Methylation of mercury and demethylation of monomethylmercury in saltmarshes – seasonal variation and plant effect

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Declaration:

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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Abstract

Saltmarshes are known accumulations areas for contaminants, namely mercury (Hg) and has been proven that these environments play a crucial role in its methylation and in monomethylmercury (MMHg) demethylation. In this study, it was used stable isotope tracers of $^{199}Hg^{2+}$ and $CH_3{}^{201}Hg^{+}$ followed by isotope-specific detection with inductively coupled plasma mass spectrometry, to determine methylation and demethylation rates simultaneously in saltmarsh sediments colonized and non-colonized by plants, in two Portuguese aquatic systems (Tagus Estuary and Ria de Aveiro). Also, ambient concentrations of total Hg (THg) and MMHg were obtained. Sediments were sampled with and without vegetation in spring and summer. Vegetated samples contained three specific species of halophyte plants: *Halimione portulacoides* (HP)*, Juncus maritimus* (JM) and *Sarcocornia fruticosa* (SF). This allowed to evaluate the plant and seasonal effect in Hg methylation and MMHg demethylation in saltmarsh sediments.

Results showed higher concentrations of ambient THg and MMHg in Ria de Aveiro. The highest concentrations of THg was found in Laranjo (LAR) saltmarsh in sediments colonized by JM (58525 ng g^{-1}) and the highest concentration of MMHg was found in Chegado (CHE) saltmarsh in sediments colonized by HP in summer (334.3 ng g^{-1}). The highest methylation rate was also observed in CHE in sediments colonized by HP in summer (0.452 day^{-1}) and the highest demethylation rate was found in Rosário (ROS) saltmarsh in Tagus estuary (25.6 day^{-1}) in spring.

In conclusion, results obtained appear to demonstrate that halophyte plants influenced Hg methylation rates and that summer conditions enhanced it possible due to higher microbial activity in the warmer season.

Keywords:

Saltmarshes, mercury methylation, monomethylmercury demethylation, sediments, estuaries

Resumo

Os sapais são reconhecidos como zonas de acumulação de contaminantes, nomeadamente de mercúrio (Hg) e já foi comprovado que estes ambientes desempenham um papel crucial na sua metilação e na desmetilação do monometilmercúrio (MMHg). Neste estudo, foram usados isótopos estáveis de $^{199}Hg^{2+}$ e de $CH_3{}^{201}Hg^{+}$ e posteriormente utilizada a deteção específica de isótopos através de espectrometria de massa com plasma indutivo acoplado para determinar as taxas de metilação e desmetilação simultaneamente em sedimentos de sapal colonizados e não colonizados por plantas, em dois sistemas aquáticos de Portugal (Estuário do Tejo e Ria de Aveiro). Além disso, as concentrações ambientais totais de Hg (THg) e MMHg foram obtidas. Os sedimentos foram amostrados com e sem vegetação na primavera e no verão. As amostras vegetadas continham três espécies específicas de plantas halófitas: *Halimione portulacoides* (HP), *Juncus maritimus* (JM) e *Sarcocornia fruticosa* (SF). Esta análise permitiu avaliar o efeito sazonal e das plantas na metilação do Hg e na desmetilação do MMHg em sedimentos de sapal.

Os resultados mostraram maiores concentrações de THg e MMHg na Ria de Aveiro. O maior valor de THg foi encontrado no sapal do Laranjo (LAR) em sedimentos colonizados por JM (58525 ng g^{-1}) e a maior concentração de MMHg foi encontrada no sapal do Chegado (CHE) em sedimentos colonizados por HP no verão (334.3 ng g^{-1}). A maior taxa de metilação foi também observada no CHE em sedimentos colonizados por HP no verão (0.452 dia^{-1}) e a maior taxa de desmetilação foi observada no sapal do Rosário (ROS) no estuário do Tejo (25.6 dia^{-1}) na primavera.

Concluindo, os resultados obtidos parecem demonstrar que as plantas halófitas influenciaram as taxas de metilação do Hg e que as condições de verão aumentaram-na possivelmente devido a maior atividade microbiana na estação mais quente.

Palavras-chave:

Sapal, metilação do mercúrio, desmetilação do monometilmercúrio, sedimentos, estuários

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List of Acronyms

ALC	Alcochete
AV	Aveiro
CAP	Chlor-alkali plant
CHE	Chegado
CRM	Certified reference materials
DMHg	Dimethylmercury
DOM	Dissolved organic matter
FeRB	Iron reducing bacteria
GEM	Gaseous elemental mercury
GOM	Gaseous oxidized mercury
H_3BO_3	Boric acid
HCl	Hydrochloric acid
HF	Hydrofluoric acid
HNO_3	Nitric acid
Hg	Mercury
HP	*Halimione portulacoides*
ICP/MS	Inductively coupled plasma mass spectrometry
JM	*Juncus maritimus*
K_D	Demethylation rate constant
K_M	Methylation rate constant
LAR	Laranjo
LOI	Loss on ignition
MMHg	Monomethylmercury
ROS	Rosário
SF	*Sarcocornia fruticosa*
SRB	Sulfate-reducing bacteria
TG	Tagus
THg	Total mercury

I. Introduction

1. Motivation

Pollution and its impact on earth and human life is without a question one of the great challenges that mankind must face. Among all types of pollution, contamination of environments with heavy metals is a pressing matter due to the severe implications it may have in ecosystems and in human health.

Saltmarshes are unique ecosystems of extreme importance, providing countless number of resources for wildlife and humans (Caçador & Vale, 2001). They are commonly near densely populated areas and because of their characteristics, they end up working as sinkholes for pollutants, namely mercury. The methylation of inorganic mercury into monomethylmercury is known to take place in saltmarshes (Canário et al. 2007b), but the mechanisms behind it, as well as the influence of environmental and biotic factors still raises many questions. Despite the study of the biogeochemistry of mercury has been developed in the last decades, the cycle of mercury in wetlands is not yet fully understood.

Knowing that monomethylmercury is a powerful neurotoxin that biomagnifies in the food web of aquatic systems (Kidd et al., 2012) is essential to understand its formation and the possible spread across the environment, once saltmarshes are known to influence adjacent ecosystems, having a tendency to export material to deeper waters (Valiela et al. 2002). The study of the formation of monomethylmercury becomes essential to prevent possible health hazards in humans and to preserve these essential ecosystems.

2. Aim of the study

The aim of this study is to evaluate the effect of three specific types of halophyte plants in two different seasons (spring and summer) in the methylation of mercury (Hg) and the demethylation of monomethylmercury (MMHg) on saltmarsh sediments. The saltmarshes of Tagus estuary and Ria de Aveiro were already studied in previous works and are known for having high levels of Hg contamination with anthropogenic origin (Figueres et al., 1985; Figueira et al, 2012).

Several studies have shown that sulfate-reducing bacteria (SRB) have a crucial role in Hg methylation (Compeau & Bartha, 1985), but the influence of other biotic components and physicochemical parameters still needs to be addressed.

Ultimately, this work's purpose is to understand the biogeochemistry of Hg in saltmarshes and the influence of biotic and abiotic components, which is vital to evaluate eventual hazards for the environment, wildlife and ultimately humans, as well as to propose hypothesis for solving the contamination problem.

3. Thesis structure

This thesis is organized as follows. Chapter one presents the motivation for this study, its aim and the explanation of its structure. Chapter two contains a review of topics of importance to understand the work conducted - first, a brief presentation about mercury, its cycle in the environment, namely in the aquatic system, its toxicity and its potential for methylation; second, a description of saltmarshes. Chapter three contains a description of the sampling sites. Chapter

four describes the sampling methods. Chapter five explains the analytical methods used in order to obtain physicochemical parameters, iron and manganese content, Hg and MMHg concentrations and the methylation and demethylation rates. Chapter six presents the results obtained and discusses them - methylation and demethylation rates in sediments colonized by different types of halophyte plants are compared with methylation and demethylation rates in non-vegetated sediments across two different seasons; the results obtained for saltmarshes of the same estuary are then compared with each other and a comparison between estuaries is also made; finally, these results are compared with the results obtained in similar studies and presented the factors that may have influenced the methylation rates. Chapter seven presents the conclusion of this study. Chapter eight concludes this thesis presenting goals for future work.

II. Literature Review

1. Mercury

1.1 Physicochemical properties, sources and uses

Mercury (Hg) is a naturally occurring element present in the environment and has been used for centuries in the most diverse applications due to its unique characteristics. In the last decades several studies have been made to understand its biogeochemical cycle and the processes that Hg undergoes in the most diverse ecosystems (e.g. Mason et al., 1994). They are of the most importance because Hg was recognized as a global pollutant by the Minamata Convention on Mercury in 2013 (UNEP, 2017), due to its ability to persist in the environment, undergoing long-range transport in the atmosphere and accumulating in the food web, endangering human and ecosystem health (Liu et al. 2012).

Mercury is the only metal that is liquid at room temperature, with a melting point of -38.89 ºC, a boiling point of 357.3 ºC, and a density of 13.6 g cm^{-3}, which is the highest density among all liquids under normal conditions (Calvo et al., 2013). It also has a rapid and uniform volume expansion and good electrical conductivity (Habaschi, 2013).

There are three states of oxidation that Hg possesses: elemental mercury (Hg^0), mercurous ion (Hg^+) and the mercuric ion (Hg^{2+}). The first is the most commonly found Hg species in atmosphere due to its volatility, the second it's an unstable species and rarely found in nature and the third is the oxidation state normally found in complexes present in water and soils. (Bigham et al 1964; Horvat, 1996)

Mercury was, and in some places of the world still is, used for medical purposes, in thermometers, in electrical circuits and in various industries. Since the realization of how harmful could be for human health, the use of Hg has diminished around the globe, specifically in consumer related products. However, the biggest sources of anthropogenic contamination are the burn of fossil fuels and the chemical and mining industries (Liu et al., 2012). Mercury easily amalgamates with other metals, including gold and silver, so it's often used in their extraction, especially in developing countries where operations tend to be more rudimentary (Liu et al., 2012). In the chemical industry, the production of caustic soda, metals and cement, has been heavily contributing to the release of Hg into the environment. (AMAP/UNEP, 2013). There are also natural sources of Hg, as is the case of volcanic emissions and natural sources of re-emission, as for example evasion from vegetation and soil or even permafrost thaw (Fitzgerald & Lamborg, 2005; Schaefer et al., 2020). Re-emission has a significant impact because once emitted, Hg enters the global atmospheric pool and after being deposited onto surfaces, can be re-emitted entering again in its biogeochemical cycle (Gustin et al., 2020).

Being a natural element, Hg can also be found in nature. Its principal ore is cinnabar (HgS), and it's normally found with pyrite, stibnite and marcasite near volcanic rocks and hot springs deposits (Rečnik 2013).

1.2 Mercury biogeochemical cycle

Mercury is considered a global pollutant due to its ability to spread in the environment. It has high mobility and is extremely toxic, being of great importance to understand its biogeochemical cycle, particularly its transport and deposition in the environment (Jitaru & Adams, 2004).

In figure 1, it's possible to see the main chemical forms of Hg found in the environment (Hg^0, Hg^{2+} and monomethylmercury - MMHg) and the two main reactions: oxidation-reduction and methylation-demethylation.

Elemental mercury (Hg^0) is easily volatilized and commonly released into the atmosphere where it has an ability to travel for long distances, reaching places far from its source of origin (Fitzgerald & Lamborg, 2005). This form of Hg is known as gaseous elemental mercury (GEM) and can stay airborne from months to a year (Travnikov, 2012). Hg^0 can be photochemically oxidized to Hg^{2+} forming gaseous oxidized mercury (GOM). Generally, it forms complexes with other ions, being the most common species $HgBr_2$ or $HgCl_2$ and because of their solubility in water, when present in the atmosphere can easily be dissolved in rain and deposited (Feng, 2015). Mercuric ion Hg^{2+} is the dominant form of Hg, being the most found in water and sediments (Jackson, 1998). The reverse can also happen, with photoreduction processes that make Hg^{2+} convert back to Hg^0 and, in general, the flux of Hg from the water and soils back into the air exceeds the deposition flux (Stein et al., 1996).

When present in water or soils, Hg^{2+} forms organometallic and/or inorganic complexes being these last ones that can be methylated by microorganisms (biomethylation) or by specific abiotic factors (abiotic methylation) originating MMHg or dimethylmercury (DMHg) (Barkay, et al., 2012). Having higher mobility and solubility, MMHg becomes more bioavailable for plants and animals, which poses a great threat because MMHg is a very toxic compound that biomagnifies in food webs (Kidd et al., 2012). Dimethylmercury can volatilize to the air, where it's photolyzed to methane and Hg^0 or can be oxidized by the hydroxyl radical (Stein et al., 1996).

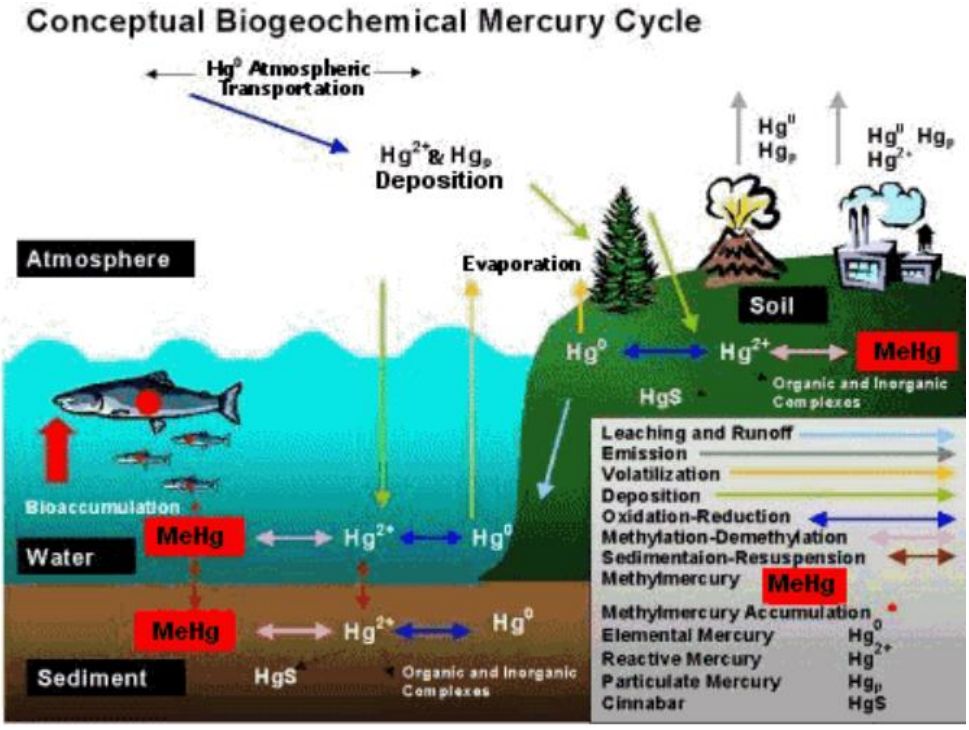

Figure 1 - Biogeochemical Mercury Cycle with MMHg represented as MeHg. (Adapted from Government of Canada, 2013)

1.3 Mercury toxicity

Mercury is considered a non-essential element because it has no biological functions, yet it accumulates within living cells and is proven to have high levels of toxicity (Oves et al., 2016). The toxicity of Hg strongly depends on its redox state, its mobility and bioavailability. Elemental mercury is poorly reactive, especially in liquid state, so the organic and inorganic forms are the ones primarily associated with Hg toxicity (Sakamoto et al., 2012). When found in the divalent oxidation state, Hg presents the higher levels of toxicity. However, inhalation of Hg^0 vapors can also have the same severe effects as the ingestion of Hg^{2+} compounds, as for example the damage of the nervous system, kidneys or dysfunction of the immune system (Jitaru & Adams, 2004).

In the divalent oxidation state, the toxicity of Hg depends on its chemical form and is the highest in organometallic species (Jitaru & Adams, 2004). Despite some inorganic heavy metal forms could be converted to less dangerous biological ones, in the case of Hg, the opposite occurs. The methylation of Hg produces the organometallic MMHg and DMHg which are the most toxic forms of Hg (Jitaru & Adams, 2004). The increased toxicity of the organic forms is related to its lipophilic character, which turns them in compounds with the ability to migrate through biological membranes and pass the blood-brain barrier as well as the placenta (Sakamoto et al., 2012).

In aquatic environments, MMHg poses a very serious threat to organisms because it bioaccumulates and biomagnifies in food webs (Kidd et al., 2012). This means that the compound is absorbed into the living bodies and because it's not processed, starts to accumulate over time. The biomagnification makes it an even bigger problem. The organisms contaminated with MMHg are the food source of the next trophic level, making the concentration increase within the food web. It can have a huge impact on apex predators and ultimately on humans (CIMI,2020). Many fish species like salmon, tuna and sharks are on the top of their food chain and may end up with high levels of Hg accumulated; when they serve as food supply for humans it can have a dramatic impact on human health. (Kidd et al., 2012; CIMI,2020)

Despite existing several environmental disasters related to Hg contamination, one of the first and most serious examples of the impact of organomercury, more specifically MMHg, in wildlife and in human health was the Minamata incident (Harada, 1995). A chemical plant (Chisso Plant) was responsible for a large-scale MMHg contamination of the Minamata Bay in Japan due to wastewater discharge between the 1930's and 1960's (Harada, 1995). The factory produced fertilizers, synthetic resins, plasticizers and other chemicals. It was thought that the main MMHg source was the wastewater discharged to the bay as well as Hg^{2+} that may have been methylated in the aquatic environment (Harada, 1995). A recent study considers that another form of organic Hg may had played an important role in the contamination (Ashley et al., 2020). The authors suggested that α-mercury-acetaldehyde, a waste product in the production of aldehyde, could also have been one of the biggest contaminants. They used computational chemistry to consider the plant chemical processes and determine the expected side-products. They found out that Chisso Plant had modified their procedures, altering a catalyst regeneration process, which may have led to the formation of α-mercury-acetaldehyde.

Due to the contamination of the food webs, not only wildlife but also humans ended up paying a high price. A lot of people developed severe symptoms and ended up dying, some developed chronicle diseases and a lot of unborn babies were seriously affected due to the ingestion of organomercury by their mothers (Harada, 1995; Sakamoto et al., 2012). The health problem remains and its' impact on the ecosystem still prevails.

1.4 Monomethylmercury formation and demethylation

It´s of extreme importance to understand the formation of the organic forms of Hg because, as it was said before, they are the most toxic ones.

Mercury methylation is known to occur in three environmental compartments: water column, sediments and biota (Li & Cai, 2013). The biomethylation of mercury is thought to be one of the main contributors in the formation of MMHg and happens, mainly, due to bacterial activity (Barkay et al., 2012). Sulfate-reducing bacteria (SRB) are the main producers of MMHg, but iron-reducing bacteria (FeRB) and methanogens are also known for being able to do it and in different types of environments (Barkay et al., 2012). Their ability to methylate Hg is related with the presence of two genes, *hgc*A and *hgc*B, presenting in Hg methylating bacteria and *archea* (Parks et al., 2013).

