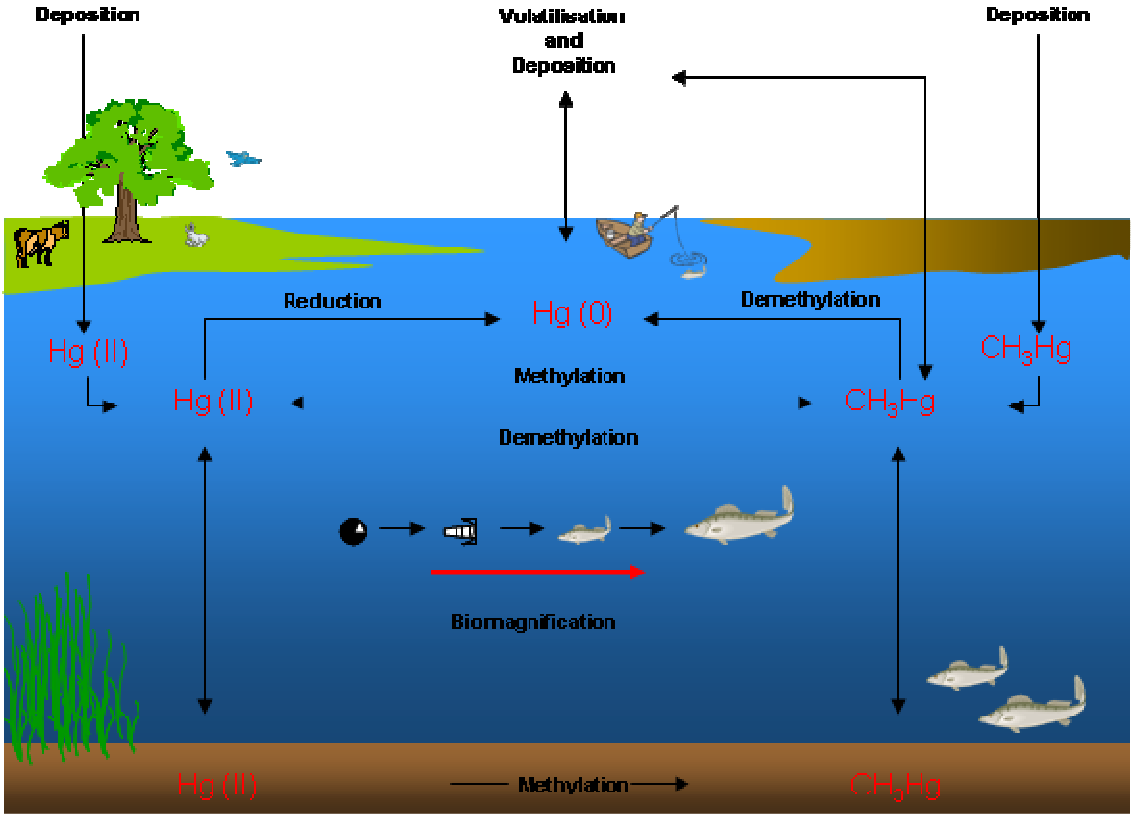


Figure 2.2 - Schematic representation of the global cycle of mercury in the aquatic environment.

The biogeochemistry of mercury in water is strongly influenced by the chemistry of the water, namely redox conditions (dissolved oxygen), salinity, pH and the presence of organic ligands (humic and fulvic acids) and inorganic ligands (Sadiq, 1992; Wiener et al., 2003). The form of which mercury is present in the sediments and water play a significant role in its bioaccumulation and consequently toxicity.

In the water column mercury can be in equilibrium between the dissolved phase and the particulate phase, associated with SPM (Ebinghaus *et al.,* 1994; Kennish, 1998; Covelli et al., 2007). According to Fitzgerald (1979) in Kennish (1998), the dissolved fraction is an operational definition for the fraction of water that can pass through a 0.45 µm filter and consequently SPM the particulate matter that remains in the filter. In the dissolved phase, mercury can be found predominantly as inorganic soluble species (e.g. $HgCl_2$, $Hg(OH)_2$, $Hg(HS)_2$, Hg^0, HgS_2^{2-} and $HgCl_4^2$) and complexed with organic ligands or in the form of organic mercury species, namely

methylmercury and dimethylmercury. The main species in the dissolved fraction are controlled by the presence of chloride anions and the pH values (Figure 2.3), thus in waters with low pH values and low chloride contents the most predominant form is the cation Hg^{2+}, which hydrolyse for higher pH values originating $Hg(OH)_2$, $Hg(OH)_3^-$, $Hg(OH)^+$ and in high salinity waters $HgCl^+$, $HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$.

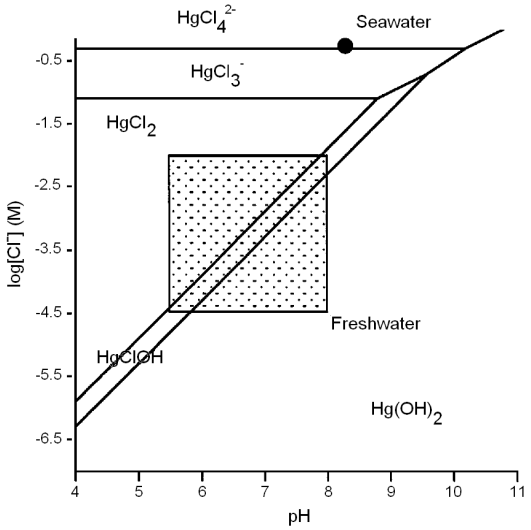


Figure 2.3 - Diagram of hydroxo- and chloro-complexes of Hg^{2+} as a function of pH and chloride concentrations (Morel et al., 1998).

Mercury species in the dissolved phase have been also classified as reactive and non reactive according to their stability. Reactive mercury is considered to be the fraction of the metal susceptible to be reduced with a chloride-stannous solution (Wiener et al., 2003) and include inorganic dissolved species, elemental dissolved mercury and labile mercury complexes. Non reactive species include organic mercury species and non-labile complexes with organic matter (Ramalhosa, 2002).

The particulate matter of estuarine systems is mainly composed by a mixture of organic and inorganic material similar to the sediments composition (Millward and Turner, 1995). In the particulate matter it is possible to find mercury species formed by adsorption and co-precipitation like HgS, $Hg(OH)_2$, $HgCl_2$ and adsorbed to organic matter (Morel et al., 1998). Due to its electrical negative charge, the adsorption of cations is promoted and plays an important role on mercury transport. Because trace metals are particle reactive and adsorb quickly to suspended particulate matter, a major fraction is deposited in the bottom sediments, being the concentrations of metals higher than those observed in the sediments (Kennish, 1998).

After the deposition of mercury in the sediments, the reactions that it is subjected are dependent on the chemical and mineralogical characteristics of the sediments (Kennish, 1998), namely pH, redox potential, organic matter content, grain size and salinity. Sedimentary compartment may be divided as solid fraction and interstitial waters being the metals in equilibrium between the two phases (Ramalhosa, 2002).

In general metals in the sediments can be as dissolved metals (soluble as free ions, inorganic and organic complexes) exchangeable ions, complexed with high molecular weight (humic materials), precipitated as insoluble sulphides, adsorbed or occluded with precipitated hydrous oxides and found within the crystalline lattice structure of primary minerals (Catallo et al., 1999). Mercury in sediments tends to form inorganic compounds, however due to their solubility, complexes with organic matter and mineral colloids can be established (USEPA, 1997a). In the sediments, mercury can also be adsorbed on the negatively charged surfaces as clay, organic particulates and oxihydroxides of Fe and Mn and Ca carbonates (Kennish, 1998; Sadiq, 2000). The adsorption of mercury in sediments is considered to be a fast reaction comparatively to desorption process, which is affected by the pH, redox conditions and salinity (Moore and Ramamoorthy, 1984).

Mercury and its inorganic compounds have a great affinity for sulphur compounds, which can reduce its mobility in the sediments. In reduced environments mercury can be found as sulphides while in more oxidic conditions can be associated to oxihydroxides of iron (Canário et al., 2003; Ramalhosa et al., 2006). Iron and manganese oxihydroxides have high superficial area (300 m^2 g^{-1}) than sediments with oxidic conditions have higher ability to adsorb metals than the reduced sediments. Disturbances of mercury contaminated sediments by physical actions including dredging operations and bioturvation can lead to mercury mobilisation which is considered to be more stable under anoxic conditions as mercury sulphides (Calmano et al., 1996, Petersen et al., 1997).

Mercury remobilisation from the sediments to the interstitial waters can occur due to the reduction of oxihydroxides of iron and manganese, dissolution/ precipitation of sulphides and bioturvation and generally mercury concentration in sediments usually reflects mercury concentrations in the interstitial waters (Bufflap and Allen, 1995), exceeding the values found in the water column (Ramalhosa et al., 2006). In interstitial waters mercury can be found associated to organic ligands, associated to chloride ($HgCl_2$, $HgCl_3^-$, $HgCl_4^{2}$) in the case of saline waters and in

the case of highly reduced sediments associated do disulphides like HgS_2H^- ou HgS_2^{2-}) (Benoit *et al.,* 1998; Jackson, 1998; Benoit *et al.,* 1999).

Trace metals may be released from particulate matter to the surrounding water by different processes, namely desorption from river borne suspended particulate matter with the contact with the seawater (chloride complexation), desorption from the sediments and also due to the release from interstitial waters due to diagenetic processes (Kennish, 1998).

Summarizing, the mobility and bioavailability of Hg and MeHg depend upon the nature and concentration of the binding phases in the sediment, which apparently are controlled by sediment redox status. Mercury associates primarily with particulate organic matter and iron/manganese oxides through adsorption and co-precipitation reactions in oxidised sediments while in anoxic sediments, mercury is adsorbed onto and co-precipitated with sulphide minerals (Gobeil and Cossa, 1993; Gagnon et al, 1997; Wang et al, 1998).

2.5 Mercury methylation in the aquatic system

The potential release of methylmercury from the sediments to the overlying column water can be a serious problem in a local/regional scale especially in areas highly dependent on fishery activities. As already mentioned, mercury can establish covalent bonds with atoms of carbon originating organic mercury species, which are extremely toxic when compared to other mercury species.

Mercury methylation is the conversion of inorganic mercury to methylmercury (or other organic mercury species) by a methyl-group donor (Wiener et al., 2003), being the most toxicologically significant transformation of inorganic mercury in the environmental cycle due to the higher the bioavailability of these compounds to wildlife and humans (Wiener et al., 2003).

Organic mercury species in environment is the result of biotic and abiotic processes, being the first ones considered the most significant pathway. Sulphate reducing bacteria (SRB) is considered the most important group of methylating bacteria; however not all SRB are able to methylate mercury, which means that the activity of the microbial community may not be enough to predict methylation activity (King et al., 1999).

As mentioned by wiener et al. (2003), methylation can occur in aerobic marine and freshwaters, floating periphyton mats, roots of some aquatic plants, the intestines of fish and

mucosal slime layer of fish; however anaerobic sediments and wetlands are considered prime sites of mercury methylation.

Methylmercury production in sediments is related with the factors that control the bioavailability of mercury in the sediments as well as with the bacterial activity responsible for mercury methylation; thus methylation of mercury is dependent on pH, temperature, chloride, organic matter, redox conditions and sulphide availability (Ullrich et al., 2001; Celo et al., 2006).

The speciation of inorganic mercury in the water and in the sediments controls the pool of mercury available for methylation (Wiener et al., 2003). For example according to Rudd et al. (1983) in Wiener et al (2003), when mercury is bound to large molecules of dissolved organic matter or to particulates (organic or clay) is considered to be unavailable for methylation. Sulphide concentrations in the sediments can also influence the methylation rates. Sulphate can stimulate both sulphate reduction and Hg methylation by sulphate reducing bacteria at relatively low sulphate concentrations (Gilmour and Henry, 1991); however according to Gilmour et al. (1998), sulphide, as a result of sulphate reduction is considered to be the most effective to reduce mercury bioavailability for methylation processes. It is referred that the presence of sulphide clearly decreases the availability of mercury for methylation and that methylmercury production is generally reduced, but not completely inhibited, at high sulphide concentrations (Kongchum et al., 2006).

Sunderland et al. (2006) observed that higher sulphide concentrations corresponded to elevated fractions of methylmercury suggesting higher methylation rates. In this study it was also concluded that this relationship is strongly associated with moderately impacted organic matter enrichment, but weak of in weak impacted, aerobic sediments. It has been shown for several ecosystems a lack of a direct dependence between methylmercury and total mercury concentrations in sediments (Heyes et al., 2006); in fact the highest percentages of methylmercury were found in sediments with lower concentrations of total mercury. Higher methylation rates occurs both in oxic and anoxic environments but in the field, methylation rates are higher in anaerobic sediments and waters (Morel et al., 1998).

Methylmercury concentrations in water are the major source for mercury concentrations in aquatic organisms (Morel et al., 1998); in fact, methylmercury in aquatic organisms corresponds to 85-90% of total mercury concentrations (Horvat, 1996). Toxicity of organic

mercury compounds is higher than inorganic mercury salts due to their higher solubility in lipids, which increases the potential for biological uptake and bio-concentration. Methylmercury can be effectively taken up by aquatic organisms with bio-concentration factors of 10^4 to 10^7 (Wiener et al., 2003).

Generally, methylation of mercury is higher in the aquatic than in terrestrial environment (Wiener et al., 2003) and is usually greater in marine environments that in freshwaters systems (Kongchum et al., 2006). Methylmercury is the most usual organic mercury species. While dimethylmercury in scarce in marine environments and when detectable it is normally in very small concentrations and in freshwater its presence has not been confirmed (Wiener et al., 2003).

Generally, methylmercury concentrations in the sediments do not exceed 1.5% of the total mercury concentrations (Horvat, 1996). As referred by Celo et al (2006) the concentration of methylmercury in the aquatic environment is a result of the natural balance between methylation and demethylation processes. While for methylation the main role is related with biotic processes in the case of demethylation both processes are important, for example in the surface water the photochemical reduction of methylmercury is pointed as one of the most important abiotic demethylation.

2.6 Mercury and human health

Mercury is the metal that is mostly associated to human health hazards. Humans can be exposed to mercury by direct contact with contaminated environmental media or ingestion of mercury contaminated food and water. The first highlights reporting mercury human health hazards occurred in Japan between the 50s and the 60s after the industrial discharges occurred in Minamata bay by Chisso Company and in Agano River (Niigata) by Showa Electrical Company's chemical plant. The mercury-rich wastewaters were a result of the acetaldehyde production using mercury sulphate as a catalyst. A side reaction of the catalytic cycle led to the production of organic mercury compounds namely methyl mercury.

Repercussions of human consumption of fish highly contaminated with mercury (Wiener, et al., 2003) had so severe in local populations that was reported an epidemic of an unknown disease of the central nervous system, known as Minamata and Niigata-Minamata disease, respectively. Later in the early 70s another poisoning accident occurs in Iraq where people use wheat seeds dressed with methylmercury, as a fungicide, for human consumption (Clarkson,

2002). Mercury poisoning through dietary products, especially fish and seafood have been reported all over the years although in a minor scale.

As referred by Wiener et al. (2003) the methylmercury concentration in fishes can exceed 10^6 to 10^7 fold the concentrations found in water. Plants and animals have the availability to regulated metals content in their tissues; however metals that cannot be excreted or have high residence times in the organism remain in the body and are continually added to over the life of the organism; this phenomenon is called bioaccumulation (Clark, 2001). The concentrations of methylmercury in fishes increase with the increment in the trophic chain position, with the increase of the size and the age due to the fact that elimination of mercury is usually slower than the accumulation. Animals which their diet is based on bioaccumultators organisms have a rich diet on conservative pollutants and in major cases they are also unable to excrete which results in a greater body burden of the metals (Clark, 2001). In consequence the top predators, including humans are subjects to large inputs of conservative substances in their diet, becoming a potential human health risk, being the reason why the conservative pollution are a great area of concern. Portuguese legislation adopted the regulatory limits of the European community (EC 466/2001) of 0.5 mg kg^{-1} for fishery products in general and 1 mg kg^{-1} for predatory fish such as tuna fish, sea bass and shark.

As referred by Gailer (2007), the health effects associate with human metals exposure, namely methylmercury is also associated to economic losses. The exposure of humans to metals increases the risk of chronic diseases.

The toxic kinetics (absorption, distribution, metabolism, and excretion) of mercury is highly dependent on the form of mercury to which a receptor has been exposed (USEPA, 1997c). Mercury accumulation in an organism occurs when the rate of uptake exceeds the rate of elimination. All forms of mercury can be accumulated; however it is methylmercury that accumulates to a greater extent (USEPA, 1997c). Despite the low percent of methylmercury in sediments, methylmercury is the mercury form that is most toxic and bioaccumulates most efficiently due to its capacity of passing the biological membrane, its high chemical stability and slow excretion from most organisms. In the past, humans were exposed to elemental and ionic mercury due to its applications in medicine and in general in industrial activities, although nowadays with the decrease of the use of mercury, the population is no more exposed to these species of mercury; although they are still exposed to methylmercury especially through the fish

and seafood consumption (Mason, 2003). All over the world, but especially in North America and Europe, there are advisories for fish consumption regarding the high levels of methylmercury in some species (Mason, 2003). According to Clarkson (2002) nowadays mercury exposure occurred especially through methylmercury in fishes, mercury vapour from amalgam tooth fillings and thimerosal in vaccines.

Elemental mercury is readily absorbed through the lungs (50-100%), but the absorption of the liquid metal in the gastrointestinal tract is less than 1%, while methylmercury is readily absorbed in the gastrointestinal tract (approx 100%) and in the lungs (Gochfeld, 2003). Studies with the administration of methylmercury nitrate in humans revealed that almost 100% of the dose is absorbed in the gastrointestinal tract into the bloodstream (Gailer, 2007). After enter in the bloodstream, mercury is subjected to complexes and still understood processes that involve binding and redox cycling (Gochfeld, 2003). The main target for methylmercury accumulation in humans is the nervous system, mainly the brain due to its capacity to transverse the blood-brain barrier (Gailer, 2007). Once inside human body methylmercury is difficult to excrete and has a half life between 44-80 days (average of approximately 70 days), while inorganic mercury is more easily to excrete (20-66 days, in blood) (USEPA, 1997c). Inorganic and methylmercury have the ability to inhibit enzymes with vicinal dithiols in their active centre. Methylmercury is known to be a potent neurotoxin and its neurotoxicity is attributed to its reaction with plasmalogens (major constituent of the phospholipids backbone in cell membranes) (Gailer, 2007). Toxicological effects of mercury include neurological damages, reproduction capacity anomalies, growth inhibition and changes in behaviour (Wiener et al., 2003). Despite of thousands of years of human exposure to mercury compounds, the mechanisms underlying to its toxicity remains to explain (Clarkson, 2002).

2.7 References

Andren, A.W., Nriagu, J.O., 1979. The Global Cycle of Mercury. In: Nriagu, J.O. (Eds). The Biogeochemistry of Mercury in the Environment. Elsevier/North-Holland Biomedical Press, Netherlands, pp. 1-21.

Benoit, J.M., Gilmour, C.C., Mason, R.P., Heyes, A., 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Environ Sci Technol 33, 951-957.

Bufflap, S.E., H.E. Allen. 1995. Sediment pore water collection methods for trace metal analysis: a review. Water Res. 1, 165-177

Calmano, W., Ahif, W., Förstner, U., 1996. Sediments quality assessment: chemical approaches. In: Calmano, W. and Förstner, U. (Eds). Sediments and Toxic Substances. Springer Verlag, Berlin, pp.1-35.

Catallo, W.J., Blankemeyer, J.T., Gambrell, R.P., Pardue, J.H., Pugesek, B., Reddy, K.R., 1999. Workgroup I Synopsis: Biogeochemical processes. In: Lewis et al. (Eds). Ecotoxicology and Risk Assessement for Wetlands. SETAC Press, USA, pp. 27-68.

Celo,V., Lean, David, R.S., Scott, S.L., 2006. Abiotic methylation of mercury in the aquatic environment. Sci Total Environ. 368 (1), 126-137.

Clark, R.B., 2001. Marine Pollution fifth edition, Oxford university press

Clarkson, T.W., 2002. The three modern faces of methylmercury. Environ. Health Persp. 110 (1), 11-23.

Covelli, S., Piani, R., Acquavita, A., Predonzani, S., Faganeli, J., 2007. Transport and dispersion of particulate Hg associated with a river plume in coastal Northern Adriatic environments. Mar Pollut Bull. 55, 436-450.

De Marco, S.G., Botté, S.E., Marcovecchio, J.e., 2006. Mercury distribution in abiotic and biological compartments within several estuarine systems from Argentina: 1980-2005 period. Chemosphere. 65, 213-223.

Ebinghaus, R., Wilken R.-D., 1996. Mercury distribution and speciation in a polluted fluvial system. In: Calmano W. and Förstner U. (Eds). Sediments and Toxic Substances: Environmental Effects and Ecotoxicity. Springer Verlag, Berlin Heidelberg, pp. 215-244.

Farago, M.E., 2000. Mercury in marine environments. In: Gianguzza, A., Pelizetti, E., Sammartano, S. (Eds). Chemical Processes in Marine Environments, Springer-Verlag, pp. 245-263.

Gailer, J., 2007. Arsenic-selenium and mercury-selenium bonds in biology. Coordin Chem Rev. 251, 234-254.

Gilmour, C.C., Henry, E.A., 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ Pollut. 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.

Gochfeld, M., 2003. Cases of mercury exposure, bioavailability and absorption. Ecotox Environ Safe. 56, 174-179.

Heyes, A., Mason, R.P., Kim, E.-H., Sunderland, E., 2006. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. Mar Chem. 102 (1-2), 134-147.

Hylander, L.D., Meili, M., 2003. 500 years of mercury production: global annual inventory by region until 2000 and associated emissions. Sci Total Environ. 304, 13-27.

Horvat, M., 1996. Mercury analysis and speciation in environmental samples. In: Regional and global mercury cycles: sources, fluxes and mass balances. Baeyens, W., Ebinghaus, R. and Vasiliev, O. (Eds.), Kluwer, Dordrecht, pp.1-31.

Jackson, T.A., 1998. Mercury in aquatic ecosystems. In: Langston, W.J. and Bebianno, M.J. (Eds). Metal metabolism in the aquatic environment. Chapman and Hall, Lda Publishers, UK, pp. 77-138.

Kennish, M.J., 1998. Trace metal-sediment dynamics in estuaries: pollution assessment. In: Ware, G.W. (Eds). Review Environmental Contamination Toxicology, vol. 155. Springer, Berlin, pp. 69-110.

King, J.K., Saunders, F.M., Lee, R.F., Jahnke, R.A., 1999. Coupling mercury methylation rates to sulphate reduction rates in marine sediments. Environ Toxicol Chem. 18(7), 1362-1369.

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, 1300-1303.

Mason, R.P., Fitzgerald, W.F., Morel, F.M.M., 1994. The biogeochemical cycling of elemental mercury: anthropogenic influences. Geochim Cosmochim Ac. 58, 3191-3198.

Mason, R.P., 1993. Mercury biogeochemical cycling in a stratified estuary. Limnol Oceanogr. 38, 1227-1241.

Millward, G.E., Turner A., 1995. Trace metals in Estuaries. In: Trace elements in natural waters. Salbu, G. and Steinnes, E. (Eds), CRC Press, Inc., Boca Raton, Florida, pp. 223-245.

Moore, J.W., Ramamoorthy, S., 1984. Mercury. In: Heavy Metals in Natural Waters- Applied Monitoring and Impact Assessement. Springer-Verlag (Eds.), New York, pp. 125-160.

Morel, F.M.M., Kraepiel, A.M.L., Amyot, M. 1998. The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst. 29, 543-566.

Petersen, W., Willer, E., Willamawski, C. 1997. Remobilization of trace metals from polluted anoxic sediments after resuspension in oxic water. Water Air Soil Poll. 99, 515-522.

Ramalhosa, E. 2002. Mercúrio na Ria de Aveiro: associações, reactividade e especiação. PhD thesis. University of Aveiro.

Ramalhosa, E., P. Pato, Monterroso, P., Pereira, E., Vale, C., Duarte, A.C., 2006. Accumulation versus remobilization of mercury in sediments of a contaminated lagoon. Mar Pollut Bull. 52, 353-356.

Ravichandran, M., 2004. Interaction between mercury and dissolved organic matter- a review. Chemophere. 55, 319-331.

Sadiq, M., 1992. Toxic metal chemistry in marine environments. Marcel Dekker, New York.

Sunderland, E.M., Gobas, F.A.P.C., Branfireun, B.A., Heyes, A., 2006. Environmental controls on the speciation and distribution of mercury in coastal sediments. Mar Chem. 102, 111– 123.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A. 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit Rev Env Sci Tec. 31(3), 241-293.

USEPA (United States Environmental Protection Agency), 1997a. Fate Transport of Mercury in Environment. In: Mercury Study Report to Congress, vol. III (EPA-452/R-97-005), U.S. Environmental Protection Agency.

USEPA (United States Environmental Protection Agency), 1997b. Executive Summary. In: Mercury Study Report to Congress, vol I (EPA-452/R-97-003), U.S. Environmental Protection Agency.

USEPA (United States Environmental Protection Agency), 1997c. Health Effects of Mercury and Mercury Compounds. In: Mercury Study Report to Congress, vol V (EPA-452/R-97-007), U.S. Environmental Protection Agency.

Wiener, J.G., Krabbenhoft, D.P., Heinza, G.H., Scheuhammer, A.M. 2003. Ecotoxicology of mercury. In: Hoffman, D.J. et al. (Eds). Handbook of Ecotoxicology. CRC Press, Boca Raton-Florida, pp. 409-463.

Yu, Ming-Ho, 2001, Environmental Metals. In: Environmental Toxicology-Impacts of Environmental Toxicants on Living Systems, Lewis publishers, CRC Press, USA, pp.151-186.

3 Salt marsh ecosystem

3.1 Introduction

The most commonly definition adopted for estuaries was given by Cameron and Pritchard (1963) who states that an estuary is a *"semi-enclosed coastal body of water, which has a free connection with the open sea, and within which sea water is measurably dilute with fresh water derived from land drainage"*; however a more recent definition doesn't taking into account the tidal action was suggested by Perillo (1995) stating that *"An estuary is a semi-enclosed coastal body of water which has free connection to the open sea, extending into the river as far as the limit of tidal influence, and within which sea water is measurably dilute with fresh water derived from land drainage"* (Dyer,1997).Wetlands ecosystems can be found in/or around estuaries environments, namely: **salt marshes**, mangroves swamps and intertidal flats (Livingston, 1993).

Wetland is a large concept used to describe a group with a high diversity of ecosystems whose formation and existence is controlled essential by water (Richardson, 1999). Located in the temperate zones of the hemisphere, salt marshes belong to this wide group. According to Mitsch and Gosselink (2000), the most known definition for salt marshes is dated from 1977 by Beeftink and states that salt marshes are "natural or semi-natural halophytic grassland and dwarf brushwood on the alluvial sediments bordering saline water bodies whose water level fluctuates either tidally or non-tidally".

Salt marshes development occurs in intertidal coastal areas where tidal action and erosion is sufficiently low enough to allow vegetation establishment (e.g. estuaries, depositional coasts, shorelines and enclosed bays) and are a result of a complex set of biological and physical interactions. The existence of a flat interface area with low tidal energy where sediments can accumulate from upland runoff and substrate availability (sediment particles) are the main requirements to its initial formation and subsequent development (Eisma and Dijkema, 1998). Salt marshes formation starts with the settling of the pioneer vegetation near the mean high level tide, where plant seeds are able to germinate. As soon as the plants start to settle, the sedimentation deposition improves and the development process starts, including the formation of creeks which play an important role in the sediment supply and stimulation of species growth. Creeks helps to transport and dewatering the sediments enhancing their aeration (Eisma and Dijkema, 1998).

Salt marsh preservation is crucial and is achieved by the balance between erosion, sedimentation and compaction (accretion). The type and density of vegetation play an important role on sedimentation. Vegetation enhances the sedimentation by reducing the flow by the leaves and stems of the plants; the deposited sediments are held by the roots. The leaves and stems of the salt marsh plants act as baffle to the incoming tidal flows, decreasing the current velocities and allows deposition of the particles, the stems of the plants can set up eddies in the tidal flow promoting high local sedimentation rates. The role of salt marsh plants on the salt marsh development is well established. Salt marsh plants can act as traps for suspended particulate matter immobilizing them beneath the bottom sediments and in their roots helping to protect the sediments from erosion promoting the development (Caçador et al., 1996; Packham and Willis, 1997; Eisma and Dijkema, 1998).Salt marsh plants can also exude salt increasing the salinity values and promoting flocculation which also may increase the deposition (Pethick, 1993). It is obvious to conclude that perennial vegetation increases the sedimentation deposition rather than annual plants (Eisma and Dijkema, 1998).

Salt marshes geomorphology is divided in four distinct zones: the pioneer zone, the lower marsh, the middle marsh and the high marsh. The pioneer zone is characterised predominantly by the existence of reduced sediments, oxidised sediment occurs only during neap tide and only in the first top centimetres of the sediment. The lower salt marsh is characterised by predominantly oxidised sediments with lower oxygen content around spring tide; and middle salt marsh zone has the longest period of aeration without fluctuations in the oxygen content, except during the highest tides (Eisma and Dijkema, 1998).

Salt marshes that are often enclosed by spits and bays are designated closed marshes. Their horizontal growth is considered to be restricted and the vertical growth is not paralleled by the area increase. On the other hand, salt marshes located on banks of estuaries or on the shores of larger coastal embayment are considered open marshes and their development can be both horizontal and vertical (Pethick, 1993).

All over the world, estuarine areas became attractive areas for human establishment due to their natural features. The high pressure caused by urban, industrial and agricultural development has increased the vulnerability of estuarine ecosystems due to the exposure to both organic and inorganic pollutants coming from anthropogenic sources. In Portugal it was estimated by 2001 that approximately 65% of the population lives in the costal zone (Rosa and Vieira, 2003).

The vital importance of salt marshes and in general of all wetlands is worldwide recognised for providing essential ecological functions to the environment; salt marshes are no longer viewed as intertidal wastelands areas. High biological productivity, regulators of biogeochemical cycles, hydrologic flux regulation, and habitats providers for fish and wildlife are among the several ecological functions mediated by these ecosystems. Salt marshes are also regarded as bio-stabilizers, due to the induced changes in the physical environment (*e.g*. reduced tidal currents, wave action and sediment resuspension, enhanced sediment cohesiveness and settling of suspended matter) (Alongi, 1997*,* Richardson, 1999, Widdows and Brinsley, 2002, Lillebø et al., 2007).

As already mentioned before, despite of all ecological values and functions, salt marshes were regarded in the past as unpleasant areas, subjected to large inputs of contaminants and reclaimed or destroyed for humans needs (e.g. new industrial, agriculture and human housing areas). These actions have resulted in the drastically reduction of their total area around the world. The lost and degradation of several wetlands, namely salt marshes, have been reported all over the world. As referred by Boorman (1999), the ecological importance of salt marshes was first described in the United States by Odum (1961) and Teal (1962) and in Europe by Chapman (1960) and Ranwell (1972). It was by 1965 that the first recommendations for their conservation appeared by Shaw and Fredin. After the 70s the role of wetlands became recognised and the first legislation actions were adopted (Mitsch and Gosselink, 2000). The rise in awareness of wetlands importance of namely has to do with an enhancement of the appreciation on their positive, ecological and environmental quality values. Nowadays endangered European and North American salt marshes have been subjected to increasing restoration programs (Scheffer et al., 2001; Lillebø et al., 2005; Elliot et al., 2007). Besides the direct anthropogenic activities several authors have been appealing for the salt marshes lost due to the sea level rise and the lack of sediments supply due to river dams (e.g. Scheffer et al., 2001; Elliot et al., 2007).

Salt marsh vegetation presents a low number of plant species, comparatively to the number of animal species. Some authors state that the vegetation type of each salt marsh is considered to be its biological fingerprint (Caçador and Vale, 2000). Intertidal species distribution shows a clearly pattern of zonation which is related to the frequency of flooding and to the elevation above mean sea level (Eisma and Dijkema, 1998). Latitude is determinant also in the succession of vegetation. In British and Western European salt marshes, the succession of plants starts from *Salicornia* on the low marsh, through *Halimione portulacoides* and *Aster tripolium* L.

on medium marsh to *Puccenelia maritima*, *Limonium vulgarae* and *Armeria maritima* in the high marsh while in the North American marshes the succession is rather different (Pethick, 1993). Salt marsh vegetation is generally dominated by grasses and shrubs, being these plants well developed to survive in this environment. Salt marshes environment is considered to be a harsh environment to species development and growth due to the high salinity values, high nutritional deficiencies, high reduced environment and waterlogging periods. Halophytes is the general classification for the plants which posses resistance mechanisms to survive in high concentrations of electrolytes in their environment, normally enriched in NaCl but also in Na_2SO_4, $MgSO_4$, CaSO4, $MgCl_2$, KCl and Na_2CO_3. Depending on their tolerance and demands for sodium salts, they may be distinguished as obligate and facultative halophytes. Obligatory means that they need some salt to their survival and facultative means they can live also under freshwater conditions. These plants have to cope with the negative osmotic potential and deal with the high levels of ions that may have a potential toxic effect on the plant metabolism. Different strategies are used to cope with high salt levels: halophytes species are often succulent (high volume/surface ratio), have reduced foliar area, many species have salt glands and others are able to store considerable concentrations of salt within their vacuole (Salisbury and Ross, 1985; Alongi, 1997).

3.2 Salt marshes biogeochemical processes

As interfacial ecosystems between the terrestrial and marine environments, salt marshes are ecotones extremely complex and dynamic (Packamn and Willis, 1997) with a high number of physical-chemical and biogeochemical reactions occurring within the water column, within the sediments and in the interface between the water and the sediments (Chenhall *et al.*, 1992).

Biogeochemical cycle of wetlands is mainly regulated by hydrological processes due to the frequency waterlogging periods that they are subjected. Salt marshes biogeochemical cycle should focus on the internal processes of the ecosystem (intrasystem cycle) as well as the processes that can occur between them and the adjacent areas (Mitsch and Gosselink, 2000). An ecosystem is classified as biogeochemically open when there is an abundant exchange of materials with its surrounding and in the other hand can be classified as biogeochemically closed when the exchange is minimal. Tidal salt marshes are usually classified as biogeochemically open systems (Mitsch and Gosselink, 2000).

Salt marsh plants grow in sediments that are waterlogged by tidal action and generally are aerobic a few millimetres after the surface; although plants are able to modify the surrounding environment of their roots or rhizomes, in order to avoid toxic ions and maximize the availability of oxygen and nutrients (Alongi, 1997). Salt marsh plants have the availability to translocation oxygen from the leaves to the roots by convective flow, rather than active pumping, through specialised channels of specialised parenchyma called aerenchyma. The oxygen is then diffused into the soils, creating an aerobic environment in the vicinity of the roots called rhizosphere (Figure 3.1) (Catallo et al., 1999; Mitsch and Gosselink, 2000). Several papers have reported chemical changes in the rhizosphere of salt marsh plants, including the redox potential (Eh), metals availability and also the oxygen and nutrient profiles (e.g. Caçador et al., 1996; Madureira et al., 1997; Alongi, 1997; Cartaxana et al., 1999; Lillebø et al., 2006, Duarte et al., 2007; Mucha et al., 2008). The oxidised environment created by the roots can influence the oxidation and subsequent mobilisation of sulphides, reduced form of iron and manganese and also promote an intense microbial activity (Sundby et al., 1998; Catallo et al., 1999; Weis and Weis, 2004). Metal remobilisation can also occur due to the acidification of the rhizosphere by the exudates released from plant roots (Doyle and Otte, 1997). Mucha et al (2008) concluded that salt marsh plants (*Juncus mar*itimus and *Scirpus maritimus*) release to the rhizosphere strong organic ligands capable to complex with Cu but also probably with other metals, forming complexes with different stability and availability. Changes in the pH and Eh sediments induce changes in metal solubility and speciation that may result in a flux from the sediments to the interstitial or water column increasing the potential uptake by the plants (Weis and Weis, 2004).

Figure 3.1- Salt marsh sediments: transition between the oxic and anoxic layer.

Internal biogeochemical cycle of salt marshes is mainly based on the mineralisation of the organic matter in the sediments. Several chemical and biological transformations occur as coupled oxidation (electron donors) and reduction (electron receptors) reactions taking place in the sediments (Mitsch and Gosselink, 2000). Wetland soils can be classified as mineral or organic soils depending on their organic matter content. Mineral soils have usually values less than 20-35% (dry weight basis) of organic matter (Mitsch and Gosselink, 2000). When sediments are inundated by tidal action, the water fills the pore spaces which drastically reduce the diffusion of oxygen into the sediments (Mitsch and Gosselink, 2000). The redox potential (or oxidation-reduction potential), can be used as an assessment tool to measure the chemical potential energy of the system by the determination of the oxidation states of the constituents. The oxidised systems are depleted in high energy chemical bonds and tend to have positive oxidation states and the free energy is low (e.g. Fe^{3+}, Mn^{4+}, S^{+6}, C^{+4}), while in the reduced systems the chemical constituents have low energy chemical bonds and tends to have low oxidation states rather than the oxidised systems (e.g. $C^{-4,}$ Fe^{+2}, Mn^{+2}, S^{-2}) and the free energy of the system is high (Catallo et al., 1999). Immediately after the sediments waterlogging, begins the oxygen depletion followed by the nitrate and sulphate reduction, on the other hand the concentrations of reduced forms of iron and manganese, hydrogen sulphide and methane arise (Figure 3.2).

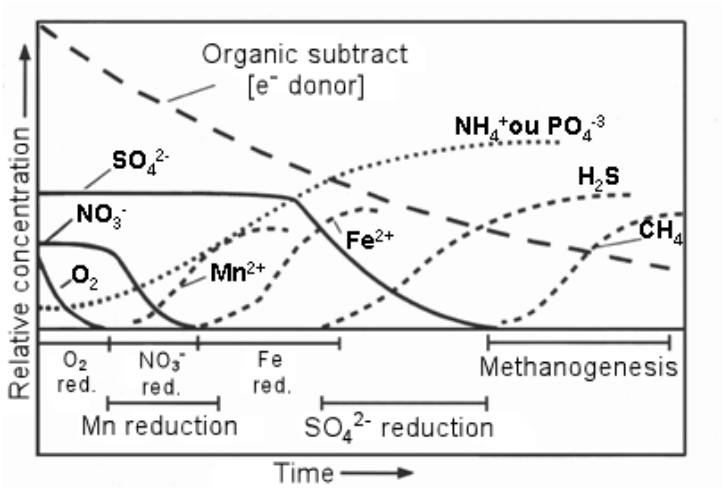


Figure 3.2- Sequence in time of transformations in sediments after flooding (adapted from Mitsch and Gosselink, 2000)

In the oxic zone, oxygen is the terminal receptor of electrons (redox potential between 400-600 mV), while in the suboxic environment the nitrate (approx. 250 mV), manganese oxides (approx. 225 mV) and iron oxides (approx. +100 to -100 mV) may act as oxidizing agents for the degradation of organic matter into inorganic carbonate species. At last in the anoxic environment sulphate (approx. -100 to -200 mV) and carbon dioxide (below -200 mV) are reduced to sulphide and methane. Nevertheless these redox values are not threshold values and can be dependent on other factor like temperature and pH. Sulphate reduction is not limited to the anoxic zone being also reported its reduction in the oxic zone in cases of higher amounts of degradable organic matter (Wallmann et al., 1996; Mitsch and Gosselink, 2000). Rates of organic matter decomposition are most rapid in the presence of oxygen as electron receptors than nitrates and sulphates (Mitsch and Gosselink, 2000).

Redox potential influences chemical and biological processes, e.g. biogeochemistry of trace metals, wetland plant distributions, productivity and physiological status, microbial and meiofaunal distributions and ecology (Catallo et al., 1999). Another important parameter is the pH of the sediments and water which is an indicative of the degree of the acidity or basicity. While the pH in terrestrial soils can range from five to nine, the pH range of wetland soils narrow and generally turns around seven. Diurnal fluctuations on pH values have been observed, with Increases during the day due to the fixation of CO_2 and diminishing during the night period due to respiration processes. These fluctuations can result in biogeochemical changes, namely microbial mediated reactions, adsorption, partitioning, and precipitation-complexation reactions and therefore toxicity and water solubility (Calmano et al., 1996).

3.3 Salt marshes and metal contamination

All soils and sediments contain many trace metals as natural constituents; however the problems arise when essential elements decreases (or became in an unavailable form), below the essential needs for the plants or when the concentrations arise (or became available) enough to cause adverse effects on the organisms (Catallo et al., 1999). Salt marshes play a significant role in metal cycling in coastal ecosystems, as they may act as sources, sinks or transformers of chemicals, depending on the wetland type, hydrologic conditions and the time of exposure to the chemical loading (Mitsch and Gosselink, 2000). Salt marshes degradation has resulted in the increase of the metals concentrations in its different biotic (plants, algae and animals species) and abiotic (sediments and pore waters) compartments.

Salt marshes may act as sinks for nutrients and contaminants (e.g. Hung and Chmura, 2005; Hwang et al., 2006; Caçador et al., 2007) but they also can act as sources, exporting organic matter (Bouchard and Lefeuvre, 2000) and nutrients which support estuarine and terrestrial food webs and they can also export metals (Montague, 1999; Weis and Weis, 2004). Several studies have been pointed also salt marshes as sinks for metals (e.g. Caçador et al., 2000; Windham et al., 2003; Spencer et al., 2003; Cundy et al., 2005; Almeida et al., 2006; Hung and Chmura et al., 2006; Hwang et al., 2006; Kongchum et al., 2006; Reboreda et al., 2008; Caetano et al., 2008) but some studies have been done in order to evaluate if they can act also as sources to the adjacent environments (e.g. Burke et al., 2000; Weis and Weis, 2004).

Metals concentrations in salt marsh sediments usually are not uniform; in fact, metals concentrations can change within salt marshes being these variations associate to the distance to the anthropogenic sources, hydrological conditions, flooding frequency and physical-chemical changes of the water quality (Williams et al., 1994). It is important to study the accumulation processes as well their distribution inside the ecosystem to predict risks and make decisions. Some metals like Hg, Sn and Pb can be converted in more toxic forms in the sediments. The introduction of toxic pollutants can influence the decomposition processes of organic matter, resulting in the change of the balance of production/decomposition, storage/release of carbon, nutrients and metals. For example the introduction of Cu can lead to the decrease of decomposition rates and influences the bacterial, algae and fungus growth (Richarson, 1999).

Metals mobility and availability for plant uptake is dependent on their chemical form in the sediments. Depending on sediments, composition, pH and redox potential, metals can be found as free cations, complexed with organic and inorganic ligands, precipitated with inorganic compounds like sulphides and occluded in iron and manganese oxides or in the primary structure of the sediments minerals and in labile forms. In general dissolved metals (soluble as free ions, inorganic and organic complexes) and exchangeable metals (metals adsorbed by electrostatic forces) are the most mobile and available for plant uptake, when compared to colloidal or precipitated forms. On the other extreme of the availability range are the metals bound within the crystalline lattice of primary minerals which are considered the most unavailable (Catallo et al., 1999). Low pH values shift metal partitioning to the ionic phase and, for negative values of redox potential, sulphide tends to precipitate with metals. Otherwise, for positives potentials, SO_4^{2-} is the predominant form and iron and manganese can occur as insoluble hydrous oxides. The

range between -150 and +200 mV is considered to be the interval where the greatest possible metal mobility exists (Jackson, 1998). Tidal action can induce changes in the salinity of interstitial water which can promote the mobilisation of metals through chloro-complexes formation, reducing their bioavailability to plants. This can be emphasised in the case of metals that can form strong complexes with chloride like mercury, lead and cadmium (Williams et al., 1994). By the other hand, salinity also influences the flocculation/coagulation of colloidal particles, adsorption/desorption reactions with organic matter and precipitation/dissociation of oxihydroxides (Williams et al., 1994).

The extent of uptake and how metals are distributed within plants can have important effects on the residence time of metals in plants and subsequently in wetlands, as well as on their potential release to the adjacent environment. If metals are accumulated into aboveground tissues, they may enter food webs, biomagnifying their effects in each level of the food chain. This information is needed in order to a better understand of these systems and to assure that the wetlands do not themselves eventually become sources of metal contamination (Weis and Weis, 2004).

With the exception of metal hyperaccumulators, most of plants restrict the movement of metals ions into their photosynthetic tissues. Metal concentrations in salt marsh plants differ according to the plant and the tissues of the plant. Studies on metal sequestration by salt marsh plants are essential to a better understand and predict the role of different plant species in metal storage and cycling. This information is important for the use of wetlands for phytoremediation as well as for marsh restoration efforts (Weis and Weis, 2004).

The oxidised rhizosphere promoted by the plants action can remobilize mercury sulphides ($HgHS^-_2$; HgS^-_2; or co-precipitated with FeS and polysulphides) (St-Cyr and Crowder, 1990; Lacerda *et al.*, 1993; Marins *et al.*, 1997). Marins et al. (1997) reported that salt marsh plants are able to mobilise deposited mercury through the exudation of oxygen. Avoiding permanent reducing environments mercury can be remobilised from stable mercury sulphides. The dynamic oxic-anoxic conditions promoted by the roots of the plants hampers the immobilisation of mercury as mercury sulphides, resulting in the migration of mercury through the sediment column with a clear accumulation of mercury in the sediments layers with higher sulphide concentrations and able to mobilise mercury.

Some salt marsh plants are able to form iron-oxide coatings surrounding their roots, called rhizoconcretions, formed by the precipitation of iron oxides in the pore of the sediments. According to Otte (1991) in Otte et al. (2000), the precipitation of iron in the rhizosphere of wetland plants results in a decreasing of the concentration gradient of soluble iron from the bulk soil towards the root surface which drive in a continuous influx of iron into the rhizosphere. Sundby et al. (1998) reported that *Spartina maritima* is able to mobilise trace metals dispersed in anoxic estuarine sediments and concentrate them into a microenvironment oxidised that surrounds the roots. The supply of oxygen trough the sediment surrounding the roots seems to be and effective mechanism for reducing the uptake of excessive amount of potentially toxic reduced species, these rhizoconcretions are sometimes enriched 5-10 times in some metals (e.g. Cd, Cu, Pb and Zn) with respect to the surrounding sediments. Thus iron chemistry plays an important and vital role on salt marshes biogeochemistry. With the oxidation of the salt marsh sediments especially during the growing season of the plants, a large percentage of sedimentary pyrite is converted to an oxidised iron mineral and the opposite reaction occurs during the periods when the plants are not active. When the high oxidation rates in the growing season are high enough to neutralize the alkalinity produced by the sulphate reduction and substantially decrease de pH, the oxidised iron minerals become increasingly soluble and the amounts of iron in the pore waters increase. (Giblin and Howarth, 1984).

Differences in uptake rates and allocation patterns between species can generate different rates of metal retention in wetland vegetation. Windham et al. (2003) reported different allocation patterns of metal accumulation in *Phragmites australis* (common reed) and *Spartina alterniflora* (salt cord grass). In this study, authors concluded that higher concentrations of mercury and chromium were found in above ground biomass (namely leaves) of *Spartina alterniflora* than in *Phragmites australis* but in the case of copper and zinc the highest values were found in the above ground tissues of *Phragmites australis*. The study concluded that mercury and chromium export from the salt marsh to the water column and/or food web is grater in stands of *Spartina alterniflora* than *Phragmites australis;* however it can be observed an enrichment of copper and zinc. Thus the replacement of *Spartina alterniflora* by *Phragmites australis* may reduce metal bioavailability of some metals by sequestering a greater proportion of its metal burden in below ground tissues that are permanently buried.

The type of the sediment in the salt marshes can affect the role of the plant on metal cycling. Almeida et al. (2006) concluded that *Juncus maritimus* (sea rush) presented a different behaviour in sandy and muddy salt marsh sediments. In the sandy sediments it was observed significant variations through the year only for Cd and Cu levels of sediments, rhizosediments and roots, while in the muddy site it was observed variations for Al, Cd, Cr, Cu, Fe, Mn, Ni and Zn. In sandy sediments the variations were the same in the biotic and abiotic compartments, with a decrease from winter to summer and an increase in autumn. In the muddy sediments the variations in Cd and Zn contents of roots were the opposite of those in the rhizosediments (increase in the roots during the summer and decrease in the sediments), while for the case of Fe and Mn similar patterns of variation for the roots and rhizosediments (increase during the summer). With respect to the remaining metals no significant correlations between rhizosediments and roots were observed.

The number of studies reporting metal contamination is high (e.g. Cundy et al., 2005; Almeida et al., 2006; Caetano et al., 2008), although the number of studies reporting mercury accumulation and especially mercury methylation in salt marshes is scarce and further limited. Most of them report total mercury concentrations in sediments (Hung and Chmura, 2006) and roots, translocation to the above ground tissues and release to the surrounding environmental compartments. Although wetlands have been pointed as prime sites for mercury methylation (Galloway and Branfireun, 2004; Holmes and Lean, 2006; Goulet et al., 2007; Hall et al., 2008), few studies have been done in order to assess methylation of mercury in salt marsh ecosystems (Heller and Weber, 1998; Kongchum et al., 2006; Canário et al., 2007). High concentrations of methylmercury in salt marshes raise the concern of bioaccumulation in salt marsh species and in general transfer of mercury compounds up the food chain. Figure 3.3 represents the hypothetical model of mercury cycling in wetlands.

Litter resultant from salt marsh plant decomposition can also contribute to the retention of metals into the ecosystem. Zawislanski et al (2001) reported the accumulation of Se and trace metals (Cu, Ni, Zn, Pb and Fe) in the litter of five common estuarine marsh plants. Metals concentrations in the litter were found 150 fold enriched relatively to the initial metal concentrations of the plant tissues.

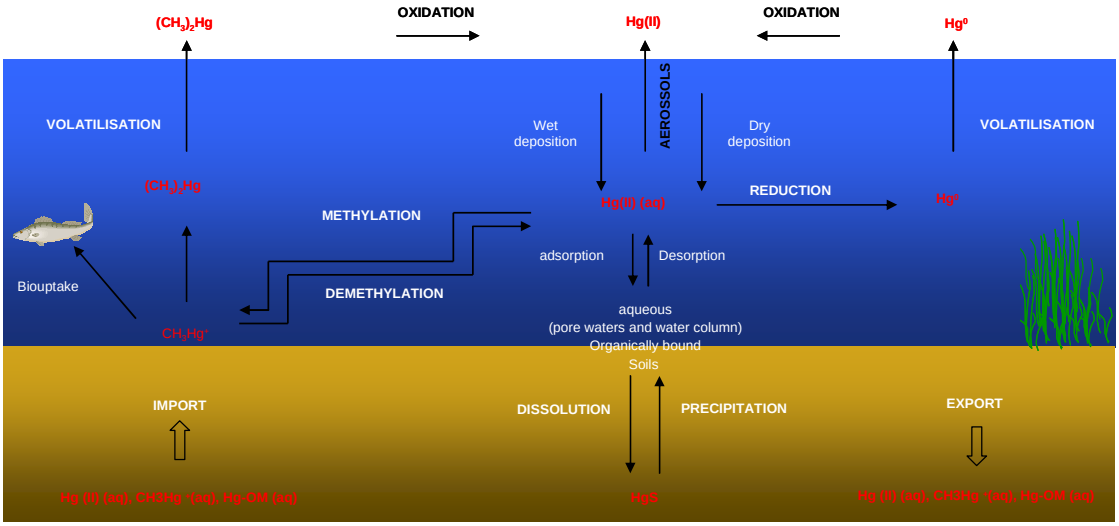


Figure 3.3 - Hypothetical model of mercury cycling in wetlands. (adapted from Richardson, 1999)

As already mentioned in chapter II, mercury is introduced in the ecosystem mainly as inorganic mercury; however mercury is bioaccumulated mainly as methylmercury. Methylmercury availability for bioaccumulation is dependent on the availability of inorganic mercury for methylation and in a final stage it is also dependent on the balance between methylation and demethylation processes. It is also important to refer that besides SRB action it is also possible to find, although in less quantities, the presence of mercury resistant bacteria, capable to detoxify ionic mercury through the production of volatile elemental mercury, thus contributing to the removal of mercury from contaminated waters. Another possible reduction of mercury in the wetlands it also due to the reduction of mercury by organic acids produced during the photosynthesis and the abiotic reduction of Hg (II) to Hg(I) with the subsequent dismutation to elemental and ionic mercury (Richardson, 1999). According to Richardson (1999), methylation and demethylation rates as well the reduction/volatilisation reactions are the key factors to understand the fate and transport of mercury in wetlands.

Heller and Weber (1998) reported high methylmercury percentages in *Spartina alterniflora* (cord grass), ranging between 6.23-48.1% of the total mercury concentration in a composite sample of leaves and stems. Kongchum et al. (2006) reported higher methylation in freshwater marshes as compared to higher salinity salt marshes. This difference is attributed to salinity effects. As referred by this author, the inhibition effect of salinity on mercury methylation

is pronounced under reducing conditions and high salinity values. Coquery et al., (1997) also reported higher concentrations of methylmercury in warmer and organic rich freshwater.

3.4 References

Almeida, C.M.R, Mucha, A.P., Vasconcelos, M.T.S.D., 2006. Variability of metals contents in the sea rush Juncus maritimus - estuarine sediments system through one year of plant's life. Mar Environ Res. 61, 424-438.

Alongi, D. M., 1997. Mangroves and Salt Marshes. In: Coastal Ecosystem Processes. Kennish, M.J. and Lutz, P.L. (Eds.), CRC Marine Science Series, CRC Press, pp. 43-92.

Boorman, L.A., 1999. Salt marshes-present functioning and future change. Mangroves and Salt Marshes. 3, 227-241.

Burdige, D.J., 1993. The Biogeochemistry of Manganese and Iron Reduction in Marine Sediments. Earth-Sci Rev. 35, 249-284.

Burke, D.J., Weis, J.S., Weis, P., 2000. Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. Estuar Coast Shelf S. 51, 153-159.

Caçador, I., Vale, C., Catarino, F., 1996. Accumulation of Zn, Pb, Cu, Cr and Ni in Sediments Between Roots of the Tagus Estuary Salt Marshes, Portugal. Estuar Coast Shelf S. 42, 393-403.

Caçador, I., Vale, C., 2001. Salt Marshes. In: Metals in the Environment- Analysis by Biodiversity. Prasad, M.N.V.(Eds.), Marcel Dekker Inc, New York, pp. 95-116.

Caçador, I., Costa, A.L., Vale, C., 2007. Nitrogen sequestration capacity of two salt marshes from the Tagus estuary. Hydrobiologia. 587, 137-145.

Caetano, M., Vale, C., Cesário, R., Fonseca, N., 2008. Evidence for preferential depths of metal retention in roots of salt marsh plants. Sci Total Environ. 390, 466-474.

Calmano, W., Ahif, W., Förstner, U., 1996. Sediments quality assessment: chemical approaches. In: Calmano, W. and Förstner, U. (Eds). Sediments and Toxic Substances. Springer Verlag, Berlin, pp.1-35.

Canário, J., Caetano, M., Vale, C., Cesário, R., 2007. Evidence for elevated production of methylmercury in salt marsh. Environ Sci Technol. 41, 7376-7382.

Cartaxana, P., Lloyd, D., 1999. N_2, N_2O and O_2 profiles in a Tagus estuary salt marsh. Estuar Coast Shelf S. 48, 751-756.

Catallo, W.J., Blankemyer, J.T., Gambrell, R.P., Pardue, J.H., Pugesek, B., Reddy, K.R., 1999. Workgroup I Synopsis: Biogeochemical Processes. In: Ecotoxicology and Risk Assessement for Wetlands, Lewis et al. (Eds.), SETAC Press, Florida-USA, pp. 9-25.

Chen, J., Goldsbrough, P.B., 1994. Increased activity of γ-glutamylcysteine synthetase in tomato cells selected for cadmium tolerance. Plant Physiol. 106, 233-239.

Chenhall, B.E., Yassini, I., Jones, B.G., 1992. Heavy metal concentrations in lagoonal saltmarsh species, Illawarra Region, Southeastern Australia. Sci Total Environ. 125, 203-225.

Coquery, M., Cossa, D., Sanjuan, J., 1997. Speciation and sorption of mercury in two macro-tidal estuaries. Mar Chem. 58, 213-227.

Cundy, A.B., Hopkinson, L., Lafite, R., Spencer, K., Taylor, J.A. Ouddane, B., Heppell, C.M., Carey, P.J., Charman, R., Shell, D., Ullyott, S., 2005. Heavy metal distribution and accumulation in two *Spartina* sp.- dominated macrotidal salt marshes from the Seine estuary (France) and the Medway estuary (UK). Appli Geochem. 20, 1195-1208.

Dyer, K.R., 1997. Estuaries; a physical Introduction, 2nd edition John Wiley and Sons Ltd

Doyle, M.O., Otte, M.L., 1997. Organism-induced accumulation of iron, zinc and arsenic in wetland soils. Environ Pollut. 96(1), 1-11.

Eisma, D., Dijkema, K. S., 1998. The influence of salt marsh vegetation on sedimentation. In: Intertidal Deposits- River Mouths, Tidal Flats, and Coastal Lagoons, Kennish, M.J. and Lutz, P.L. (Eds.), CRC Press, Florida, pp. 403-414.

Duarte, B., Delgado, M., Caçador, I., 2007. The role of citric acid in cadmium and nickel uptake and translocation, in *Halimione portulacoides*. Chemosphere. 69, 836-840.

Elliott, M.D. Burdon, Hemingway, K.L., Apitz, S.E., 2007. Estuarine, coastal and marine ecosystem restoration: Confusing management and science-A revision of concepts. Est Coast Shelf S. 74, 349-366.

Galloway, M.E., Branfireun, B.A., 2004. Mercury dynamics of a temperate forested wetland. Sci Total Environ. 325, 239-254.

Giblin, A.E., Howarth, R.W., 1984. Porewater evidence for a dynamic sedimentary iron cycle in salt marshes. Limnol Oceanogr. 29, 47-63.

Goulet, R.R., Holmes, J., Page, B., Poissant, L., Siciliano, S.D., Lean, D.R.S., Wang, F., Amyot, M., Tessier, A., 2007. Mercury transformations and fluxes in sediments of a riverine wetland. Geochim. Cosmochim. Ac. 71, 3393-3406

Hall, B.D., Aiken, G.R., Krabbenhoft, D.P., Marvin-DiPasquale, M., Swarzenski, C.M., 2008. Wetlands as primcipal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region. Environ Pollut. 154(1), 124-134.

Heller, A.A., Weber, J.H., 1998. Seasonal study of speciation of mercury (II) and monomethylmercury in *Spartina alterniflora* from the great Bay estuary, N.H. Sci Total Environ. 221, 181-188.

Holmes, J., Lean, D., 2006. Factors that influence methylmercury flux rates from wetland sediments. Sci. Total Environ. 368, 306-319.

Hung, G.A., Chmura, G.L., 2006. Mercury accumulation in surface sediments of salt marshes of the Bay of Fundy. Environ Pollut. 142, 418-431.

Hwang, H.M., Green, P.G., Higashi, R.M., Young, T.M., 2006. Tidal salt marsh sediment in California, USA. Part 2: Occurrence and anthropogenic input of trace metals. Chemosphere. 64, 1899-1909.

Jackson, T.A., 1998. Mercury in aquatic ecosystems. In: Langston, W.J. and Bebianno, M.J. (Eds). Metal metabolism in the aquatic environment. Chapman and Hall, Lda Publishers, UK, pp. 77-138.

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, 1300-1303.

Lacerda, L.D., Carvalho, C.E.V., Tanizaki, K.F., Ovalle, A.R.C., Rezende, C.E., 1993. The biogeochemistry and trace metal distribution of Mmngrove rhizospheres. Biotropica. 25(3), 252-257.

Lillebø, A.I., Neto, J.M., Martins, I., Verdelhos, T., Leston, S., Cardoso, P.G., Ferreira, S.M., Marques, J.C., Pardal, M.A., 2005. Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system nutrient loading. Est Coast Shelf S. 65, 697-707.

Lillebø, A.I., Flindt, M.R., Pardal, M.A., Marques, J.C., 2006. The effect of *Zostera noltii, Spartina maritima* and *Scirpus maritimus* on sediment pore-water profiles, in a temperate intertidal estuary. Hydrobiologia. 555,175-183.

Lillebø, A.I., Flindt, M.R., Pardal, M.A., Cardoso, P., Ferreira, S., Marques, J.C., 2007. The faunal role on the degradation of the common intertidal salt-marsh plant *Scirpus maritimus*. Hydrobiologia. 579, 369-378.

Madureira, M.J., Vale, C., Simões Gonçalves, M.L., 1997. Effect of plants on sulphur geochemistry in the Tagus salt-marshes sediments. Mar Chem. 58, 27-37.

Marins, R.V., Lacerda, L.D., Gonçalves G.O., Paiva, E.C., 1997. Effect of Root Metabolism on the post-depositional mobilization of mercury in salt marsh soils. B Environ Cont Toxicol. 58, 733-738.

Mitsch, W.J.,Gosselink, J. G., 2000. Wetlands, 3 ed., John Wiley and Sons, Inc. USA.

Montague, C.L., 1999. Encyclopedia of Environmental Science, Kluwer Academic Publishers.

Mucha, A.P., Almeida, M.R., Bordalo, A.A., Vasconcelos, M.T.S.D., 2008. Salt marsh plants (*Juncus maritimus* and *Scirpus maritimus*) as sources of strong complexing ligands. Estuar Coast Shelf S. 77, 104-112.

Otte, M.L., Kenny, F.J., Doyle, M.O., 2000. Accumulation of Iron and other metals in the rhizosphere of wetland plants- implications for biogeochemical processes. Verh Internat Verein Limnol., 27, 1730-1733.

Packamn, J.R., Willis, A.J., 1997. Ecology of Dunes, Salt Marsh and Shingle, Published by Chapman and Hall, UK.

Pethick J., 1993. An introduction to coastal geomorphology. Arnold, E. (Eds.), Great Britain.

Piechalack, A. Tomaszewska, B. Baralkiewicz, D. Malecka, A., 2002. Accumulation and detoxification of lead ions in legumes. Phytochemistry. 60, 153-152.

Reboreda, R. Caçador, I., Pedro, S., Almeida, P.R. 2008. Mobility of metals in salt marshes sediments colonised by *Spartina maritima* (Tagus estuary, Portugal). Hydrobiologia. 606, 129-137.

Reis, A., 1993, Ria de Aveiro- Memórias da Natureza. Câmara Municipal de Ovar

Richardson, C.J., 1999. Plenary Session Presentation: Ecological Functions of Wetlands in the Landscape. In: Ecotoxicology and Risk Assessement for Wetlands, Lewis et al. (Eds.), SETAC Press, Florida-USA, pp. 9-25.

Rosa, M.J.V., Vieira, C. 2003. A população portuguesa no século XX-Análise dos Censos de 1900-2001. Instituto da Ciências sociais da Universidade de Lisboa (Eds), Liboa.

Salisbury, F.B., Ross, C.W**.,** 1985. Plant Physiology, 3 ed., Chp. 24.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature. 413, 591-596.

Spencer, K.L., Cundy, A.B., Croudace, I.W., 2003. Heavy metal distribution and early-diagenesis in salt marsh sediments from the Medway estuary, Kent, UK. Estuar Coast Shelf S. 57, 43-54.

St-Cyr, L., Crowder, A. A., 1990. Manganese and copper in the root plaque of *Phragmites australis* (Cav) Trin, ex Steudel. Soil Science. 149, 191-198.

Sundby, B., Vale, C., Caçador, I., Catarino, F., 1998. Metal-rich concretatios on the roots of salt marsh plants: mechanism and rate of formation. Limnol Oceanogr. 43(2), 245-252.

Wallman, K., Petersen, W., Reiners, C., Gramm, H. 1996. Trace element diagenesis in polluted sediments of the river Elbe estuary. In: Calmano, W. and Förstner, U. (Eds). Sediments and toxic substances: environmental effects and toxicity Springer Verlag Berlin Heidelberg, pp. 197-214.

Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implication for phytoremediation and restoration. Environ Int. 30, 685-700.

Widdows, J., Brinsley, M., 2002. Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. J Sea Res. 48, 143-156.