Biomethylation tends to be higher in suboxic/anoxic conditions and dependent of several factors, such as: microbial activity, abundance of electron receptors, organic matter content, nutrient availability, bioavailability of inorganic Hg and its methylation potential (Barkay et al., 2012). There is also a significant number of abiotic factors that seem to be relevant in the methylation of Hg, such as: pH, temperature, redox shifting, dissolved oxygen and the presence of complexing agents (Ullrich et al., 2001). All these factors should be taken into consideration when trying to evaluate the environmental factors and seasonal variations in Hg methylation.

Another very important factor is the presence of sulfate (SO_4^{2-}), once SRB are one of the main contributors for MMHg formation. The amount of SO_4^{2-}, as an electron acceptor during organic matter degradation, seems to have direct relation with the MMHg production (Ullrich et al., 2001). At low concentration, the increase of SO_4^{2-} seems to enhance Hg methylation, but at high concentrations the sulfide (S^{2-}) generated by SO_4^2 respiration can have an adverse effect, limiting MMHg production. This limitation is thought to happen because of the reaction of Hg with S^{2-}, occurring HgS precipitation or due to Hg-S charged complexes (Ullrich et al., 2001).

The MMHg content in the environment results from the balance between the methylation and demethylation processes. MMHg demethylation can also occur due to biotic or abiotic decomposition processes (Ullrich et al., 2001). Biotic demethylation occurs due to microbial activity and can happen with reductive or oxidative processes (Barkay et al., 2012). In the reductive one, bacteria use organomercurial lyase enzymes to decompose MMHg obtaining methane and Hg^{2+}, that is later reduced to Hg^0 by the mercuric reductase enzyme (Ullrich et al., 2001). Once methylation and demethylation processes occur simultaneously, it has already been proven that SRB and methanogens also play a part in oxidative demethylation, but it´s still uncertain the faith of the Hg^{2+} produced (Ullrich et al., 2001). The abiotic process is the photolytic decomposition (Barkay et al., 2012). In the atmosphere, DMHg can be demethylated giving origin to Hg^0. In surface waters, ethyl- and monomethylmercury can be photodegraded by singlet oxygen (Ullrich et al., 2001). While photolytic decomposition could be a determining factor of the demethylation of MMHg on seawater, in sediments where sunlight exposure is less significant, biological demethylation tends to be more important (Ullrich et al., 2001).

1.5 Mercury in aquatic environments

Mercury in aquatic systems can be a consequence of direct anthropogenic inputs, hydrological transport or atmospheric deposition (Fitzgerald & Lamborg, 2005). It can have different mobility and solubility in water and in sediments, depending on the Hg species. The most commonly form of Hg in aquatic environments is Hg^{2+} and its mobility and ability to form complexes is dependent of several factors, such as: pH, temperature, redox conditions and availability of complexing agents (Ullrich et al., 2001).

Due to its tendency to be sorbed on surfaces, Hg normally bounds with sediments or attaches itself to suspended particles in water (Jackson, 1998). Normally, it bounds with inorganic sulphides and thiol groups of humic organic matter, but it can also be sorbed by mineral particles, principally Fe and Mn oxyhydroxides (Gagnon et al. 1997). In freshwater systems, most of the Hg is complexed by organic matter or hydroxides, especially in oxic conditions, but in anoxic conditions, sulphide seems to mainly control mercury's chemistry (Ullrich et al., 2001). In seawater, due to the presence of chloride ions, Hg can also be complexed by chloride (Morel et al., 1998).

Once most of the Hg is associated to sediments and suspended particles, processes like erosion, river currents, floods and storms can have an important impact in the resuspension, transport and deposition of its carrier particles (Jackson, 1998). River basins, lakes, estuaries and wetlands tend to trap Hg, especially when HgS is formed, but the above-mentioned processes can subject Hg to remobilization, resuspension and its returning to the aquatic environment (Sunderland et al., 2004).

According to Ullrich et al (2001) sediments are considered to be the main reservoir of Hg in freshwater systems. Commonly being in river basins and estuaries, sediments in saltmarshes tend to be a place for the accumulation of Hg (Jackson, 1998). The interactions between metals and the constituents of sediments depends on several physicochemical processes, such as: physical adsorption of metals to clay particles, humic matter and organic residues; physical, chemical and coprecipitation of Fe and Mn oxyhydroxides; precipitation/solubilization of carbonates, sulphides and hydroxides of metals. (Canário, 2004)

The accumulation of metals, more specifically Hg, as well as metal speciation in saltmarsh sediments has been proven to be related with the vegetation, more specifically the halophyte plants that colonize these environments (Figueira et al. 2012). Plants promote changes in the rhizosphere that influence several physical, chemical and biological processes (Pedro et al., 2015). The release of oxygen by the plant roots in anoxic sediments, creates a shift in redox conditions that oxidize the sediment and potentially affects mobility and availability of metals (Williams et al., 1994).

1.6 Mercury isotope tracer studies

The fate of Hg in the environment and its methylation rate, as well as the MMHg demethylation rates have been object of study for the past decades. The use of isotope tracers in Hg transformations started approximately 50 years ago (Akagi et al. 1979) and has greatly evolved. In the past, studies were conducted using radioactive Hg isotopes requiring the use of high concentrations which represented danger for those conducting the experiments and weren't representative of the concentrations in the environment (Hintelman & Evans, 1997). Results started being questioned because saturation effects in studying partitioning or toxic effects in uptake experiments were a possibility (Hintelman & Evans, 1997). In Hg methylation and MMHg demethylation the use of radiotracers raised doubts because high concentrations weren't like those found in the environment and could mislead to false conclusions.

In this study, the Hg methylation and MMHg demethylation rates were determined using stable Hg isotopes (Hintelmann, 2012). Mercury has seven natural stable isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg and ^{204}Hg) with a relative mass span of 4% (Yin et al. 2016). By using the Inductively coupled mass spectrometry (ICP-MS) technique, Hg isotopes can provide multi-dimensional tracers to discriminate sources, transport, transformation and bioaccumulation of Hg in the environment (Yin et al. 2016), as well as discriminate and quantify each Hg isotope individually, allowing very precise and accurate measurements (Hintelmann & Ogrinc, 2003).

The (ICP-MS) has high levels of sensitivity and proves to be suitable because it allows to study the fate of Hg species in the environment at tracer levels, allowing to investigate both processes simultaneously, reducing errors (Hintelmann et al., 2000). Due to the high level of precision of this method, stable Hg isotopes can be used at natural levels, reducing the use of high concentrations. Because it can detect different types of isotopes, it´s possible to evaluate the transformations that each isotope is subjected and determine the individual isotope contributions from the different sources. (Hintelmann & Ogrinc, 2003) This characteristic is very important because Hg methylation and MMHg demethylation occurs simultaneously.

This technique has been used in various studies and proven to provide good and reliable results (e.g., Hintelmann et al., 2000; Cesário et al., 2017). Therefore, it was chosen for this study in the expectation of providing solid outcomes about the Hg methylation and MMHg demethylation in sediments collected in the chosen saltmarshes for this study.

2. Saltmarshes

Saltmarshes are a very important and specific type of ecosystems that occurs worldwide in middle to high latitudes. They are present in estuaries, deltas, lakes and bays in intertidal zones being the transition between coastal and marine environments and playing an important part in coastline protection (Silva et al 2013). They are flooded and drained by the tide and occur mainly in areas protected from the direct mechanical effect of waves, as for example sheltered margins of estuaries where river sediments can be deposited (Silva et al 2013). These areas are of great importance because they are home to rich ecosystems. They are a place of primary production and where many species of plants, aquatic birds, fishes and crustaceans thrive and use as nursery (Antunes Dias & Marques, 1999; Caçador & Vale, 2001).

The saltmarsh soils are composed by mud, silt, peat, layers of deposited sediments due to fluvial deposition or waves and dissolved and particulate material from estuarine waters (NOAA, 2020). Due to playing an important role in the exchange of sediments, saltmarshes are a place where accumulation of organic matter and contaminants commonly happens, including heavy metals (Antunes Dias & Marques, 1999; NOAA, 2020). This characteristic is of special importance for the adjacent environments. Being a sinkhole, saltmarshes act as filter for contaminants, but due to human pressure and climate changes scenarios, these vulnerable ecosystems are increasingly in danger (Kirwan et al., 2016). The sea level rise, changes in water temperature and salinity or coastal erosion are some of the environmental impacts that can disrupt saltmarshes and remobilize contaminants to adjacent ecosystems, such as the aquatic system.

The main characteristic for plants to thrive in saltmarshes is their ability to live in environments with high values of salinity as is the case of Halophyte plants (Chapman, 1974). Saltmarsh vegetation is divided spatially in low, mid and high marsh communities. This difference in designation is related to the frequency and duration of the tide submersion. Low marsh community is submerged most of the time and high marsh community is only submerged for brief periods of time (Silva, 2012). *Halimione portulacoides*, *Sarcocornia fruticosa* and *Juncus maritimus* were the halophyte plants used in this study and they colonize low and mid marsh, mid to high marsh and low to high marsh, respectively (Sousa et al., 2010). Being of great importance in these ecosystems, they play an important part in the biogeochemistry of nutrients and metals (Reboreda & Caçador, 2006; Caffrey et al., 2007). Halophytes have developed an aerenchyma system that allows the plant to live in hypoxic soils, as is the case of wetlands (Caçador & Vale, 2001). This system allows the plant to take oxygen from the leaves to the roots, where it´s diffused to the surrounding sediments. This process is of great importance, because it enhances nutrients availability, creates an oxidized microenvironment where the redox potential of sediments is higher and where speciation of metals may occur (Caçador & Vale, 2001).

Considering the geological and ecological importance of saltmarshes, these ecosystems should be preserved. The location of cities and industrialized areas near these environments, as well as the contamination of sea and river waters poses a great threat with several potential nefarious effects. In the case of Hg contamination, the uptake by saltmarsh vegetation could lead to bioaccumulation in the food webs, to its re-emission through plant leaves (Windham et al. 2001) and, as it has been proven in previous studies, saltmarshes could be a place where favorable conditions for methylation of Hg and demethylation of MMHg may occur (Canário et al. 2007b; Cesário et al., 2017). In fact, on saltmarsh sediments, the presence of plant roots can increase the organic matter content and the availability of nutrients, which may enhance microbial activity (SRB, FeRB and/or methanogens) and consequently promote conditions for the methylation of Hg (Canário et al., 2010; Sun et al., 2011). Previous studies have showed that colonized sediments contain up to 70 times more MMHg than non-vegetated sediments (Canário et al., 2010) and that the presence of vegetation in sediments favors the formation of MMHg (Cesário et al., 2017).

III. Study Area

The field work took place in four different saltmarshes, from two different estuaries in Portugal: Tagus estuary (TG) and Ria de Aveiro (AV).

Figure 2 shows the geographic location of the estuaries as well as the specific location of saltmarshes in each one. In Tagus estuary, the saltmarshes chosen were Alcochete (ALC) and Rosário (ROS); in Ria de Aveiro were Laranjo (LAR) and Chegado (CHE). These estuaries, and more precisely these saltmarshes, were selected for this study because they are in places already known for its contamination with anthropogenic Hg. In Ria de Aveiro, LAR was chosen as the more contaminated site and CHE as the reference one; in Tagus estuary, ROS is in the more contaminated area and ALC was chosen has reference site.

Figure 2 – Geographic location of the two estuaries in Portugal chosen for this study. Tagus estuary (TG) and Ria de Aveiro (AV). The location of each saltmarsh inside each estuary is also presented: In Tagus estuary are Rosário (ROS) and Alcochete (ALC) and in Ria de Aveiro are Laranjo (LAR) and Chegado (CHE) saltmarshes.

1. Tagus estuary

The Tagus estuary is one of the biggest in western Europe and has an area of approximately 320 km^2 (Taborda et al., 2009). Located in the most populated area of the country, near the capital Lisbon, it's a very important natural resource in the region and an important asset in commercial, urban and recreation activities (APA, 2010).

Inside the estuary, Tagus Estuary National Reserve is one of the most important protected areas in Europe that spans across 14563 ha with a high diversity of coastal environments. The estuarine waters are home to extensive tidal mudflats, reed beds and saltmarshes. Human presence can be found inside the reserve, in the form of salt pans, agricultural polders and inland cultivated areas (RAMSAR, 1992).

Most of the estuary is occupied by extensive tidal mudflats which when exposed to low tides are drained naturally by a network of channels. On the margins of the mudflats there is a significant number of saltmarshes. They are of extreme ecological importance to the environment, once marshes are very rich in biodiversity. In these areas, different numbers of crustaceans and mollusks thrive, a lot of marine fish species use it as place to lay their eggs and it's also an important transition area for freshwater fishes that migrate to the sea (NaturalPT, 2020). Another very important aspect of the biodiversity of Tagus estuary is its importance to many aquatic migratory bird species. One major example is the Pied Avocet (*Recurvirosta Avossetta*) that uses the estuary as a place to spend the winter and to nest. The wintering population in Tagus estuary comes to an amount of 25% of all the European population (RAMSAR, 1992; NaturalPT, 2020).

In Tagus estuary the two saltmarshes chosen to collect the samples are in different areas. ALC, in the northern part of the estuary, is in the border of Tagus Estuary National Reserve, and presents lower to moderate Hg contamination (Canário et al., 2010; Cesário et al., 2016). In contrast, ROS saltmarsh, which is on one of the many coves on the left side of the river with moderate to high Hg contamination (Canário et al., 2007a). It is located between Moita and Barreiro, an area that once was very industrialized. Despite Hg having almost no part in industries nowadays, this area still has a significant presence of the contaminant, making Rosário a more polluted site (Micaelo et al., 2003).

2. Ria de Aveiro

Located on the cost of the center region of Portugal, Ria de Aveiro is one of the most important geological formations in the country (Bioria, 2020). It is the river mouth of Vouga River and it is mainly characterized by the extensive network of islands and channels that stretch inland from a big lagoon that was formed parallel to the sea (Dias & Dekeyser, 1999). Occupying an area of 11000 ha, with 6000 of them being submerged all the time, its formation occurred due to the retreat of the sea, having a connection with the Atlantic Ocean made by men in the 18th century. (Bioria, 2020) This makes Ria de Aveiro a perfect example of an environment where there is a balance between man and nature and today it is a very important ecosystem and an area of extreme importance to the population.

Due to the connection with the sea, Ria de Aveiro, just as Tagus estuary, is occupied by extensive mud flats inserted in intertidal areas. On the shores of the mudflats can be found the saltmarshes spreading along an extensive area of the Ria. Two different areas were chosen to collect samples: CHE and LAR. These sites are located between Cacia and Estarreja (Figure 2). Both places are home to several industries that were responsible for the pollution of Ria de Aveiro. In the Chemical Complex of Estarreja, just a few kilometers northeast of the study sites, UNITECA had a mercury-cell chlor-alkali plant (CAP) that operated for 50 years (Reis, 2008; Figueira et al., 2012). Despite the factory no longer uses this technology, Hg contamination in the environment is still significant. LAR site is in greater proximity with the CAP, where effluent discharge of factories took place and it is a more contaminated area (Figueira et al., 2012), compared with CHE, that was chosen to be a reference site inside Ria de Aveiro.

IV. Sampling

The samples for this study were collected in depth using specific metallic corers. In two different seasons, spring and summer of 2019, two types of sediments were sampled: vegetated and non-vegetated. The vegetated cores contained specific species of plants that were chosen for this work. The plant species *Halimione portulacoides* (HP) was chosen in common in both aquatic systems, for better comparison between sites. In the two saltmarshes of Ria de Aveiro the collected vegetated cores were sampled in areas colonized by the plant species HP and *Juncus maritimus* (JM) and in Tagus estuary saltmarshes, the vegetated species in study were *Sarcocornia fruticosa* (SF) and (HP).

Figure 3 - (Right) Special metallic corer used to collect sediments for the isotope incubation experiment (Center) Metallic corer used to collect sediments for measuring physicochemical parameters, amount of biomass and content of metals (Left) injection of the isotopic solution in the inner PVC slices.

In each site five sediment cores for the incubation experiment were collected (Figure 3): two for each colonized plant and one in a non-vegetated area. These cores were obtained with a special metallic sediment corer (Figure 3 (right)) with a PVC tube composed by 3-cm slices with a diameter of 6 cm, previously glued with duck-tape with a total length of 30 cm, inside the metallic corer. In the case of the vegetated samples, the corer was put on top of a plant and pressed to the ground; but with the attention to keep intact the aerial part of the plant. In this way, the core contained the entire plant, it's roots (up to 30 cm in depth) and the adjacent sediment.

After the collection of the sediment cores, samples were spiked *in situ* with an isotopic solution of Hg ($^{199}Hg^{2+}$) and MMHg ($CH_3{}^{201}Hg^{+}$) previously prepared in the laboratory. Using needles, predeterminate amounts of the solution were injected trough previously made holes in the inner PVC slices (Figure 3 (left)). The amount of isotopes added were previously calculated taking into account the natural Hg and MMHg concentrations find in each site, which were determined in a background survey made before this fieldwork.

After the injection of the spike solution the PVC tubes were placed in the field (the hole where they were extracted) and were left for a period of 5 hours (incubation time previously defined according to (Cesário et al., 2017). After the incubation period, the PVC tubes were taken from the field, sliced in 3 cm layers, placed on plastic zipper bags and kept immediately in dry ice to stop the incubation and transported back to laboratory.

Additionally, three sediment cores were sampled with a different metallic corer with 7 cm diameter (Figure 3 (center)) and 30 cm depth. One sediment core from each plant and another with non-

vegetated sediment were sampled to determine the amount of biomass, to measure physicochemical parameters and to analyze the content of metals.

These samples, destinated to determine the amount of biomass and to measure physicochemical parameters, were also sliced in 3 cm layers. Temperature and pH were measured *in situ* for each depth. After, the sediment layers were placed on plastic zipper bags and kept in a cooler until they arrived at the laboratory where they were conserved in a normal freezer.

V. Analytical Methods

1. Sediment Analysis

1.1 Measurement of temperature and pH of sediment samples

Temperature was measured with a probe thermometer immediately after the cores were retrieved from the saltmarshes (see values in Annex A) and the pH was measured using a benchtop pH meter model Basic 20, Crison, with a glass electrode (Mettler) previously calibrated (20 ^{0}C) with pH 4, 7 and 10 buffer solutions, while waiting for the incubation time to end. Values were obtained for every 3 cm sediment layer of a selected core.

1.2 Water content (Humidity)

To evaluate the amount of water present in the sediments, approximately 1.5g of sample were weighed in a small aluminum crucible. The samples were put to dry at 105º C for 24h and weighed again.

The humidity was calculated using the following equation:

$$\%H = \frac{m_{wet\ sed} - m_{dry\ sed(105ºC)}}{m_{wet\ sed}} \times 100 \quad (1)$$

Where %H is the mass of water lost in the drying process, $m_{wet\ sed}$ is the mass of the wet sediment and $m_{dry\ sed(105ºC)}$ is the mass of dry sediment at 105º C.

1.3 Loss on Ignition (LOI)

To determine the amount of organic matter in the sediment samples, it was used the method of Loss on Ignition. This method allows to determine the weight change of the samples after some of its content has been burned at high temperatures, in this case the organic matter. The previously dried samples were put in a muffle furnace at 450º C for 2h and then weighed again.

The Loss on Ignition (%LOI) was calculated with the following equation:

$$\%LOI = \frac{m_{dry\ sed\ (105ºC)} - m_{dry\ sed(450ºC)}}{m_{dry\ sed\ (105ºC)}} \times 100 \quad (2)$$

Where %LOI is the percentage of mass burned in the heating process, $m_{dry\ sed\ (105ºC)}$ is the sediment mass after 24 hours at 105º C and $m_{dry\ sed(450ºC)}$ is the sediment mass after 2h at 450º C in the muffle furnace.

1.4 Biomass content

To determine the amount of biomass in each layer of each vegetated core, it was used the following equation:

$$\%Biomass = \frac{m_{dry\ roots}}{m_{(roots+sed)dry}} \times 100 \quad (3)$$

Where $m_{dry\ roots}$ is the mass of roots weighed after being dry and $m_{(roots+sed)dry}$ is the mass of sediment and roots after drying. Because it's impossible to have a direct measurement of $m_{(roots+sed)dry}$, this parameter is the sum between $m_{dry\ roots}$ and $m_{dry\ sediment}$. To determine $m_{dry\ sediment}$ it was used the following equation:

$$m_{dry\ sediment} = m_{(roots+sed)wet} \times \left(1 - \frac{\%H}{100}\right) - m_{dry\ roots} \quad (4)$$

It is subtracted the amount of water and the weight of the dry roots to the mass of sediment and roots while wet, obtaining the mass of dry sediment.

1.5 Total Iron (Fe) and Manganese (Mn) sediment content

To determine the total Fe and Mn concentrations was necessary to digest the sediment samples and then proceed to metal quantification by flame atomic absorption spectroscopy (AAS-F).

The digestion was made accordingly to the method described by Loring & Rantala (1992). It consists in using a total decomposition method with hydrofluoric acid (HF) and aqua regia, that is a mixture of hydrochloric (HCl) and nitric (HNO_3) acids in a proportion of 3:1. The HF is used due to its ability to completely dissolve the silicate lattices and therefore being able to release the associated metals, such as Fe, Mn and Al (Loring & Rantala, 1992). Aqua regia is used to solubilize the metals due to the strong oxidizing power. In the end of the procedure, boric acid (H_3BO_3) is added to neutralize the HF and to prevent the precipitation of fluoride (Loring & Rantala, 1990).

First, 100 mg of sediment sample were weighed and in Teflon containers. Then, 1 ml of aqua regia and 6 ml HF were added to the containers. The same process was also made without any sediment sample (blanks) and with standard reference materials. The Teflon containers were then placed in an oven during 1h at 100ºC. After the acid digestion, and with the samples cooled, the content transferred to 50 ml propylene tubes. The tubes already contained 5.6 g of H_3BO_3 and were filled with Milli-Q water to the mark and left to rest for further analysis.

After completing the digestion, the samples were analyzed in a Thermofisher S Series AA Spectrometer with a Mn hallow cathode lamp for Mn determination and a Fe hallow cathode lamp for Fe determination, in both cases the standard addition method was applied using Fe and Mn standard solutions of 100 µg mL^{-1}.

1.6 Determination of the total mercury (THg) concentration in sediments

In order to use an ICP-MS detector for measuring mercury isotopes, the samples were first subjected to a digestion. Approximately 100 mg of dry sediment was placed in a clear, labelled vial after being spiked with $^{198}Hg^{2+}$ as an internal standard, where were added 7 mL of 7_2:3 HNO_3

(aq):H_2SO_4(aq) mixed acid solution for complete digestion. The vials were placed on a hot plate which was pre-warmed in increments from 75 °C to 110 °C and covered with marbles that were soaked in HNO_3 acid and rinsed with deionized water. The vials were left overnight for at least 24 hours. Once the solutions become clear, they were diluted with deionized water. Then, the samples were left to cool down and stored at room temperature in the dark until further mercury analysis. The same process was also done with replicates of the standard reference material (PACS-2) and with blanks for every set of samples.

To quantify the mercury isotopes in the digested samples was used a continuous flow cold-vapor generation with ICP-MS (8800 ICP-MS Triple Quad Agilent Technologies) detection. The sample was continually mixed with a solution of stannous chloride 3% (w/v) in 10% (%v/v) HCl by means of a peristaltic pump and the formed mercury vapors were separated from the liquid in a gas-liquid separator (Model L1-2) and the elemental mercury swept into the plasma of the ICP-MS. The concentrations of the mercury isotopes were calculated according to the method described in Hintelmann and Ogrinc (2003) and the following isotopes: $^{202}Hg^{2+}$ (added isotope for the methylation determination), $^{198}Hg^{2+}$ (internal standard) and $^{199}Hg^{2+}$ (to calculate ambient THg) were determined.

1.7 Determination of monomethylmercury (MMHg) concentration in sediments

To evaluate the amount of MMHg in the sediment samples it was used water vapor distillation (Hintelmann et al., 2000). Approximately 200-500 mg of wet sample was weighed into Teflon vials and then added 10ml of distilled water. Prior to distillation the samples were spiked with CH_3HgCl enriched with $^{198}Hg^{2+}$ as an internal standard and then 500 mL of H_2SO_4 (9 M) and 200 mL of KCl (20%) were added to the vials. The teflon vials were put into a heating block at 140ºC and a continuous stream of nitrogen (60 ml min^{-1}) was passing through the sample into to the receiving vials. The distillation was considered finished when at least 85% of the sample was distilled. The same process was also done with blank samples and with a certified reference material (IAEA-158).

The quantification of the MMHg in the samples was done by species-specific isotope dilution inductively coupled plasma mass spectrometry using an automated Tekran 2700 system coupled to ICP- MS (8800 ICP-MS Triple Quad Agilent Technologies) and allowed to measured four different isotopes: $^{202}Hg^{2+}$ (methylated Hg), $^{200}Hg^{2+}$ (MMHg demethylation assay), $^{198}Hg^{2+}$ (internal standard) and $^{199}Hg^{2+}$ (to calculate ambient MMHg). The concentration of the individual mercury isotopes was calculated with an Excel spreadsheet that uses matrix algebra, as described in Hintelmann and Ogrinc (2003).

1.8 Stable mercury isotope tracer study

This work used stable isotopes of Hg at tracer levels to measure the Hg methylation and MMHg demethylation rates. Using a cocktail solution of stock solutions of 511 µg mL^{-1} of $^{202}HgCl_2$ with 91.5% purity (10mL) and 55.7 µg mL^{-1} of $CH_3{}^{200}Hg^+$ in ethanol (0.368mL), several injections were made in the sediment cores. The cores were collected and sustained inside PVC tubes that were already prepared with pre-drilled ports, enabling the injection of the solution. The sediments were injected with 25, 100, 300 or 750 µL of the cocktail solution (spike solution) at 0-3 cm,6-9 cm and 21-24 cm depths and incubated *in situ* for approximately 5 hours. This means that the injected amount represented 12322, 49286, 147859 and 369647 ng of $^{202}Hg^{2+}$ and 49, 198, 593 and 1483 ng of $CH_3{}^{200}Hg^+$, respectively. For each saltmarsh, different spikes were chosen accordingly to the already proven Hg contamination of the site. 750 µL spikes in LAR, 300 µL spikes in CHE, 100 µL spikes in ROS and 25 µL spikes in ALC were introduced into the layers mentioned above. To ensure the dilution of the spiked solution, upon injection, the isotopes were dispersed as evenly

as possible into each 1 cm layer and then vertical migration within the core diluted the spike further. On average, the total Hg concentration increased less than 10% and MMHg levels increased by a factor of 1.7. In the most contaminated sites, ROS and LAR, increases were lower and in the less contaminated sites, ALC and CHE, increases were relatively higher. Once methylation and demethylation rates were measured during the same period of time and in the same volume of sediment, they are directly comparable.

1.9 Mercury methylation and monomethylmercury demethylation rates

The mercury methylation and monomethylmercury demethylation rates were calculated based on the assumption that both processes have first-order kinetics (Cesário et al., 2017). To evaluate the rates, it was calculated the methylation rate constant (K_M) and the demethylation rate constant (K_D), both of them expressed in day^{-1}.

To determine the methylation rate constant K_M (day^{-1}), it was used the following equation:

$$K_M = [MM^{202}Hg^{+}]/([^{202}Hg^{2+}] \times t) \quad (5)$$

Where, [$MM^{202}Hg^{+}$] is the concentration of monomethylmercury (ng g^{-1}) that was formed due to the methylation of the spiked mercury, [$^{202}Hg^{2+}$] is the total concentration (ng g^{-1}) of this mercury isotope and t is the incubation time (day).

To determine the monomethylmercury demethylation rate its necessary to take into account that the concentrations of the spiked $MM^{202}Hg^{+}$ decrease exponentially over time, due to the fact that this is a first-order kinetic process. The following equation was used:

$$c\,(t) = c(0)\ \times\ e^{(-K_D \times t)} \quad (6)$$

Where c (0) is the starting concentration of $MM^{202}Hg^{+}$ in each sample, c (t) is the initial concentration at time of spiking and t is the incubation time. By solving the prior equation, the following equation is obtained:

$$K_D = \frac{(Ln[c(0)] - L[c(t)])}{t} \quad (7)$$

To solve equation 7 it is first necessary to determine c (0). This value cannot be obtained directly and therefore needs to be estimated by using the ratio (r) of $^{202}Hg^{2+}$ by $MM^{200}Hg^{+}$ in the spike solution. By knowing the measured concentration of $^{202}Hg^{2+}$ in each sample and then diving it by r, the concentration of $MM^{200}Hg^{+}$ at the start of the incubation is obtained, so:

$$c(0) = \frac{[^{202}Hg^{+2}]}{r} \quad (8)$$

1.10 Quality assurance and control

Quality control is very important to assure valid results. In the determination of Hg and MMHg, it was conducted by using a defined set of samples for each batch. For Hg determination, two reagent blanks and one certified reference material (PACS – 2) was used in each acid digestion. For MMHg quantification by distillation, one instrument blank (blubber) and one certified reference material (IAEA- 158) were used in each distillation procedure.

The results obtained for Hg and MMHg analysis of the certified refence materials for sediments is showed in table 1.

Table 1 - Mercury (µg/g) and monomethylmercury (ng/g) certified reference material for sediment concentrations and obtained concentrations

	Hg(µg/g)	*MMHg(ng/g)*
	PACS – 2	IAEA - 158
Certified	3040 ± 200	1410 ± 400
Obtained	2900 ± 41	1750 ± 20

To guarantee the results obtained in both Hg and MMHg determinations it was used a solution of an internal isotope standard ($^{198}Hg^{2+}$), that in turn was also validated. The validation was similar in both cases, only changing the concentrations of the isotopic solution and the isotope itself ($^{198}Hg^{2+}$ for Hg determination and $MM^{198}Hg^{2+}$ for MMHg determination). In the case of Hg, samples with 100 mL of 1% HCl were made, along with three replicates blanks, another with 100 pg mL^{-1} ambient mercury (II) and three replicates of 100 pg mL^{-1} ambient mercury (II) added with 40 µL of the internal standard solution ($^{198}Hg^{2+}$ solution with 33.4 µg mL^{-1}). In the case of MMHg, five replicates were made containing a total of 500 pg ambient MMHg and another four replicates containing the same amount plus 25 µL of internal standard solution ($MM^{198}Hg^{2+}$ solution with 9 ng mL^{-1}). In both cases, to determine the concentration of the used internal standard, it was used the matrix calculation mentioned in subchapters 1.5 and 1.6.

In the determination of total Fe and Mn contents, duplicates were done to guarantee accuracy of all results. Once both analyses were done from the same sediment sample, in both cases was used reagent blanks and certified refence materials (PACS-2, MESS-4 and IAEA- 457).

Table 2 - Total Fe and Mn (mg/g) certified reference material for sediment concentrations and obtained concentrations

	Fe(mg/g)		
	PACS – 2	MESS - 4	IAEA-457
Certified	60.9 ± 0.6	37.8 ±1.6	41.45 ± 2.24
Obtained	41.1	37.7 ±0.2	41.15 ± 1.05
	Mn(mg/g)		
	PACS – 2	MESS - 4	IAEA-457
Certified	0.44 ± 0.019	0.298 ± 0.014	0.427 ± 0.030
Obtained	0.457	0.283 ± 0.0015	0.399 ±0.003

It was obtained the Limit of detection (LOD) and the limit of quantification (LOQ), from the calibration curves, using the following equations (Harris, 2010):

$$LOD = 3\frac{S_y}{m} \tag{9}$$

$$LOQ = 10\frac{S_y}{m} \tag{10}$$

Where S_m is the standard deviation determined by equation X and m is the slope of each curve.

$$S_y = \sqrt{\frac{\sum(y_i - y)^2}{N - 2}} \tag{11}$$

VI. Results and Discussion

1. Sediment Characterization

1.1 Sediment pH and temperature

After the sediment cores were collected, pH and temperature were measured *in situ* in each sediment slice.

Figure 4 presents the measured pH values for sediments collected in Laranjo (LAR), Ria de Aveiro, during spring and summer campaigns. The sediments colonized with *H. portulacoides* presented values between 5.33 and 6.58 in the spring and from 5.59 to 6.19 in the summer. The pH values for *J. maritimus* colonized sediments varied between 5.17 and 6.18 in the spring and from 5.71 to 6.09 in the summer. In non-vegetated sediments the pH values ranged between 5.59 and 6.27 in the spring and from 5.20 to 6.49 in the summer.

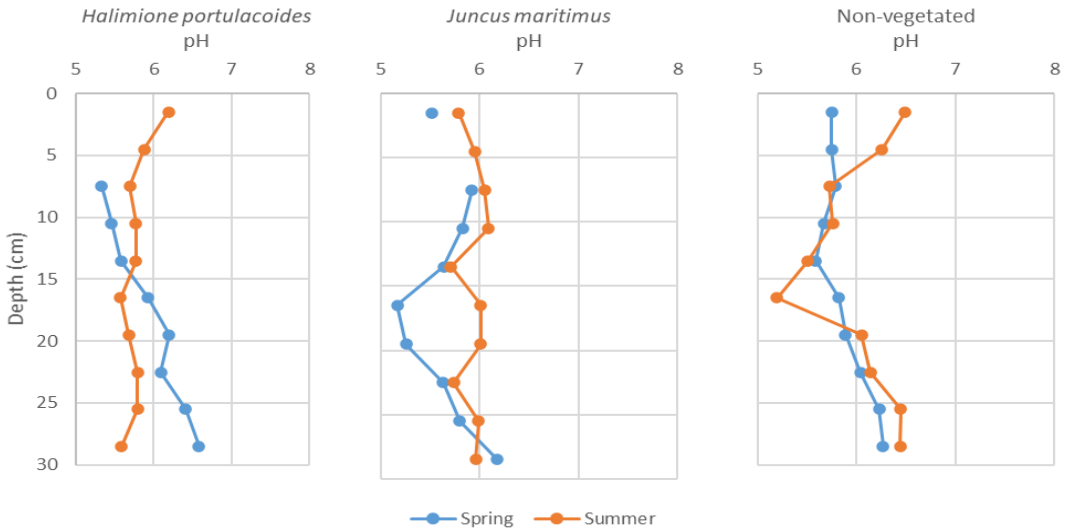

Figure 4 - Vertical profiles of pH on sediment samples collected in Laranjo (LAR) saltmarsh, Ria de Aveiro.

The pH values obtained in sediments of Chegado (CHE), Ria de Aveiro, are in figure 5. In sediments colonized by *H. portulacoides*, the pH values varied between 5.62 and 6.15 in the spring and from 5.60 to 6.15 in the summer. In the sediments colonized by *J. maritimus*, pH recorded was between 5.34 and 6.06 in the spring and from 5.52 to 6.43 in the summer. In non-vegetated sediments the pH values ranged between 6.30 and 7.07 in the spring and between 6.53 and 6.91 in the summer.

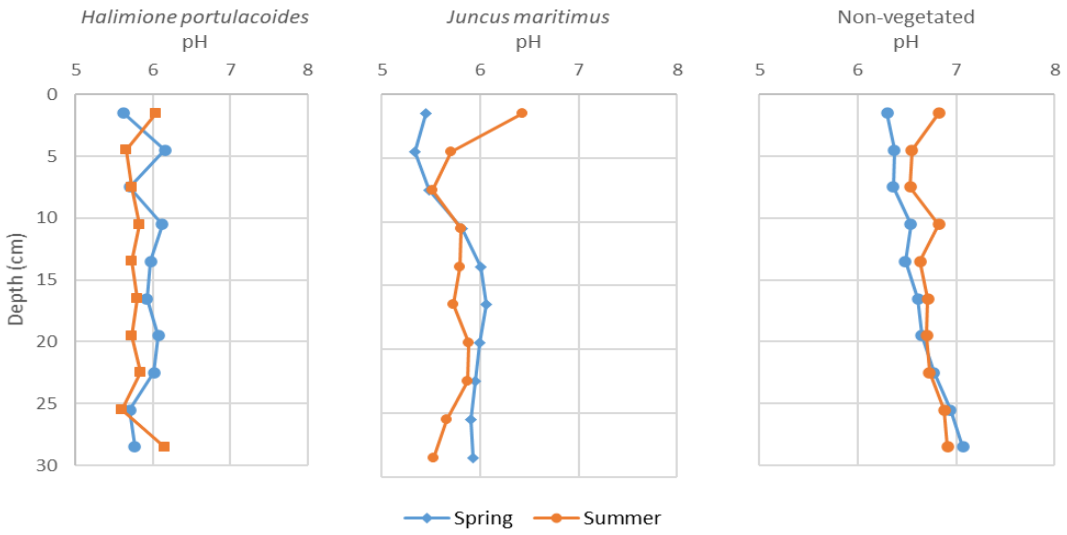

Figure 5 - Vertical profiles of pH on sediment samples collected in Chegado (CHE) saltmarsh, Ria de Aveiro

Analyzing the pH values from the two saltmarshes of Ria de Aveiro, it seems that pH tends to be slightly higher in non-vegetated sediments, as opposed to colonized ones. Doesn't seem to exist any seasonal variation and profiles tend to be regular, with smaller deviations normally happening near the surface.

The pH values from sediments collected in Rosário (ROS) site, Tagus estuary are presented in figure 6. The sediments colonized with *H. portulacoides* presented pH values between 6.87 and 7.34 in the spring and from 6.59 to 6.98 in the summer. In sediments that contained *J. maritimus*, pH presented values between 6.57 and 7.14 in the spring and from 6.55 to 7.48 in the summer. For non-vegetated sediments, the pH values ranged between 6.81 and 7.30 in the spring and from 6.80 to 7.40 in the summer.