Williams, T.P., Bubb, J.M., Lester, J.N., 1994. Metal accumulation within salt marsh environments: a review. Mar Pollut Bull. 28, 277-290.

Windham, L., Weis, J.S., Weis, P., 2003. Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). Est Coast Shelf S. 56, 63-72.

Zawislanski, P.T., Chau, S., Mountford, H., Wong, H.C., Sears, T.C., 2001. Accumulation of selenium and trace metals in plant litter in a tidal marsh. Estuar Coast Shelf S. 52, 589-603.

4 Methods and experimental section

4.1 Introduction

To achieve a good quality and trustable data, some cautions must be undertaken especially with mercury studies. The collection of representative samples and its preservative treatment and subsequently store are crucial steps for a good quality assurance program that must be implemented. In mercury studies some aspects regarding the sample preservation must be undertaken and emphasised to avoid cross contamination and/or volatilisation processes and conversion processes of mercury species (Horvat, 1999).

This chapter describes the experimental methods adopted, as well all the operational conditions, sample treatment and quality control assurance. Detailed information about the sampling design is described in the correspondent chapter since the different studies discussed in this thesis required different sampling strategies.

4.2 Cleaning procedures

Special attention should be taken with material cleaning procedures, especially when low contents of mercury contents samples are manipulated. In this work ultra clean protocols were adopted from Bloom (1995) and USEPA (1996). All glassware material was firstly washed with tap water followed by immersion (24 h) into a solution of Decon (5%). After the Decon immersion, the material was over again immersed (24 h) into an acid solution (HNO_3, 4 M). Finally all the material is rinsed with distilled water.

All material that will be handling with low mercury content samples (namely waters) was processed apart from the rest of the material. This material was pre-treated with HNO_3 (conc.) before the immersion into the diluted acid solution of HNO_3 (4 M). Different bath solutions were used and distilled water was replaced by ultra pure water (<18 MΩcm, Millipore Milli-Q model 185 system).

4.3 Sample treatment

4.3.1 Sediments

Immediately after the collection the sediments were transferred to polyethylene bags and transported to the laboratory under refrigerated conditions. Sediments were homogenised, freeze-dried (Christ Alpha 1-4 lyophilizator) and manually sieved using a 1 mm size nylon sieve. All

chemical analysis performed in vegetated sediments were done in sediments previously cleaned from roots and debris.

Sediments for AVS analysis were preserved in aluminium foil and always manipulated under N_2 atmosphere to avoid oxidation processes. The preservation was made by freezing at-20 ^{o}C until analysis.

4.3.2 Water samples

Water samples were collected in acid-cleaned polyethylene bottles and rinsed with the sample prior to the filling. After the collection, samples were transported to the laboratory under refrigerated conditions. In the laboratory, water samples were filtered through a Millipore 0.45 µm filter (Millipore), acidified with nitric acid (Hg-free) to pH<2 and stored in borosilicate acid-cleaned bottles at 4^{o}C. The filtration process occurred within a few hours after the collection.

Pore waters were extracted from the sediments and always manipulated under N_2 atmosphere. The extraction from the sediments was performed by centrifugation at 3783 g (6000 rpm) during 30 min (B. Braun, model Sigma 4-10). After centrifugation, the pore waters samples were filtered (Millipore 0.45 µm), acidified with HNO_3 (Hg-free) to pH<2 and stored in borosilicate acid-cleaned bottles at 4 ^{o}C.

In order to assess any possible contamination during the filtration process, procedural blanks were carried out between the samples. Procedural blanks were performed by passing through the filtration unit system, Milli-Q water, which was treated as an ordinary sample.

4.3.3 Biomass

Below ground biomass (Figure 4.1) were carefully sorted from the sediment under a flux of water using a 250 µm mesh size and rinsed with ultra pure water to remove any adhering particles of sediments.

Figure 4.1-Representative figure of below ground biomass samples.

Above ground biomass (Figure 4.2) was also carefully rinsed with distilled water separated in leaves and stems. Any adhering material to the external surfaces like epiphytes was removed by moderate scraping.

Figure 4.2- Representative figure of above ground biomass samples.

Biomass material for mercury analysis was oven dried at 45-60 ^{o}C (maximum) until reaching a constant weight and homogenised for further analysis. Biomass samples for mercury intracellular partitioning and chelation studies were immediately frozen at -80 ^{o}C after the cleaning process and processed in a few days after the collection.

4.4 Sample analysis

4.4.1 Sediments

4.4.1.1 Water and organic matter content

Water content in the sediments samples was determined by oven drying 5 g of wet sediments at 120 ^{o}C (until constant weight-usually 24h) and the results were expressed as percentage. The dried sediments were used to determine the organic matter content lost on ignition (LOI) placing the sediments at 500 ^{o}C during 4 hours in a muffle and the result was also expressed as percentage.

4.4.1.2 Fine fraction

Fine particles (<63 μm) of the sediments were determined by wet sieving. This method consisted in weighing 5 g of dried sediments (duplicate) and sieving the sediment through a 63 μm sieve under a gently water flux (Pereira, 1996). The major advantage of this method is the disaggregation of the sediments clusters, formed in the drying process of the sediments, which sometimes it is impossible with the dry sieving. After the sieving the retained fraction (>63 μm) is dried at 120 ^{o}C until constant weight and the fraction <63 μm it is estimated by the difference.

4.4.1.3 Total mercury

Mercury concentrations in sediments samples were determined in triplicate by atomic absorption spectrometry (AAS) with thermal decomposition of the sample (LECO AMA 254) according to Costley et al. (2000). This methodology is simple and based on a thermal decomposition of the sample and collection of the mercury vapour on a gold amalgamator. The sample is firstly dried at 120 ^{o}C prior the combustion at 680-700 ^{o}C in an oxygen atmosphere. The mercury vapour is collected in a gold amalgamator and after a pre-defined time (45 s) the gold amalgamator is heated at 900 ^{o}C. The released mercury is transported to a heated cuvette (120 ^{o}C) and then analysed by AAS using a silicon UV diode detector. The major advantage of using this technique is that it is not require complex manipulation of the sample, such as digestion processes, avoiding contaminations issues.

Operational conditions used included a drying time: 10 s; decomposition time: 150 s; waiting time: 45 s and the amount of samples used ranged between 50 and 500 mg.

4.4.1.4 Methylmercury

Methylmercury was extracted from the samples according to Cai et al. (1997) which is based on the treatment of the samples (aprox. 2 g) with 8 mL of an acidic potassium bromide and 1 M $CuSO_4$ mixture (2:1) and 10 mL of dichloromethane. Vials are left to shaken overnight and after that, centrifuged, at least 2000 rpm for 10 min. The organic phase is carefully removed and 2 mL of Na_2SO_3 are added followed by the shaken for 30 min and mixed on a vortex genie for 30 s. A known volume (approx 1.5 mL) of the sodium thiosulphate layer (aqueous layer) is transferred carefully and stored, while another 2 mL portion of sodium thiosulphate is added to the vial containing the dichloromethane extract and sodium thiosulphate. This is shaken for a further 30 min then mixed on a vortex genie for 30 s. A second known volume (approx 1.5 mL) of the upper sodium thiosulphate layer is removed and added to the first portion. The next step consists in the addition of 1.2 mL of acidic potassium bromide and $CuSO_4$ solution to the sodium thiosulphate solution and 1 mL of dichloromethane is added and the solutions shaken for 30 min then mixed on a vortex genie for 30 s. The organic phase is then analysed by 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 m $\times$ 0.53 mm ID-Megabore) coated with a 1.5 µm film thickness of DB-1 (JandW Scientific) was used. Calibration was performed using liquid standards, prepared from a 1000 mg L^{-1} methylmercury chloride standard solution, diluted in methanol.

4.4.1.5 Iron oxihydroxides

Iron oxihydroxides content in sediments were determined after extraction (duplicate) with 20 mL of a hydroxylammonium chloride solution (0.04 M in 25% acetic acid) for 6 h at room temperature with continuous mechanic agitation (Chester and Hughes, 1967). The extracts were separated from the solid residue by centrifugation at 3783 g (6000 rpm) for 30 min, and then filtered (Millipore 0.45 µm). Extracted solutions were analysed F-AAS (Perkin-Elmer, model AAnalyst 100). Calibration was performed using liquid standards, prepared from a 1000 mg L^{-1} iron standard solution (BDH), diluted in acidifies ultra pure water.

4.4.1.6 Acid volatile sulphides

The analytical methodology for releasing sulphide from the sediments and trapping the evolved H_2S consists in the digestion of wet sediment samples (Figure 4.3), about 70 mg on a 2 cm

× 2 cm piece of aluminium foil, for 40 min at room temperature with deaerated 3 M HCl in a system previously flushed with N_2 for 20 min. The evolved H_2S was trapped in 20 mL of a deaerated solution of NaOH (pH10-11) (Allen et al., 1993). Particular attention was paid to the recovery of sulphide from standards in evaluating the used methodology.

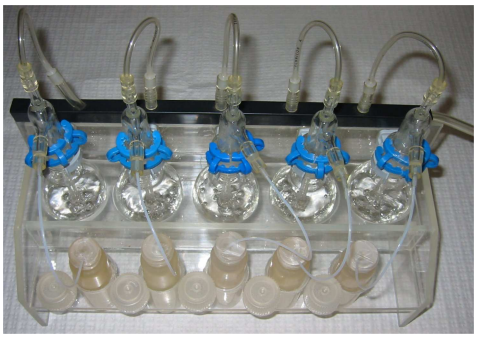


Figure 4.3- Digestion apparatus for AVS extraction from sediments.

The H_2S formed was measured by fast linear scan cathodic stripping voltammetry in 20 mL of NaOH cell solution (pH 10-11), previously purged with N_2 for 5 min, to which an appropriate volume (ca. 20-100 µL) of the deaerated sulphide sample or standard was added. The deposition step lasted 5 s at a deposition potential, E_{dep}, of −100 mV, while the solution was stirred (rotational frequency 25 s^{-1}). After a 5 s quiescent time, the stripping step was performed from −100 to −1000 mV. The scan rate was 1000 mV s^{-1}. Calibration was carried out with Na_2S standardised solutions diluted in NaOH (pH 10-11) (Carapuça et al., 2004).

4.4.2 Biomass samples

4.4.2.1 Total mercury

Total mercury concentrations in biomass samples were determined in triplicate by AAS with thermal decomposition (LECO AMA 254) according to Costley et al. (2000). The operational conditions were the same as the used for sediments.

4.4.2.2 Methylmercury

The method used for methylmercury extraction from the biomass (roots and leaves) was the same that was used for sediments.

4.4.2.3 Extraction of the cystosolic fraction of Halimione portulacoides tissues

Frozen root tissues were homogenised essentially as described by Rauser (2000), with 100 mM HEPES (pH 8.6), 1 mM PMSF (phenylmethylsulfonyl fluoride) and 0.2% Tween 20 (v/v), at a ratio of 1g of tissue to 1 mL buffer. The extracted material was centrifuged at 52 000 g, during 10 min at 4 ^{o}C and the supernatant, designed Extract 1, was sub-sampled for metal quantification. The pellet obtained was resuspended in 10 mM HEPES (pH 8.6), 0.04% Tween 20 (v/v), with a volume of 1.5 times the fresh weight. The suspension was centrifuged once again and the supernatant was designed Extract 2. This procedure was then repeated four times until 6 extracts were obtained, which together provided the material for the peptide-Hg complex characterisation and mercury analysis. Buffer extractions 1-6 were pooled, constituting the soluble (cytosolic) fraction, and freeze-dried. The pellet (insoluble fraction) was also freeze-dried.

4.4.2.4 Size exclusion chromatography of the cytosolic fractions

Freeze-dried extracts 1-6 were resuspended in 7 mL of aqueous 0.2% Tween 20, (v/v) and centrifuged at 48 000 g, during 6 min at 4 ^{o}C. The volume of supernatant was measured, subsampled for mercury analysis and fractioned by gel filtration in a Sephacryl S-100 column (326 i.d. $\times$ 112 mm; 119 mL, Amershan Biosciences). The gel bed was equilibrated with degassed elution buffer 10 mM HEPES (pH 8.0) and 300 mM KCl. Elution was achieved with an injection of 2 mL of sample and at a flow rate of 0.8 mL min^{-1}, at room temperature. The absorbance was registered (Amersham Biosciences detector) at 254 nm (A254) and fractions were collected (Gilson 201 Fraction Collector) every 3 min (approximately 2.4 mL). All fractions were sub-sampled for mercury quantification and those corresponding to PC-Hg complexes were combined and frozen for metal and thiol analysis.

4.4.2.5 Separation and determination of thiol compounds

For thiol analysis, selected fractions were collected and complexes were dissociated by acidification, as described by Rauser (2000). Monothiols and polythiols in both complexes were separated by HPLC with pre-column derivatisation with monobromobimane (mBBr), as described previously (Lima et al., 2006). Samples (100 µL) were neutralised with 0.1 M NaOH, after the addition of 200 µL of 0.1 M Tris-HCl buffer (pH 8.0), 1 mM EDTA and 25 µL of 2 mM DTE (Ditioeritritol). After incubation for 1 h at room temperature, 50 µL of 20 mM mBBr (monobromobimane-Calbiochem) were added. Derivatisation was performed in the dark, for 40 min at a temperature of 35 ^{o}C. The reaction was stopped by the addition of 5% (v/v) acetic acid,

up to a total volume of 1.5 mL. Samples were stored at 4 ºC before HPLC-RP analysis (Klapheck, 1988).

The highly fluorescent bimane derivatives were separated by RP-HPLC (Gilson liquid chromatograph, model 306), as described earlier (Lima et al., 2006). Thiols were resolved and eluted at a flow rate of 1 mL min^{-1} and detected by fluorescence (Jasco 821-FP Intelligent Spectrofluometer) with excitation at 380 nm and emission at 480 nm (Klapheck, 1988, Sneller et al., 2000). PC peaks were identified with synthesised PC standards, as described in Lima et al (2006).

4.4.2.6 Determinations of mercury concentrations in cytosolic fractions

Extracted solutions were also directly analysed AAS with thermal decomposition, using an Advanced Mercury Analyser (AMA) LECO 254. Since extracted solutions are liquid samples the operational conditions used are different regarding the drying time step. For these determinations the operation conditions were: drying time: 350-700 s (depending on the sample volume: 500-1000 µL); decomposition time: 150 s; waiting time: 40 s. The quality control of these determinations were performed using liquid standards, prepared from a 1000 mg L^{-1} mercury nitrate standard solution (BDH), diluted in similar matrices of the different extraction solutions used.

4.4.3 Water samples

Reactive mercury in waters and pore waters was determined in triplicate by CV-AFS (PSA model Merlin 10.023 equipped with a detector PSA model 10.003) using $SnCl_2$ (2% m/v) as the reducing agent.

Total dissolved mercury concentrations were also determinate by cold vapour atomic fluorescence spectrometry (in triplicate), according to the method described by Mucci et al., 1995. This determination is based on the addition of potassium persulphate (500 µL) to the filtered sample (50 mL) and subsequent UV irradiation (1000 W) for 30 min. The excess of oxidant is reduced with hydroxylamine solution (12%, w/v). After this photochemical oxidation, total dissolved mercury was determined using the same instrumental conditions as used for reactive mercury. Procedural blanks were carried out to avoid reagents and material contamination. Calibration was performed using liquid standards, prepared from a 1000 mg L^{-1} mercuric nitrate standard solution (BDH), diluted in Milli-Q water acidified with HNO_3 (2% Hg-free).

4.5 Quality control

A good quality control is needed to ensure the integrity of the results. Accuracy, precision, limit of detection and linearity of the working range are parameters that should be carefully monitorised.

Accuracy of the results can be achieved by the use of Certified Reference Materials (CRMs); however due to the lack of these materials for some matrices, another option is the use of spiked samples with the analyte in study. This procedure is controversy due to the fact that the interactions between the analyte and the sample are not the same from those observed if the analyte was present naturally in the matrix. In this work certified reference material were always preferred with the exception of methylmercury in biomass samples due to the lack of CRMs for methylmercury determinations in plants. To assure accuracy of the results the recovery of the certified reference material were compared with the certified values.

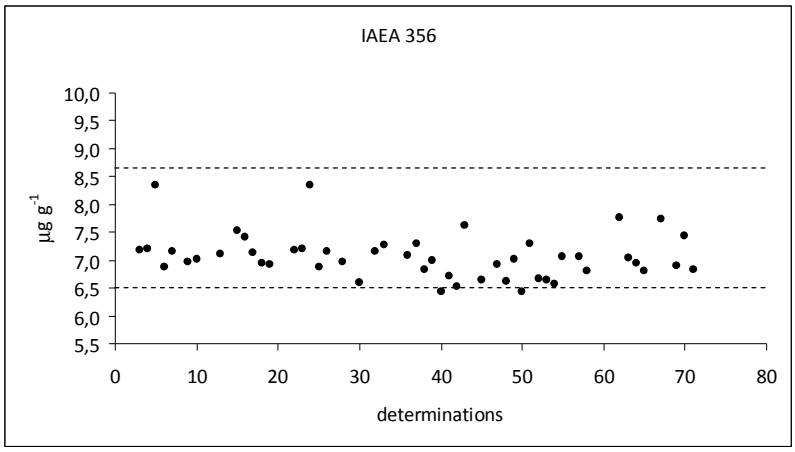
The precision of a method relies on the dispersion of the replicate results. In this work each sample was analysed at least in duplicate and the results rejected if the coefficients of variation were higher than 10%. The linearity of the working range was assed by linear regression analysis and evaluated by the Pearson product moment coefficient (R).

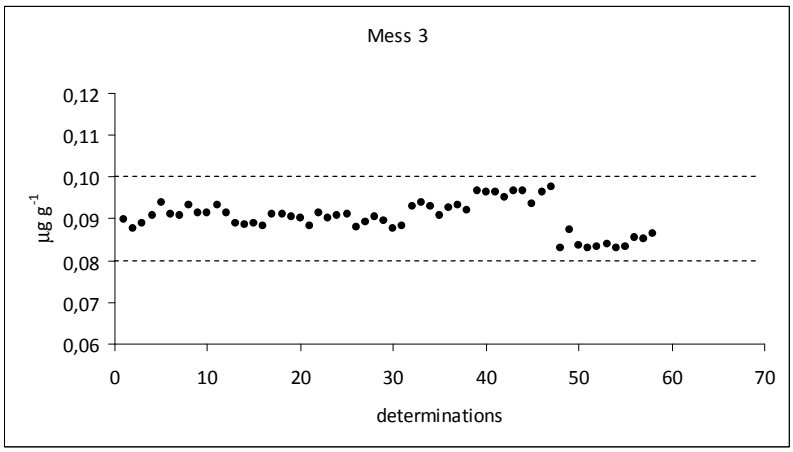
4.5.1 Quality control of operational procedures in the laboratory

4.5.1.1 Mercury determinations

The mercury analyser (LECO AMA 254) has an internal calibration which was daily checked, at the beginning and at the end of the day, by the analysis of certified reference materials and periodically checked with liquid standard solutions of mercury nitrate (BDH 1000 mg L^{-1}).

According to the type of the sample it was used a CRM with a similar matrix; in this case, for sediments, IAEA 356 (marine sediment) PACS 2 (marine sediment), Mess 2 (marine sediment) and Mess 3 (marine sediment) were the adopted reference materials while for biomass it was used the BCR 60 (aquatic plant-Lagarosiphon major). The accuracy and precision of the results were assed and control charts for each CRM were elaborated (Figure 4.4; Figure 4.5). Memory effects of the equipment were controlled, especially when higher mercury concentrations were manipulated, by performing blank analysis between samples.





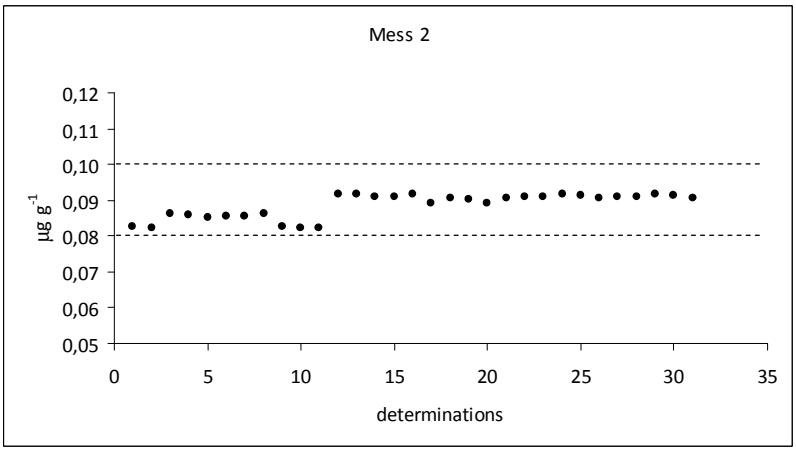


Figure 4.4- Control charts of the certified reference materials.

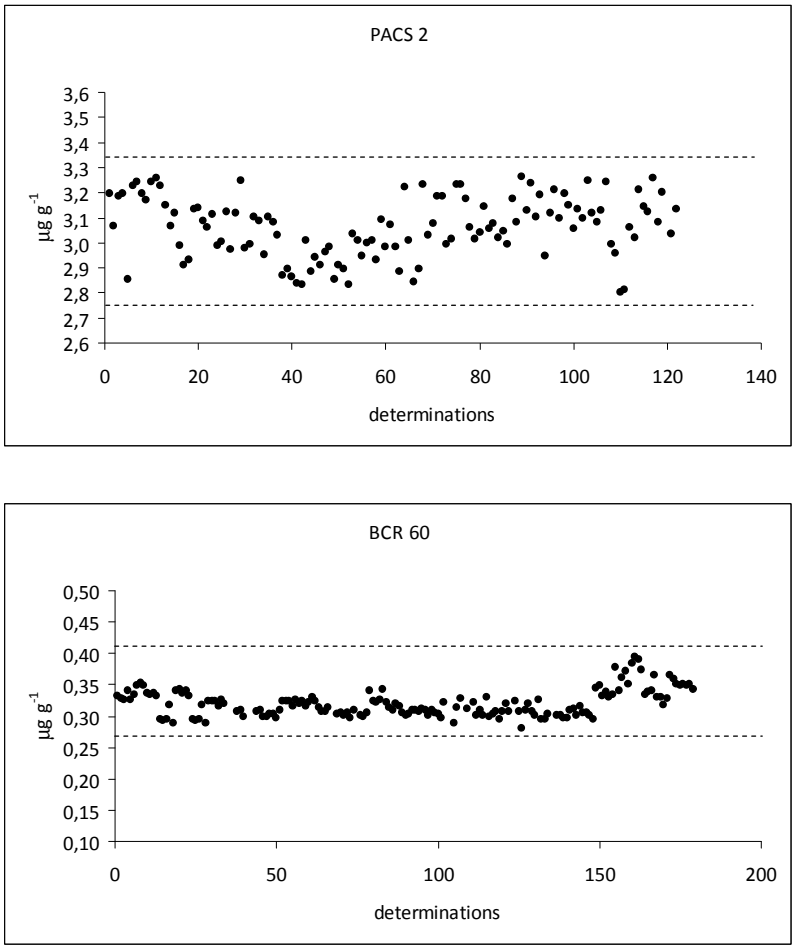


Figure 4.5- Control charts of the certified reference materials (cont.).

For dissolved mercury in water and pore waters the CV-AFS instrument was calibrated every day with at least five mercury standards between the working range concentrations. The calibration was checked through the day and at the end of the day. Extreme control was taken and the concentrations of mercury in the filtrate blanks were always below the detection limit. For each sample three measurements were performed (coef. Var.< 10%) and memory effects of the equipment were controlled when higher mercury concentrations were manipulated. Blank analyses were always performed between the samples.

4.5.1.2 Iron oxihydroxides determinations

The F-AAS instrument (Perkin–Elmer, model AAnalyst 100) was daily calibrated, at least, with five standards between the working concentrations range. Two different calibration methods were adopted, the method of calibration curve and the method of standard addition. The determinations of total concentrations of iron in the sediments were assessed by the method of

calibration curve while in the case of the water samples and the extraction of oxihydroxides method used was the standard addition to eliminate matrix effects. CRM PACS 2 was used to control the digestion processes of the sediments.

4.5.1.3 Methylmercury determinations

The GC-AFS instrument was daily calibrated every with at least five freshly methylmercury standards between the working ranges of concentrations. The calibration was checked through the day and at the end of the working day. The calibration curve was the adopted method for the calculation of the lod since the signal of the blank was not distinguishable from those the baseline. The certified reference material CRM 580 was used for control the extraction recoveries of the methylmercury of the sediments while in the case of biomass due to the lack of these types of materials we adopted to perform spikes of different methylmercury concentrations. Methylmercury recovery ranged between 73-91% for sediments and 100-105% for plant biomass (spiked samples).

4.5.2 Limits of detection

According to Miller and Miller (2000), the lod of an analyte may be described as the concentration which gives an instrument signal significantly different from the blank signal or the background signal (Miller and Miller, 2000). Limit of detection can be estimated by the blank signal and its standard deviation or by the calibration curve (Miller and Miller, 2000) when it is not possible to distinguish the signal blank from the baseline fluctuation. Limits of detection for each instrumental technique are described the different in Table 4.1.

Table 4.1- Limits of detection for the different quantification methods.

Method	Analyte	Limit of detection
AAS-Thermal decomposition	Hg	0.1 ng absolute mercury
CV-AFS	Hg (total and reactive)	0.5-2.8 ng L^{-1}
F-AAS	Fe	0.2-0.4 mg L^{-1}
Voltammetry	AVS	0.34 µmol L^{-1}
GC-AFS	MeHg	2.8-5.0 µg L^{-1}

4.6 References

Allen, H. E., Fu, G., Deng, B., 1993. Analysis of acid volatile sulphide (AVS) and simultaneously extracted metals (SEM) for the estimation of potential toxicity in aquatic sediments. Environ Toxicol Chem. 12, 1441-1453.

Bloom, N.S., 1995. Mercury as a case-study of ultraclean sample handling and storage in aquatic trace metal research. Environmental Laboratory March/April, 20.

Cai, Y., Jaffe, R., Jones, R., 1997. Ethylmercury in the soils and sediments of the Florida Everglades. Environ Sci Technol. 31(1), 302-305.

Carapuça, H.M., Válega, M., Pereira, E., Duarte, A.C., 2004. Monitoring acid-volatile sulphide by a fast scan voltammetric method: application to mercury contamination studies in salt marsh sediments. Anal Chim Acta 524, 127-131.

Chester, R., Huges M. J., 1967. A chemical technique for the separation of ferromanganese minerals, carbonate minerals and adsorbed trace metals from pelagic sediments. Chem Geol. 2, 249-262.

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. Anal Chim Acta. 405, 179-183.

Horvat, M., 1999. Current status and future needs for biological and environmental reference materials certified for methylmercury compounds. Chemosphere. 39(7), 1167-1179.

Klapheck, S., 1988. Homoglutathione: isolation, quantification and occurrence in legumes. Plant Physiol. 74, 727-732.

Lima, A.I.G.L., Pereira, S.I.A.P., Figueira, E.M.A.P, Caldeira, G.C.N., Caldeira, H.D.S.Q., 2006. Cadmium detoxification in roots of *Pisum sativum* seedlings: relationship between toxicity levels, thiol pool alterations and growth. Environ Exp Bot. 55, 149-162.

Mucci, A., Lucotte, M., Montgomery, S., Plourde, Y., Pichet, P., VanTra, H., 1995. Mercury remobilisation from flooded soils in a hydroelectric reservoir of northern Quebec, La Grande-2: results of a soil resuspension experiment. Can J Fish Aquat Sci. 52, 2502-2517.

Pereira, M.E.C., 1996. Distribuição, reactividade e transporte do mercúrio na Ria de Aveiro. Tese de Doutoramento. Departamento de Química, Universidade de Aveiro. Aveiro, Portugal.

Rauser, W.E., 2000. Roots of maize seedlings retain most of their cadmium through two complexes. J Plant Physiol. 156, 545-551.

Sneller, F.E., van Heerwaarden, L.M., Koevoets, P.L., Vooijs, R., Schat, H.,Verkleij, J.A., 2000 Derivatization of phytochelatins from *Silene vulgaris*, induced upon exposure to arsenate and cadmium: comparison of derivatization with Ellman's reagent and monobromobimane. J Agric Food Chem. 48, 4014–4019.

USEPA (United States Environmental Protection Agency), 1996. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. July.

5 Long term effects of anthropogenic mercury discharges in salt marsh vegetation

5.1 Introduction

In systems historically contaminated, particularly by metals, biota may develop metal tolerance (Ashmore, 1997), yet if the resistance capacity of the system is exceeded, the new stable state tends to favour only the more resistant genotypes, and thus, metals can act as potential agents for natural selection, and consequently only metal-tolerant species may survive (Crawley, 1997).

Scheffer et al. (2001) showed that from the point of view of ecosystem management, increasing environmental change requires a focus on building and maintaining the resilience of a desired stable state. If an ecosystem has shifted into a contrasting state, restoration objectives seek to return to the previous stable state (e.g. Zhang et al., 2003; Webster and Harris, 2004; Lillebø et al., 2005). This means that, if recovery is successful, then the community established will be similar (species composition, population size and density, and biomass structure) to a comparable or previous non-impacted condition (Elliot et al., 2007). Thus, recovery implies that a system will return to a previous condition either following a passive recovery, once stressors have been removed, or after an active recovery by human-mediated actions (Elliot et al., 2007). However, there may be a lag in recovery, meaning that the response of an ecosystem following the removal of the stress may be very different between the driving variables and those caused by the perturbation, showing hysteresis in response (Scheffer et al., 2001; Beisner et al., 2003; Webster and Harris, 2004; Elliot et al., 2007).

The impact of mercury contamination has been reported in different compartments (biotic and abiotic) of the Ria de Aveiro (e.g. Ramalhosa et al., 2001; Coelho et al., 2005; Pato et al., 2008). Yet, as ten years have passed since the cessation of mercury loading, we aim to address the resilience of the salt marsh vegetation, here defined as "*the ability of an ecosystem to return to its original state after being disturbed*" (Elliot et al., 2007), through a passive recovery. To accomplish these objectives two approaches were used: a) a spatial/horizontal assessment following the contamination gradient and the change in the diversity of salt marsh plants (salt marsh species richness) as a function of distance from the mercury source and b) a temporal/vertical assessment to track the temporal changes in the richness of the salt marsh species as a function of mercury loading.

5.2 Sampling details

Five sampling stations were selected in the Laranjo Bay (Figure 5.1) along a transect defined by the distance from the mercury point source: Station A was considered to be at the point source in the estuary; stations B (450 m), C (1000 m), D (1250 m) and E (2500 m). Salt marsh species were identified and classified as: I-*Halimione portulacoides;* II-*Sarcocornia perennis;* III-*Triglochin maritima*; IV-*Juncus maritimus;* V-*Phragmites australis*; VI-*Scirpus maritimus*.

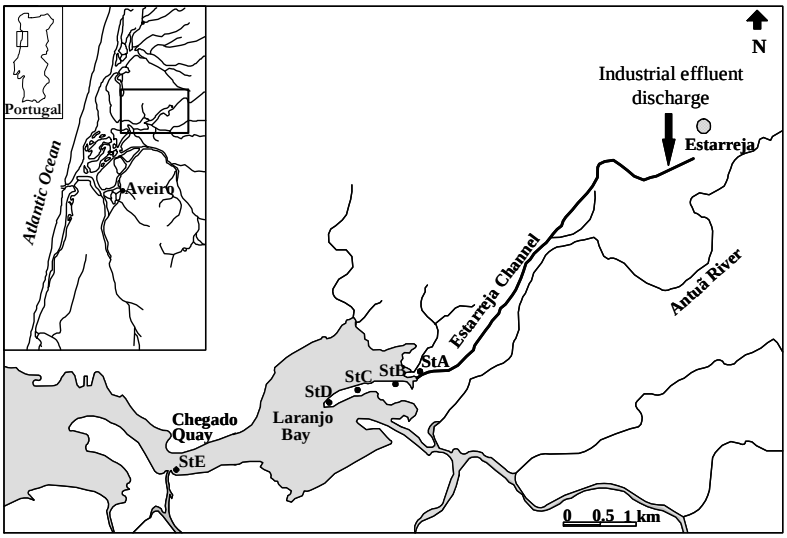


Figure 5.1- Map of Ria de Aveiro (Largo do Laranjo bay) with the sampling sites.

Sediment cores (Figure 5.2) with different lengths were collected to perform the two approaches to this study. For the spatial/horizontal, sediment cores (Ø 7 cm; n=3) of 15 cm depth were collected at each station in monotypic stands of each species and in adjacent areas without vegetation, while for the temporal/vertical study, sediment cores of 50 cm depth were collected at the same sites. Sediment samples were sliced in the field into layers of 5 cm thickness and sub-samples stored in plastic bags for transportation to the laboratory. Previous to sediment segmentation and *in situ*, redox potential (Eh) and pH (WTW–pH 330i/set equipped with SenTix® 41 and SenTix® ORP) were measured at six replicates in each layer, using calibrated sensors. The conductivity of the water was measured in the surface layers of the salt marsh at each sampling point (WTW Cond 330i/set equipped with Tetracon® 325 probe). Samples for sediment analysis were homogenised, freeze-dried and sieved (1 mm) in order to eliminate roots/rhizomes and other debris. Samples for roots/rhizomes quantification and identification were separated from each sediment layer by wet-sieving through a 250 µm mesh size sieve. At each sampling station,

six transects (50 m long) were defined in two different seasons to assess the percentage of coverage of each salt marsh species.

Figure 5.2- Collection of cores in sediments vegetated by *Halimione portulacoides*.

5.3 Statistical analysis

The significance of the differences in sediment environmental parameters between vegetated and the adjacent unvegetated sediments, and along the defined transect, was assessed with a two-way ANOVA test performed with SigmaStat version 3.1. Normality and equal variance tests were carried out before the application of the two-way ANOVA test. A constrained linear ordination method (redundancy analysis, RDA) was performed using the CANOCO version 4.5 software program.

5.4 Results

5.4.1 Spatial/horizontal study

All of the Laranjo Bay sediments consisted of a mixture of sand and mud. Physicochemical parameters for sediments with and without vegetation are shown in Table 5.1.

Table 5.1- A) Sediment physicochemical parameters with and without vegetation (mean values); **B)** Statistical analysis (Two-way ANOVA) of the differences within stations (vegetated and adjacent unvegetated sediment) and between stations. *no statistically significant differences

A	Vegetated sediments					Unvegetated sediments				
	Ø<63 µm (%)	LOI (%)	Eh (mV)	pH	Conducitvity (µs/cm)	Ø<63 µm (%)	LOI (%)	Eh (mV)	pH	Conductivity (µs/cm)
St A	79.3	15.2	-17	5.7	45.7	55.9	13.6	-45	6.2	47.2
St B	80.5	19.2	-54	6.3	46.3	89.2	15.0	-344	7.5	44.9
St C	78.6	22.1	-172	6.4	47.8	75.0	16.7	-332	7.0	47.4
St D	71.3	20.6	-220	6.4	47.9	66.7	16.7	-348	7.2	47.4
St E	84.3	17.1	112	5.7	52.8	76.9	14.4	-173	6.6	56.7

B	Significance within stations (vegetated/unvegetated sediments)	Significance between stations (StA, B, C, D and E)
Ø<63µm (%)	n.s.* (P=0.306)	n.s.* (P=0.277)
LOI (%)	P=0.005	P=0.035
Eh	P=0.023	n.s.*(P=0.08)
pH	P=0.003	P=0.025
Conductivity	n.s*. (P=0.548)	P= 0.017

The percentage of LOI, the redox potential (Eh) was significantly higher in vegetated sediments (P>0.05), while the pH was significantly lower in vegetated sediments (P>0.05), comparative to adjacent unvegetated sediments. No significant differences were found for the percentage of fine particles (less than 63 µm) nor for conductivity (P<0.05). Along the transect without vegetation, no significant differences were found between stations (two-way ANOVA, P>0.05), while the transect with vegetation showed significant differences (P>0.05) for %LOI, pH and conductivity. Statistically significant differences (P>0.05) were found between mercury concentrations in the unvegetated sediments along the transect, showing a mercury concentration gradient decrease from the point source (St A and B mean concentration = 19.2±4.0

mg kg^{-1}) towards Station E (mean concentration= 8.34±2.8 mg kg^{-1} for St D and 1.8±1.1 mg kg^{-1} for St D and E) (Figure 5.3).

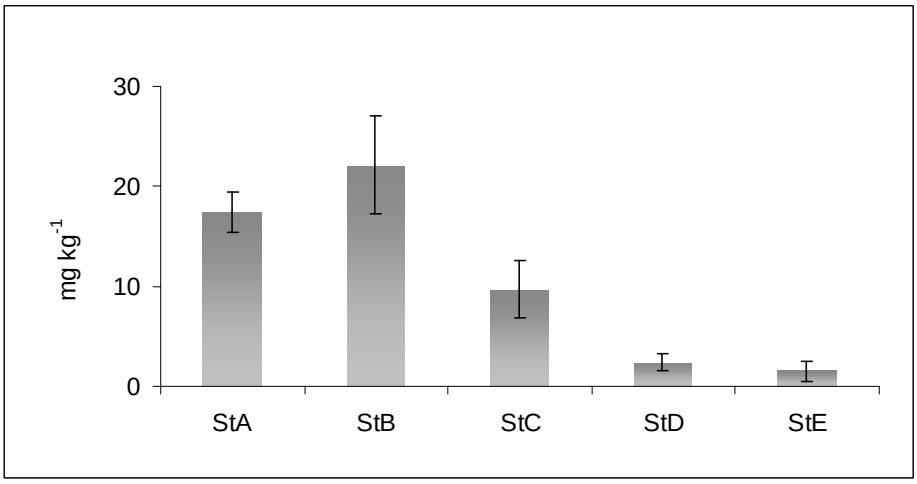


Figure 5.3- Mercury concentrations (mg kg^{-1} Dwt±stdev) in superficial unvegetated sediments along the contamination gradient, ten years after the ending of mercury discharges.

Salt marsh species diversity changed sharply along the gradient of mercury (Figure 5.4). Station A had lower species richness, with *Phragmites australis* as the dominant species (64% of coverage). At station B *Phragmites* coverage was reduced to less than 1%, and vegetation was dominated by *Scirpus maritimus* (54%) and *Juncus maritimus* (30%). At station C *Juncus maritimus* was more abundant (54%), followed by *Scirpus maritimus* (25%). At station D, *Phragmites* was absent and at Station E it represented 1% of plant coverage. At these two far end stations, vegetation was dominated by *Juncus maritimus* (58% Station D and 47% Station E), followed by *Triglochin maritima* (18% Station D and 19% Station E) and *Halimione portulacoides* (15% and 14% at Stations D and E, respectively).

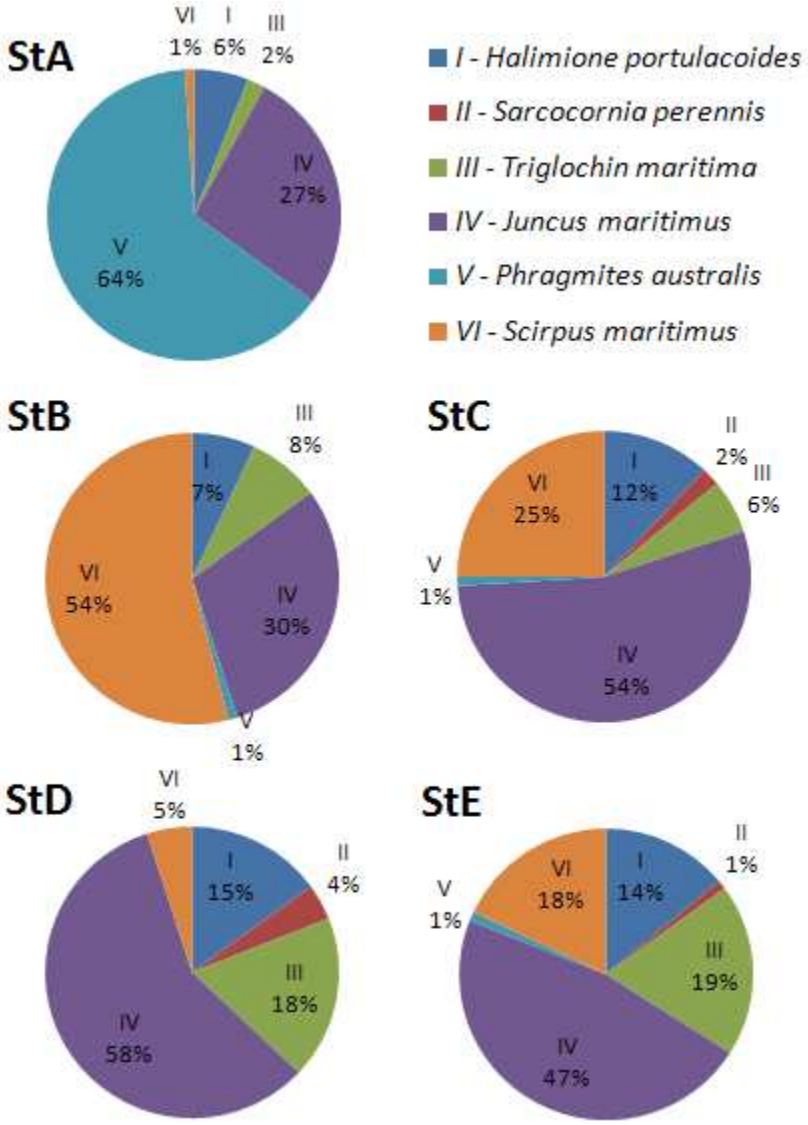


Figure 5.4- Salt marsh species composition along the disturbance gradient, ten years after the ending of mercury discharges.

The first two axes of the RDA triplot of stations, species and environmental factors (variables), performed in order to evaluate distribution and abundance patterns, accounted for 89.2% of the total variance (eigenvalues of 0.61 and 0.28) and 96.6% of the variance due to species–environmental relations (Figure 5.5), respectively. Mercury was the environmental factor with the highest magnitude while pH and salinity were the weakest ones. Distribution of *Phragmites australis* appeared strongly related to mercury concentrations in the sediments, implying that this species is the most mercury-tolerant.

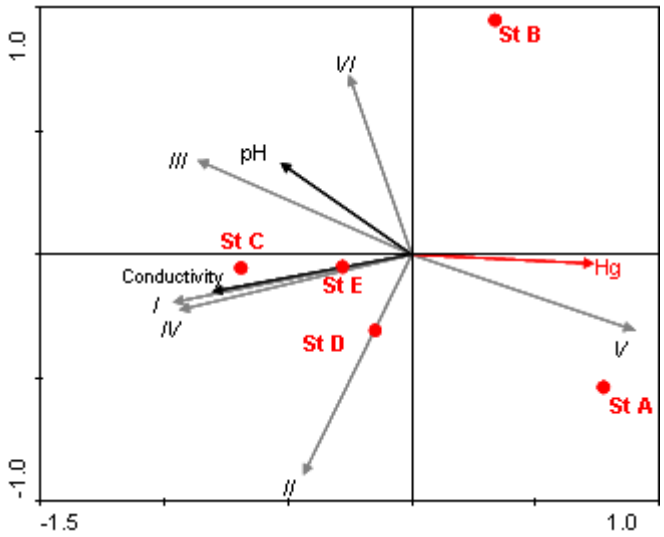


Figure 5.5- Redundance analysis (RDA) results of salt marsh vegetation distribution with the environmental factors included. St A-point source; St B-450m; St C-1000m; St D-1250m and St E-2500m; species composition: I-*Halimione portulacoides*; II-*Sarcocornia perennis*; III-*Triglochin maritima*; IV-*Juncus maritimus*; V-*Phragmites australis*; VI-*Scirpus maritimus*.

5.4.2 Temporal/vertical study

The analysis of the vertical profile of mercury concentrations in the sediment cores allows us to reconstruct the historical inputs of the mercury loading into the system. The results show an increase of mercury associated with the start of the chlor-alkali plant production, with the highest mercury concentrations found at deeper layers and subsequent decreasing towards the surface (Table 5.2).

Table 5.2- Vertical distribution (50 cm) of total mercury concentrations (mg kg^{-1} Dwt) and plant species roots/rhizomes structures in vegetated sediments along the transect define by the distance to the mercury point source, 15 years after the ending of mercury discharges: St A-point source; St B-450 m; St C-1000 m; St D-1250 m and St E-2500 m. [a]Others can be: *Halimione portulacoides; Juncus maritimus; Scirpus maritimus; Triglochin maritima*.

Depth	StA		StB		StC		StD		StE	
(cm)	Species	[Hg] (mg kg^{-1})±stdev	Species	[Hg] (mg kg^{-1})±stdev	Species	[Hg] (mg kg^{-1})±stdev	Species	[Hg] (mg kg^{-1})±stdev	Species	[Hg] (mg kg^{-1})±stdev
0-5	*P. aust.*	16.0±0.14	Others[a]	15.6±1.78	Others	7.7±0.5	Others	5.0±0.33	Others	2.3±0.17
5-10	*P. aust.*	21.5±0.06	*P. aust. vs* others	14.3±0.10	Others	8.7±0.90	Others	6.4±0.05	Others	2.8±0.05
10-15	*P. aust.*	19.6±0.12	*P. aust.*	16.9±0.14	*P. aust.*	19.1±0.25	Others	18.5±0.03	Others	2.1±0.05
15-20	*P. aust.*	35.4±0.11	*P. aust.*	40.4±0.34	*P. aust.*	41.3±0.65	Others	17.3±0.08	Others	2.7±0.05
20-25	*P. aust.*	125.5±0.89	*P. aust.*	110.3±0.46	*P. aust.*	16.1±0.14	Others	0.63±0.02	Others	2.9±0.11
25-30	*P. aust.*	223.2±1.29	*P. aust.*	101.0±1.67	*P. aust.*	2.9±0.3	*P. aust. vs* others	0.17±0.01	Others	4.8±0.23
30-35	*P. aust.*	180.8±0.81	*P. aust.*	34.9±0.24	*P. aust.*	0.31±0.00	*P. aust. vs* others	0.16±0.00	Others	6.3±0.36
35-40	*P. aust.*	86.4±0.48	*P. aust.*	0.94±0.02	*P. aust.*	0.13±0.01	*P. aust. vs* others	0.20±0.00	*P. aust. vs* others	9.2±0.10
40-45	*P. aust.*	17.2±0.08	*P. aust.*	1.2±0.14	*P. aust.*	0.09±0.00	*P. aust. vs* others	0.35±0.01	*P. aust. vs* others	5.2±0.01
45-50	*P. aust.*	2.2±0.02	*P. aust.*	1.5±0.05	*P. aust.*	0.09±0.00	*P. aust.*	0.04±0.00	*P. aust.*	6.4±0.25

In addition, it is also possible to establish a correspondence between time and depth, since the year of 1985 was, potentially, the most productive of the chlor-alkali plant (1100 kg Hg $year^{-1}$), and thus it may correspond to the highest mercury concentrations in the vertical profile. Therefore, assuming that the observed mercury peak for each station corresponds to the year of 1985, we can reach a rough value of the sedimentation rate for each station. Station A and B, located close to the entrance of the Laranjo bay, have sedimentation rates in the order of 1.3 and 1.1 cm y^{-1}, while the two stations located in the middle of the bay present the lowest values (0.83 and 0.59 cm y^{-1}, respectively). Station E, which is at the far extremity of the Laranjo Bay, presented the highest value of 1.78 cm y^{-1}.

Roots/rhizomes debris composition varied with depth in the sediments (Table 5.2). The highest mercury concentrations in sediments were associated with layers dominated by the presence of *Phragmites australis*. As the mercury concentrations in sediments decrease, the root/rhizomes debris from other species increases, namely *S. maritimus* and *J. maritimus* (named as "others" in Table 5.2). The vegetation material below ground was easy to clean from the sediment but species composition was sometimes difficult to identify, except for *P. australis, S. maritimus* and *J. maritimus* roots/rhizomes. Thus, "others" may also include the roots/rhizomes of *Halimione portulacoides, Sarcocornia perennis* and of *Triglochin maritima*.

5.5 Discussion

The variability in environmental parameters (e.g. higher Eh, lower pH, higher %LOI content) as well as the significant differences between the vegetated and the adjacent unvegetated sediments were due to induced changes in the rhizosphere by salt marsh plants (Cartaxana and Lloyd, 1999; Azzoni et al., 2001), while the significant differences between vegetated sediments along the transect result from the species-specific interaction with the sediments (Wigand et al., 1997; Lillebø et al., 2006). Since differences among environmental parameters in relation to unvegetated sites were not significant, this transect was assumed to represent reference conditions for the environmental parameters, with a gradient of mercury decreasing from the stations closer to the point source to the far end stations. Presently, ten years after the cessation of mercury discharges, stations A and B have the same mean concentration of mercury (19.2±4.0 mg kg^{-1}) in the top 15 cm of sediments, while stations D and E have lower concentrations (1.8±1.1 mg kg^{-1}). One would expect that, following the removal of mercury (stressor), the salt marsh community would recover and contain similar species richness as the far end stations. Yet, station A is still dominated by *Phragmites australis* (64% of coverage).

In station B *Scirpus maritimus* (54%) and *Juncus maritimus* (30%) are the most representative species despite the same mean concentration of mercury in the top 15 cm, while in the two far end stations *Juncus maritimus* coverage varies from 47% to 58% and the two other species are practically absent. These qualitative data along the gradient of mercury suggest that the recovery in species composition, after the cessation of mercury loading, may not be following the same decline trajectory, suggesting hysteresis in the response. This concept has been applied previously to characterise the trajectory of recovery of vegetation in shallow lakes (Scheffer et al., 2001; Zhang et al., 2003) and in estuaries (Lillebø et al., 2005), although their conclusions were supported by quantitative data concerning cultural eutrophication. Nevertheless, authors consider that the Ria de Aveiro case study fits the conceptual model of changes to the state of a system with increased pressure proposed by Elliot et al., (2007). Furthermore, the vertical/temporal assessment suggests that the loading of mercury affected the resistance to change of the salt marsh at the Laranjo Bay by inducing a shift in salt marsh species diversity. The alternative state is then characterised by the dominance of the species *Phragmites australis*, possibly due to its relative tolerance to metals, namely, Zn, Pb, Cd and Cu (Ye et al., 1997; Ali, 2004), which has enabled its use in phytoremediation (Massacci, 2001) and in pthytostabilizitation (Weis and Weis, 2004) programmes. Although our vertical/temporal assessment is also supported by qualitative data (Table 5.2), the vertical profile of mercury concentrations indicates that higher species diversity corresponded to lower mercury concentrations, which also corresponded to comparatively higher sedimentation rate. In fact, previous works have shown that most of the mercury accumulated in the sediments over the years is efficiently retained in the Laranjo Bay, suggesting that it is mostly buried in deeper layers due to sedimentation (Ramalhosa et al., 2001), although there might also be an export of mercury from this bay to the main system (Pereira et al., 1998).

Resistance to change and *resilience* are inherent properties of the ecosystem being the ability of the ecosystem to recover dependent on the stressor, the impacted species or community and the spatial and temporal intensities of the stressor (Elliot et al., 2007). In the Ria de Aveiro case study the passive recovery may be dependent on the interaction of the salt marsh plants, their biology, morphology and physiology, and the possible difference in the species-specific interactions with the biogeochemical cycle of mercury. In the Weis and Weis (2004) review, different plant species having different allocation patterns of metals is specifically discussed, predicting, as an example, that *Phragmites australis* would lead to a reduction in mercury availability comparative to *Spartina*

alterniflora marshes, as a result of *Spartina* higher mercury above ground standing stock of mercury (Windham et al., 2003).

The effect of mercury in primary producers has been assessed essentially through laboratory tests to screen its genotoxic effects (reviewed in Table 5.2 of Patra et al., 2004) and through toxicological bioassays, as summarised in Table 5.3. However, the number of studies reporting the direct effects of mercury on marine and salt marsh plants *in situ* is scarce, meaning that this is an open topic for further research in order to achieve a better understanding of the salt marsh ecosystem structure and functioning. Furthermore, studies concerning the effect of mercury on primary producers have raised several unanswered questions, namely whether mercury induces phytochelatins, which sequester and detoxify metals in plants and algae. For example in *Posidonia oceanica* (Ferrat et al., 2003) and in *Sesbania drummondii* (Israr et al., 2006) mercury induces glutathione metabolism, and in *Potamogeton crispus* (Ali et al., 2000) increases the content in non-protein thiol, which represents the major proportion of phytochelatins. Moreover, another question concerns the impact of primary producer bioaccumulation of mercury on higher trophic levels (e.g. Gupta and Chandra, 1998; Windham et al., 2003; Weis and Weis 2004) and which plants might be used for phytoremediation of mercury-polluted habitats (Ali et al., 2000; Weis and Weis 2004).

Table 5.3- Summary of the specific biological, morphological and physiological effects of mercury in primary producers (dw - dry weight).

Species	Mercury levels	Effect of mercury exposure	System/experiment	Reference
Posidonia oceanica (marine phanerogam)	Blade 24-252 ng g^{-1} dw	Physiologic/metabolic (induce of glutathione metabolism)	North-Western Mediterranean	Ferrat et al., 2003
Vallisneria spiralis (fresh water rooted macrophyte)	Leaf 0.25 µmol g^{-1} dw Root 1.12 µmol g^{-1} dw	Physiologic/metabolic (decrease in Chlorophyll and nutrients, NPK, content)	Toxicological bioassay	Gupta and Chandra, 1998
Plumaria elegans (read algae sporelings)	Water 0.25-1.0 mg L^{-1}	Biologic/morphologic (50% growth inhibition)	Toxicological bioassay	Boney, 1971 *in* Boening 2000
Sesbania drummondii (medium-sized perennial shrub)	Shoot 998 mg Kg^{-1} dw Root 41 403 mg Kg^{-1} dw	Physiologic/metabolic (none or very little photosynthesis stress at 10 mg Hg L^{-1}; enhanced the glutathione GSH/GSSG ratio)	Toxicological bioassay	Israr et al., 2006
Potamogeton crispus (fresh water rooted macrophyte)	125 µg g^{-1} dw at 10 µM Hg^{2+} after 96 hours exposure	Physiologic/metabolic (decrease in Chlorophyll, increase malondialdehyde (MDA) content, K^{+} loss, increase in cysteine and non-proteine thiol contents)	Toxicological bioassay	Ali et al., 2000
Bacopa monnieri (emergent rooted macrophyte)	Shoot 48.7 µg g^{-1} dw (maximum) Root 273.7 µg g^{-1} dw (maximum) at 5 µg mL^{-1} Hg after 14 days exposure	Physiologic/metabolic (increase in cysteine, total -SH and GSH and decrease of MDA at the initial exposure period; decrease in Chlorophyll an protein content at higher concentration and time exposure)	Toxicological bioassay	Sinha et al., 1996

5.6 References

Ali, M.B., Vaipayee, P., Tripathi, R.D., Rai, U.N., Kumar, A., Singh, N., Behl, H.M., Singh, S.P., 2000. Mercury bioaccumulation induces oxidative stress and toxicity to submerged macrophyte Potamogeton crispus L. B Environ Contam Tox. 65, 573-582.

Ashmore, M., 1997. Plants and Pollution. In: Crawley, M.J. (Eds.), Plant Ecology, Blackwell Science, UK, pp. 568-581.

Azzoni, R.G., Giordani, M., Bartoli, D.T.W., Viaroli, P., 2001. Iron, sulphur and phosphorus cycling in the rhizosphere sediments of an euthrophic *Ruppia cirrhosa* meadow (Valle Smarlacca, Italy). J Sea Res. 45, 15-26.

Beisner, B.E., Haydon, D.T., Cuddington, K., 2003. Alternative stable stages in ecology. Front Ecol Environ. 1(7), 376-382.

Cartaxana, P., Lloyd, D., 1999. N_2, N_2O and O_2 profiles in a Tagus estuary salt marsh. Estuar Coast Shelf S. 48, 751-756.

Crawley, M.J., 1997. Life History and Environment. In: Crawley, M.J. (Eds.), Plant Ecology, Blackwell Science, UK, pp. 73-131.

Elliott, M.D. Burdon, Hemingway K.L., Apitz, S.E., 2007. Estuarine, coastal and marine ecosystem restoration: Confusing management and science-a revision of concepts. Est Coast Shelf S. 74, 349-366.

Ferrat, L., Gnassia-Barelli, M., Pergent-Martini, C., Roméo, M., 2003. Mercury and non-protein thiol compounds in the seagrass *Posidonia Oceanica*. Comp Biochem Phys. C 134, 147-155.

Gupta, M., Chandra, P., 1998. Bioaccumulation and toxicity of mercury in rooted-submerged macrophyte *Vallisneria spiralis*. Environ Pollut. 103, 327-332.

Israr, M., Sahi, S., Datta, R., Sarkar, D., 2006. Bioaccumulation and physiological effects of mercury in *Sesbania drummondii*. Chemosphere 65, 591-598.

Lillebø, A.I., Neto, J.M., Martins, I., Verdelhos, T., Leston, S., Cardoso, P.G., Ferreira, S.M., Marques, J.C., Pardal, M.A., 2005. Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system nutrient loading. Est Coast Shelf S. 65, 697-707.

Lillebø, A.I., Flindt, M.R., Pardal, M.A., Marques, J.C., 2006. The effect of *Zostera noltii*, *Spartina maritima* and *Scirpus maritimus* on sediment pore-water profiles, in a temperate intertidal estuary. Hydrobiologia, 555,175-183.

Massacci, A., 2001. Remediation of wetlands by *Phragmites australis*, the biological basis. Minerva Biotecnol. 13(2), 135-140.

Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A., 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ Exp Bot. 52, 199-223.

Pato, P., Válega, M., Pereira, E., Vale, C., Duarte, A.C., 2008. Inputs from a mercury-contaminated lagoon: impact on the nearshore waters of the Atlantic Ocean. J Coastal Res. 24, 2B, 28-38.

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. Sci. Total Environ. 213, 157-163.

Ramalhosa, E., Monterroso, P., Abreu, S., Pereira, E., Vale, C., Duarte, A.C., 2001. Storage and export f mercury from a contaminated bay (Ria de Aveiro, Portugal). Wetlands Ecology and Management. 9, 311-316.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature. 413, 591-596.

Sinha, S., Gupta, M., Chandraet, P., 1996. Bioaccumulation and biochemical effects of mercury in the plant Bacopa monnieri (L). Environ Toxic Water. 11, 105-112.

Webster, I.T., Harris, G.P., 2004. Anthropogenic impacts on the ecosystems of coastal lagoons: modelling fundamental biogeochemistry process and management implications. Mar Freshwater Res. 55, 67-78.

Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implication for phytoremediation and restoration. Environ Int. 30, 685-700.

Widdows, J., Brinsley, M., 2002. Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. J Sea Res. 48, 143-156.

Wigand, C., Stevenson, J.C., Cornwell, J.C., 1997. Effects of different submersed macrophytes on sediment biogeochemistry. Aquat Bot. 56, 233-244.

Windham, L., Weis, J.S., Weis, P., 2003. Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). Est Coast Shelf S. 56, 63-72.

Ye, Z.H., Baker, A.J.M., Wong, M.H., Willis, A.J., 1997. Zinc, Lead and Cadmium Tolerance, Uptake and accumulation by the Common Reed, *Phragmites australis* (Cav.) Trin. Ex Steudel. Ann Bot-London 80, 363-370.

Zhang, J., Jørgensen, S.E., Beklioglu, M., Ince, O., 2003. Hysteresis in vegetation shift-Lake Mogan prognoses. Ecol Model. 164, 227-238.

6 Mercury mobility in salt marsh colonised by *Halimione portulacoides*

6.1 Introduction

Salt marshes play a significant role in metal recycling in the coastal ecosystems, as they may act as sources, sinks or transformers of chemicals. Some salt marshes may act as sinks for nutrients and pollutants (e.g. Hung and Chmura, 2006; Hwang et al., 2006; Caçador et al., 2007), and sources of organic matter (Bouchard and Lefeuvre, 2000) and nutrients, supporting estuarine and terrestrial food webs. The problems arise when salt marshes act as sources exporting metals (Montague, 1999) to the adjacent environments. The outwelling hypothesis was first introduced by Odum in 1968 and states that marsh-estuarine systems produce more material than can be degraded or stored within the systems, and that the excess material is being exported to the coastal ocean supporting near-coastal ocean productivity (Dame and Allen, 1996).

Once released into the aquatic environment, inorganic mercury salts can be converted into more toxic forms, such as organic mercury compounds, particularly methylmercury. Physicochemical and biological processes (e.g. erosion, dredging, early-diagenesis, bioturbation) may enhance the presence of organic mercury compounds in the overlying water column affecting the environment on a local/regional scale, endangering the system ecologically and economically, particularly in areas highly dependent on fishery activities. The primary route for human's exposure to mercury is through the consumption of contaminated fish and shellfish. Therefore, the knowledge of mercury biogeochemistry in the different compartments of salt marsh environment is extremely important. As already mentioned, inputs of mercury into the aquatic systems has been reduced during the last decades although mercury pools in sediments are still a worrying problem due to its potential release to other environmental compartments. The number of studies highlighting the potential role of salt marshes as sinks for several metals, namely mercury (Weis and Weis, 2004; Hung and Chmura, 2006; Kongchum et al., 2006; Válega et al., 2008) has been increasing over recent years; however, most of these studies report only the potential role of vegetation in metal speciation and availability, especially when this is due to root activity (Alloway, 1995; Mendelssohn et al., 1995).

The present study intends to increase the knowledge on the mobility of mercury in a salt marsh vegetated by *Halimione portulacoides* (L.) Aellen (Caryophyllales: Chenopodiaceae), its redistribution in the sediment layers containing plants and its incorporation into below ground biomass. *Halimione portulacoides* (Figure 6.1) plays an important role in the floristic coverage of the European salt marshes; in fact this species is noted as being one of the most abundant in European salt marshes and one of the most productive species (Bouchard et al., 1998). For these

purposes, mercury pools in *Halimione portulacoides* biomass and in sediments were assessed, as well the potential export of mercury from the contaminated salt marsh to the adjacent areas. Thus, this study provides important data about the dynamic of mercury inside the salt marsh, comprising the below ground and the above ground system.

Figure 6.1- *Halimione portulacoides* (L.) Aellen.

6.2 Sampling details

Samples were collected bi-monthly during a one-year period between April 2003 and April 2004 at the highly contaminated area of Laranjo Bay salt marsh (Figure 6.2) during low tide. *Halimione portulacoides* biomass (above and below ground biomass), sediments and water samples from intertidal water pools were collected in monotypic stands, uniform in size and in density of stems. Above ground material was collected at ground level in squares of 50 cm (n=3) and, after cutting the above ground material, three sediment cores of Ø 7 cm and 15 cm depth were taken. Below ground biomass of *Halimione portulacoides* is more abundant in upper layers (<15 cm), since the effect of root-sediment interactions in the first 15 cm layers can be more clearly observed. Sediment cores were sliced into 5 cm layers and one of the cores was preserved under nitrogen environment for pore waters extraction and acid volatile sulphide analysis. Adjacent sediments without vegetation were also collected and sliced at the same depth intervals.

Previous to sediment segmentation and *in situ*, redox potential (Eh) and pH were measured at six replicates in each layer, using calibrated sensors (WTW–pH 330i/set equipped with SenTix® 41 and SenTix® ORP). The salinity of the intertidal water pools was also measured (WTW Cond 330i/set equipped with Tetracon® 325 probe).

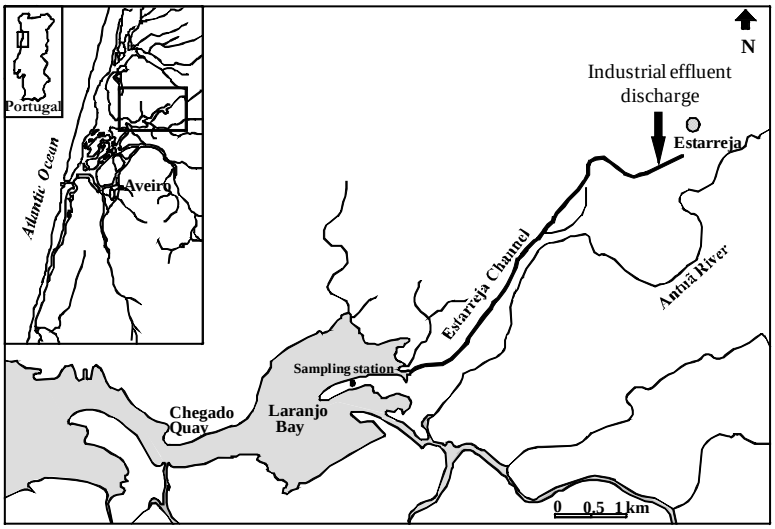


Figure 6.2- Location of the sampling station in the Laranjo bay.