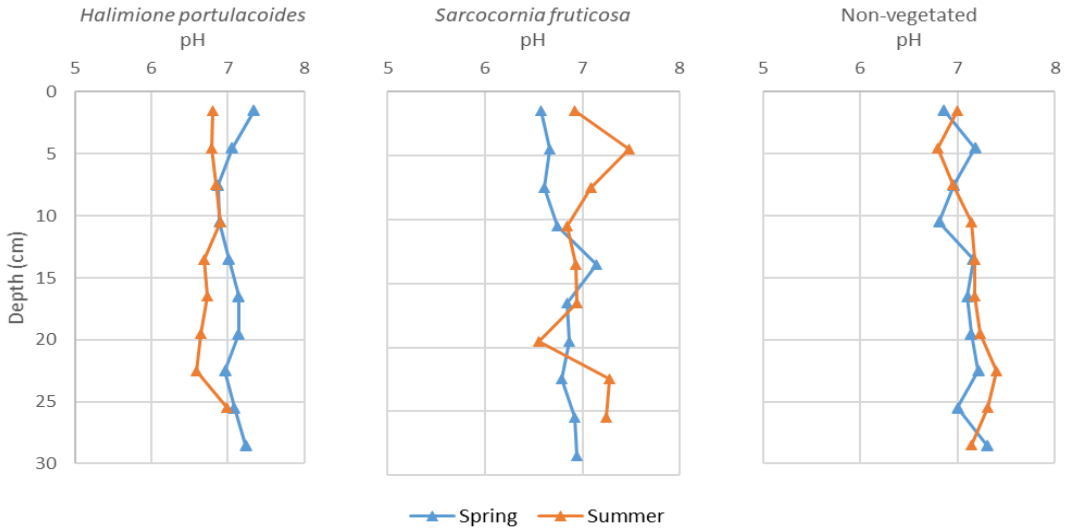

Figure 6 - Vertical profiles of pH on sediment samples collected in Rosário (ROS) saltmarsh, Tagus estuary

In Alcochete (ALC) site, Tagus estuary, the sediments colonized by *H. portulacoides* showed pH values between 5.98 and 6.24 in the spring and from 6.03 to 6.53 in the summer. The sediments containing *J. maritimus* had pH values between 6.46 and 7.09 in the spring and from 6.66 to 6.89 in the summer. In non-vegetated sediments, pH values ranged between 6.62 and 7.46 in the spring and from 6.68 to 7.50 in the summer (Figure 7).

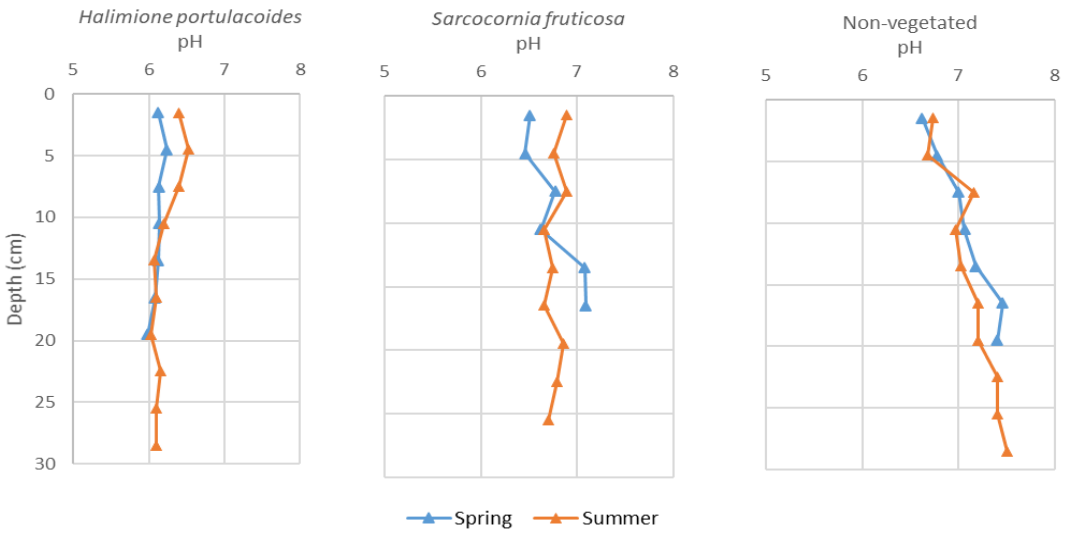

Figure 7 - Vertical profiles of pH on sediment samples collected in Alcochete (ALC) saltmarsh, Tagus estuary.

In the saltmarshes of Tagus estuary, pH values are higher in ROS than in ALC and in both cases doesn't seem to exist seasonal variation. Profiles are regular, with no specific variation with depth, and non-vegetated sediments also have higher pH values than those found in colonized sediments.

The pH may be an important factor when studying the methylation of Hg and demethylation of MMHg, because is one of the many factors that have an influence on these reactions. However, literature data on effects of pH on the mobility and methylation of Hg are still contradictory (Frohne et al., 2012). According to Ramlal et al (1985) Hg may be less available to be methylated at low pH because of the increase sorption to particles, but Winfrey & Rudd (1990) hypothesized that, in sediment pore waters, the likely decrease in dissolved organic matter (DOM) binding sites at low pH values resulting from the protonation of functional groups may stimulate methylation by promoting Hg binding directly onto microbial cells (Ullrich et al., 2001). Also, in anoxic sediments, low pH can decrease Hg methylation maybe due to the suppression of bacterial activity (Gilmour & Henry, 1991). On the other hand, demethylation is also pH sensitive and variations may increase or decrease its rates, however it's thought to be less affected than methylation rates (Ullrich et al. 2001).

Comparing the two estuaries, it's possible to see that in Tagus Estuary pH values found in sediments are higher than those found in Ria de Aveiro. While in LAR and CHE, pH values varied approximately between 5 and 7, in ROS and ALC varied mostly between 6 and 8. Overall, the variations in all four saltmarshes of both estuaries don't appear to be significantly higher to determine if pH values induce variations in methylation and demethylation rates.

1.2 Humidity

The percentage of humidity represents the amount of water present in the sediment relative to its weight. The following figures (8 to 11) represent the vertical profiles of humidity in sediment samples colonized by *Halimione portulacoides*, *Juncus maritimus* and *Sarcocornia fruticosa* as well as non-vegetated sediments collected at the four different sites in spring and in summer.

In the sediments collected in LAR, the humidity values in *H. portulacoides* colonized sediments varied between 61.1% and 70.5% in the spring and from 60.7% to 70.9% in the summer. In *J. maritimus* colonized sediments, the water content varied between 62.4% and 81.8% in the spring, with an abnormally low value at 30 cm depth of 46.2%, and from 60.8% to 74.1% in the summer. For non-vegetated sediments, the humidity values varied between 60.7% and 78.1% in the spring and from 57.7% to 78.1% in the summer (Figure 8).

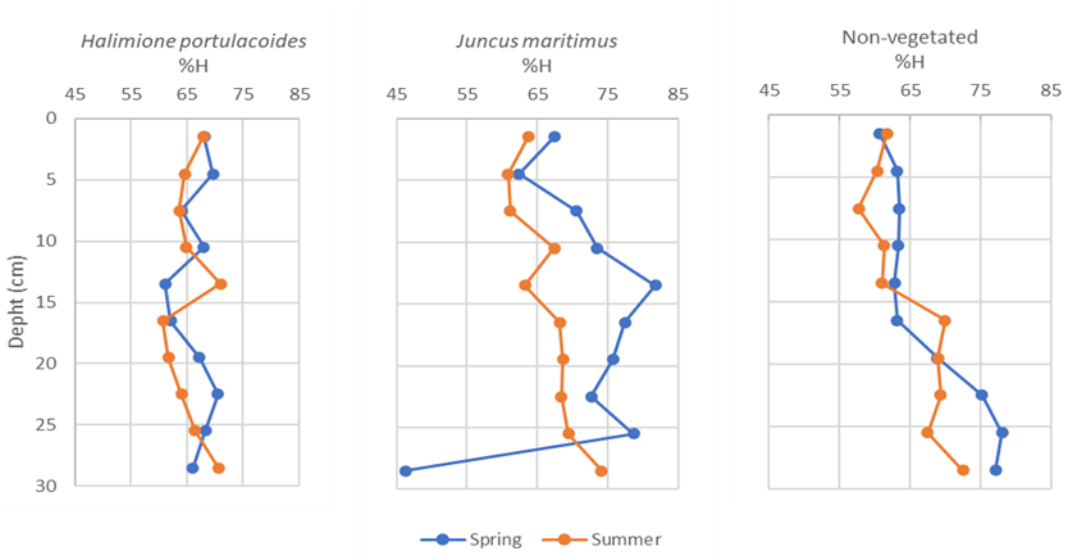

Figure 8 - Vertical profiles of humidity values (%) of sediment samples collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

The values of humidity obtained in CHE are presented in figure 9. For sediments that *contained H. portulacoides*, the humidity varied between 53.0% and 68.2% in the spring and from 56.1% to 67.4% in the summer. Sediments colonized by *J. maritimus*, presented water content ranged between 52.9% and 71.9% in the spring and from 54.4% to 64.7% in the summer. For non-vegetated sediments, these values varied between 52.8% and 76.5% in the spring and from 52.0% to 75.1% in the summer.

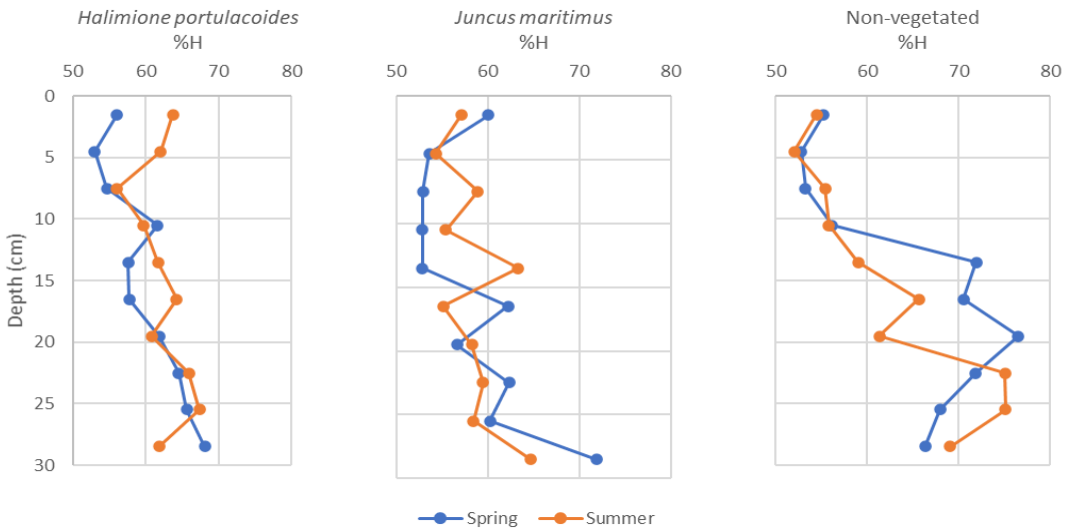

Figure 9 - Vertical profiles of humidity values (%) of sediment samples collected in Chegado (CHE) saltmarsh, Ria de Aveiro.

Comparing the two saltmarshes of the same estuary - Ria de Aveiro - it´s noticeable that don't seem to exist significant seasonal variations. Only in the sediment samples colonized by *J. maritimus* from LAR and in the non-vegetated ones from CHE seems to exist a small difference between spring and summer, with sediments from spring having higher water content, probably related to the rain season. It's also possible to see that, in general, humidity appears to have a small decrease until approximately 3 to 10 cm in depth and then slowly increases, indicating a high permeability of the sediments.

Figure 10 shows the percentage of humidity in sediments collected in ROS. In sediments colonized by *H. portulacoides* the values varied between 20.6% and 55.8% in the spring and from 19.7% to 48.6% in the summer. Water content in S*. fruticosa* colonized sediments varied between 50.6% and 66.6% in the spring and from 20.5% to 61.6% in the summer. For non-vegetated sediments, water content varied between 27.5% and 61.6% in the spring and from 29.0% to 52.8% in the summer.

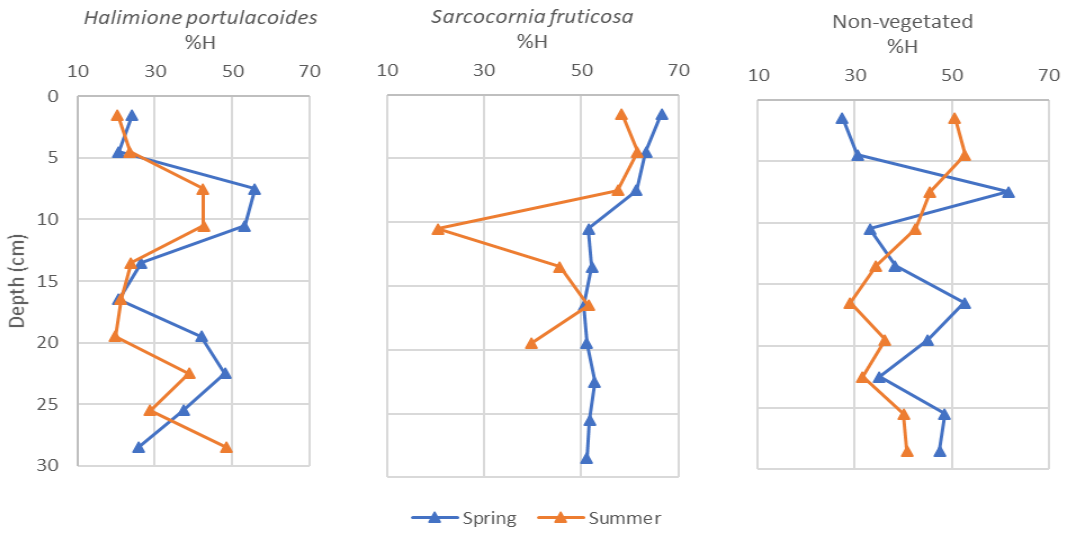

Figure 10 - Vertical profiles of humidity values (%) from sediment samples collected in Rosário (ROS) saltmarsh, Tagus estuary.

In ALC, *H. portulacoides* colonized sediments water content varied between 40.7% and 50.9% in the spring and from 19.2% to 48.2% in the summer. In sediments colonized by *S. fruticosa* the

humidity varied between 20.1% and 50.0% in the spring and from 17.8% to 57.0% in the summer. For non-vegetated sediments, humidity varied between 14.9% and 39.4% in the spring and from 30.7% to 48.0% in the summer (Figure 10).

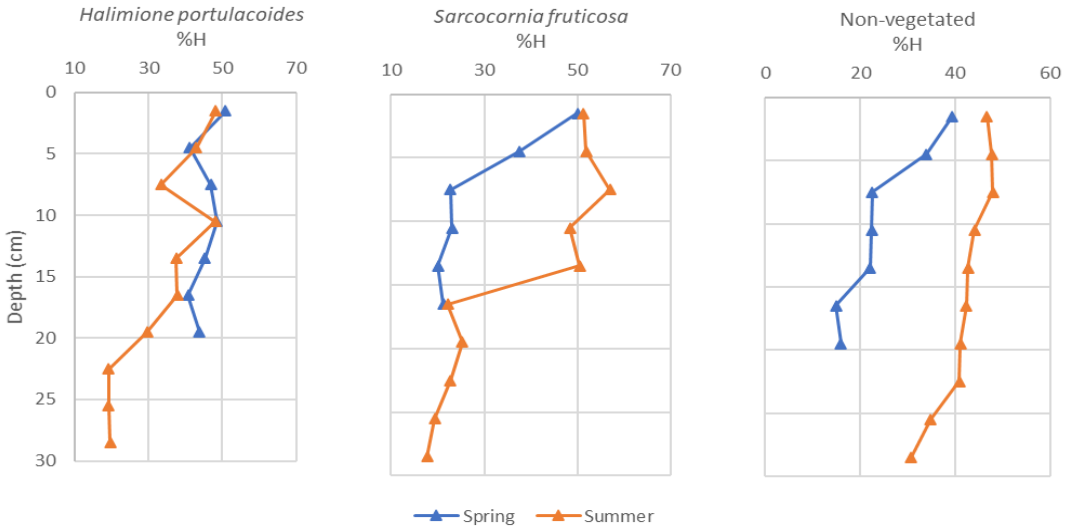

Figure 11 - Vertical profiles of humidity values (%) from sediment samples collected in Alcochete (ALC) saltmarsh, Tagus estuary.

Considering the humidity values of the sediments from Tagus estuary it´s possible to see some differences between saltmarshes. In ALC, humidity decreases with depth, but in ROS values were more irregular and isn't possible to say the same, except for the sediments colonized by *S. fruticosa*. In terms of seasonal variation, in ROS it doesn't seem to exist, but in ALC the humidity values were higher in the summer for sediments colonized by *S. fruticosa* and for non-vegetated sediments

Comparing the percentages of humidity obtained in the sediments of the two estuaries, is noticeable that sediments from Ria de Aveiro normally present higher values than those observed in Tagus estuary. The sediments collected in LAR and CHE, normally present values between 50 and 80%, and the ones collected in ROS and ALC, normally present values between 20 and 50%. The difference is probably related to grain size of the soil. In Ria de Aveiro the sediments are mostly constituted by mud and silt and in Tagus estuary sediments are a mixture of mud, silt and sand with the presence of small stones, more pronounced in ALC. In any of the saltmarshes has been noticed a difference between colonized sediments and non-vegetated sediments. There is also another factor could have influenced the measurements of water content, that was the tide submersion. Saltmarsh halophyte plants colonize different areas of the saltmarshes and can sustain different times of submersion. Sediments collected in low marsh areas can be prone to have higher humidity values as opposed to sediments that colonize high marsh areas.

1.3 Loss on Ignition (LOI)

In the following figures (12 to 15) are presented the vertical profiles of the amount of organic matter, measured as LOI, present in the sediment samples collected in Ria de Aveiro and in Tagus estuary saltmarshes.

Figure 12 presents the vertical profiles of LOI (%) in sediment samples collected in LAR. The *H. portulacoides* colonized sediments showed similar LOI percentages in both seasons ranged between 13.1% and 22.9%. The same pattern was observed in non-colonized sediments where LOI percentages varied from 12.3% to 29.5%. Sediments colonized by *J. maritimus* presented different LOI vertical profiles between seasons with higher range during the spring (between 10.1% and 35.7%) decreasing in summer for LOI percentages of 11.9% and 21.6%.