6.3 Biomass production and mercury pools calculation

Biomass net primary production (above ground-NPP_{Above} and below ground-NPP_{Below}) was estimated according to the differences between the maximum and minimum biomass recorded during the survey period, as described by Caçador et al. (2004). Turnover rate of biomass (above ground and below ground) was estimated according to the ratio between biomass production and the maximum biomass. The macro-detritus annual production was estimated as the product between NPP_{Above} and the turnover rate of above ground biomass. The mercury pool of the biomass material was calculated by multiplying biomass (g DW m^{-2}) and mercury concentrations (mg kg^{-1}). Mercury bioaccumulation of *Halimione portulacoides* was estimated considering the maximum and the minimum of mercury pools in the different tissues of the plant. Mercury turnover was calculated by the ratio between the mercury bioaccumulation and maximum of the mercury pools.

In the sediments, mercury pool was calculated per square meter using the bulk density value (g cm^{-3}) and mercury concentrations in sediments (mg kg^{-1}).

The significance level of the differences between sediment layers and environmental parameters under the surveyed period was assessed with ANOVA test after normality and equal variance tests had been performed.

6.4 Results

6.4.1 Sediments characterisation

Sediment characterisation revealed that the Laranjo Bay sediments are a mixture of sand and mud containing 35-74% of fine particles (less than 63 µm) (Figure 6.3), with no statistically significant differences between depths. Bulk density was generally lower at vegetated sediments comparative to sediments without vegetation (0.42±0.01 and 0.84±0.09, mean±stdev respectively). Acid volatile sulphides (AVS) of vegetated sediments (Figure 6.4) were below 7.3 µmol g^{-1}, ranging between 0.2 and 7.3 µmol g^{-1} (median value 0.83 µmol g^{-1}) and did not show significant differences between depths or between months ($P>0.05$); however the highest values were found in the deepest layer (10-15 cm). The iron oxides concentrations of vegetated sediments (Figure 6.5) were not statistically different, neither between depths nor between months ($P>0.05$); however the highest values were found in the uppermost layers (0-5 and 5-10 cm), where the values ranged between 0.6 and 3.2 mg g^{-1}, while in the deepest layer (10-15 cm) the values ranged between 0.3-1.4 mg g^{-1}. Salinity values of the intertidal low-water pools ranged between 14 and 37 over the year, with the lowest values being recorded in January and the highest values in July. Physical and chemical parameters (median values of the three studied depths) of the salt marsh sediments are described in Table 6.1. Statistically significant differences were only observed in pH (Figure 6.6) and Eh (Figure 6.7) values between depths, and for pH values between months ($P<0.05$), the highest pH values being registered in the winter months. The uppermost layers (0-5 and 5-10 cm) present lower pH and higher Eh values than the 10-15 cm layer. Organic matter content (% LOI) and water content (% water) were higher in vegetated sediments comparative to non-vegetated sediments ($P<0.05$). Median values of organic matter content for vegetated sediments were 24.2% while the non-vegetated sediments presented lower values (9.1%). The same pattern was observed for the water content of sediments with the highest values in the vegetated sediments (median values 73.5%) and the lowest in the non-vegetated sediments (52.2%).

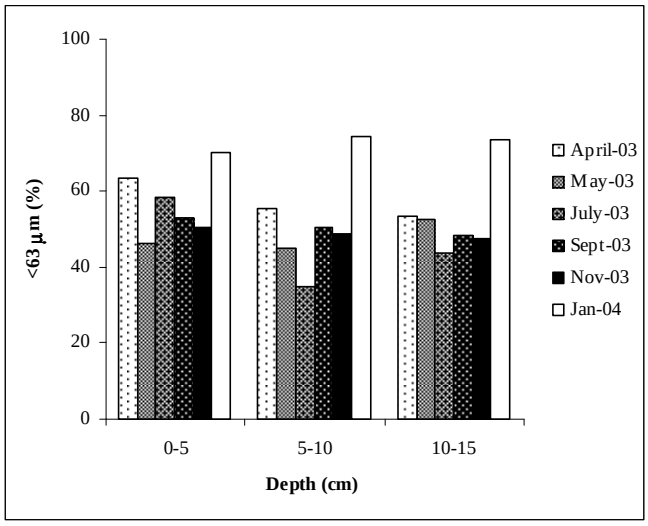


Figure 6.3- Percentage of fine particles (<63 µm) of sediments vegetated by *Halimione portulacoides*.

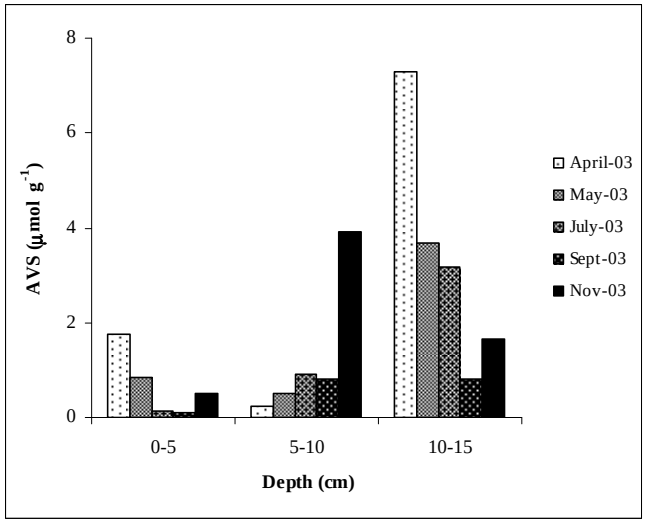


Figure 6.4- Acid volatile sulphides (µmol g^{-1}) concentrations in sediments of Laranjo salt marsh and vegetated by *Halimione portulacoides*.

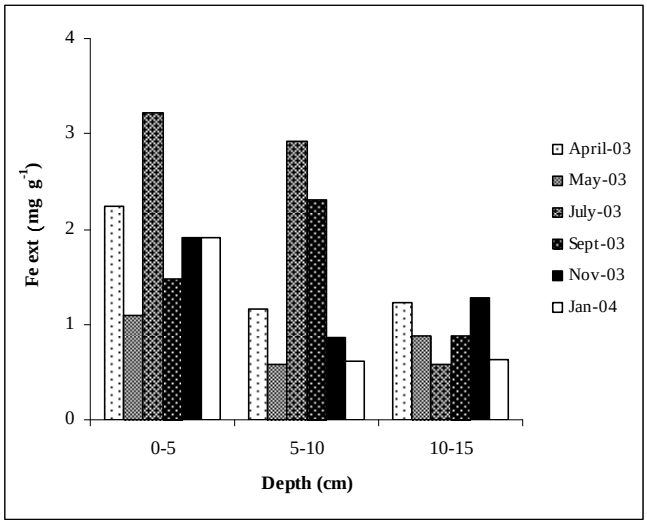


Figure 6.5- Iron oxides extracted with hydroxylammonium chloride solution (mg g^{-1}) in sediments of Laranjo salt marsh and vegetated by *Halimione portulacoides*.

Table 6.1- Physical and chemical parameters (median values and standard error of the three studied depths) of the Laranjo salt marsh sediments during the surveyed period.

Type of sediment / Parameter	Vegetated		Non-vegetated	
	Median	Standard error	Median	Standard error
pH	6.2	0.1	7.0	2.8
Eh (mV)	102	31	-165	-67
Fraction <63µm (%)	51.5	12.1	31.2	10.4
LOI (%)	24.2	7.9	9.1	3.7
Moisture (%)	73.5	1.0	52.2	21.3

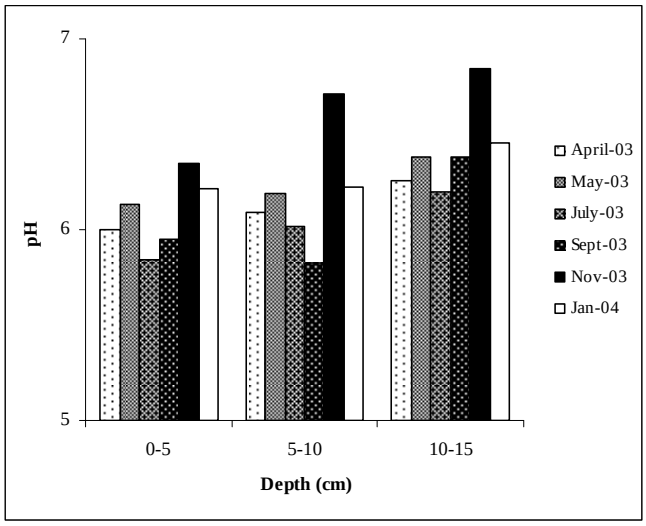


Figure 6.6- pH values of the sediments vegetated by *Halimione portulacoides* collected in the Laranjo salt marsh.

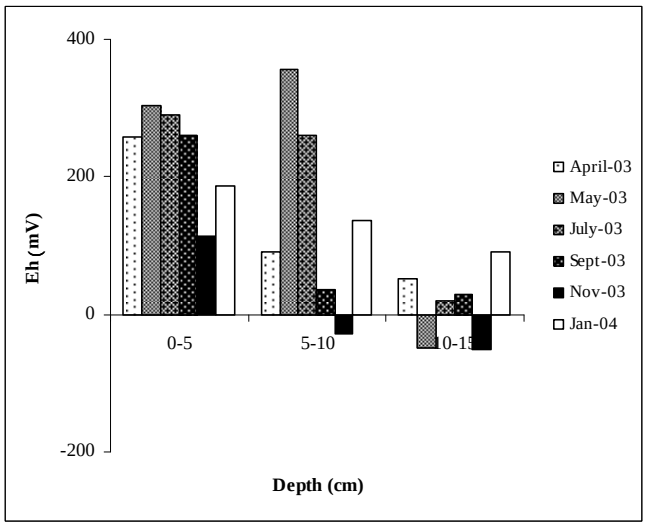


Figure 6.7- Eh (mV) values of the sediments vegetated by *Halimione portulacoides* collected in the Laranjo salt marsh.

6.4.2 *Halimione portulacoides* biomass net primary production

Results showed that the *Halimione portulacoides* below ground annual biomass production was higher than above ground (Figure 6.8). The above ground biomass ranged between 978 and 1804 g DW m^{-2} (Figure 6.9) while below ground biomass (Figure 6.10) for the 15 cm considered (sum of the three layers 0-5, 5-10 and 10-15 cm) ranged between 2781 and 4839 g DW m^{-2}, this being within the range described for other European salt marshes (e.g. Bouchard and Lefreuve, 2000; Caçador et al., 2004). At the Aveiro system, *Halimione portulacoides* biomass

generally increased from late spring to early summer, and then decreased gradually until late winter. The highest values of below ground biomass were observed from May to July and the lowest values in autumn. The highest values of above ground biomass were observed in July, which corresponded to the flowering period and November was the month in which the lowest values were registered.

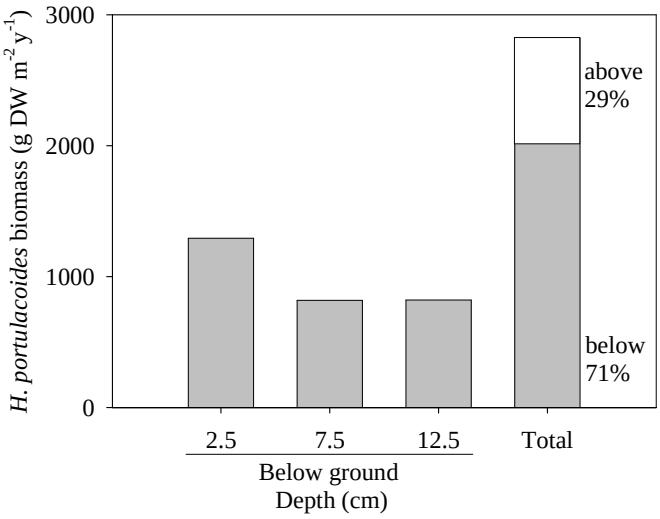


Figure 6.8- Annual biomass production of *Halimione portulacoides* (g DW m^{-2} y^{-1}).

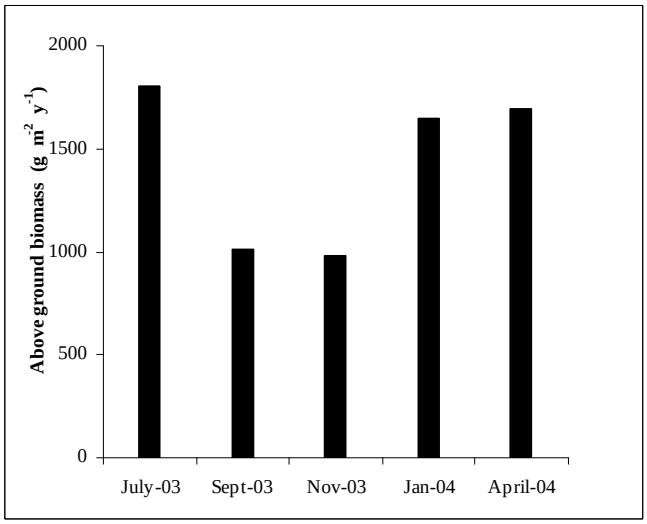


Figure 6.9- Above ground biomass of *Halimione portulacoides* collected in Laranjo salt marsh.

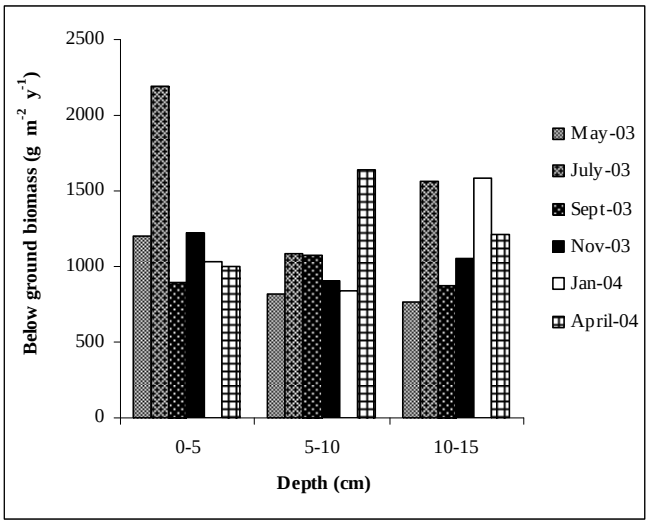


Figure 6.10- Below ground biomass of *Halimione portulacoides* collected in Laranjo salt marsh.

Turnover rates for below ground biomass were higher than for above ground biomass. The first layer (0-5 cm) presented the highest turnover rate (0.59 y^{-1}); however, similar rates were observed for deeper layers (0.50 and 0.52 for 5-10 and 10-15 cm layers, respectively) while, for above ground biomass, turnover rates of 0.46 y^{-1} were estimated (Figure 6.11).

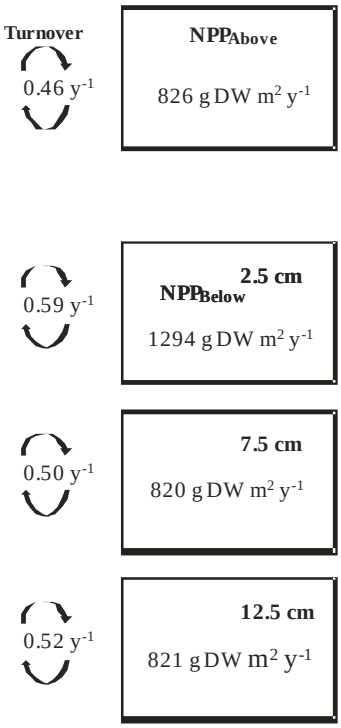


Figure 6.11- Schematic representation of *Halimione portulacoides* net primary production (NPP) and turnover rates for above and below ground biomass.

6.4.3 Mercury concentrations in the studied matrices

Mercury concentrations in above ground biomass tissues (Figure 6.12) were lower and ranged between 0.04-0.10 mg kg^{-1} (average value for stems (young and old stems), leaves and flowers). Higher mercury concentrations (0.06-0.14 mg kg^{-1}) were found in the leaves (0.03-0.08 mg kg^{-1}) than in the stems ($P<0.05$) and, in general, higher concentrations were found in April and the lowest in May and July, which correspond to the months with higher biomass values. Statistical tests were performed excluding mercury concentrations in flowers since they only occurred during July and September (although mercury concentrations in flowers presented similar values to the leaves). No statistically significant differences were found in the mercury concentrations of below ground biomass throughout the period of the survey ($P>0.05$) although significant differences were found between depths ($P<0.05$). Below ground biomass (Figure 6.13) of the first two layers (0-5 and 5-10 cm) presents similar mercury concentrations, ranging between 6.0-21.5 mg kg^{-1}, the highest concentrations being found at 10-15 cm layers (12.4-61.1 mg kg^{-1}).

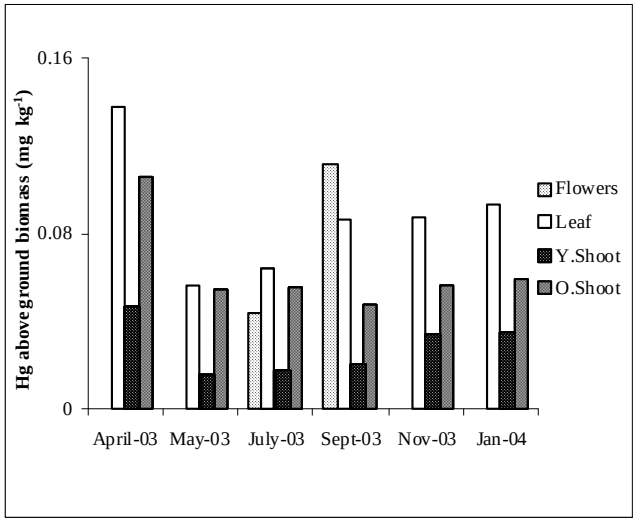


Figure 6.12- Mercury concentrations in above ground biomass (mg kg^{-1}) of *Halimione portulacoides* collected in the Laranjo salt marsh.

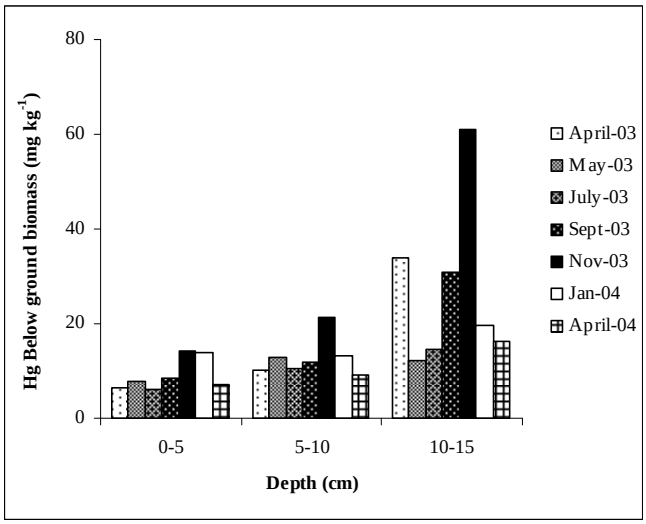


Figure 6.13- Mercury concentrations in below ground biomass (mg kg^{-1}) of *Halimione portulacoides* collected in the Laranjo salt marsh.

Mercury concentrations in the sediments (Figure 6.14) did not show statistically significant differences throughout the period of the survey ($P>0.05$); however, significant differences were observed between layers ($P<0.05$), the highest concentrations (average±stdev) being recorded at 10-15 cm layers (19.1±5.6 mg kg^{-1}); in fact 0-5 and 5-10 cm layers presented similar mercury concentrations (8.2±1.4 mg kg^{-1}).

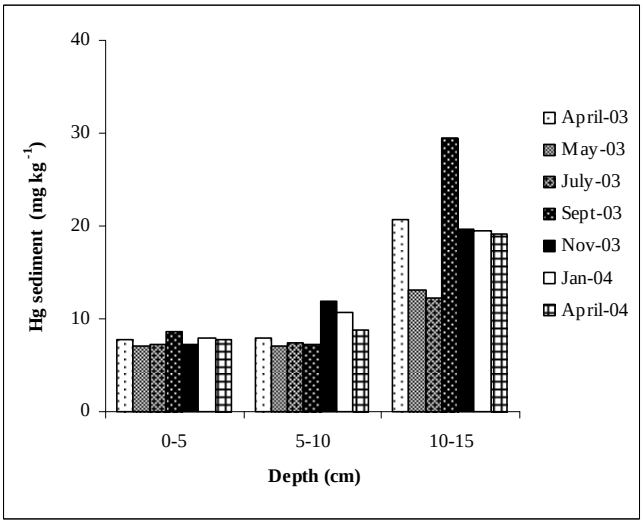


Figure 6.14- Mercury concentrations in sediments (mg kg^{-1}) of Laranjo salt marsh vegetated by *Halimione portulacoides*.

Mercury concentrations in non-vegetated sediments collected in Laranjo Bay were generally lower than in sediments with vegetation and ranged between 6.6±2.2 mg kg^{-1} and 9.7±2.4 mg kg^{-1} for 0-10 and 10-15 cm layers, respectively.

Total dissolved and reactive mercury concentrations in pore waters extracted from the sediments did not show statistically significant differences between depths and between months ($P>0.05$). Reactive mercury concentrations (Figure 6.15) ranged between 1.4% and 17.8% of the total dissolved mercury, which varied between 4 and 68 ng L^{-1}. Total mercury concentrations (60-835 ng L^{-1}) in pore waters (Figure 6.16) were significantly higher than those observed for reactive mercury. Although dissolved organic mercury concentrations were not measured in this study, pore waters are naturally enriched in organic compounds, especially in vegetated sediments which presents higher organic matter contents due to the roots (Lopes et al., 2006), which can explain the high total mercury concentrations.

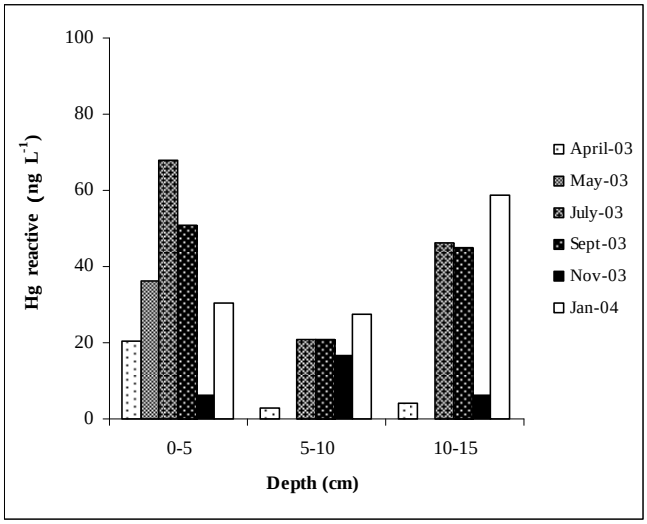


Figure 6.15- Reactive mercury concentrations (ng L^{-1}) in pore waters extracted from Laranjo salt marsh sediments, vegetated by *Halimione portulacoides*.

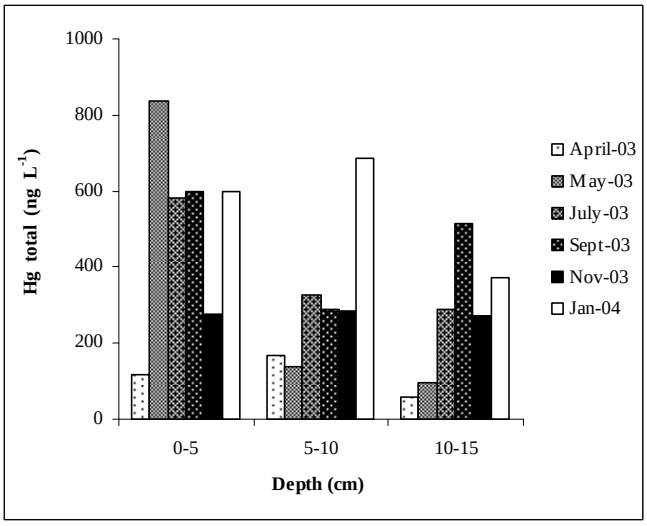


Figure 6.16- Total mercury concentrations (ng L^{-1}) in pore waters extracted from Laranjo salt marsh sediments, vegetated by *Halimione portulacoides*.

Diffusive fluxes across the water-sediment interface were calculated using Fick's first law, considering mercury concentrations of the pore waters in the first centimetres of the sediments and in supernatant water collected in the intertidal low-water pools above the sediment.

Concentrations of reactive mercury in the intertidal water pools ranged between 4.4 and 5.6 ng L^{-1}. Estimated diffusive fluxes of reactive mercury varied between 1 and 103 ng m^{-2} d^{-1} with the highest values recorded in July and September.

6.4.4 Mercury pools and mercury bioaccumulation by *Halimione portulacoides*

Mercury pools in above ground biomass ranged between 0.06-0.16 mg m^{-2} while in below ground biomass these values ranged between 29-102 mg m^{-2} (for the three studied depths) the highest values being recorded in the 10-15 cm depth layer (10-64 mg m^{-2}). Mercury pools estimated for the macro-detritus were much lower (0.051 mg m^{-2} y^{-1}). The annual bioaccumulation of mercury in above ground tissues was estimated in 0.11 mg m^{-2} y^{-1}, while in below ground biomass, considering the total mercury pools of the three studied depths, it was 72 mg m^{-2} y^{-1}. The highest values of annual mercury bioaccumulation were found at 10-15 cm depth (55 mg m^{-2} y^{-1}), which also corresponded to the highest concentrations of mercury in the sediment (Figure 6.17). Mercury pools calculated for the three studied depths ranged between 560-943 mg m^{-2}, which is considerably higher when compared to mercury pools found in *Halimione portulacoides* biomass. Mercury pools of *Halimione portulacoides* below ground biomass represent only 4-13% of the mercury pool of the sediments. Mercury pools in sediments without

vegetation were higher (1045-1672 mg m^{-2}) due to the differences of the bulk density of the sediments.

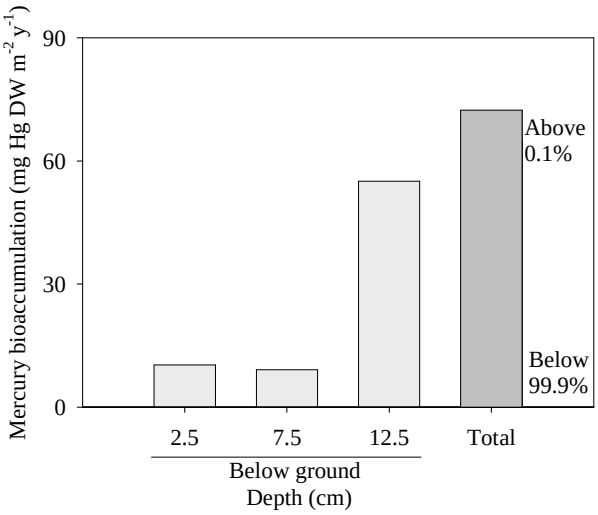


Figure 6.17 - Annual mercury bioaccumulation by *Halimione. portulacoides* (mg Hg DW m^{-2} y^{-1}).

6.5 Discussion

According to our results, the presence of vegetation clearly affects sediment parameters, such as Eh, % LOI, pH, moisture content values and also the mercury concentrations of the sediments (Válega et al., 2008). Vegetated sediments of the Laranjo Bay salt marsh were generally more acidic and Eh values tended to be positive compared to sediments without vegetation. Results suggest that the presence of the root system also seems to contribute to the retention of water in the sediments. Plant roots can interact with the surrounding sediments, pumping oxygen into the root zone through the aerenchyma tissue and acidifying the rhizosphere by the exudation of organic compounds which influence the distribution and availability of trace metals (Alloway, 1995; Mendelssohn et al., 1995; Duarte et al., 2007). In the present study, *Halimione portulacoides* seems to be active all year, since Eh values showed that in general the rhizosphere is always enriched with oxygen. Eh and pH have an important role in the bioavailability of metals such as mercury, as partitioning between dissolved and solid fraction is dependent on these parameters. It is stated that anoxic sediments may contain very high concentrations of metals in a reduced state associated with sulphides, reducing their bioavailability when compared to systems with oxidised sediments. The range between -150 and +200 mV is considered to be the interval where the greatest possible metal mobility exists (Jackson, 1998), which corresponds to the range

recorded at the Laranjo Bay salt marsh vegetated by *Halimione portulacoides*. The results of pH and Eh obtained in this study reflect the mobility of mercury in contaminated salt-marshes. Marins et al. (1997) have reported that salt marsh plants are able to mobilize deposited mercury through the release of oxygen into the sediments. As plant roots excrete molecular oxygen, the surrounding sediment sulphides are oxidised and iron, manganese and sulphur interact repeatedly in redox reactions. Acid volatile sulphide values of the Laranjo Bay salt marsh sediments were generally low due to the presence of oxygen delivered by the roots. Scavenging of mercury by iron oxides has been reported in a marine environment presenting high contents of iron (Gobeil and Cossa, 1993; Gagnon et al., 1997). As shown by Eh values, sediments at the Laranjo Bay salt marsh are enriched through oxygen being delivered by the roots of *Halimione portulacoides*, so that iron can precipitate as oxides and contribute to the retention of mercury at the rhizosphere. The results obtained for iron oxides and AVS showed that the different mercury concentrations between depths is not related with the plant activity during the entire year by the delivery of oxygen, and that mercury is not stabilised as sulphides.

Mercury concentrations in sediments and below ground biomass were higher in the deepest layer (10-15 cm) and in fact this can be explained in relation to the historical mercury contamination of the studied system. The sampling station presents a sedimentation rate of approximately 0.83 cm y^{-1}, which means that 10-15 cm layers correspond to periods of mercury discharges from the industry. The chlor-alkali plant started to reduce their discharges in 1994, which explains the lower values of mercury in surface sediment layers.

Furthermore, the results obtained also suggest that the pools of mercury in vegetated sediments are largely associated with the solid fraction, with only a small amount associated with the pore waters. A previous work performed in Laranjo Bay by Ramalhosa et al. (2006) in non-vegetated sediments has reported higher values, approximately 5-270 ng m^{-2} d^{-1}. The present study suggests that the contribution of vegetated sediments to reactive mercury concentrations in the water column is not significant, which is particularly relevant since reactive mercury is pointed out as a suitable measure of the metal substrate available for methylation, elemental mercury formations and other conversion processes of mercury within the aquatic environment (Mason et al., 1993).

Pools of mercury bioaccumulated by *Halimione portulacoides* was much lower at above ground biomass (0.1%), suggesting that mercury mobility is mainly between sediments and roots and only a very small fraction is translocated to the above ground parts of the plant. Mercury

budgets clearly show that roots are the main organ for mercury accumulation. Below ground material presented higher concentrations of mercury by 174 to 545 fold than above ground tissues and by 0.8 to 8.4 fold than sediments. According to a recent study performed in two Portuguese salt marshes of the Tagus estuary (approximately 250 km south from Ria de Aveiro) (Pereira et al., 2007), the decomposition of the below ground biomass of *Halimione portulacoides* corresponded closely to 42-46% of mass lost (dry weigh) in the first months. This suggests that the pool of mercury bioaccumulated in the below ground part of the plant is quite mobile, being able to return to the sediment pool throughout the mineralisation process and/or contribute to reactive mercury concentrations in water pore waters.