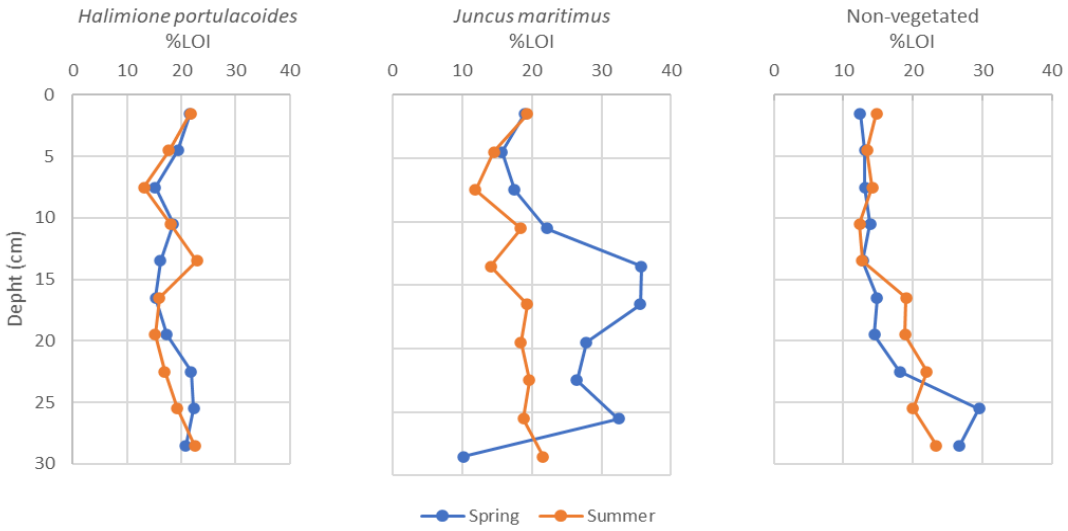

Figure 12 - Vertical profiles of Loss on Ignition (LOI) values (%) of sediment samples collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

In CHE site, LOI vertical profiles of sediments colonized by *H. portulacoides* presented similar results from those collected in LAR, where LOI percentages varied between 10.8% and 24.2% in both seasons. Additionally, sediments colonized by *J. maritimus* also had similar results between seasons where LOI vertical profiles varied between 3.9% and 21.8%. In non-vegetated sediments LOI vertical profiles were similar between seasons above 10 cm depth. Below this depth LOI had a considerable increase in spring season (from ~10% to ~20%) until the endo of the core. The same pattern was observed for the summer core but only below the 20 cm depth (Figure 13)

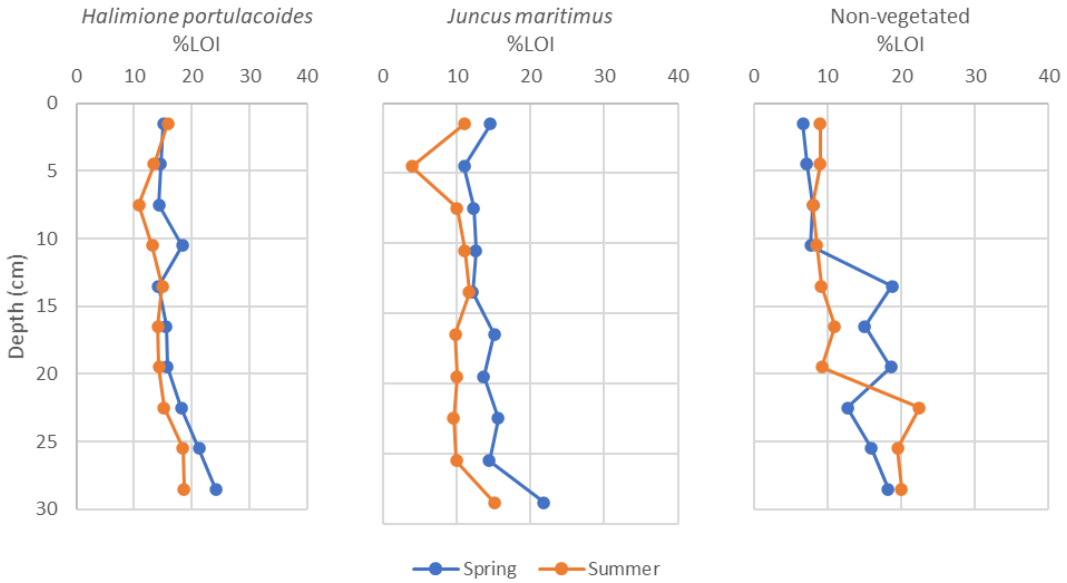

Figure 13 - Vertical profiles of Loss on Ignition (LOI) values (%) from sediment samples collected in Chegado (CHE) saltmarsh, Ria de Aveiro

Analyzing the results obtained in the two saltmarsh areas from Ria de Aveiro it's possible to see that there aren't seasonal changes, except for the sediments colonized by *J. maritimus* collected in LAR. They present slightly irregular values and show higher content of organic matter in the spring. In fact, further ahead (Figure 16) it's possible to see that this increase in organic matter corresponds with an increase of the percentage of belowground biomass. This shows that the presence of belowground biomass increases the organic matter content in sediments. All the other vertical profiles are regular, only with small variations at different depths, which indicates that relation between depth and organic matter content doesn't seem to exist. Comparing the two plants, don't exist visible differences in organic matter content, but non-vegetated samples, especially near the surface, present smaller values, probably due to the lack of plant matter.

Figure 14 shows the organic content of the samples collected in ROS. The LOI values for *H. portulacoides* colonized sediment were between 2.0% and 13.9% in the spring and from 0.9% to 12.1% in the summer. Sediments colonized by *S. fruticosa* presented higher LOI in both seasons, ranged between 6.4% and 18.9% in the spring and 1.9% and 27.0% in the summer. In non-vegetated samples, values obtained were between 3.0% and 15.5% in the spring and 2.3% and 8.7% in the summer.

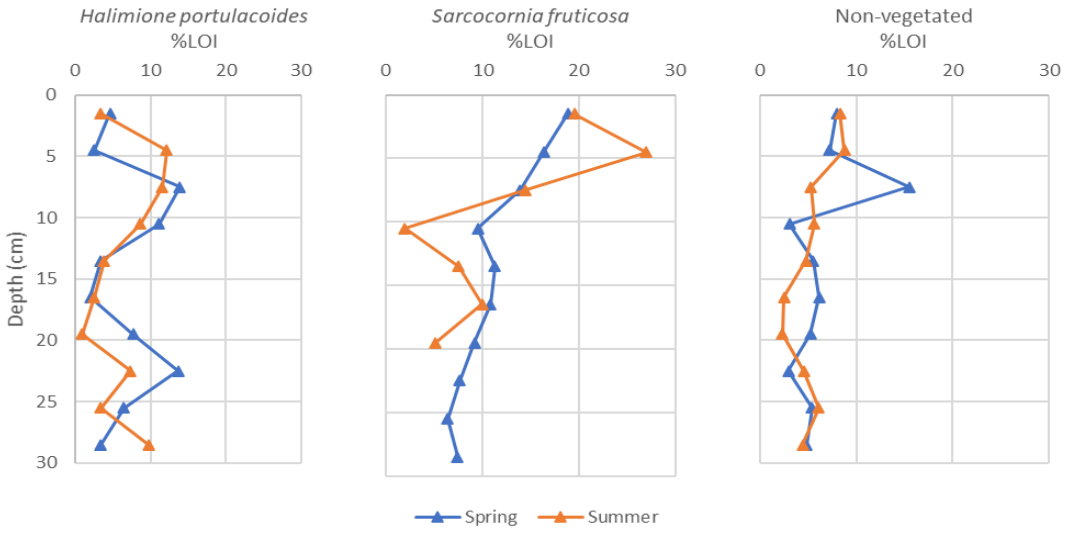

Figure 14 - Vertical profiles of Loss on Ignition (LOI) values (%) from sediment samples collected in Rosário (ROS) saltmarsh, Tagus estuary.

The LOI values obtained in sediments from ALC are presented in figure 15. For *H. portulacoides* colonized sediments, LOI vertical profiles were similar between seasons with the values ranged between 8.4% and 16.4% in the spring and 1.8% and 11.3% in the summer. The same pattern was observed in non-colonized sediments where LOI percentages ranges between 0.6% and 2.7% in the spring and from 1.9% to 6.1% in the summer. However, a different pattern was observed in LOI percentages of sediments colonized by by *S. fruticosa* where the values ranged between 3.7% and 12.8% in the spring and from 1.6% to 20.7% in the summer.

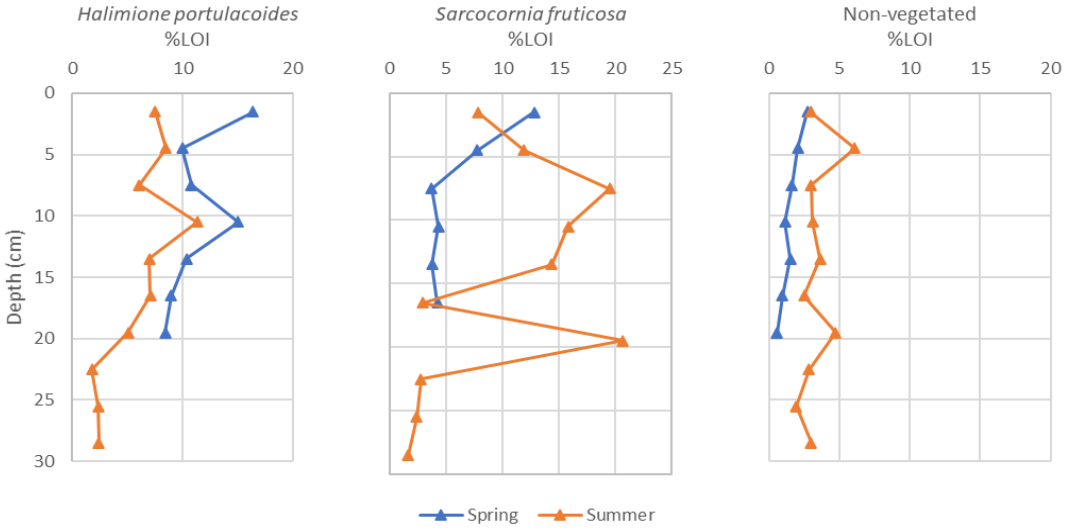

Figure 15 - Vertical profiles of Loss on Ignition (LOI) values (%) from sediment samples collected in Alcochete (ALC) saltmarsh, Tagus estuary.

Comparing the LOI vertical profiles of sediments from the saltmarshes of Tagus estuary it's possible to see that in both sites a decrease of LOI with depth was observed and that profiles from sediments colonized by *S.fruticosa* are more irregular than the others. In the sediments colonized by *S.fruticosa* collected in ALC exists a peak at 20 cm in depth that, due to its difference, can be related with process errors, an organism or a shell present in the sediment. Comparing the two plants, despite having values in the same range, *S. fruticosa* colonized sediments tend to present slightly higher values of organic matter than those colonized by *H. portulacoides*. Doesn't seem to exist any significant seasonal variations of LOI in none of the saltmarshes. In opposite

to what was observed in Ria de Aveiro, non-vegetated sediments showed smaller organic matter content, which is particularly noticeable in the sediments from ALC.

Organic matter is an important parameter to consider when trying to access conditions for the methylation of Hg. It's role still needs further studies to be completely understood, but in general, is a very important factor. High organic matter contents it's associated with high increases in methylation rates, which is normally linked with the effect it has on enhancing microbial methylation activity (Ullrich et al., 2001). In fact, some studies already showed that organic matter content may be an important parameter to try to predict MMHg production in the environment (e.g. Meng et al. 2015). Organic matter plays a major role on the mobilization of Hg, due to its ability to form organic complexes, which in turn could favor its accumulation, but on the other hand, organic matter enhances the solubility of HgS and may lead to a significant release of Hg into solutions (Ravichandran et al. 1998).

Analyzing the results of both estuaries, the sediments of saltmarshes in Ria de Aveiro present higher values than those recorded in Tagus estuary. Sediments collected in LAR and CHE, had LOI percentages ranged between 10% and 25% but in the sediments obtained in ROS and ALC LOI these values were normally between 1% and 20%.

1.4 Belowground biomass

The following figures (16 to 19) represent the vertical profiles of the amount of belowground biomass in the four saltmarshes of this study. It is compared the amount of belowground tissues between seasons, as well as their variation with depth.

Figure 16 shows the percentage of belowground biomass in the sediment samples collected in LAR. Sediments colonized by *H. portulacoides* presented values between 7.5% and 20.4%. Sediments colonized by *J. maritimus* the belowground biomass varied between 5.8% and 26.4%. In both cases, the values are only for spring.

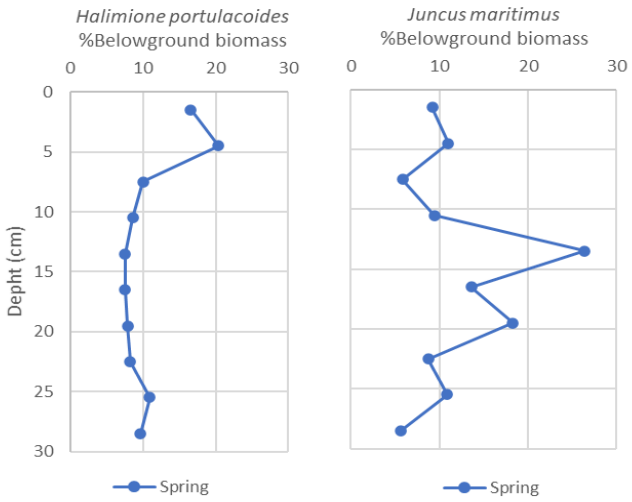

Figure 16 - Vertical profiles of belowground biomass (%) from colonized sediments collected in Laranjo (LAR) saltmarsh, Ria de Aveiro.

In the sediments collected in CHE, belowground biomass varied between 4.3% and 13.6% in sediments colonized by *H. portulacoides* during the spring. Sediments colonized by *J.maritimus* presented values between 1.4% and 12.2% in the spring and from 1.5% to 2.5% in the summer (Figure 17).

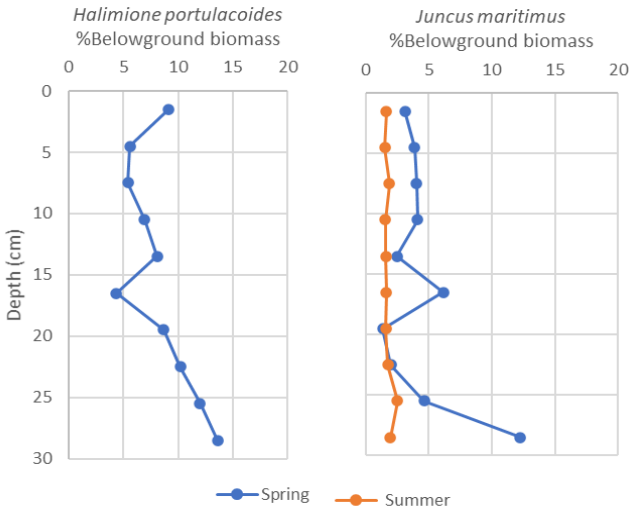

Figure 17 - Vertical profiles of belowground biomass (%) from colonized sediments collected in Chegado (CHE) saltmarsh, Ria de Aveiro.

Comparing the two saltmarshes of Ria de Aveiro it´s possible to see that the sediments with *H. portulacoides* as well as sediments with *J. maritimus* showed higher values in LAR than in CHE. Another difference, that can only be considered in the sediment samples from CHE is that exists seasonal variation, with *J. maritimus* colonized sediments having more belowground biomass in the spring than in the summer.

Figure 18 shows the belowground biomass values obtained from the samples collected in ROS. In sediments colonized by *H.portulacoides*, biomass varied between 0.1% and 7.9% in the spring and 0.6% and 1.3% in the summer. In *S. fruticosa* colonized sediments these values varied between 0.7% and 6.9% in the spring and 0.6% and 3.1% in the summer.

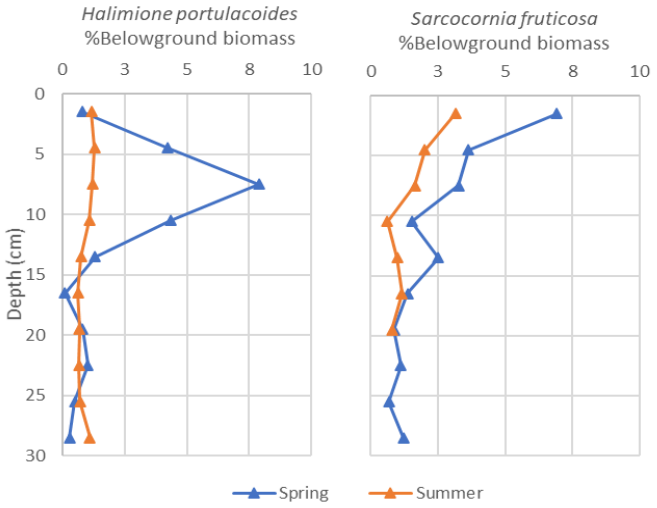

Figure 18 - Vertical profiles of belowground biomass (%) from colonized sediments collected in Rosário (ROS) saltmarsh, Tagus estuary.

In ALC, belowground biomass in the sediments colonized by *H. portulacoides* ranged between 0.9% and 2% and from 0.4% to 1.4% in spring and summer, respectively. For those colonized by *S. fruticosa* values varied between 0.01% and 4.7% in the spring and from 0.5% to 2.9% in the summer.

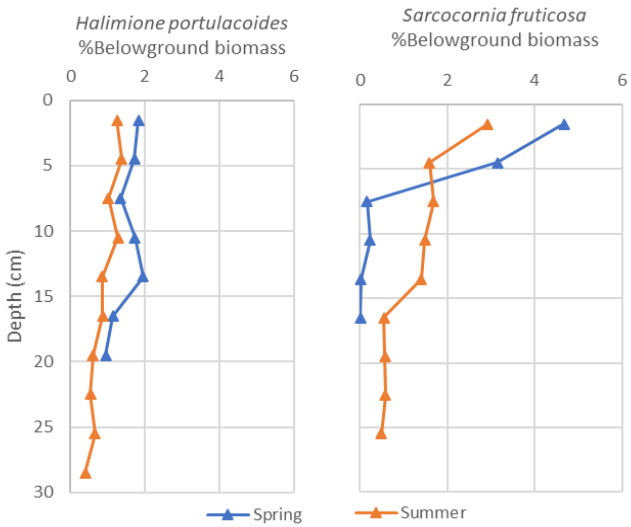

Figure 19 - Vertical profiles of belowground biomass (%) from sediments collected in Alcochete (ALC) saltmarsh, Tagus estuary

Comparing the two saltmarshes from Tagus estuary it's possible to see that the values for belowground biomass tend to decrease with depth and are generally higher in ROS. In the sediments colonized by *H. portulacoides* from ROS there is an abnormal increase in biomass until 7.5 cm in depth and then a steady decrease. One possible explanation is that when the samples were retrieved, at that depth, were also present roots from a nearby plant. Appears to exist a small seasonal variation, which suggests that occurred a degradation of the roots from spring to summer.

Evaluating the values from the two estuaries, it´s clear that Ria de Aveiro presents higher values of belowground biomass. The sediments collected in LAR and CHE normally present values between 1.5% and 15%, while sediments collected in ROS and ALC normally present values between 0% and 7%. In LAR and CHE doesn't seem to exist a significant decreased with depth, showing that the root system is well developed in the rhizosphere even at bigger depths unlike what happens in ROS and ALC, where soil characteristics don't allow for such a big development of roots.