Macro-detritus production of above ground biomass of *Halimione portulacoides* was estimated to be 378 g m^{-2} y^{-1}, which means that about 46% of the biomass can be rapidly transformed into necromass. The detrital material can be exported from the contaminated inner bay, the Laranjo Bay, or redistributed within the salt marsh. According to the studies of Bouchard and Lefeuvre (2000), a significant fraction of the detritus is usually redistributed inside the salt marsh; however, due to the hydrodynamics characteristics of Laranjo Bay, it seems that the export hypothesis is more likely. The potential of the system to export macro-detritus (either to coastal waters or to other areas of the system) appears to be strongly influenced by its proximity to the creek network and the Laranjo Bay salt marsh has a high number of creeks in which water flows. For each tidal cycle at Laranjo Bay, it has been estimated that approximately 1.5×10^{6} m^3 of water (mean value) flows out of the bay during the ebb tide and about 0.5×10^{6} m^3 remains in the main channel between Chegado Quay and Bico Quay, which flows in the incoming tide. The volume of water that flows out from Laranjo Bay during neap tide was estimated as being approximately 3% of the total volume of water that is changed in each tidal cycle between the entire system of the Ria de Aveiro lagoon and the Atlantic Ocean. The potential of this inner bay to export macro-detritus appears to confer some mobility to the pool of mercury bioaccumulated in the above ground part of *Halimione portulacoides* (the above ground corresponded to 29% of the plant total biomass). The mercury pool in this macro-detritus was comparatively low (the annual mercury pool of above ground biomass represented 0.1% of the total pool of mercury in the plant), yet it constitutes a source of mercury to be exported from the contaminated inner bay, the Laranjo Bay into the Ria system. Figure 6.18 shows a schematic representation of the mobility of mercury in the Laranjo Bay salt marsh colonised by *Halimione portulacoides*.

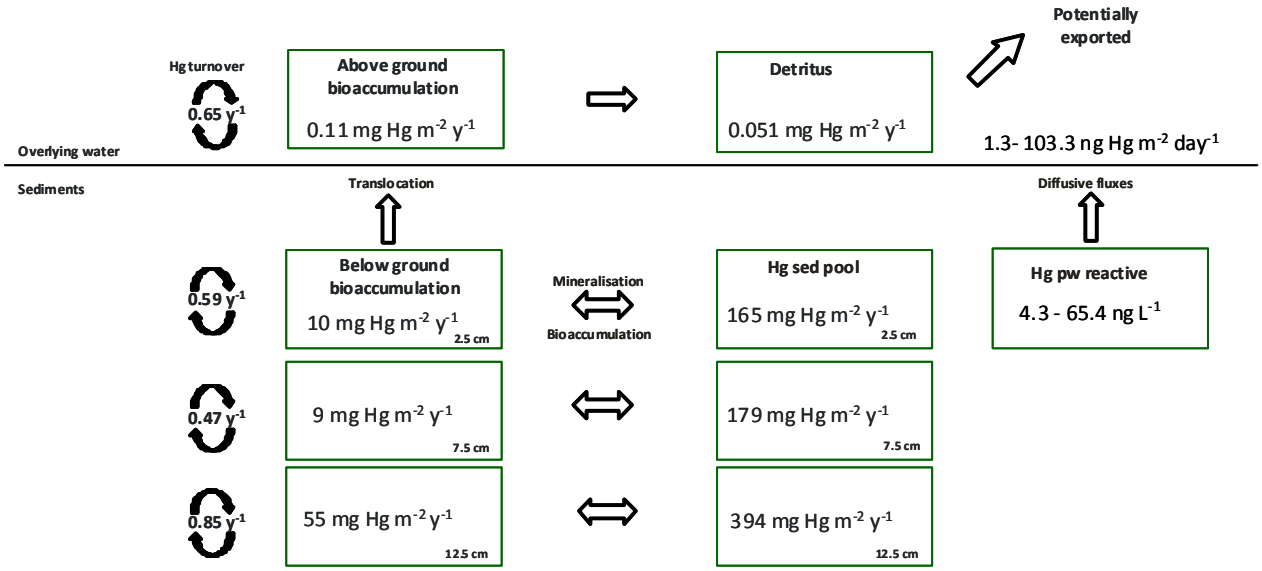


Figure 6.18- Schematic representation of mercury mobility in the Laranjo Bay salt marsh colonised by *Halimione portulacoides.*

6.6 References

Alloway, B.J., 1995. Soil processes and the behaviour of heavy metals. In: Alloway, B.J. (Eds). Heavy Metals in Soils. Blackie Academic Publications, UK

Bouchard, V., Creach, V., Lefeuvre, J.C., Bertru, G., Mariotti, A., 1998. Fate of plant detritus in a European salt marsh dominated by *Atriplex portulacoides* (L.) Aellen. Hydrobiologia 373/374, 75-87.

Bouchard, V., Lefeuvre, J.C., 2000. Primary production and macro-detritus dynamics in a European salt marsh: carbon and nitrogen budgets. Aquat. Bot. 67, 23-42.

Caçador, I., Costa, A.L., Vale, C., 2004. Carbon storage in Tagus salt marsh sediments. Water Air Soil Poll. 4, 701-714.

Caçador, I., Costa, A.L., Vale, C., 2007. Nitrogen sequestrion capacity of two salt marshes from the Tagus estuary. Hydrobiologia. 587, 137-145.

Dame, R.F, Allen, D.M., 1996. Between estuaries and the sea. J. Exp. Mar. Biol. Ecol. 200, 169-185.

Duarte, B., Delgado, M., Caçador, I., 2007. The role of citric acid in cadmium and nickel uptake and translocation, in *Halimione portulacoides*. Chemosphere. 69, 836-840.

Hwang, H.M., Green, P.G., Higashi, R.M., Young, T.M., 2006. Tidal salt marsh sediment in California, USA. Part 2: Occurrence and anthropogenic input of trace metals. Chemosphere. 64, 1899-1909.

Hung, G.A., Chmura, G.L., 2006. Mercury accumulation in surface sediments of salt marshes of the Bay of Fundy. Environ. Pollut. 142, 418-431.

Jackson, L.J., 1998. Paradigms of metal accumulation in rooted aquatic vascular plants. Sci. Total Environ. 219, 223-331.

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, 1300-1303.

Lopes, C.B., Abreu, S., Válega, M., Duarte, R.M.B.O., Pereira, M.E., Duarte, A.C., 2006. The assembling and application of an automated segmented flow analyzer for the determination of dissolved organic carbon based on UV-persulphate oxidation. Anal Lett. 39, 1979–1992.

Marins, R.V., Lacerda, L.D., Gonçalves, G.O., Paiva, E.C., 1997. Effect of root metabolism on the post-depositional mobilisation of mercury in salt marsh soils. B Environ Contam Tox. 58, 733-738.

Mason, R.P., 1993. Mercury biogeochemical cycling in a stratified estuary. Limnol Oceanogr. 38, 1227-1241.

Mendelssohn, 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.

Pereira, P., Caçador, I., Vale, C., Caetano, M., Costa, A.L., 2007. Decomposition of belowground litter and metal dynamics in salt marshes (Tagus estuary, Portugal). Sci Total Environ. 380, 93-101.

Ramalhosa, E., Segade, S.R., Pereira, E., Vale, C., Duarte, A., 2006. Mercury cycling between the water column and surface sediments in a contaminated area. Water Res. 40, 2893-2900.

Roulet, M., Lucotte, M., Saint-Aubin, A., Tran, S., Rheault, I., Farella, N., De Jesus Da Silva, E., Dezencourt, J., Sousa Passos, C.J., Santos Soares, G., Guimaraes, J.R., Mergler, D., Amorim, M., 1998. The geochemistry of mercury in central Amazonian soils developed on the Alter-do-Chao formation of the lower Tapajos River Basin, Brazilian Amazon. Sci. Total Environ. 27, 19-27.

Válega, M., Lillebø, A.I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Long-term effects of mercury in a salt marsh: Hysteresis in the distribution of vegetation following recovery from contamination. Chemosphere. 71, 765-772.

Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implication for phytoremediation and restoration. Environ Int. 30, 685-700.

7 Assessment of methylmercury production

7.1 Introduction

Attending to salt marshes biogeochemistry, where the sulphate reduction plays an important role, these ecosystems are among the environments with good conditions for mercury methylation (Hines et al., 1999). Salt marshes have high microbial activity especially during the warm season. Redox transitional areas appear to be ideals for methylation where organic carbon and sulphate for bacterial respiration are available. During daylight the first 5 cm are potential zones for methylmercury production in estuarine sediments are since supports high sulphate reducing rates and have relatively sulphide concentrations (Langer et al., 2001).

Holmes et al. (2006) describe sediment-water fluxes of methylmercury between 1.6-10.02 ng $m^{-2}day^{-1}$ for a freshwater marsh. Freshwater marshes appear to have higher methylmercury concentrations than salt marshes probably to the fact that of salinity and high values of sulphate may inhibit methylation processes (Kongchum et al., 2006). Higher percentages of methylmercury are usually found in freshwater marshes. Kongchum et al. (2006) reported for fresh marshes percentages of 3% for methylmercury while this value is considerable low for salt marshes (1.7%). These differences may be related to the higher concentrations of sulphate and consequently higher concentration of sulphide resulting from the sulphate reduction, which can restrict the formation of methylmercury due to the reducing of available mercury substrate. Gilmour and Henry (1991) suggest 200-500 μM sulphate for methylation; however Langer et al (2001) have demonstrated high methylation in high sulphate environments (≥28 mM). Methylmercury studies in salt marshes are scarce (Langer et al., 2001; Hung and Chmura, 2006; Kongchum et al., 2006).

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., 2006). Methylmercury studies in salt marshes are scarce and very recent (Langer et al., 2001; Hung and Chmura, 2006; Kongchum et al., 2006).

It is important to understand the mechanism of metals storage in salt marsh sediments and plants to avoid them becoming a metal 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). 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.

Metals that are accumulated in the root system are considered to be phytostabilised, 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; Mendelssohn et al., 1995).

7.2 Sampling details

Three sediment cores samples of 12 cm depth were collected in August 2005 during ebb tide in monotypic stands of *Halimione portulacoides* and *Sarcocornia perennis* (Figure 7.1) and in the adjacent sediment without vegetation (at a distance of approximately 20 m) of Laranjo bay (Figure 7.2). Above ground plant materials were 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.

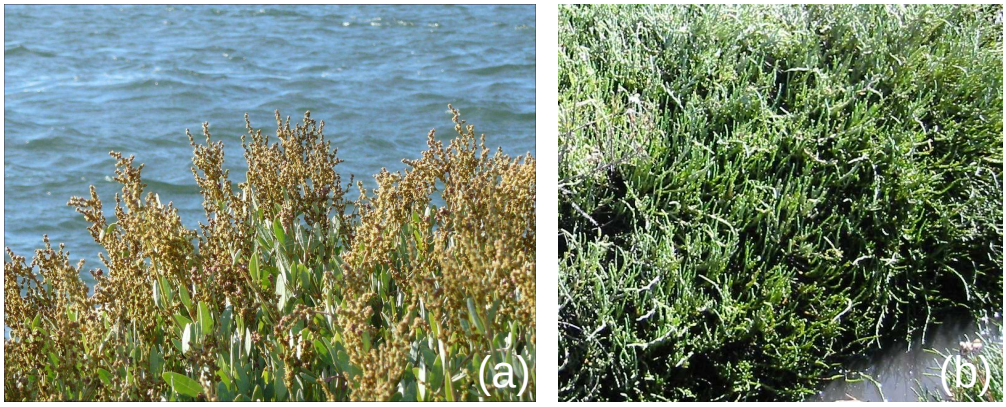


Figure 7.1- Photographs of *Halimione portulacoides* (a) *Sarcocornia perennis* (b).

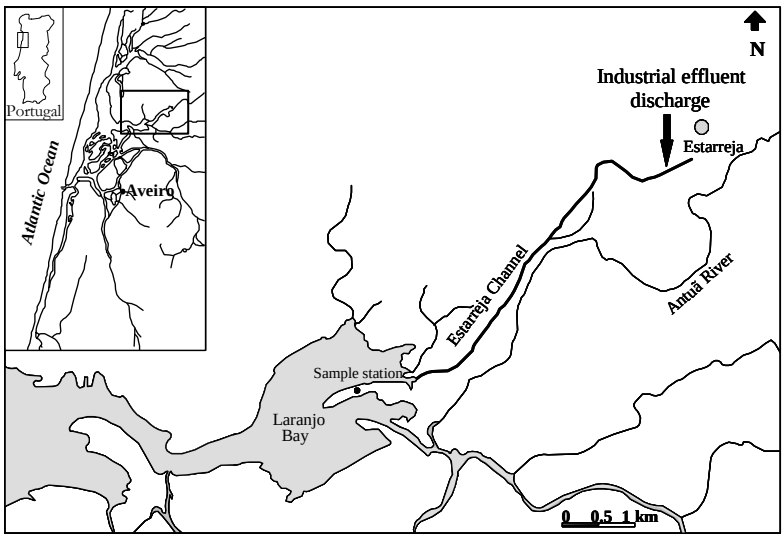


Figure 7.2- Location of the sampling station in the Laranjo Bay.

7.3 Results and discussion

All Laranjo Bay sediments consisted of a mixture of sand and mud. Considering the percentage of fine particles (53.9±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 ±3.2%) than in non-vegetated (mean value for the whole core, 8.7±0.5%) ($P<0.05$). Vegetated sediments were generally more acidic ($P\leq0.001$, 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 (Figure 7.3).

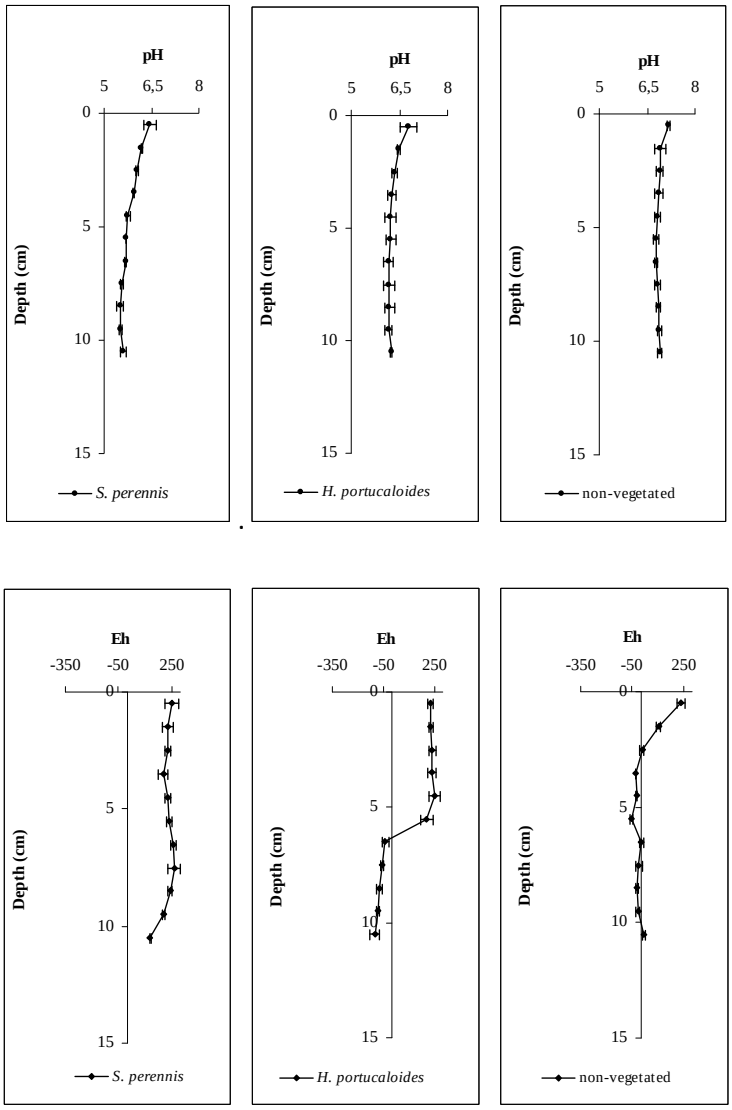


Figure 7.3- Eh (mV) and pH values (mean±standard deviation) of Laranjo Bay salt marsh sediments colonised by *Halimione portulacoides* and *Sarcocornia perennis* and in non-vegetated sediments.

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 7.1) were approximately two times higher

than the concentrations found in vegetated sediments (Figure 7.4), which indicates bioaccumulation of mercury by both studied plants.

Table 7.1- Total mercury concentrations (ng g^{-1}) and methylmercury (MeHg, ng g^{-1}) in above ground biomass and roots of *Halimione portulacoides* and *Sarcocornia.perennis* collected at Laranjo Bay salt marsh.

	Total Hg (ng g^{-1})	**MeHg (ng g^{-1})**
S. perennis		
Shoots	95.9	13.3
Roots	11350	80.6
H. portulacoides		
Stem	26.5	4.4
Leaves	54.1	<Lod
Roots	14770	73.1

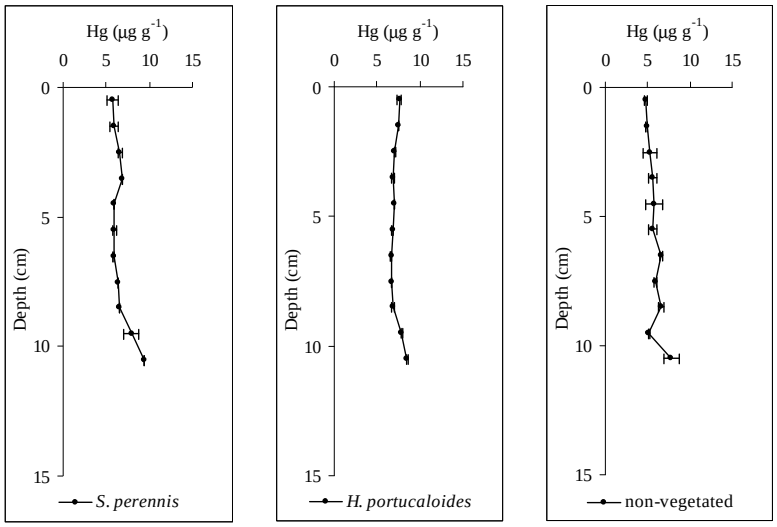


Figure 7.4- Total mercury concentrations (µg g^{-1}±standard deviation in Laranjo Bay salt marsh sediments colonised by *Halimione portulacoides* and *Sarcocornia perennis* and in non-vegetated sediments.

Total mercury concentrations in the above ground biomass (Table 7.1) 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 16.2-56.4 ng g^{-1} were found in sediments of *Halimione portulacoides* and 10.2-75.8 ng g^{-1} for *Sarcocornia perennis*, while in non-vegetated sediments the values ranged between 1.2-14.7 ng g^{-1} (Figure 7.5).

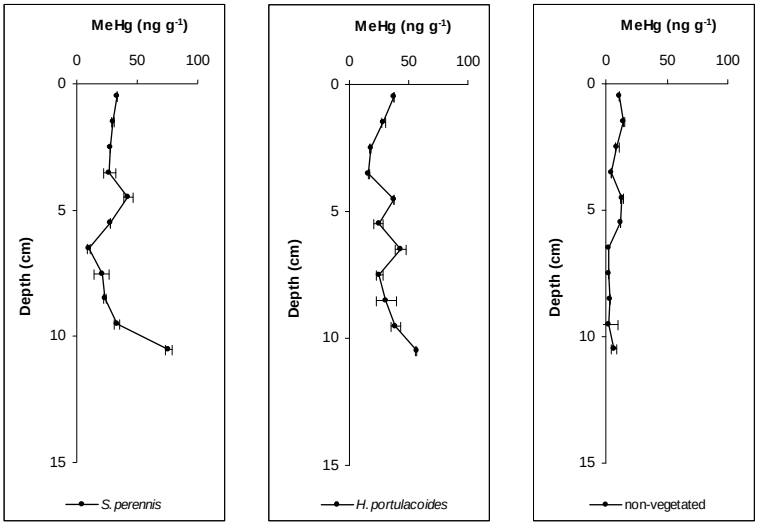


Figure 7.5- Methylmercury (MeHg, ng g^{-1}±standard deviation) in Laranjo Bay salt marsh sediments colonised by *Halimione portulacoides* and *Sarcocornia perennis* and in non-vegetated sediments.

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 (Figure 7.6).

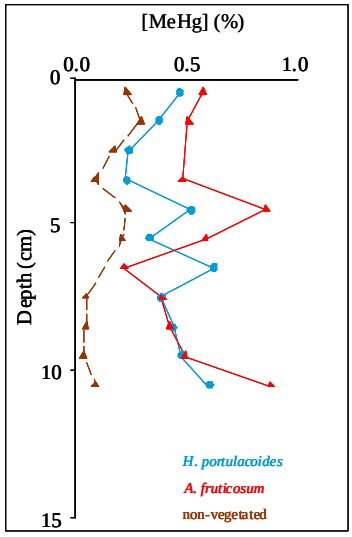


Figure 7.6- Values of methylmercury (MeHg, %) in Laranjo Bay salt marsh sediments colonised by *Halimione portulacoides* and *Sarcocornia perennis* and in non-vegetated sediments.

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 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. 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 *Halimione portulacoides* and 14% in the shoots of *Sarcocornia perennis* (Table 7.1). 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 0.5% of the total mercury for *Sarcocornia perennis* and *Halimione 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 et al. (2006) describe sediment-water fluxes of methylmercury between 1.6-10.02 ng m^{-2} day^{-1} for a freshwater marsh. Although *Halimione portulacoides* and *Sarcocornia perennis* marshes in Ria the Aveiro presented similar methylation ratios at the root system, other salt marsh plants with more distinct physiology, depth of active roots, life cycle, and tolerance to 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.

7.4 References

Alloway, B.J. 1995. Trace metals in soils. Chapman and Hall (Eds.), Great Britain, pp. 11-35.

Ebinghaus, R., Wilken R.-D. 1996. Mercury distribution and speciation in a polluted fluvial system. In: Calmano W. and 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. Environ Pollut. 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. Mar Chem. 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.

Hung, G.A., Chmura, G.L., 2006. Mercury accumulation in surface sediments of salt marshes of the Bay of Fundy. Environ Pollut. 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.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit Rev Env Sci Tec. 31(3), 241-293.

8 Mercury intracellular partitioning and chelation in *Halimione portulacoides*

8.1 Introduction

When metal concentration is above a certain threshold level, which is variable depending on the metal itself, phytotoxicity induced processes can take place in the plant, such as: changes in the permeability of the membrane cell, reactions with sulphydril groups, affinity for reacting with phosphate groups and active groups of ADP or ATP and replacement of essential ions. According to Clarkson (1972) in Patra et al. (2004), mercury has a high affinity for sulphydril groups and consequently can disturb cellular functions where critical or non-protected proteins are involved, especially those having sulphydril groups in sites important for protein composition or activity. Several studies report that plants can respond to metal stress by several mechanisms, which may include metal immobilisation in root cell walls, exclusion or intracellular chelation (Hall, 2002). Some works have demonstrated that metals can be retained by means of extracellular carbohydrates (Wagner, 1993), such as pectic sites or the hystidyl group of the cell wall (Leita et al., 1996), forming very stable complexes. Under metal stress conditions, plants usually respond by synthesizing specific peptides or organic acids (Rauser, 1999). A common response is the synthesis of phytochelatins (PCs: $(\text{-Glu-Cys})_n\text{-Gly}$, where *n* is generally in the range of 2 to 5), small metal-binding polypeptides, enzymatically produced from glutathione that sequester metals through thiol coordination, reducing the damage to metabolic processes (Zenk, 1996; Rauser, 1999; Cobbett and Goldsbrough, 2002). When in the presence of toxic metal concentrations, PCs form complexes with the metal ions, preventing them from interfering with the cellular metabolism (Vögelli–Lange and Wagner, 1990; Ortiz et al., 1995; Zenk, 1996).

Although PCs have already been implicated into mercury tolerance (Zenk, 1996), studies of mercury sequestration by PCs are very scarce. Gupta et al. (1998) showed that two aquatic plants (*Hyrilla verticillata*) and rooted (*Vallisneria spiralis*) synthesised different species of phytochelatins, during mercury exposure. These studies are relevant to understand the roles that phytochelatins play in mercury detoxification. Few reports focus on metal distribution through the subcellular fractions of salt marsh plants and fewer analyse the role of PCs in their metal detoxification, particularly in the field. This is of paramount importance, since most studies on metal tolerance mechanisms rely on laboratory experiments, and such laboratory results are difficult to extrapolate due to several limitations associated to the test conditions which may be from the natural environment, namely the bioavailable fraction of the metal in the sediments. Laboratory tests also tend to be more conservative and usually are performed in a short period of time and with young plants (Powell, 1997). In order to evaluate the real role of these mechanisms in stress coping, field

studies should be undertaken and this is particularly true for salt marshes, were a complex equilibrium of different factors may take place.

This work was carried out with samples collected from their natural habitats. The aim of this paper was to clarify the biochemical processes behind mercury tolerance in *Halimione portulacoides*. With this purpose, two fractions of mercury were separated: buffer-soluble (mainly cytosolic) and insoluble mercury (mainly associated with membranes and cell walls). The amount of both fractions of metal was compared and related to metal distribution within plant organs. It was also assessed if the tolerance of this species was associated with the induction of metal chelation by phytochelatins. With this purpose, protein-mercury complexes were isolated and analysed for their thiol content.

8.2 Sampling details

Sediments vegetated by *Halimione portulacoides* were collected in five stations of Laranjo bay (Figure 8.1) with different levels of mercury contamination, during low tide in monospecific stands of *Halimione portulacoides* and then transported to the laboratory under refrigerated conditions. Roots were carefully sorted from the sediments and washed with distilled water to assure that no sediment particles were in the roots. Leaves were also rinsed with distilled water. After the washing process, roots and leaves, were immersed in 5 mM $CaCl_2$, during 10 min, and after washed with distilled water to remove the extracellular metal.

Sediment samples were homogenised freeze-dried and sieved (1 mm) in order to eliminate root debris. Biomass samples were homogenised and divided for total mercury analysis and cytosolic extraction. For total mercury analysis biomass samples were oven dried at 45 ^{o}C during several days until constant weight, while the samples for cytosolic extraction of the biomass samples were immediately frozen (-80 ^{o}C) and processed in a few days after the collection.

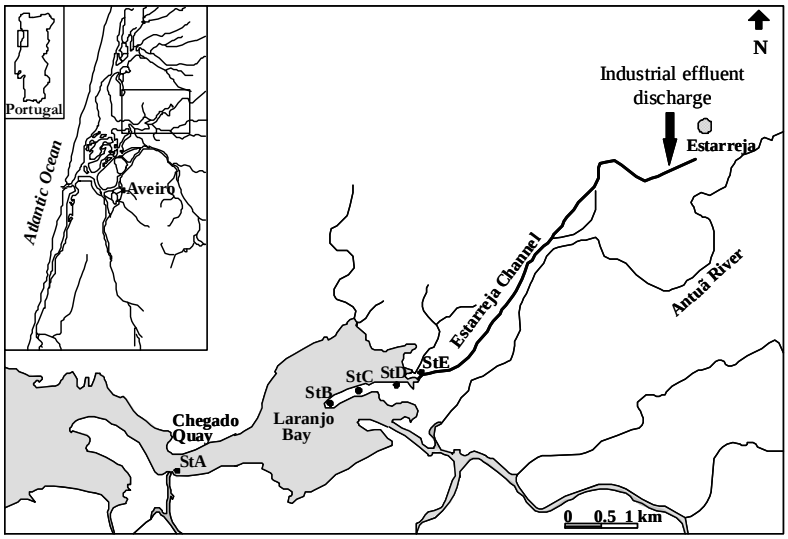


Figure 8.1- Map of Laranjo bay with the respective sampling stations.

8.3 Results

8.3.1 Mercury concentrations in sediments and biomass

The highest mercury concentrations in the sediments (Figure 8.2) were found in StD and StE (15.7±4.2 mg kg^{-1} dry weight (dw) and 15.2±1.7 mg kg^{-1} dw, respectively ± stdev), which are the closest to the source of mercury contamination and decreased towards StA (1.9±0.01 mg kg^{-1} dw).

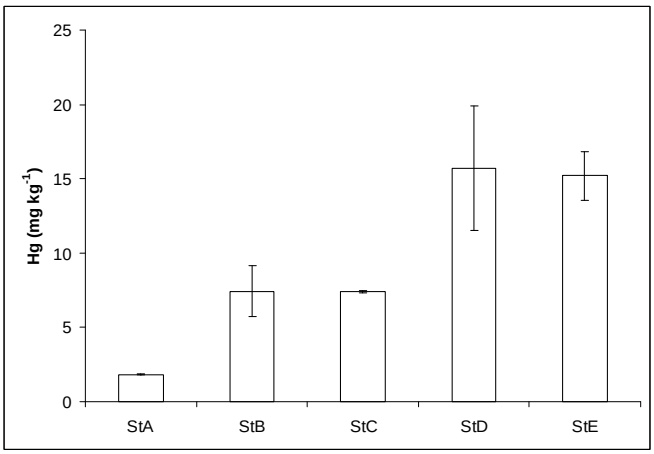


Figure 8.2- Mercury concentrations (mg kg^{-1}±stdev) in the sediments vegetated by *Halimione portulacoides* collected in the Laranjo bay salt marsh.

Mercury concentrations in the roots were significantly higher than those found in leaves. Mercury concentrations in the roots ranged between 0.14-2.8 mg kg^{-1} (fresh weight-fw) while in

the leaves the values ranged between 0.005-0.024 mg kg^{-1} (fw), being the highest concentrations found in the stations with higher mercury concentrations in the sediments.

According to a recent study (Válega et al. 2008) *Halimione portulacoides* may be used as a biomonitor for mercury contamination in salt marshes ecosystems where leaves responded following a positive linear model for a sediment contamination range between 0.03 and 17.0 µg g^{-1}, while roots responded according to a sigmoidal model.

8.3.2 Plant mercury partitioning

Figure 8.3 shows the partitioning of mercury in roots and leaves of *Halimione portulacoides*. The Hg present in the six pooled buffer extractions and the remaining pellet allowed assessing the partitioning of metal through the different cellular fractions.

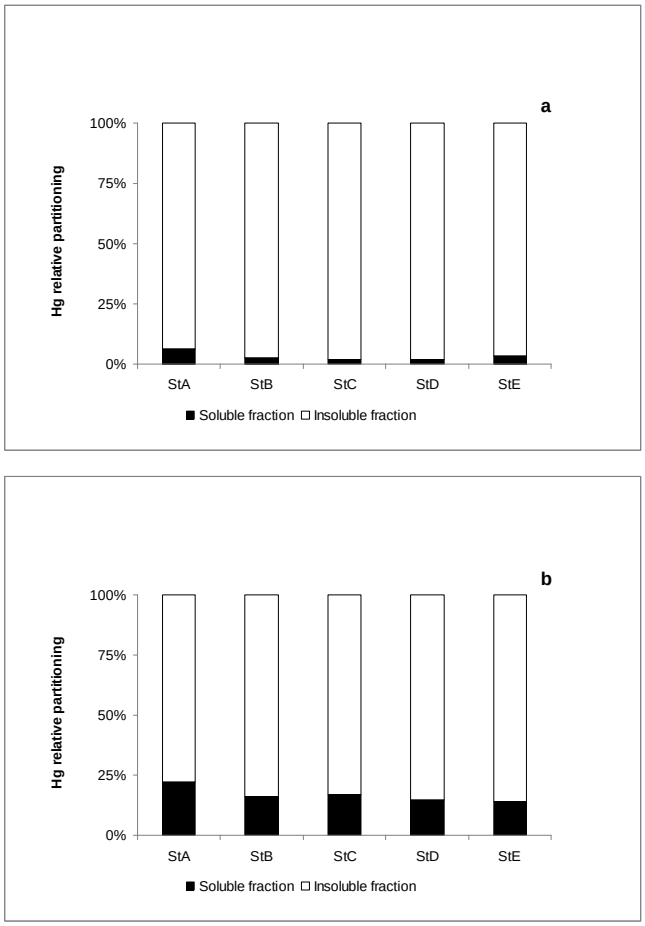


Figure 8.3- Plant mercury cellular partitioning between the soluble (cytosol) and insoluble (membranes, cell walls and hydrophilic compounds) fractions in *Halimione portulacoides* in five stations with different levels of mercury contamination of Laranjo bay salt marsh. The results are the media of three replicates collected in each station. a) roots; b) leaves

Results show that the most significant amount of the metal was retained in the insoluble fraction. Differences were observed between organs, with leaves presenting higher mercury concentrations in the soluble fraction. The soluble fraction represents only 2-7% of the total mercury in the roots (Figure 8.3a) and 17-28% in the leaves (Figure 8.3b). Despite of buffer soluble concentrations are very low, statistical correlations were found between the soluble fraction of the metal and the total concentrations of the roots (Figure 8.4a) and the leaves (Figure 8.4b).