Belowground biomass can be an important factor in the fate of Hg in saltmarshes. Recent studies suggested that plants can have an influence in Hg speciation due to its ability to promote changes in the rhizosphere (Canário et al., 2007b; Figueira et al., 2012; Cesário et al., 2017). The capacity to introduce O_2 in anoxic sediments creates shifts in redox conditions (Sundby et al., 2003) and the presence of roots also enhances microbial activity by providing nutrients and organic matter (Canário et al 2010). The previous authors suggested that these factors contributed for the methylation of Hg in saltmarshes. Also, it was found that concentrations of Hg and MMHg in belowground tissues could be considerably higher than the concentration in adjacent sediments, which indicates that the presence of belowground biomass contributes to the uptake and retention by plant species (Canário et al., 2007b; 2010) which could bring consequences in plant health or even for the food chain. However, for the halophyte plant species in study, it has already been proven poor translocation of Hg and MMHg from roots to above ground tissues (Canário et al., 2007b; Figueira et al. 2012; Cabrita et al., 2019).

1.5 Total Iron and Manganese contents in sediments

The total Fe and Mn concentrations was analyzed in order to understand if their presence in the rhizosphere could affect the speciation of Hg and thus influence the Hg methylation or MMHg demethylation. In the study, values for Fe and Mn concentrations were only measured for the approximate depths were MMHg concentrations were obtained.

1.5.1 Total Iron (Fe) content

Figure 20 shows the total iron (Fe) content of the sediments collected in LAR. In sediments colonized by *H. portulacoides*, values varied between 21.7 and 46.7 mg g^{-1} in the spring and from 27.6 to 40.3 mg g^{-1} in the summer. For sediments containing *J. maritimus*, values were similar between seasons and varied between 27.3 and 39.9 mg g^{-1}. Similar patterns were observed in non-vegetated sediments, where Fe contents were slightly higher in spring (37.5 to 42.2 mg g^{-1}) when comparing with summer (27.4 and 33.5 mg g^{-1}).

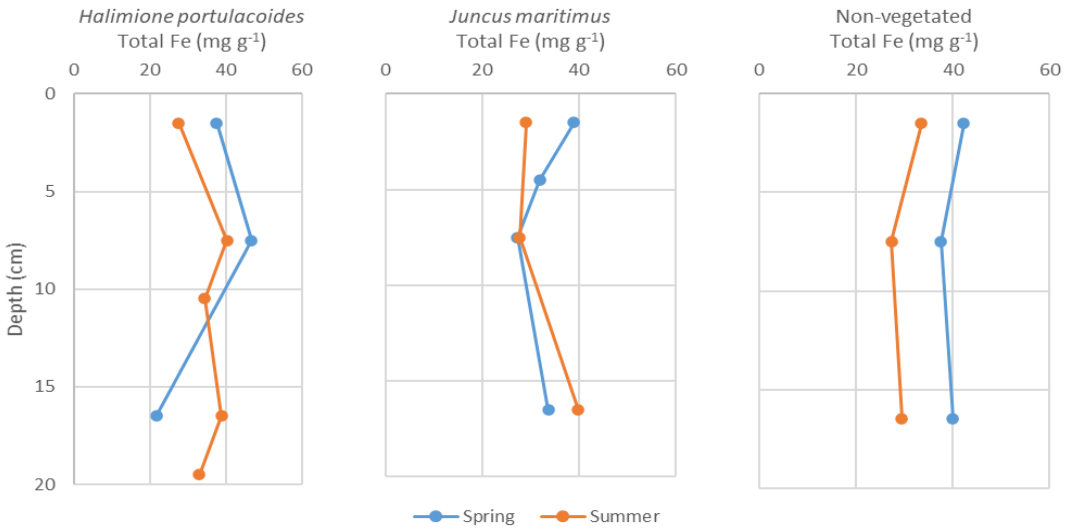

Figure 20 - Vertical profiles of total Fe content (mg g^{-1}) from sediments collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

In CHE, the sediments colonized by *H. portulacoides* exhibited a total Fe content between 31.7 and 42.1 mg g^{-1} in the spring and 5.7 and 47.0 mg g^{-1} in the summer. *J. maritimus colonized* sediments presented values between 26.8 and 37.7 mg g^{-1} in the spring and 24.5 and 37.1 mg g^{-1} in the summer. In non-vegetated sediments values varied between 21.8 and 50.6 mg g^{-1} in the spring and 34.5 and 49.2 mg g^{-1} in the summer (Figure 21).

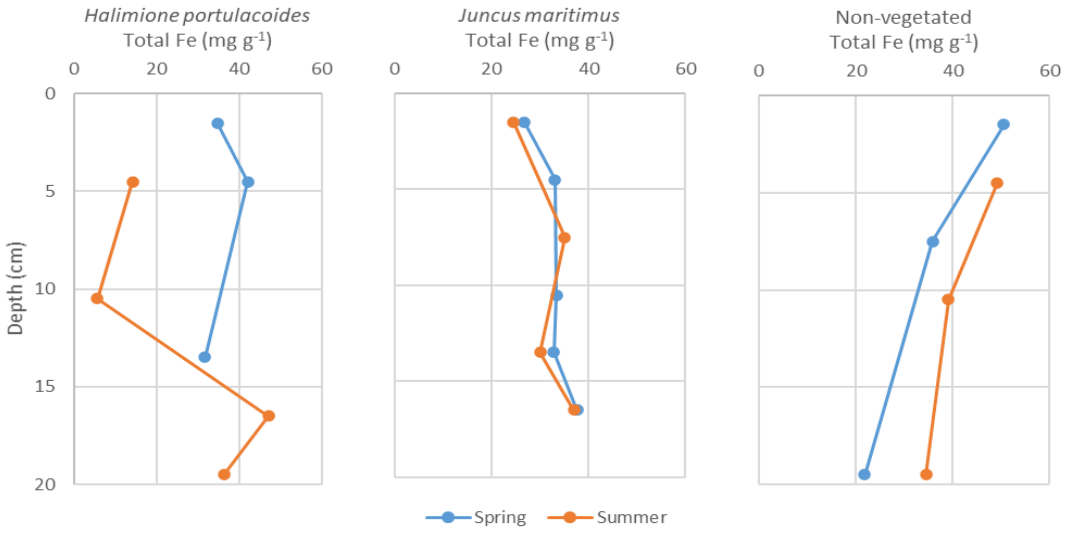

Figure 21 -- Vertical profiles of total Fe content (mg g^{-1}) from sediments collected in Chegado (CHE) saltmarsh, Ria de Aveiro

Comparing the two saltmarshes of Ria de Aveiro, it's noticeable that the range of total Fe content is very similar in both. The total Fe vertical profiles from sediments colonized with *H. portulacoides* obtain in CHE are the most irregular in depth and between seasons, which may be due to spatial variations. In both saltmarshes don't seem to exist seasonal variations, quantitative difference between colonized and non-vegetated sediments and a difference between sediments colonized by the two different types of plants.

The following figure (22) represent the values of total Fe content in the sediments of ROS. For the sediments colonized by *H. portulacoides*, levels varied between 2.3 and 46.7 mg g^{-1} in the spring and from 17.9 to 47.2 mg g^{-1} in the summer. In *S. fruticosa* colonized sediments total Fe content varied between 26.7 and 42.1 mg g^{-1} in the spring and from 24.0 to 52.3 mg g^{-1} in the summer. Non-vegetated sediments presented total Fe contents between 2.4 and 31.2 mg g^{-1} in the spring and from 19.2 to 50.5 mg g^{-1} in the summer.

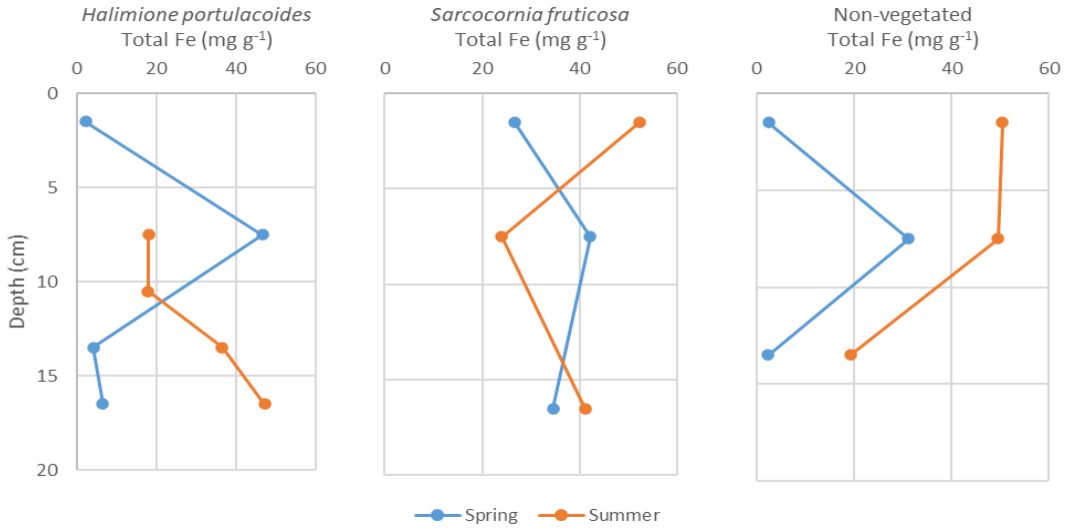

Figure 22 - Vertical profiles of total Fe content (mg g^{-1}) from sediments collected in Rosário (ROS) saltmarsh, Tagus estuary

Figure 23 shows the total Fe content exhibited in sediments collected in ALC. For sediments colonized by *H. portulacoides*, values varied between 52.1 and 65.5 mg g^{-1} in the spring and from 32.4 to 43.0 mg g^{-1} in the summer. In *S. fruticosa*, colonized sediments total Fe concentrations were lower in both seasons and ranged between, 11.0 and 42.6 mg g^{-1} in the spring and 32.5 and 40.6 mg g^{-1} in the summer. For non-vegetated sediments, total Fe contents varied between 14.5 and 27.4 mg g^{-1} in the spring and 38.1 and 43.3 in the summer, being the lowest when compared with colonized ones.

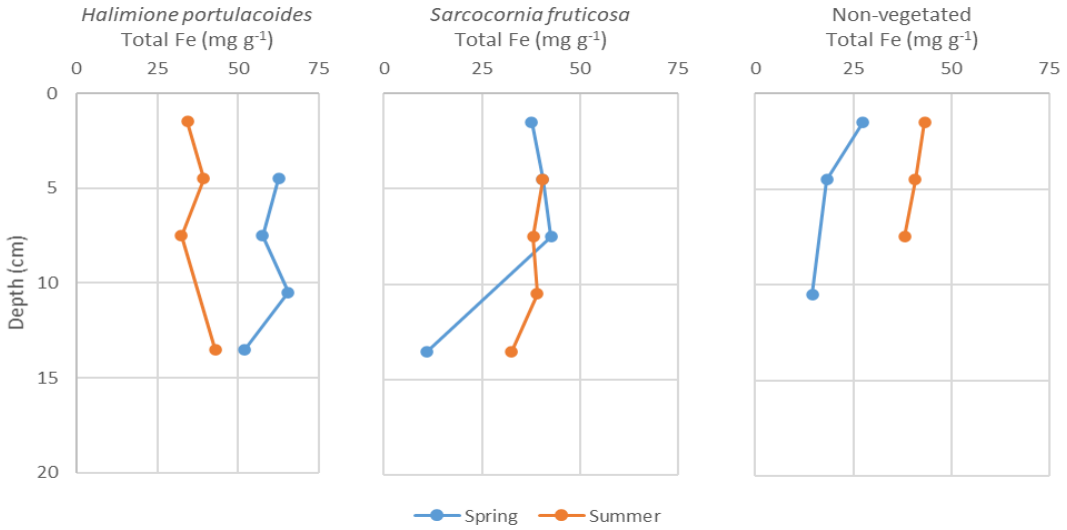

Figure 23 - Vertical profiles of total Fe content (mg g^{-1}) from sediments collected in Alcochete (ALC) saltmarsh, Tagus estuary

Analyzing the total Fe contents in sediments from ROS and ALC, it's possible to say that in ALC the values were more regular, than those obtained for ROS, in which there is a broader range of values and it's possible to see that total Fe content in spring tends to increase at a depth of approximately 7.5 cm and then decreases again at bigger depths. It doesn't seem to exist significant seasonal variations, differences between vegetated or non-vegetated sediments, neither differences between sediments colonized by different plants.

1.5.2 Total Manganese (Mn) content

Figure 24 shows the total Mn content in sediments collected in LAR saltmarsh. For sediments colonized by *H. portulacoides*, values varied between 0.23 and 1.02 mg g^{-1} in the spring and from 0.16 to 0.68 mg g^{-1} in the summer. In *J. maritimus* colonized sediment values varied in a lower range, between 0.22 and 0.30 mg g^{-1} in the spring and from 0.16 to 0.47 mg g^{-1} in the summer. For non-vegetated sediments, values varied between 0.27 and 0.56 mg g^{-1} in the spring and from 0.16 to 0.35 mg g^{-1} in the summer.

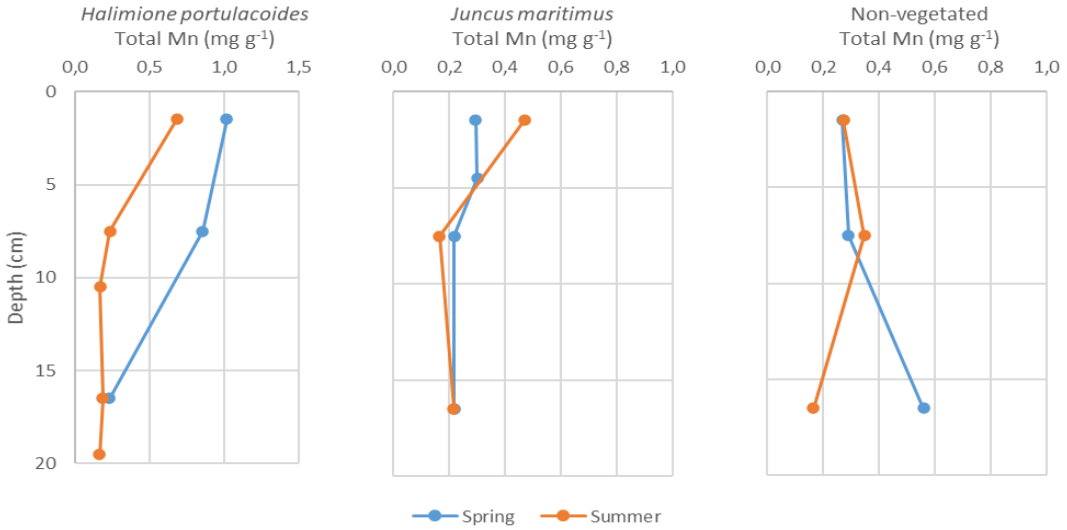

Figure 24 - Vertical profiles of total Mn content (mg g^{-1}) from sediments collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

For samples retrieved in CHE, total Mn concentrations in sediments colonized by *H. portulacoides* ranged between 0.24 and 0.48 mg g^{-1} and 0.18 and 0.20 mg g^{-1} in the spring and summer, respectively. Sediments colonized by *J. maritimus,* exhibited total Mn contents in a similar range in summer and varied between 0.25 and 0.37 mg g^{-1} in the spring. The same pattern was observed in non-vegetated sediments, where total Mn concentrations varied between 0.22 and 0.35 mg g^{-1} in the spring and from 0.17 to 0.21 mg g^{-1} in the summer (Figure 25).

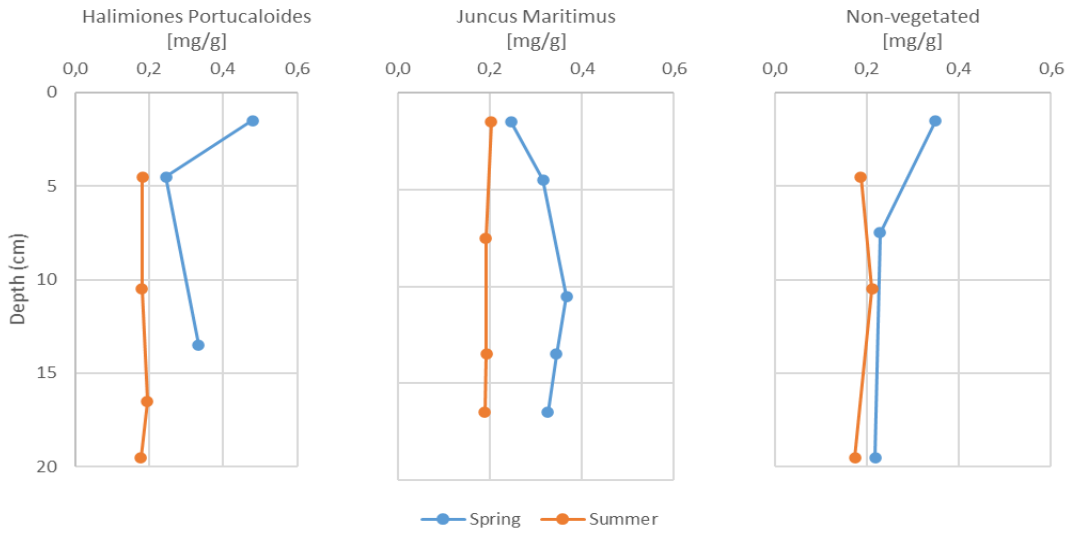

Figure 25 - Vertical profiles of total Mn content (mg g^{-1}) from sediments collected in Chegado (CHE) saltmarsh, Ria de Aveiro

Comparing the two saltmarshes from Ria de Aveiro, it's possible to observe that total Mn content is higher in LAR and that in none of the saltmarshes appears to exist seasonal changes. Values tend to be higher near the surface and don't seem to exist a difference between colonized and non-vegetated sediments.

Figure 26 shows the values for total Mn concentrations in the sediments collected in ROS saltmarsh. *H. portulacoides* colonized sediments presented values between 0.06 and 0.32 mg g^{-1} in the spring and ranged between 0.14 and 0.20 mg g^{-1} in summer. Non-vegetated sediments presented a similar pattern in both seasons, ranged between 0.06 and 0.37 mg g^{-1} in the spring and from 0.17 to 0.22 mg g^{-1} in the summer. For sediments colonized by *S. fruticosa*, values varied between 0.29 and 0.56 mg g^{-1} in the spring and decreasing in summer ranged between 0.08 and 0.15 mg g^{-1}.