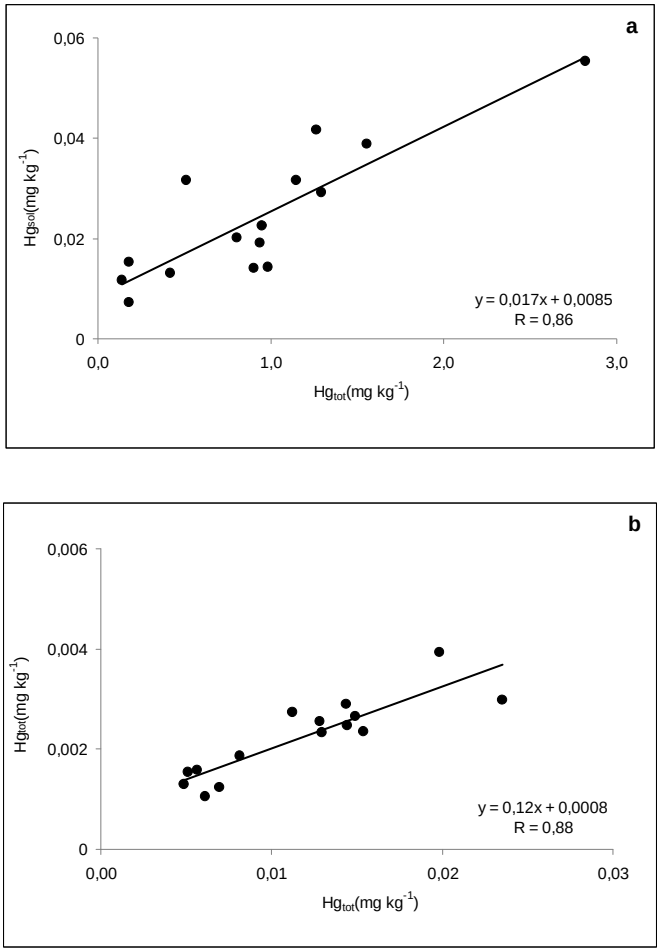


Figure 8.4- Correlations between the mercury concentrations (mg kg^{-1}) of the buffer-soluble fraction and the total mercury concentrations (mg kg^{-1}) in the organs of *Halimione. portulacoides*. a) roots; b) leaves.

8.3.3 Mercury binding complexes

Roots were washed with 5 mM $CaCl_2$ in order to remove all extracellular Hg from the root surfaces as described for other metals (Meuwly and Rauser, 1992; Rauser, 2000).

The freeze-dried material of buffer-soluble mercury was separated by gel filtration. Figure 8.5 shows an example of the chromatographic profile obtained for the buffer-soluble mercury from roots of *Halimione portulacoides*. The mercury distribution among the collected fractions is represented by the histogram. It was observed one major mercury peak, which matched a higher portion of the eluted proteins. Chromatographic profiles show that practically all mercury was concomitant to specific protein peaks instead of eluting as free ions, revealing that all Hg was present in association with proteins. It was observed Hg was eluted in a broad peak, matching a wide portion of proteins and that it appeared in the higher molecular weigh fraction of the chromatogram.

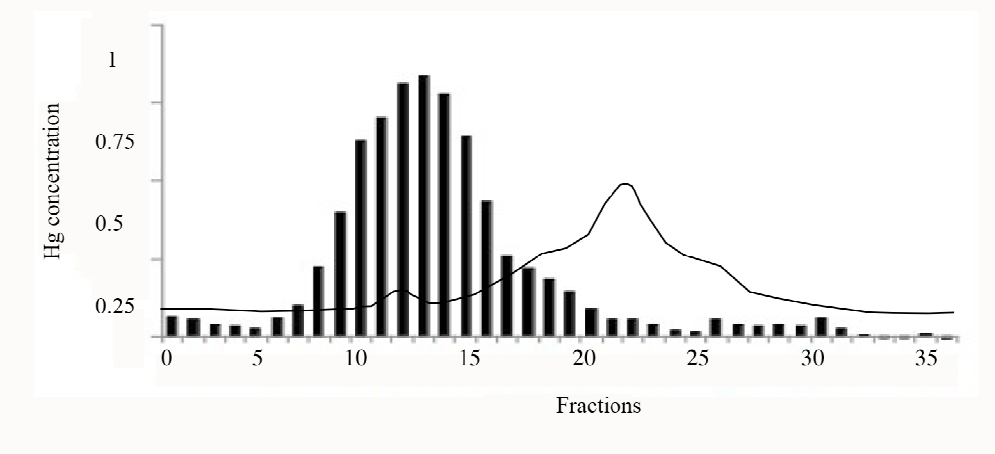


Figure 8.5- Example of a size exclusion chromatography of the buffer soluble mercury. The continuous line corresponds to the absorbance of the eluted proteins (254 nm) and the histogram corresponds to the mercury concentrations (μg L^{-1}) present in each collected fraction.

8.3.4 Phytochelatins analysis

In order to analyse the process of metal chelation in *Halimione portulacoides*, we set out to analyse whether PCs were present in the mercury complexes isolated by gel filtration. As a basis for comparison, we also analysed the thiol profiles in the other areas of the chromatogram (Figure 8.6): area I (fractions 1-6); area II (fractions 7-20) and area III (fractions 21-35), where PCs complexes were reported in previous similar studies (Rauser, 2000). Area III comprised polythiols equivalent to phytochelatins (PCs) with 2 to 5 olygomeric repeats (PC2, PC3, PC4 and PC5), hence demonstrating the presence of PCs, but not associated with mercury. Area I presented low amounts of thiols, comprising some unknown peptides and no PCs. Finally, the mercury containing zone presented the same unknown thiol present in Area I, but also contained few PCs,

namely PC3, PC4 and PC5.

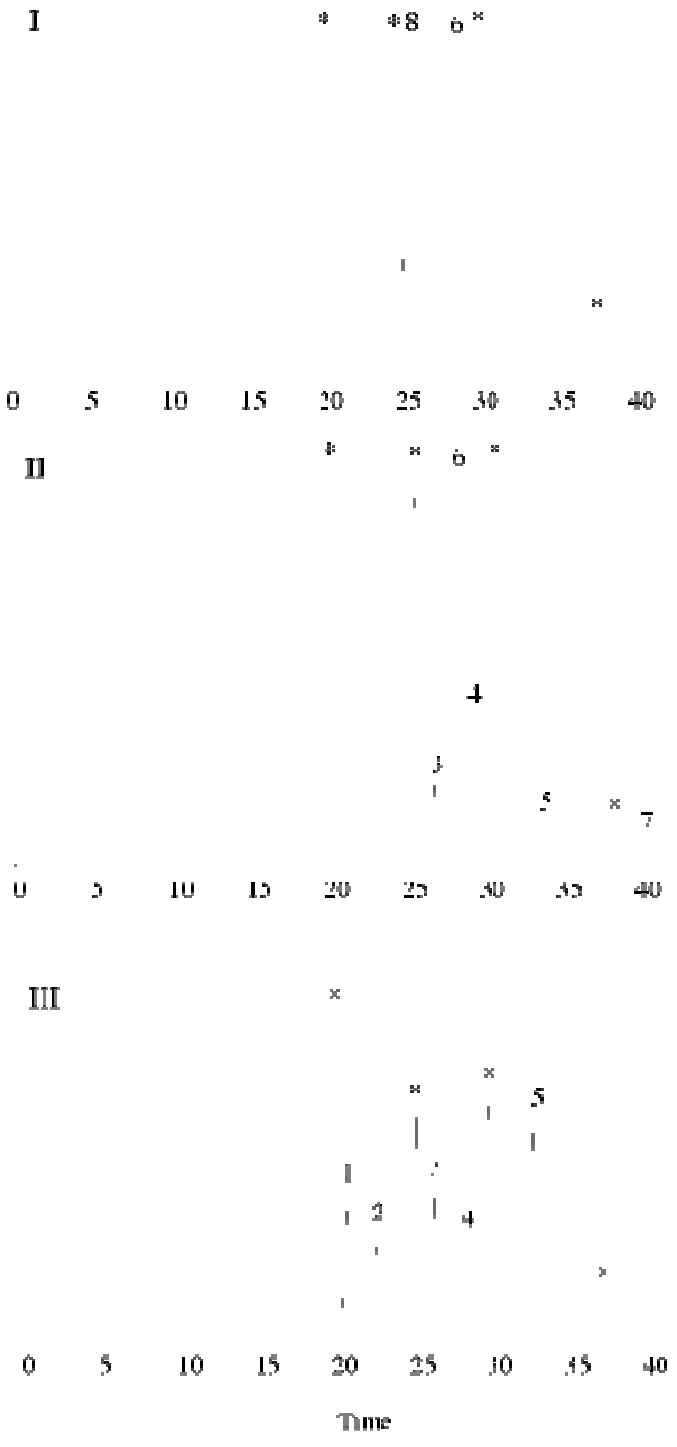


Figure 8.6- Example of a chromatographic profile of HPLC analysis of thiol compounds present in the different areas obtained in the separation of the peptide-mercury complexes. Isolated peaks are as follows: (1) PC_2; (3) PC_3; (4) PC_4, (5) PC_5, (2), (6) and (7) unknown thiols. Peaks marked with (*) correspond to monobromobimane hydrolysis peaks.

8.4 Discussion

Our results clearly show that roots were the main organs for mercury retention in *Halimione portulacoides*, which is in agreement with previous studies (Válega et al., 2008). Metal retention in roots can be a strategy for protecting the more sensitive aerial parts from the deleterious effects induced by metal stress (Lozano-Rodriguez et al., 1997) and it has been reported that in most plant species, metal ions are preferably retained in the root tissues and only small portions are translocated to leaves.

Besides organ partitioning, the subcellular distribution of metal ions within a certain organ is also a critical factor in tolerance; in fact intracellular free mercury (or metal) ions are reported to be much more toxic than complexed mercury (Cavallini et al., 1999). The knowledge about the metal partitioning through cytosol and cell walls can not only provide information on the degree of toxicity the plant is experiencing, besides allowing to speculate about the metal tolerance mechanisms that plants use to cope with long-term mercury exposures.

According to Nishizono et al. (1989) it is possible for a plant to accumulate large amounts of metal in the root cell walls, without noticeable translocation to the intracellular fractions. Our results show that more than 93% of the total mercury found in the roots was immobilised in the cell walls and 72% of the total mercury found in the leaves were in the also in the cell walls. Sousa et al. (2008) found that in the presence of other metals (Zn, Pb, Co, Cd, Ni and Cu) similar results were obtained, with metals being mostly retained in the cell wall compartments. According to Zornoza et al. (2002), a higher accumulation of metals in cell walls can function as a protection barrier by reducing the metal concentration in the cytoplasm.

When comparing these results with those observed in metal exposures in controlled laboratory experiments, they differ substantially. In most of these works, the effectiveness of cell wall retention is reduced, possibly because most of them use very young plantlets and very high metal exposures (Sanitá di Toppi and Gabrielli, 1999), which enhance the entry of metal ions into the cell, instead of its accumulation in the cell walls (Lima et al., 2006).The allocation of most of the metal in the cell walls is an efficient and low energy-consuming mechanism, particularly for long-term exposure to metals. Nevertheless, very small mercury amounts could also be found in intracellular fractions. The comparison of the mercury partitioning between the soluble and insoluble fractions allows us to estimate the importance of cell wall retention when facing different

toxicity levels. Good correlations were found between the mercury concentrations in the sediments with the soluble mercury in the organs of the plant ($r= 0.82$; $n=15$; $p<0.05$ and $r= 0.67$; $n=15$; $p<0.05$, for roots and leaves, respectively) along the degree of mercury contamination. Thus the amount of buffer-soluble mercury increases with higher mercury concentrations in the sediments; however when observing the percentages of soluble mercury (Figure 3), StA which presents the lowest values of mercury contamination in sediments shows the higher values of buffer-soluble mercury as a percentage of the total mercury retained in the respective organs while in the other stations with higher mercury contamination the percentage values are lower, which suggests a defence mechanism of the plant to avoid the presence of cytosolic mercury, which would be much more harmful.

When facing the presence of toxic metals in the cytosol, plants tend to use chelation mechanisms, which usually involve small peptides and organic acids. Even though PCs have been detected on a variety of plant species, their role in metal detoxification is still unclear. For example, in *Lycopersicon esculentum* and Arabidopsis plants, PCs seem to be the major mechanism for Cd tolerance (Chen and Goldsbrough, 1994), but in *Silene vulgaris* their synthesis does not protect plants from exposure to the pressure of metal (De Knecht et al., 1992). Other works (Leopold et al., 1999; Piechalack et al., 2002) suggest that PC formation may only have a partial role in metal resistance and that in higher degrees of stress other mechanisms may be activated. In *Pisum sativum*, the synthesis of PCs is in fact dependent on the time and degree of exposure (Lima et al., 2006), being reduced with higher and more prolonged exposures. Furthermore, up until now, PCs role in real tolerance, under environmental exposures is a controversial subject.

The plain analysis of PCs production in plants under environmental exposures does not comprove per se that the specific metal in study was chelated by PCs since other metals are usually present in the environment and may interfere with PC synthesis and the complexation process. The best way to study if mercury chelation by PCs is occurring in environmental exposures is through the separation of mercury-peptide complexes. Analysis of the chromatographic profiles reveals that practically all of the scarce intracellular mercury was eluted in the first high-molecular weight protein peak. Previous studies with in vitro exposures of Cd, Zn and other metals usually report that metal–phytochelatin complexes are eluted in the final fractions of the chromatogram, in one or more peaks, because of their low molecular weight (Rauser, 2000), and that an occasional peak in the higher molecular weight area is usually due to non-specific adsorption of the metal ions to higher weighted proteins (Meuwly and Rauser, 1992; Rauser, 2000). This peak usually tends to loose

its importance throughout the time of exposure. However, in this work, practically all of the buffer-soluble mercury was presented as one individual peak co-eluting with heavier proteins. These results seem to indicate that PCs do not represent the main role in mercury chelation. With this in mind, we set out to analyse the thiol content of the different portions of the chromatogram.

When observing the HPLC separations we can see that different types of PCs were present in the last eluted proteins, clearly demonstrating that *Halimione portulacoides* did produced PCs. This result can indicate the presence of other metal exposures than mercury in the sample sites, since these PCs were not associated with mercury. Nevertheless, the mercury peak presented some PCs in its constitution, namely with 3, 4 and 5 olygomeric repeats. These results are of significant importance because they show that mercury can be complexed by PCs in environmental prolonged exposures. Due to the high molecular weights of the proteins it is plausible that other molecules may be assisting the mercury complexation and that PCs are not the main mercury chelators. A recent work (Marentes and Rauser, 2007) has analysed cadmium speciation in wheat plants and concluded that phytochelatins were not necessarily the major ligands, particularly in leaves. In fact, a cystein-rich protein metallothionein was also shown to be present in the higher molecular weight fractions of gel filtrations of leaf extracts (concomitant to a cadmium peak that differed from the classic phytochelatin-binding complexes). It is therefore possible that higher molecular weighted metallothioneins could also be playing a role in mercury chelation sequestration, in *Halimione portulacoides* instead of only phytochelatins.

It has also been hypothesised that in the vacuoles, metal ions can also be chelated by other molecules, such as organic acids, sulphide etc, forming heavier clusters, and also possibly releasing some thiols back to the cell (Zenk, 1996; Rauser 2000). For cadmium, it has been demonstrated that a process of bio-mineralisation enhances the Cd-binding ability of higher weighted complexes (Mehra et al., 1994; Kneer and Zenk, 1996) and hence increases tolerance.

A replacement of mercury chelators in the vacuoles could also explain the presence of PCs not associated to mercury. This is particularly plausible when roots are more developed and vacuolated, hence presenting higher levels of citrate, malate and oxalate (Sanitá di Toppi and Gabrielli, 1999). But whether the remaining PCs in the chromatogram were released PCs from the mercury complexes or were complexed to other metals remains to be elucidated. However, it seems largely improbable that the higher amount of PCs in the last fraction of the chromatogram would not be associated with any metal cations. It is more likely that in the presence of multi metal

exposures, such as the case of environmental contaminations, PCs are synthesised and bind those metal ions that have higher affinity to GSH.

Parallel studies were conducted in Laranjo bay salt marshes which indicate high concentrations of Pb, Cd, Cu and Zn in the sediments (12.4; 0.47; 58.8 and 246.7 μg g^{-1}, respectively) (Monterroso, 2005). This selectivity would yield a group of different complexes, with different molecular weights and containing different metal ions. A different approach, with concomitant metal exposures would be very interesting in order to understand the role and effectiveness of the PC-based mechanism. This work clearly demonstrates that PC-metal chelation in the environment can be a complex phenomena, possibly with different efficiency rates according to the metal in question.

8.5 References

Cavallini, A., Natali, L., Durante, M., Maserti, B., 1999. Mercury uptake, distribution and DNA affinity in durum wheat (Triticutum durum Desf.) plants. Sci. Total Environ. 243/244, 119-127.

Cobbett, C.S., Goldsbrough, P., 2002. Phytochelatins and methalothioneins: Roles in heavy metal detoxification and homeostasis. Ann Rev Plant Biol. 53, 159-182.

De Knecht, J.A., Koevoets, P.L.M., Verkleij, J.A.C., Ernst, W.H.O., 1992. Evidence against a role for phytochelatins in naturally selected increased cadmium tolerance in *Silene vulgaris* (Moench) Garcke. New Phytol. 122, 681-688.

Gupta, M., Tripathi, R.D., Rai, U.N., Chandra, P., 1998. Role of glutathione and phytochelatin in *Hyrilla verticillata* (l.f.) royle and *Vallisneria spiralis* L. under mercury stress. Chemosphere, 37, 785-800.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot. 366, 1-11.

Kneer, R., Zenk, M.H., 1996. The formation of Cd-phytochelatin complexes in plant cell cultures. Phytochemistry 44, 69-74.

Leita, L., De Nobili, M., Cesco, S., Mondini, C., 1996. Analysis of intercellular cadmium forms in roots and leaves of bush bean. J Plant Nutr. 19, 527-533.

Leopold, I., Gunther, D., Schmit, J., Neumann, D., 1999. Phytochelatins and heavy metal tolerance. Phytochemistry 50, 1323-1328.

Lima, A.I.G.L., Pereira, S.I.A.P., Figueira, E.M.A.P, Caldeira, G.C.N., Caldeira, H.D.S.Q., 2006. Cadmium detoxification in roots of *Pisum sativum* seedlings: relationship between toxicity levels, thiol pool alterations and growth. Environ. Exp. Bot. 55, 149-162.

Lozano-Rodriguez, E., Hérnandez, L.E., Bonay, P., Carpena-Rui, R.O. 1997. Distribution of cadmium in root tissues of maize and pea plants: physiological disturbances. J Exp Bot. 306, 123-128.

Marentes, E., Rauser, W.E., 2007. Different proportions of cadmium occur as Cd-binding phytochelatin complexes in plants. Physiol Plantarum. 131, 291–301.

Mehra, R.J., Mulchandani, P., Hunter, T.C., 1994. Role of CdS quantum crystallites in cadmium resistance in *Candida glabrata*. Biochem. Bioph. Res. Co. 200, 1193-1200.

Meuwly, P., Rauser, W.E., 1992. Alteration of thiol pools in roots and shoots of maize seedlings exposed to cadmium: adaptation and developing cost. Plant Physiol. 99, 8–15.

Monterroso, P., 2005. Distribuição e comportamento do cádmio, chumbo, cobre e zinco nos sedimentos e coluna de água da Ria de Aveiro. PhD Thesis.

Nishizono, H., Kubota, K., Suzuki, S., Ishii, F., 1989. Accumulation of heavy metals in cell walls of *Polygonum cuspidatum* roots from mettalliferous habitats. Plant Cell Physiol. 30, 595-598.

Ortiz, D.F., Ruscitti, K.F., McCue, K.F., Ow, D.W., 1995. Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein. J Biol Chem. 27, 4721-4728.

Patra, M.m Bhowmik, N., Bandopadhyay, B., Sharma, A., 2004. Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Env Exp Bot. 52, 199-223.

Powell, R.L., 1997. The use of vascular plants as “field” monitors. In: Wang, W. et al. (Eds.), Plants for Environmental Studies. CRC Press, Lewis publishers, Boca Raton, Florida, pp. 335-365.

Rauser, W.E., 1999. Structure and function of metal chelators produced by plants. The case of amino acids, organic acids, phytin and methalothioneins. Cell Biochem Biophys. 31, 1-31.

Rauser, W.E., 2000. Roots of maize seedlings retain most of their cadmium through two complexes. J. Plant Physiol. 156, 545-551.

Sanitá di Toppi, L., Gabrielli, R., 1999. Response to cadmium in higher plants. Environ Exp Bot. 41, 105-130.

Sneller, F.E., Van Heerwaarden, L.M., Koevoets, P.L., Vooijs, R., Schat, H.,Verkleij, J.A., 2000 Derivatization of phytochelatins from *Silene vulgaris*, induced upon exposure to arsenate and cadmium: comparison of derivatization with Ellman’s reagent and monobromobimane. J Agric Food Chem. 48, 4014–4019.

Sousa, A.I., Caçador, I., Lillebø, A.I., Pardal, M.A., 2008. Heavy metal accumulation in H*alimione portulacoides:* Intra -and extra-cellular metal binding sites. Chemosphere. 70, 850-857.

Wagner, G.J., 1993. Accumulation of cadmium in crop plants and it consequences to human health. Adv Agron. 51, 173-212.

Válega, M., Lillebø, A.I., Caçador, I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Mercury mobility in a salt marsh colonised by *Halimione portulacoides*. Chemosphere. 72, 1607-1613.

Zenk, M.H., 1996. Heavy metal detoxification in higher plants- a review. Gene. 179, 21-30.

Zornoza, P., S. Vazquez, E. Esteban, M. Fernandez-Pascual, and R. Carpena., 2002. Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiol Biochem. 40, 1003-1009.

9 Salt marsh plants as biomonitor tools: *Halimione portulacoides* as case study

9.1 Introduction

Environmental monitoring is essential to identify hazards to human health and also to assess environmental cleanup efforts to prevent further degradation of the ecosystems (Butterworth, 1995). The European Water Framework Directive (WFD) highlights the establishment of monitoring programmes to transitional and coastal waters in order to assess their ecological and chemical status. The chemical analysis approach by itself may not be enough to predict the effects of a certain pollutant (the bioavailable fraction) on the living organisms and usually a complex set of speciation and fractionation studies are required.

According to Markert (2007) a bioindicator is an organism, a part of an organism or a society of organisms which gives information on the quality of its environment (qualitative information) and a biomonitor is an organism (part of an organism or a society of organisms) that quantifies the quality of its environment (quantitative information). The use of biomonitors has been increasing over the last years acting as a promising tool to identify potential hazards to human health and to provide new environmental quality assessment approaches (Butterworth, 1995; Ferrat et al., 2003; Melville, 2006). Biomonitors have also been pointed out as important tools to assess geographical and/or temporal variations of the bioavailable fraction of the contaminants in the ecosystems (Rainbow and Phillips, 1993); in fact it is the bioavailable fraction that has more toxicological relevancy from the ecological point of view. According to Zhou et al. (2008) the physico-chemical analysis can give detailed information about the metal species and contamination levels of the ecosystem while biomonitoring studies go further giving information about the bioaccumulation level and integrated toxicological effects. Metals are preferentially accumulated in the sediments but the bioavailable fraction is affected by its physico-chemical characteristics such as pH, salinity, particle size and organic matter (Rainbow, 1995). The exposure of an organism to a contaminant is regulated by the amount of the contaminant but especially by its bioavailability and time of exposure (Powell, 1997).

The use of biomonitors in the field has several advantages since contaminants bioavailabilities are highly dependent on environmental conditions where abiotic and biotic factors are integrated. As already referred metal availability and subsequent uptake is dependent on physico-chemical parameters of the sediment and since plants can modify the surrounding environment and consequently influence the bioavailable fraction, the bulk sediment analysis by itself may not measure the processes that may occur at the membrane surface where the changes occur (Powell, 1997).

Macrophytes have been suggested as bioindicators of metal contamination in coastal regions (Ferrat et al., 2003; Melville, 2006). A good candidate as a biomonitor should obey a certain set of features such as: a wide geographical distribution; to be easy to identify and sample; it should not be seasonal and it should be sufficiently long-lived, sedentary and available in such a reasonable size that could provide enough tissues for analysis and easily measurement of contaminants in their tissues without risk of approaching detection limits and contaminations issues (Rainbow 1995).

Several attempts have been made to quantify the bioavailable fraction of mercury in the sediments; however they always result in a complex set of speciation and fractionation studies with several sequential extraction procedures and controversy discussions of the results (Bacon and Davidson, 2008). The speciation of mercury it is not a simple procedure (Válega et al., 2008) and the application of sequential extraction procedures can have some disadvantages namely the low concentrations of the bioavailable fraction of mercury. Total concentrations of the bioavailable fraction represent low percentages values of the total mercury concentrations in the sediments. In a study performed for a wide range of mercury concentrations in soils (5-1710 μg g^{-1}) of Almaden area (Spain) the bioavailable fraction of mercury for the plants represents less than 6.5% of the total concentrations (Millán et al., 2006) which can represent some difficulties with detection limits in low to moderate mercury contaminated systems.)

Plants have been used for biomonitoring processes giving a more time-integrated picture of the metal concentrations in the sedimentary compartment (Rainbow, 1995). There is evidence that plants may accumulate metals in their tissues (Weis and Weis 2004; Válega et al., 2008a) and that rooted submerged macrophytes take up the bioavailable fraction of the metals from the sediments and interstitial waters.

Halimione portulacoides presents a clear seasonal variation in its growing cycle, ranging between 978 and 1804 g DW m^{-2}. Above ground biomass increased from late spring to early summer, and then gradually decreased until late winter (Válega et al., 2008c). The same pattern was observed in French salt marshes (Bouchard and Lefreuve, 2000).

The aims of this study were: i) to determine mercury concentrations in salt marsh sediments vegetated by *Halimione portulacoides* along the Portuguese coast; ii) to evaluate mercury concentrations in the different organs of the plant; iii) to evaluate the potential use of *Halimione portulacoides* as mercury biomonitor of salt marsh sediments. To achieve the above

mentioned objectives *Halimione portulacoides* was sampled from North to South of Portugal in eight estuaries with different degrees of mercury contamination, and in each system two or more stations were assessed.

9.2 Sampling details

This study was carried out along the coastal zone of Portugal (Figure 9.1). Seventeen salt marsh stations from eight distinct estuarine systems were selected, namely, Cávado (41°31'N 8°46'W- S1), Ria de Aveiro (40°43'N 8° 37'W-S2; S3; S4; S5), Mondego (40°07'N 8°48W- S6; S7), Tagus (38°38'N 9º07W- S8; S9; S10), Sado (38°31'N 8°46'W- S11; S12), Mira (37°43'N 8°46'W- S13; S14), Ria Formosa (37°00'N 7°58'W- S15; S16) and Guadiana estuary (37°13'N 7°25'W- S17). With the exception of Mira (S13; S14) and Guadiana (S17), all stations are located nearby large urban areas and subjected to industrial, agricultural and fisheries activities. Cávado, Tagus and Sado are also subjected to harbour associated activities, like naval industry and Ria de Aveiro and Tagus estuaries were in the past subjected to mercury contamination due to the presence of chlor-alkali plants.

Sampling took place during late autumn and winter seasons (to exclude the growing season of the plants) and the samples were randomly collected in the salt marsh areas during low tide. Five replicates of sediments (15 cm depth) and plants biomass (below ground and above ground biomass) distanced by 15 m at least were always sampled. Previously, a sampling program was carried out bimonthly at Ria de Aveiro in three different stations for the characterisation of *Halimione portulacoides* annual growing cycle. The above ground biomass (leaves and stems) was collected at the ground level (25×25 cm).

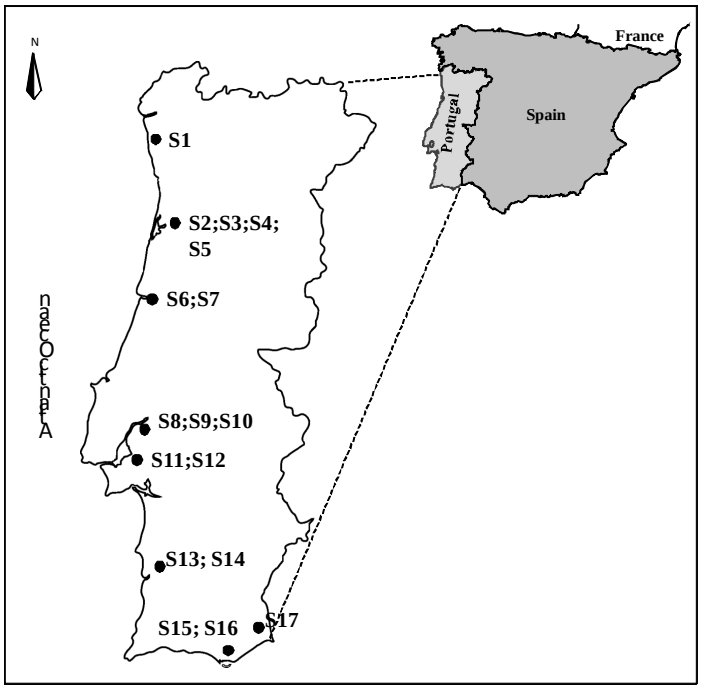


Figure 9.1- Map of Portugal with the sampling stations along the Portuguese coast.

9.3 Results

9.3.1 Mercury concentrations in sediments

Significant differences were found between the sampling stations ($p \leq 0.001$). The range of mercury concentrations found in the salt marsh sediments along the Portuguese coast was high (Figure 9.2). Mercury concentrations ranged between 0.03-17 μg g^{-1} being the highest concentrations found in four stations (S2; S3; S4; S5) of the Ria the Aveiro followed by one station located in the Tagus estuary (S8- Rosario salt marsh). From the stations located in the Ria de Aveiro it was possible to observe a gradient of metal contamination. Station 2 presented the highest values (15.6±1.0 μg g^{-1}) while station 5 the lowest one (1.9±0.1 μg g^{-1}). Station 3 and station 4 presented similar concentrations of mercury, 7.5±1.9 and 7.7±0.4 μg g^{-1}, respectively. These differences denote a contamination gradient according to the distance to the point source of mercury into the system, S2 being the closest and S5 the furthest as observed in a previous work (Válega et al., 2008b). In Tagus estuary significant differences were observed between the three sampling stations although the mercury values found in Rosario (S8) and Corroios (S9) salt marshes were much higher than those observed in the Hortas station (S10). The lowest mercury concentrations were found in Cávado, Mondego, Mira and Ria Formosa salt marshes. According to

the Portuguese classification for mercury concentrations in dredged sediments it was possible to identify different types of sediment classes along the Portuguese coast. The classification of sediments is made in classes from 1 to 5 regarding their degree of contamination: the least contaminated is classified as class 1 (<0.5 μg g^{-1}), followed by class 2 (0.5-1.5 μg g^{-1}), class 3 (1.5-3.0 μg g^{-1}), class 4 (3.0-10 μg g^{-1}); the most contaminated is class 5 (>10 μg g^{-1}). Most of the stations were classified as class 1 (S1; S6; S7; S10; S12; S13; S14; S15; S16), three as class 2 (S9; S11; S17), one as class 3 (S8), three as class 4 (S3; S4; S5) and only one station was classified as class 5 (S2). The values found in S2, S3 and S4 (7-17 μg g^{-1}) located in Ria Aveiro can be considered extremely high and according to the Portuguese classification reveals that is an area requiring a special concern in order to avoid future environmental problems.

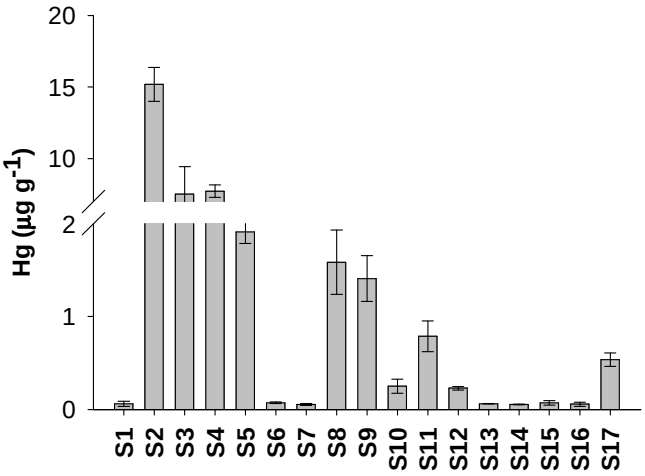


Figure 9.2- Total mercury concentrations in the sediments (μg g^{-1}±stdev) colonised by *Halimione portulacoides*.