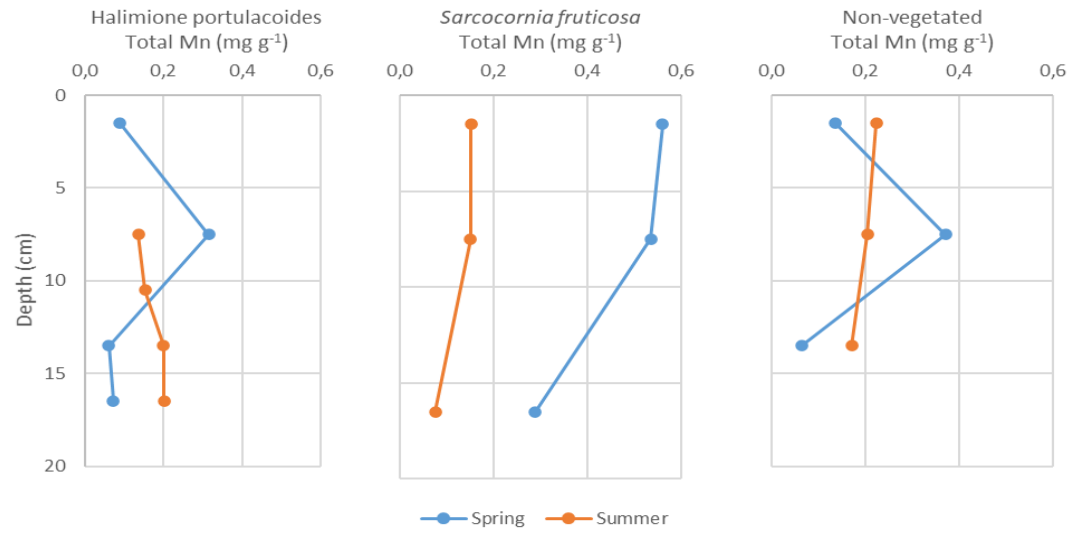

Figure 26 - Vertical profiles of Mn content (mg g^{-1}) from sediments collected in Rosário (ROS) saltmarsh, Tagus estuary

In ALC saltmarsh, the total Mn concentrations in sediments colonized by *H. portulacoides* varied between 0.45 and 1.85 mg g^{-1} in the spring and from 0.61 to 0.83 mg g^{-1} in the summer. In *S. fruticosa*, colonized sediments total Mn concentrations varied in a lower range in both seasons, 0.18 to 0.61 mg g^{-1} in the spring and between 0.11 and 0.48 mg g^{-1} in the summer. The non-

vegetated sediments presented similar patterns in both seasons with the same range of total Mn concentrations (Figure 27).

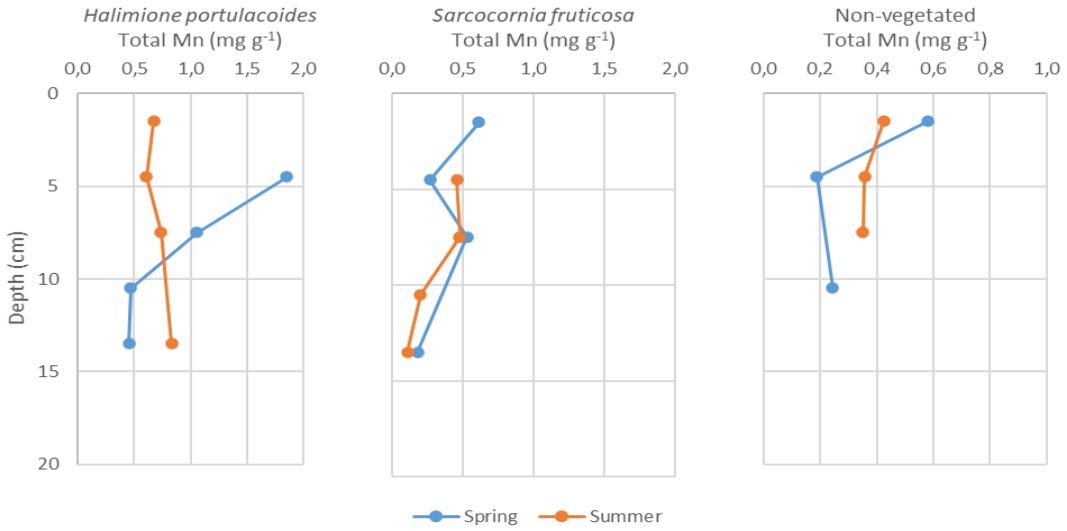

Figure 27 - Vertical profiles of total Mn content (mg g^{-1}) from sediment collected in Alcochete (ALC) saltmarsh, Tagus estuary

Profiles are regular and seem to indicate a higher concentration of total Mn near the surface in the sediments from ALC saltmarsh. The *H. portulacoides* colonized sediments present the highest values in the spring. The same pattern was observed in ROS where higher concentrations of Mn were recorded in the spring between 5 to 10 cm belowground. Total Mn concentrations were lower in comparison with ALC and also don't appears to exist relevant seasonal change, neither a difference between colonized sediments with both plant species and between non-vegetated ones.

The impact of Fe and Mn in Hg biogeochemistry can be of great importance. In this study weren't tested the sediments to determine if conditions belowground were oxic or anoxic. However, a common situation in estuaries and lakes is the formation of an oxic-suboxic-anoxic interface (Skyllberg, 2012). In that interface, Fe can react with H_2S, produced by SRB, occurring the formation of organic thiols by the incorporation of H_2S into organic substances, which can increase the solubility of Hg, due to the formation of Hg-polysulfides (Skyllberg, 2012). The formation of Fe and Mn oxyhydroxides that are known to accumulate Hg is also very important (Gagnon et al., 1997). When Hg is bounded to them, they can act as sink for Hg and prevent it from being release to the water column by precipitating (Canário, 2004), leaving Hg less available for methylation. However, with the changes of redox conditions, Fe and Mn oxides can be subjected to reductive dissolution due to microbial degradation of organic matter and Hg mobility increases (Gagnon et al., 1997), being released back into sediments and pore waters enhancing potential for methylation.

2. Mercury and Monomethylmercury

The first analysis to be done is on the ambient concentrations of Hg and MMHg. They are essential to understand the degree of contamination of the sites and to evaluate the potential for Hg methylation and MMHg demethylation. In this study, because some of the duplicated vegetated cores presented different patterns between each other, it was chosen to represent them both alongside the non-vegetated one. This difference between duplicated cores may exist due to spatial variation. When collecting environmental data, despite trying to obtain similar and close samples, sometimes spatial variation may have a big impact in results.

2.1 Ambient total Hg (THg) concentrations

In table 3 are presented the concentration range of ambient THg found in the saltmarsh sediments of LAR and CHE, in Ria de Aveiro.

Table 3 – Range of ambient THg concentrations (ng g^{-1}) in sediments from Laranjo (Lar) and Chegado (CHE) saltmarshes, Ria de Aveiro, colonized by Halimione portulacoides (HP1 and HP2), by *Juncus maritimus* (JM1 and JM2) and non-vegetated ones (NV)

	Ambient THg (ng g^{-1})			
	Laranjo (LAR)		Chegado (CHE)	
Sediment Cores	Spring	Summer	Spring	Summer
HP1	3428 – 11629	6393 – 27360	289 – 1003	99 – 1514
HP2	5800 – 17892	3117 – 21354	555 – 695	52 – 1321
JM1	10362 – 58525	88 – 18275	659 – 1046	726 – 1942
JM2	853 – 24030	69 – 14148	584 – 1319	1208 – 4263
NV	264 – 29698	466 – 26881	47 – 1588	629 – 4462

First, the results obtained clearly show that LAR saltmarsh is a much more Hg contaminated area than CHE saltmarsh. In LAR, sediments showed some values that were more than ten times higher than those recorded in CHE. This was not unexpected, due to the location chosen for the sampling sites. As mentioned before, LAR saltmarsh is in Laranjo bay, downstream of Estarreja channel a place where effluent discharges of a mercury-cell chlor-alkali plant once took place. Despite the values from LAR being high, they are in line with values obtained in previous studies at the same site (Pereira et al., 1998; Micaelo et al., 2003). The proximity between sites and the high discrepancy in Hg concentrations shows the retention capacity of saltmarshes and the ability of Hg to bind with sediments. Despite having high levels of ambient Hg, contamination problem in Ria de Aveiro is mainly confined to Laranjo bay and so long that don't exist major disturbances to sediments, enhancing re-suspension of contaminants (Pereira et al., 2009), the cycling of mercury doesn't appear to be promoting increases in THg concentrations in places as close as CHE.

Second, the results indicate that ambient THg concentrations generally increase with depth as observed in figures 28 and 29. Sediments from LAR colonized by *H. portulacoides* showed an increase in concentration between 5 to 10 cm depth, coincident with a peak in the amount of belowground biomass obtained in the spring, and then the highest values appear between 20 to 30 cm depth. In the sediments colonized by *J. maritimus,* the increase of ambient THg relative to the upper layers seems to happen between 18 to 21 cm depth (noticeable in JM1-Summer and JM2-Spring cores). However, in the JM1 – Spring core it's observed an increase in THg concentration between 13 to 20 cm, with values of 55335 and 58525 ng g^{-1}. This can be an indication of a preferential layer for Hg accumulation. Soil organic matter exhibits a capacity for strong Hg binding (Aiken et al., 2012) and results show an increase in organic matter in *J. maritimus* colonized sediments at approximately the same depth (Figure 12). Additionally, the amount of belowground biomass obtained in this depth for *J. maritimus* colonized sediments

presented the highest values in spring exactly at the same depth (Figure 16). More belowground biomass raises the organic matter content which in turns increases the accumulation of Hg. This indicates that retention occurs mainly in the rooting sediment layers, which shows the influence of plants in the sequestration of Hg. In a similar study, conducted by Micaelo et al (2003) also in Laranjo bay, higher results of Hg concentration were also found to be related with the presence of roots in the sediments.

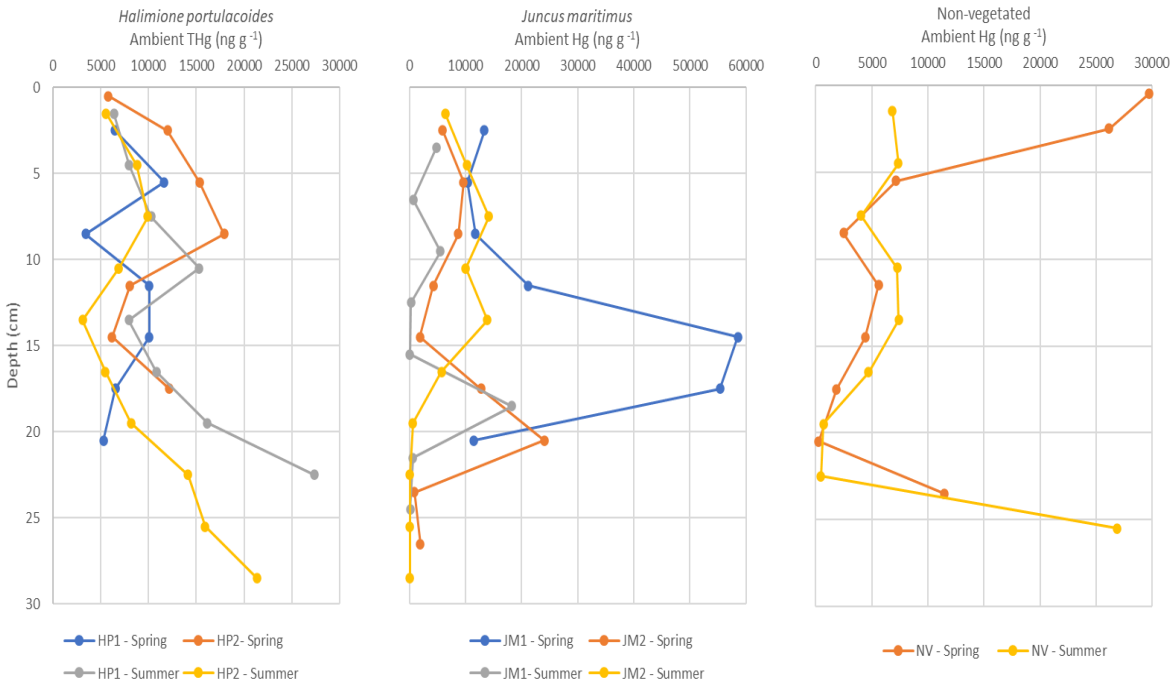

Figure 28 - Vertical profiles of ambient THg (ng g^{-1}) from sediments collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

In CHE saltmarsh, the sediments colonized by *J. maritimus* presented regular values of ambient THg in both seasons until approximately 15 cm and then, for the only core with deeper samples, higher THg concentrations were found at approximately 30 cm depth. The ambient THg vertical profiles of *H. portulacoides* colonized sediments were more irregular and with peaks at 5 cm, 15 to 20 cm and at approximately 30 cm depth. Looking at the profiles obtained for organic matter content it's possible to see that in spring and summer the highest values were recorded at approximately 30 cm in depth, like what happened with THg concentration (Figure 13).

In the non-vegetated sediments, it was expected to find less concentration of ambient THg, yet that wasn't the case. In LAR sediments, values were regular, lower than vegetated ones and similar in spring and summer, between 5 to 25 cm in depth. However, in spring there is a peak in the two first layers of sediment with high THg concentrations of 29698 and 26152 ng g^{-1} and high concentrations were also found at approximately 25 cm depth, which coincides with the higher values of organic matter content (Figure 12). In the non-vegetated sediments of CHE, the amount of ambient THg concentration didn't present significant differences in comparison with colonized sediments. In both seasons there is a steady increase in concentration until 18 cm depth, but in deeper sediments a different pattern was observed. In summer, ambient THg concentration increases to higher levels (4462 ng g^{-1}) at the same depth where is observed and increase of the percentage of LOI (Figure 13) before decreasing to spring-like concentrations and in spring concentrations decrease significantly (47 ng g^{-1}).

One hypothesis for why higher concentrations were normally found in the deepest sediments (25 to 30 cm below ground), despite being colonized or non-vegetated, could be the cease of the contamination source. Natural sedimentation of both saltmarshes can explain why concentrations are higher at bigger depths. Probably, values recorded in past years were closer to the surface and contaminated sediments have become buried. This historical record is consistent with what was found by Pereira et al. (1998).

Finally, it doesn't seem to exist significant seasonal variation. In some cases, the highest values were found in summer, but many times they correspond to depths where only summer samples were collected. According to Stoichev et al (2019), seasonal variations in the concentration of ambient THg found in sediments from Ria de Aveiro, usually follows concentrations changes in geochemical variables when noticeable.

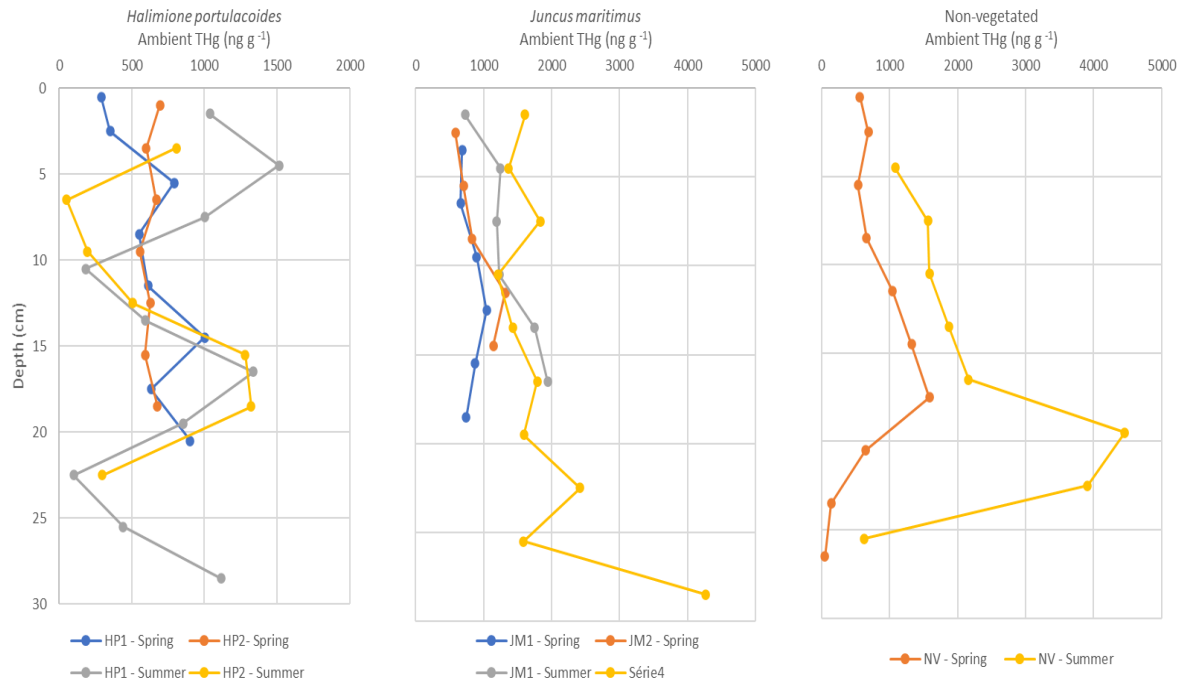

Figure 29 - Vertical profiles of ambient THg (ng g^{-1}) from sediments collected in Chegado (CHE) saltmarsh, Ria de Aveiro

In the following table are presented the range of ambient THg concentrations found in the saltmarshes of Tagus Estuary.

Table 4 - Range of ambient THg concentrations (ng g^{-1}) in sediments from Rosário (ROS) and Alcochete (ALC) saltmarshes, Tagus estuary, colonized by Halimione portulacoides (HP1 and HP2), by *Sarcocornia fruticosa* (SF1 and SF2) and non-vegetated ones (NV)

	Ambient THg (ng g^{-1})			
	Rosário (ROS)		*Alcochete (ALC)*	
Sediment Cores	Spring	Summer	Spring	Summer
HP1	5 – 455	33 – 721	12 – 219	240 – 674
HP2	26 – 843	115 – 2363	7 – 213	184 – 747
SF1	48 – 457	171 – 612	90 – 252	218 – 489
SF2	92 – 890	84 – 3762	9 – 251	244 – 455
NV	49 – 2071	657 – 1227	72 - 186	214 – 388

Comparing the two saltmarshes in terms of degree of contamination, the results are the ones expected from Tagus estuary. In ROS concentrations of ambient THg are higher, showing that the site is more contaminated than ALC – located on the border of the Tagus National Reserve. This degree of contamination is in line with previous studies conducted at these locations (Canário et al., 2007a; Cesário et al., 2017). As expected, the difference in concentration is due to the distance between the contamination source and the sampling site. However, differences are of minor degree that those observed in Ria de Aveiro where locations were closer, showing that morphology and hydrodynamics of Tagus Estuary has a greater impact in the influence of Hg dynamics, for sites with lower contamination (Cesário, et al., 2016).