9.3.2 Mercury concentrations in *Halimione portulacoides*

Results concerning mercury accumulation in *Halimione portulacoides* showed that it occurs mainly in the roots. Mercury concentrations in the roots ranged between 0.01-12.9 μg g^{-1} (Figure 9.3a), followed by the leaves, 0.009-0.14 μg g^{-1} (Figure 9.3b) and stems, 0.002-0.056 μg g^{-1} (Figure 9.3c). The highest values of mercury in the different organs of the plant were found in Ria de Aveiro and in one station of Tagus estuary (S8- Rosario salt marsh), as observed for the mercury concentrations in the sediments.

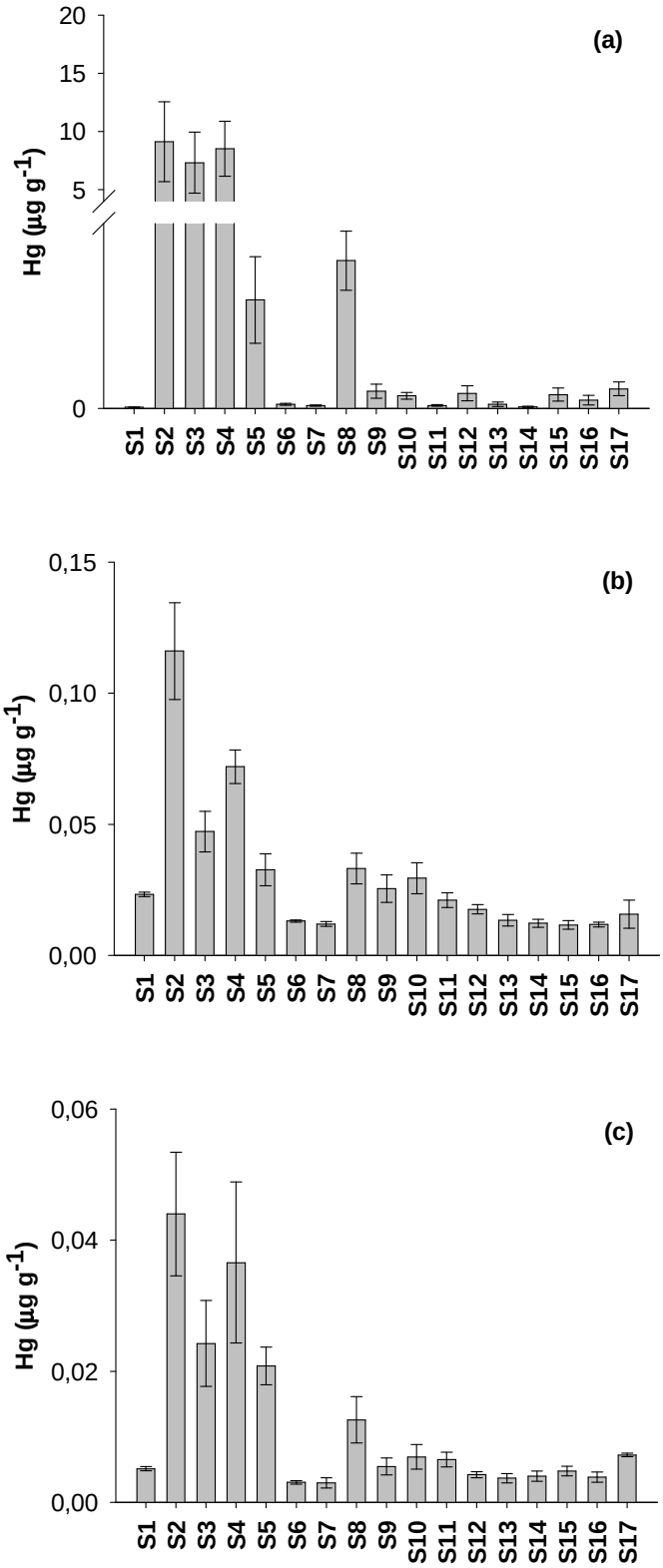


Figure 9.3- Total mercury concentrations (µg g^{-1}±stdev) in the different organs of *Halimione portulacoides* (a) roots; (b) leaves; (c) stems.

A linear regression model was found between the concentrations of mercury in the different plant organs: between the mercury concentrations in the roots and stems (Figure 9.4a, R^2_{adj}=0.75), stems and leaves (Figure 9.4b, R^2_{adj}=0.85) and roots and leaves (Figure 9.4c, R^2_{adj}=0.78).

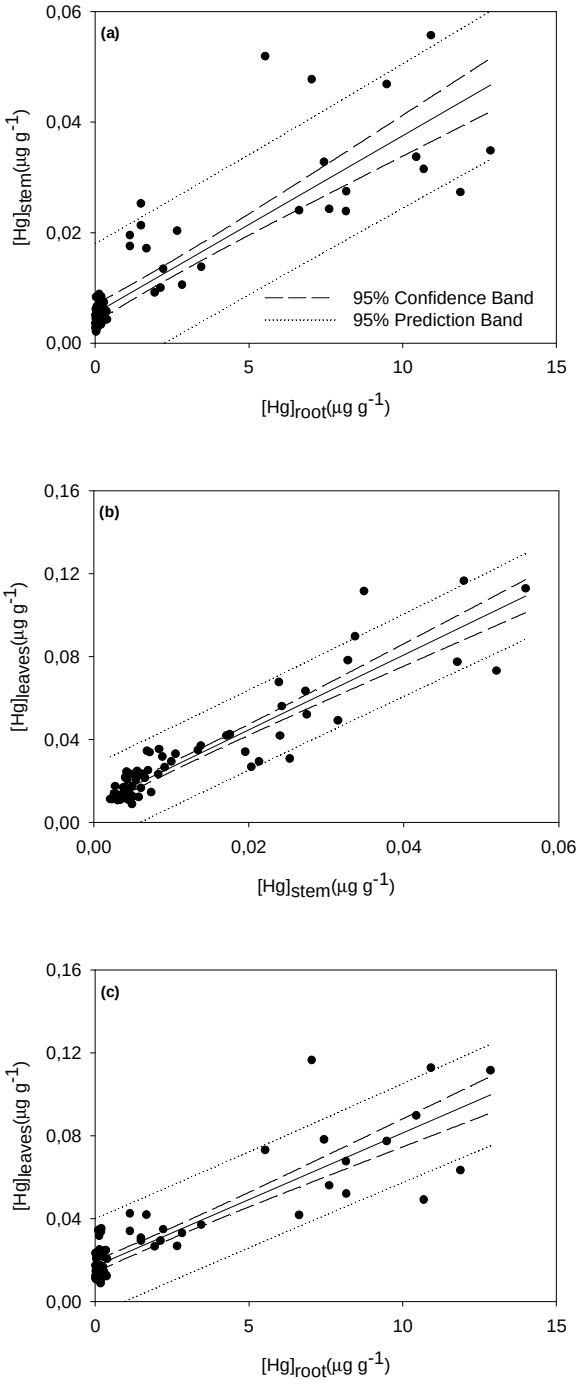


Figure 9.4- Correlation coefficients between: a) roots and stems of *Halimione portulacoides*, b) stems and leaves of *Halimione portulacoides*, c) roots and leaves of *Halimione portulacoides*.

9.3.3 Halimione portulacoides as a biomonitor

In order to evaluate the response of *Halimione portulacoides* (roots and leaves) to mercury concentrations in sediments a linear model and a sigmoidal model were tested and compared. The adjusted correlation coefficients (R^2_{adj}) were calculated between: a) roots and sediments and between leaves and sediments, in order to find the model with the best fit to our data. All independent values were used for each station and not the mean values. The variation of mercury concentration in the roots versus mercury concentration in the sediments was best fitted by a sigmoidal model equation (Figure 9.5a, R^2_{adj}=0.89).

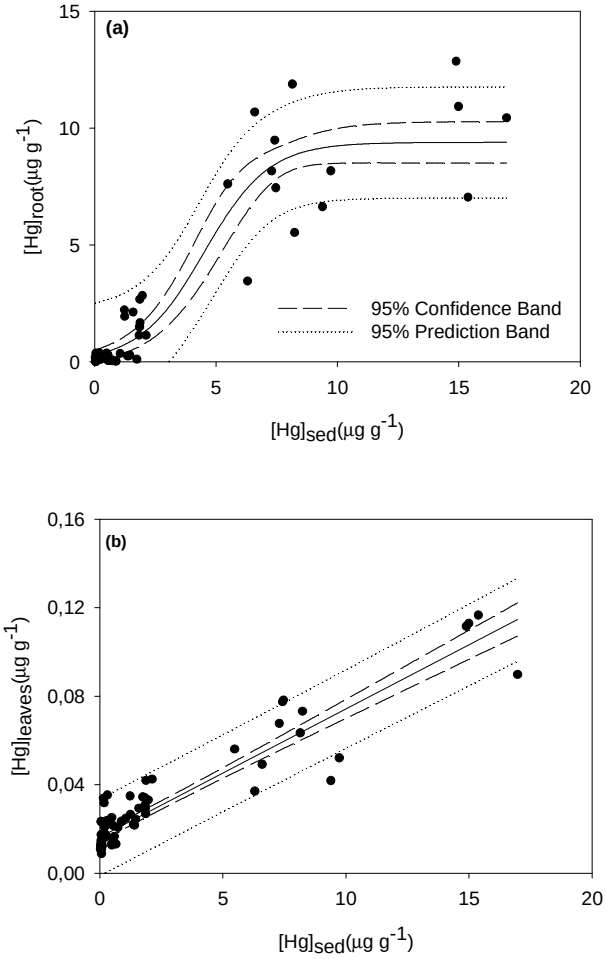


Figure 9.5- Correlation coefficients between: a) sediments and roots of *Halimione portulacoides*, b) leaves of *Halimione portulacoides* and sediments vegetated by *Halimione portulacoides*.

As shown in Figure 9.5a, mercury accumulation in the roots can be described in three steps: at a low range of mercury concentrations in the sediments (from 0.03 up to 2 µg g^{-1}), the

accumulation of mercury in roots is also low reaching a maximum concentration of 1.3 μg g^{-1} in a second step the rate of mercury accumulation in roots attains its maximum value until the concentration of mercury in sediments reach approximately 4.5 μg g^{-1}; after this maximum the accumulation of mercury in the roots slows down leading to a plateau in the concentration of mercury in the roots of about 9.4 μg g^{-1}, which corresponds to a concentration of mercury of about 11 μg g^{-1}.

With respect to the relation between the mercury concentrations in leaves versus the concentrations in sediments the best model explaining was the linear regression model (R2adj =0.88) as shown in Figure 9.5b.

The ratios of mercury concentrations between the organs of the plant and sediments are indicative of the metal transfer from the sediment to the plant and gives information about the accumulation of the metal by the plant. Accumulation factors were calculated by the ratio between metal concentrations in roots and sediments as well between as roots and leaves and between leaves and stems (Table 9.1). *Halimione portulacoides* does not accumulate high amounts of mercury in the different organs. The bioaccumulation factor observed for the roots with respect to the sediments ranged between 0.1 and 2.2 (median value 0.8). Accumulation factors of mercury in the roots relatively to the leaves ranged between 0.7-152 (median value 12) which is an indicative of the low translocation of mercury inside the plant. Lowest differences were observed between the stems and the leaves; however higher accumulation factors were found in leaves ranging between 1.6 and 4.8 (median value 3.2).

Table 9.1- Accumulation factors of mercury (average values for each station) for the different organs of *Halimione portulacoides*.

Station	Accumulation factor		
	Roots/Sed	Roots/Leaves	Leaves/Stems
S1	0.3	0.7	4.5
S2	0.7	91.1	2.8
S3	1.0	152.4	2.0
S4	0.6	120.3	2.1
S5	0.8	51.8	1.6
S6	0.8	4.4	4.4
S7	0.7	3.3	4.3
S8	1.4	66.9	2.8
S9	0.2	8.9	4.8
S10	0.8	6.4	4.4
S11	0.1	2.0	3.3
S12	1.0	12.3	4.2
S13	1.1	4.7	3.8
S14	0.4	1.8	3.1
S15	2.2	14.4	2.5
S16	1.7	12.0	3.2
S17	0.6	24.9	2.8

9.4 Discussion

Mercury concentrations in the sediments clearly identify the Portuguese hotspots of mercury: the Ria de Aveiro and the Tagus estuary, due to chlor-alkali plant discharges in the past (Pereira et al., 1998; Canário et al., 2005). As mentioned by Covelli et al. (1999) marine sediments contaminated by industrial effluents can constitute a secondary source of mercury to the aquatic ecosystems even after the discharges have been ceased. The results show that sediments are the main compartment for mercury accumulation in salt marshes and represent the major reservoir of the metal. The highest levels of mercury contamination were observed in stations S2, S3 and S4 which are located in a confined area of Ria de Aveiro where several works have been reporting the contamination levels in the different compartments of the system (Abreu, et al., 2000; Coelho et al., 2006; Ramalhosa et al., 2006, Válega et al., 2008a). Concerning mercury accumulation in *Halimione portulacoides* results showed that it occurs mainly in the roots which is also in agreement with observations for other salt marsh species (Weis and Weis 2004; Válega et al., 2008a). The lower mercury accumulation in leaves is probably due to their constant renovation,

while the lowest accumulation in stems is probably due to the fact that this organ is considered to be a transport organ for fluids and not a storage organ. The good correlations found between the stems and the leaves are an indicator that mercury concentrations in the leaves are probably due to translocation from the roots and not due to deposition of atmospheric mercury or absorption by the leaves during high tide. Nevertheless the deposition process can not be excluded but probably is not a major process.

Despite the fact that mercury concentrations in the organs (roots and leaves) of H. portucaloides increased with the increment of mercury in the sediments, two different response models were observed. Mercury concentrations in t roots versus the concentration in sediments followed a sigmoidal model. Similar to a calibration curve (imaging *Halimione portulacoides roots* as a biological sensor) at low concentrations the accumulation in the roots is low and does not show a linear response, suggesting mechanisms of defence from the plant to avoid toxic concentrations. Above the concentration of 9.4 μg g^{-1} the concentration of mercury in the roots no longer increases with the increment of mercury in the sediments, showing a maximum constant response. This is probably due to the turnover rate of mercury between roots and the sediments. On the other hand, mercury concentrations in leaves versus the concentration at the rhizosphere followed a linear model. Differences in responses of roots and leaves are explained by the dynamics of the plant organs. Mercury accumulated in the below ground part of the plant is quite mobile and mostly returns to the sediment pool throughout the mineralisation process. According to Pereira et al. (2007) the decomposition of roots of *Halimione portulacoides* corresponded closely to 42-46% of mass lost (dry weigh) in the first months.

Due to the positive linear correlation coefficients between the mercury concentrations in roots, leaves and sediments, *Halimione portulacoides* can be suggested as a suitable biomonitor for mercury in salt marshes. However, the application of the model should be cautious and some aspects should be taken into account namely: the life cycle of the species, and the variability in environmental contamination. Explicitly, during the growing season results may be underestimated due to the fast increase in biomass which may promote a dilution in the effect on the accumulated mercury, whilst the spatial variability of the system should be taken into account during sampling procedure, especially in areas with a high level of contamination. On the other hand, although leaves showed higher correlations coefficients with mercury concentrations in sediments, roots should be preferred for environmental evaluation whenever atmospheric deposition of mercury is significant.

The results obtained in this work show that the sampling of salt marsh plants must be done carefully and a high number of field replicates is recommended in order to reflect the high spatial variability of these ecosystems. Coefficients of variation (standard deviation/average*100) were calculated for the results in order to assess the spatial variability of each system. According to the results it was possible to conclude that in some systems, independently of the degree of contamination, the spatial variability can be extremely high. In sediments the coefficients ranged from 4 to 31% while in roots the coefficients were found to vary between 20 and 54%. Low values were found in the leaves (3-21%) and stems (4-34%). This variability can be related with the system variability caused by the dispersion of the contamination into the system and with the genetic variability of the plants besides their age.

An important advantage of this work is the fact that it is a field work. Laboratory tests have their limitations and the results are usually dependent on several variabilities including the test conditions which may be different from the natural conditions. Laboratory test tends to be more conservative and usually are performed in a short period of time (Powell, 1997).

9.5 References

Abreu, S., Pereira, E., Vale, C., Duarte, C., 2000. Accumulation of mercury in sea bass from a contaminated lagoon (Ria de Aveiro, Portugal). Mar Pollut Bull. 40, 293-297.

Bouchard, V., Lefeuvre, J.C., 2000. Primary production and macro-detritus dynamics in a European salt marsh: carbon and nitrogen budgets. Aquat Bot. 67, 23-42.

Butterwoth, F.M., 1995. Introduction to biomonitors and biomarkers as indicators of environmental change. In: Butterwoth et al (Eds), Biomonitors and biomarkers as indicators of environmental change: a handbook, Plenum press, New York.

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

Coelho, J.P., Rosa, M., Pereira, E., Duarte, A., Pardal, M.A., 2006. Pattern and annual rates of *Scrobicularia plana* mercury bioaccumulation in a human induced mercury gradient (Ria de Aveiro, Portugal). Estuar Coast Shelf Sci. 69, 629-635.

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. Anal Chim Acta. 405, 179-183.

Covelli, S., Faganeli, J., Horvat, M., Brambati, A., 1999. Porewater distribution and benthic flux measurements of mercury and methylmercury in the Gulf of Trieste (Northern Adriatic Sea). Estuar Coast Shelf Sci. 48, 415-428.

Markert, B., 2007. Definitions and principles for bioindication and biomonitoring of trace metals in the environment. J Trace Elem Med Bio. 21, 77-82.

Melville, F., Pulkownik, A., 2006. Investigation of mangrove macroalgae as bioindicators of estuarine contamination. Mar Pollut Bull. 52, 1260–1269.

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. Sci Total Environ. 213, 157-163.

Pereira, P., Caçador, I., Vale, C., Caetano, M., Costa, A.L., 2007. Decomposition of belowground litter and metal dynamics in salt marshes (Tagus estuary, Portugal). Sci Total Environ. 380 (1-3), 93-101.

Powell, R.L., 1997. The use of vascular plants as "field" monitors. In: Wang, W. et al. (Eds.), Plants for Environmental Studies. CRC Press, Lewis publishers, Boca Raton, Florida, pp. 335-365.

Rainbow, P.S, Phillips, D.J.H., 1993. Cosmopolitan biomonitors of trace metals. Mar Pollut Bull. 26, 593–601.

Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. Mar Pollut Bull. 31, 183-192.

Ramalhosa, E., Pato, P., Monterroso, P., Pereira, E., Vale, C., Duarte, A.C., 2006. Accumulation versus remobilization of mercury in sediments of a contaminated lagoon. Mar Pollut Bull. 52, 353-356.

Válega, M., Lillebø, A.I., Pereira, M.E., Corns, W.T., Stockwell, P.B., Duarte, A.C., Pardal, M.A., 2008. Assessment of methylmercury production in a temperate salt marsh (Ria de Aveiro Lagoon, Portugal). Mar Pollut Bull. 56, 136–162.

Válega, M., Lillebø, A.I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Long-term effects of mercury in a salt marsh: Hysteresis in the distribution of vegetation following recovery from contamination. Chemosphere. 71,765-772.

Válega, M., Lillebø, A.I., Caçador, I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008. Mercury mobility in a salt marsh colonised by *Halimione portulacoides*. Chemosphere. 72, 1607-1613.

Weis, J.S., Weis, P., 2004. Metal uptake, transport and release by wetland plants: implication for phytoremediation and restoration. Environ Int. 30, 685-700.

10 Final considerations

The first studies of mercury contamination in Ria de Aveiro reported essentially mercury concentrations in the different biotic and abiotic compartments of the system. At that time, as obvious, the main concern was about mercury exportation from outside Laranjo bay to the rest of the system. Over all these long years of mercury research in Ria de Aveiro direction of the questions changed and new issues arise. After the cessation of mercury discharges new research guidelines emerged focused in the effects and consequences of the discharges into the system. One of the questions raised in the beginning of this research work has to do with floristic coverage of the salt marsh along the years. Local population claims that salt marsh flora have changed drastically since the time when chlor-alkali plant was labouring from now. The first sampling of cores of sediments in depth corroborates the theory that nowadays flora seems to be different from those existent in the past. Salt marshes plants form a characteristic tangle hiding several species of animals in various stages of life.

The case study of the Ria de Aveiro shows how a considerable loading of mercury into a salt marsh for four decades has affected its resistance, inducing a change from salt marsh plants species richness into an alternative state dominated by one species in this case, *Phragmites australis*. Despite of nowadays concentrations in the superficial sediments be much lower than those found in the past, the sediments of Laranjo salt marsh records a strongly pronounced gradient of mercury contamination. Salt marsh species diversity is very different along the gradient of mercury contamination with a high number of species in the more remote stations from the contamination source. It is important to denote that these differences were observed in a reduced special scale within a few kilometres (approximately 2 km). The reconstruction of the historical inputs of mercury by the vertical profiles of mercury concentrations allows us to conclude that the highest mercury concentrations in sediments, corresponding to the years of high volume of mercury discharges, were associated with layers dominated by the presence of *Phragmites australis* which is pointed as a metal-tolerant species for a high number of metals (Válega et al., 2008a).

Nowadays, approximately ten years after the cessation of mercury loading and based on the salt marsh plants species richness, the system still shows an incomplete resilience due to the lag in recovery, named hysteresis. The study suggests that the recovery of marshes historically contaminated with mercury may also depend on its species-specific composition. The passive recovery may be dependent on the interaction of the salt marsh plants, their biology, morphology

and physiology, and the possible difference in the species-specific interactions with the biogeochemical cycle of mercury (Válega et al., 2008a).

By this point we are able to conclude that salt marsh plants accumulate mercury in their organs. Assuming that, a new issue related with mercury mobility inside the salt marsh arise. Once in the salt marsh, mercury can enter in a complex set of biogeochemical reactions. The major fraction of mercury remains associated to the solid fraction of the sediments; the other is incorporate in the roots being only a small fraction of mercury translocated to the above ground parts of the plant. Laranjo salt marsh plants are not available for direct grazing; however it is known that several species, namely young fishes, have a varied diet looking for food in the salt marsh mud during the high tide period.

Once in the plant, mercury can enter rapidly in the food chain; with the plant decay and subsequently decomposing, organic detritus are generated and become a source of food for many salt marsh dwellers and can be consumed by detritivores and fishes. Tidal action contributes to the dispersion of the detritus throughout the estuary. On the other hand with the decomposition of the root system mercury that may be phytostabilised can be released from sediment and subsequent to pore waters and overlying waters, through mineralisation processes of organic matter. The turnover rates for below ground biomass of *Halimione portulacoides* were higher than those observed for above ground biomass, corresponding to higher mercury mobility within the rhizosphere. Mercury pools in the below ground biomass are significantly higher than those found for aboveground biomass as well the annual bioaccumulation of mercury in the below ground biomass. Despite of salt marsh sediments without vegetation had generally lower concentrations the mercury pools were higher due to physical characteristics of the sediments. Temporal differences between the mercury pools correspond to the mercury that can be mobilised within the salt marsh, namely being bioaccumulated by the plant or being potentially exported from the contaminated inner bay. Taking into account the pool of mercury in above ground biomass of *Halimione portulacoides*, the export of mercury by macro-detritus following the “outwelling hypothesis” is not significant for the mercury balance in the studied system (Válega et al., 2008b).

The bioaccumulated mercury in the below ground part of the plant is quite mobile, being able to return to the sediment pool throughout the mineralisation process and/or contribute to reactive mercury concentrations in pore waters. During low tide, the chemical equilibrium between the sediments and the sediment interstitial water will control the availability of reactive

mercury and/or total mercury to efflux during the flooding period. The contribution of vegetated sediments to reactive mercury concentrations in the water column is not significant, which is particularly relevant since reactive mercury is pointed out as a suitable measure of the metal substrate available for methylation, elemental mercury formations and other conversion processes of mercury within the aquatic environment (Válega et al., 2008b).

Being the mercury phytostabilized in the sediments vegetated by plants, the potential conversion of inorganic mercury into organic forms is of great concern and remains an open question. Salt marsh rhizosphere seems to have good conditions to enhance mercury methylation and in fact higher methylmercury concentrations were found in vegetated sediments. Despite of the percentages of methylmercury found in this work were low, it represents high values of methylmercury in sediments and special attention should be paid in order to avoid potential releases to the overlying waters. This study reinforces the idea of a lack of direct dependence between methylmercury and total mercury concentrations in sediments, being the highest percentages of methylmercury found in sediments with lower concentrations of total mercury. In this study was also possible to confirm that the difference in the mean values of the sediments colonised by the two studied plants was not significantly different No other organic mercury species rather than methylmercury were found in the sediments and plants. It is also important to denote that despite the fact that the above ground biomass of the plants have very low total mercury concentrations, a significant fraction is methylmercury which must be taken into account in the macro-detritus exportation or animal feeding as a source of methylmercury (Válega et al., 2008c).

The stress term is usually used to refer changes in environmental conditions outside the normal range encountered by plants in its initial stage development. The presence of metal in salt marsh sediments can trigger a series of tolerance mechanisms by the plant to survive in metal contamination environments, which means that the plant can adapt to the environments which can be found, beyond the stressor.

This study intended to study the molecular mechanisms underlying metal stress coping in *Halimione portulacoides*. Several studies about have been undertaken in this research area in laboratory conditions; however it has been identified substantial differences in the responses between the natural and laboratory controlled conditions. The partition of mercury in *Halimione portulacoides* showed that the most significant amount of the metal was retained in the insoluble fraction and differences were observed between organs, with leaves presenting higher

concentrations in the soluble fraction, therefore the general strategy to cope with environmental mercury exposure seems to be its retention in the insoluble fractions. The biochemical processes behind mercury tolerance in *Halimione portulacoides* collected in Laranjo bay salt marsh seems to involve the immobilisation of the metal in the root cell wall as the major mechanism of metal resistance, rather than metal chelation in the cytosolic fraction. The isolation of protein-mercury complexes showed that the tolerance strategy of *Halimione portulacoides* to mercury was associated with the induction of metal chelation by phytochelatins in a very small extent, bringing out new light on the mercury tolerance strategies of *Halimione portulacoides* and on the role of phytochelatins in environmental mercury contaminations. Mercury chelation in environmental exposures seems to be a more complexed phenomenon than those observed in laboratory controlled experiments, involving the formation of different types of complexes. Phytochelatins chelation was demonstrated to occur in mercury exposures in the environment but possibly assisted by other molecules (Válega et al., 2009).

Biomonitors are considered to be an appealing tool for pollution assessment, namely mercury which speciation studies require very complex procedures. Given the importance of *Halimione portulacoides* in mercury accumulation and be considered a mercury bioindicator it was also important to study its potential value for mercury biomonitoring. This study conducted along the Portuguese coast give us a general idea of the Portuguese mercury contamination in the main estuaries. The concentration of mercury in the sediments is low in the entire coast with the exception of Ria de Aveiro lagoon.

The relation between the concentrations of mercury in the different plant organs was better explained by a linear model while the variation of mercury concentration in the roots versus mercury concentration in the sediments was best fitted by a sigmoidal model. Mercury accumulation in the roots can be described in three steps. At a low range of mercury concentrations in the sediments, from 0.03 up to 2 μg g^{-1}, where the accumulation of mercury in roots is low reaching a maximum concentration of 1.3 μg g^{-1} followed by the highest rates of mercury accumulation in the roots until the concentrations of mercury in the sediments reach approximately 4.5 μg g^{-1}. Finally, after reaching this maximum value, the rate of mercury accumulation in the roots slows down leading to a plateau in the concentration of mercury in the roots of about 9.4 μg g^{-1} (Válega et al., 2008d).

The application of models in biological systems must be cautious and some aspects concerning the life cycle of the species and the spatial variability of the system must be taken into

account. In the case of plants the growing season must be avoided and therefore it is extremely necessary to know the life cycle of the plant; on the other hand, salt marshes have a high spatial variability; within a few meters, it is possible to obtain very high coefficients of variation. A good sampling plan is necessary in order to collect representative samples of the system which can sometimes be traduced in a large volume of laboratory work (Válega et al., 2008e).

It is never too much highlight the importance and reinforce the necessity to preserve the integrity of Laranjo bay salt marsh. Their destruction may potential release high concentrations of mercury and methylmercury to the adjacent coastal waters. Two different scenarios emerge from this study. If by one side salt marsh contributes to the retention and phytostabilisation of mercury in the plants root system, reducing contamination of the adjacent coastal waters and consequently be directly available for bioaccumulation they can be potential sources of methylmercury to the adjacent environments which have higher bioavailability and toxic effects in biota.

10.1 Further research suggestions

During the course of this work some aspects about the cycle of mercury in salt marshes have been clarified, however as a research work new questions were raised and new challenges have been proposed for even a better understanding. Each chapter/issue of this work has generated new questions that should be further investigated.

Since that the recovery of salt marshes historically contaminated with mercury seems to be dependent on its species-specific composition, further research should be accomplished to evaluate how complete the Ria de Aveiro salt marsh resilience can be in the future.

Mercury mobility studies in salt marsh sediments should be accomplished with fractionation studies of the metal. The sequential extractions of mercury by various chemical reagents in order to determine the different mercury species and partition can provide useful information about toxicology, bioavailability and biogeochemical reactivity.

Since 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 of vegetated sediments have lower pH values compared to non-vegetated sediments, desorption processes of methylmercury could possibly take place with subsequent increases of methylmercury in pore

waters. Although *Halimione portulacoides* and *Sarcocornia perennis* marshes in Ria the Aveiro presented similar methylation ratios at the root system, 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. The study of microbial community depth profiles could also be interesting to relate with methylation rates.

During the studies of mercury partitioning inside the plant it was observed one major mercury peak which matched to a higher portion of the eluted proteins. The result was a broad peak corresponding to a wide portion of proteins of higher molecular weight than phytochelatins. Therefore more studies should be undertaken in order to evaluate which are the molecules associated to mercury.

On the other hand, the analysis of phytochelatins production in plants does not prove *per se* that the specific metal in study was chelated by phytochelatins since other metals are usually present in the environment and may interfere with phytochelatins synthesis and complexation processes.

The mercury concentrations found in the salt marshes along the Portuguese coast ranged between very high to low levels of contamination, resulting in a lack of intermediate levels which could be useful to adjust our model. It would be interesting to study other salt marshes preferentially with medium levels of contamination, even if necessary outside from Portugal. It was also be interesting investigate the potential use of *Halimione portulacoides* for other metals rather than mercury.

10.2 References

Válega, M., Lillebø, A.I., Pereira, M.E., Duarte, A.C. Pardal, M.A., 2008a. Long-term effects of mercury in a salt marsh: hysteresis in the distribution of vegetation following recovery from contamination. Chemosphere, 71, 765-772.

Válega, M., Lillebø, A.I., Pereira, M.E., Corns, W.T., Stockwell, P.B., Duarte, A.C., Pardal, M.A., 2008b. Assessment of methylmercury production in a temperate salt marsh (Ria de Aveiro Lagoon, Portugal). Marine Pollution Bulletin/Baseline, 56, 136-162.

Válega, M., Lillebø, A.I., Caçador, I., Pereira, M.E., Duarte, A.C., Pardal, M.A., 2008c. Mercury mobility in a salt marsh colonised by *Halimione portulacoides*. Chemosphere, 72, 1607-1613.

Válega, M., Lillebø, A.I., Pereira, M.E., Caçador, I., Duarte, A.C., Pardal, M.A., 2008d. Mercury in salt marshes ecosystems: *Halimione portulacoides* as biomonitor. Chemosphere 73, 1224-1229.

Válega, M., Lima, A.I.G., Figueira, E.M.A.P., Pereira, E., Pardal, M.A., Duarte, A.C., 2009. Mercury intracellular partitioning and chelation in a salt marsh plant, *Halimione portulacoides* (L.) Aellen: strategies underlying tolerance in environmental exposure. Chemosphere, 74, 530-536.