The results show that variation with depth presented different patterns between saltmarshes. In ROS, ambient THg concentration tends to increase with depth, but in ALC values show an opposite trend (Figure 30 and 31). In ROS, sediments colonized by *H. portulacoides*, showed an increase in concentration starting at 5 cm depth and reaching the highest values at 15 cm depth. This increase was especially high in the HP2 – Summer core. Despite no seen relation with a specific increase of belowground biomass or an increase in organic matter, this depth corresponds to the highest concentration of Fe in the *H. portulacoides* core collected in summer. Fe can play an important role in Hg accumulation. The formation of iron plaques and Fe-oxides concretions around roots was observed by Vale et al (2003) in Tagus estuary as a consequence of the oxidation of iron sulfides and rapid precipitation of Fe (III) (Canário et al., 2007b). The presence of Fe around roots may enhance the accumulation of Hg, because, as previously mentioned, Hg can precipitate with Fe oxyhydroxides. The ambient THg content in sediments colonized by *S. fruticosa* presented similar trends in all four cores until 20 cm depth varying between 32 ng g^{-1} and 880 ng g^{-1}. Below this depth a sharp increase of ambient THg is observed until 3762 ng g^{-1} at approximately 30 cm depth for the only core with samples at deeper depths (SF2 – Summer core). The same pattern could be expected in the other cores of *S. Fruticosa* colonized sediments if they were the same size than this one.

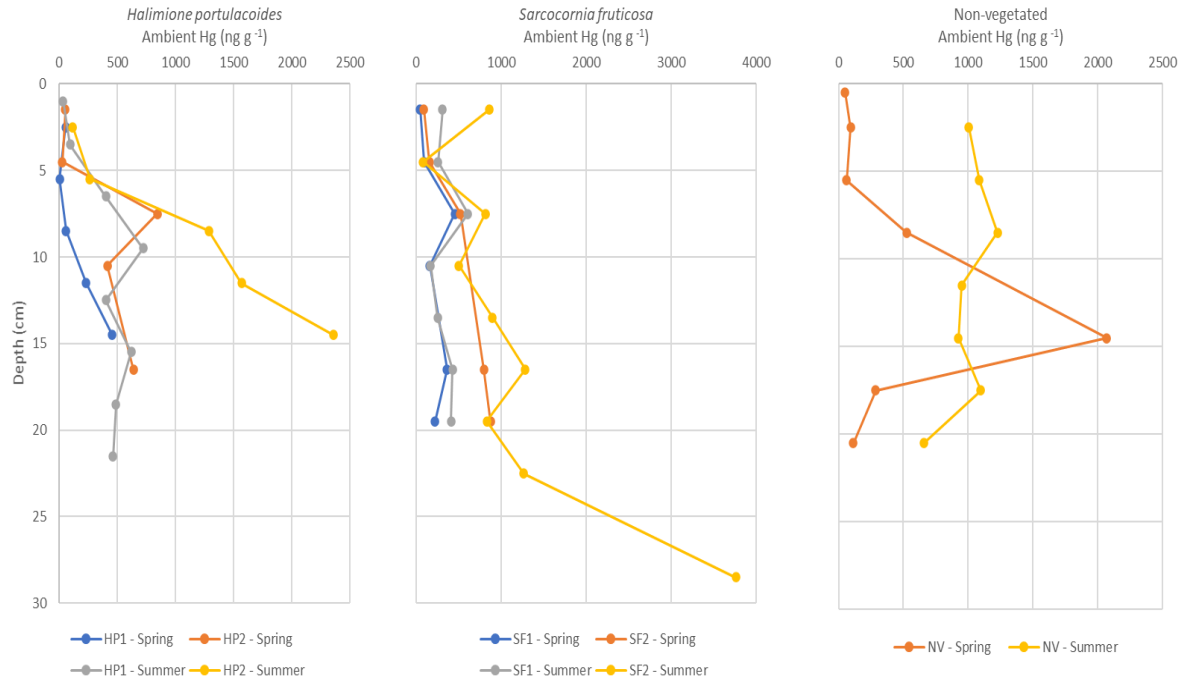

Figure 30 - Vertical profiles of ambient THg (ng g^{-1}) from sediments collected in Rosário (ROS) saltmarsh, Tagus estuary

In ALC, vegetated sediments presented smaller values of ambient THg concentration at bigger depths (Figure 31). The decrease of THg was accompanied by a decrease in organic matter in the case of *H portulacoides* and in the case of *S. fruticosa* colonized sediments, it seems that the decrease in the amount of belowground biomass may have had an influence (see Figure 19). Belowground biomass diminished clearly with depth in both seasons, what can explain higher values of ambient THg near the surface.

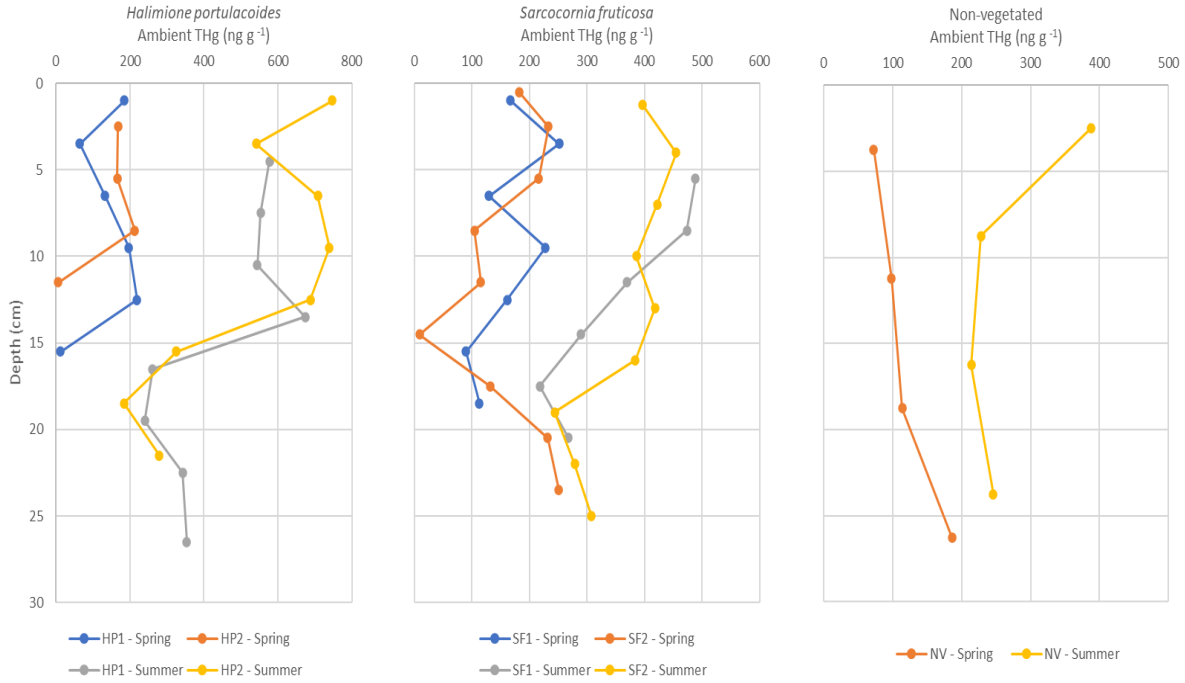

Figure 31 - Vertical profiles of ambient THg (ng g^{-1}) from sediments collected in Alcochete (ALC) saltmarsh, Tagus estuary

In the non-vegetated sediments of ROS, the highest concentration coincides with a peak recorded at approximately 15 cm depth and in the non-colonized sediments of ALC, the highest concentration was recorded near the surface, in the first 5 cm of sediment. Without plant presence, the concentrations in the non-vegetated sediments from ALC were steadier and don't seem to decrease with depth. Comparing the ambient THg concentrations of colonized and non-vegetated sediments, doesn't seem to exist a significant difference, but the range of values for non-vegetated sediments in ALC was smaller when compared with colonized sediments. The different patterns of ambient THg concentration in depth observed on the two saltmarshes could also be explained by historical reasons. Because ROS was closer to the ancient contamination sources, it became polluted earlier and the high values registered at deeper depths are probably related to that period. In the case of ALC, values are closer to the surface because contamination is probably more recent and happened due internal estuarine water circulation resultant from the dynamics of Tagus estuary (Cesário et al., 2016).

Analyzing the results in terms of seasonal variation, it seems that ROS doesn't show any significant differences in values between spring and summer, but in ALC, ambient THg concentrations appears to be higher in summer. Looking at figure 31, concentration within seasons appear to be closer to each other in both replicated cores, with summer ones always having higher ambient THg concentrations. The same pattern was observed in colonized cores and in the non-vegetated one.

2.2 Ambient MMHg concentrations

Table 5 has the ambient MMHg concentrations in the sediment collected in the saltmarshes of Ria de Aveiro.

Table 5 - Range of ambient MMHg concentrations (ng g^{-1}) in sediments from Laranjo (LAR) and Chegado (CHE) saltmarshes, Ria de Aveiro, colonized by Halimione portulacoides (HP1 and HP2), Juncus maritimus (JM1 and JM2) and non-vegetated ones (NV)

	Ambient MMHg concentrations (ng g^{-1})			
	Laranjo (LAR)		Chegado (CHE)	
Sediment Cores	Spring	Summer	Spring	Summer
HP1	6.3 – 17.2	65.5 – 115.5	4.6 – 17.7	9.9 – 18.9
HP2	7.7 – 39.1	34.5 – 101.1	6.9 – 37.5	15.7 – 334.3
JM1	22 – 77.6	143.9 – 260.5	18 – 27.2	25.3 – 42.3
JM2	27.5 – 80.8	75.2 – 256.5	13.8 – 23.4	18.2 – 67.7
NV	8.3 – 27.9	31.4 - 165	3 - 7	2.6 – 12.8

As expected, MMHg concentrations were higher in LAR in comparison with CHE. Concentrations presented a broad range of values, with the highest ones being found in the summer in all sediment cores. In both saltmarshes, appears to exist seasonal changes, with higher temperatures having an impact in MMHg production, especially in colonized sediments. These results are in line with other studies where MMHg concentrations were also found to be higher in summer months (Hiltelmann & Wilken, 1995; Canário et al., 2007a; Monteiro et al., 2016; Cesário et al., 2016; 2017). Warmer temperatures may enhance microbial activity and, as a result, increase the methylation of available Hg to MMHg. Another possible explanation is that demethylation of MMHg could be affected in summer months. In fact, Mason & Benoit (2003) reported that methylation rate in sediments was positively related to temperature while demethylation rate was negatively related.

In the non-vegetated sediments, the increase of ambient MMHg in LAR during the summer season was significant and comparable to the colonized sediments, however in CHE the ambient MMHg concentration only varied slightly between seasons. Various studies already showed that the presence of vegetation in sediments appears to impact Hg methylation and increase MMHg concentrations (Canário et al., 2007a; Cesário et al, 2017). One possibility, that can explain the differences in ambient MMHg concentration between CHE and LAR non-vegetated sediments is that the non-vegetated core from LAR collected in summer, was not completely devoid of roots. In fact, during the sampling procedures, it was observed a big difficulty to achieve a truly non-vegetated sediment core. Despite no presence of aboveground plant parts, many times roots were found in some layers of sediments due to the immense network of roots systems existing belowground. If so, this could be a possible reason why there is a significant difference in ambient MMHg concentrations with the change of season in the non-vegetated sediments from LAR, as opposed to CHE where the non-vegetated cores were truly sediments without belowground biomass.

Comparing the vegetated cores by plant species, the ones colonized by *J. maritimus* normally have higher ambient MMHg concentrations. These happened in both saltmarshes, with different degrees of contamination, which appears to indicate that this specific plant species enables better conditions for the methylation of Hg. In the case of CHE, ambient THg was also found to be higher in vegetated sediments of *J. maritimus*, but the same did not happen in LAR. This can corroborate the previous statement because of the non-existing correlation between THg and MMHg in sediments from this estuary ($r=0.005$, $p>0.05$). *J. maritimus* root system may have greater microbial activity and/or provide better conditions for the methylation of Hg to occur. Its well-developed aerenchyma system could be specifically effective at oxidizing sediments and creating conditions for Hg mobility (Figueira et al. 2012), leaving it more available for methylation.

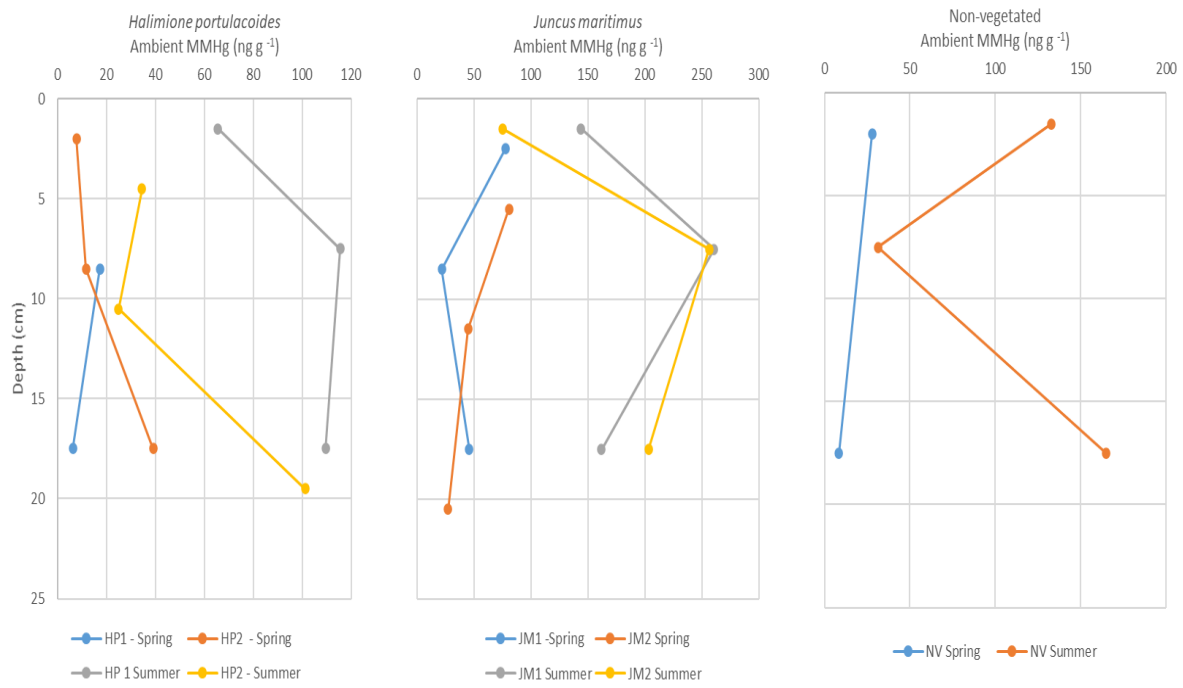

Figure 32 - Vertical profiles of ambient MMHg (ng g^{-1}) from sediment samples collected in Laranjo (LAR) saltmarsh, Ria de Aveiro

In terms of ambient MMHg variation with depth, the higher values recorded in LAR were generally between 5 to 10 cm depth (Figure 32). In CHE, ambient MMHg concentrations were normally higher closer to the surface, in the first 5 cm of the sediment, but it was detected a high concentration of MMHg at ~15 cm depth (HP2 – Summer Core) (Figure 33). In this layer, the percentage of ambient MMHg was 26.1% of ambient THg. This may corroborate the hypothesis that, in colonized sediments, exists preferential layers of retention of Hg or MMHg with optimal zones for methylation (Canário et al. 2007b). The way roots distribute themselves in the sediment can create heterogeneous patterns of sediment redox conditions (Sundby et al., 2003) and improve or not, conditions for microbial activity that will enhance methylation.

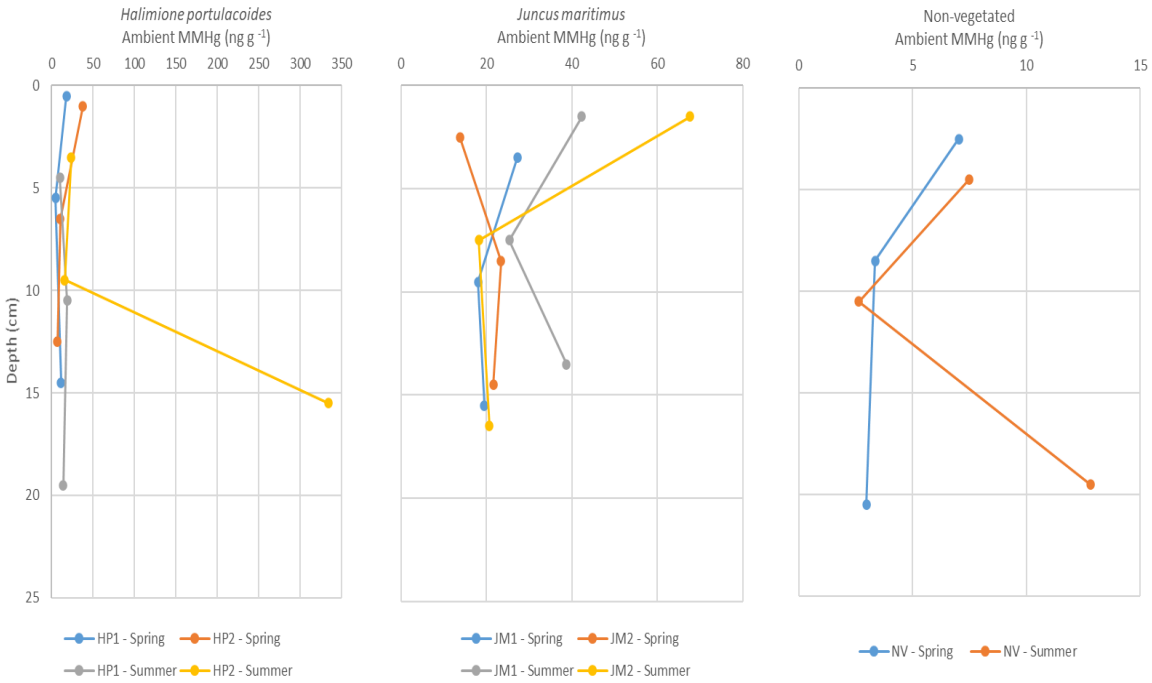

Figure 33 - Vertical profiles of ambient MMHg (ng g^{-1}) from sediment samples collected in Chegado (CHE) saltmarsh, Ria de Aveiro

In the following table are presented the values for ambient MMHg concentrations in the sediments of ROS and ALC saltmarshes from Tagus estuary.

Table 6 - Range of ambient MMHg concentrations (ng g^{-1}) in sediments from Rosário (ROS) and Alcochete (ALC) saltmarshes, Tagus estuary, colonized by Halimione portulacoides (HP1 and HP2), Sarcocornia fruticosa (SF1 and SF2) and non-vegetated ones (NV)

	Ambient MMHg concentrations (ng g^{-1})			
	Rosário (ROS)		*Alcochete (ALC)*	
Sediment Cores	Spring	Summer	Spring	Summer
HP1	1.3 – 6.0	3.7 – 24.0	0.79 – 0.46	1.65 - 6
HP2	3.4 – 4.7	6.4 – 14.0	0.97	5.95 – 8.29
SF1	1.7 – 6.6	20.2 – 131.5	1.88 – 5.36	2.54 – 6.60
SF2	1.4 – 3.0	10.3 – 12.5	1.7 – 3.4	1.71 – 5.63
NV	0.9 – 3.5	14.3 – 42.4	-	1.79 – 17.86

Comparing the ambient MMHg concentrations between both sites, the more contaminated site – ROS – shows higher ambient MMHg concentrations. The relation between seasons observed in Ria de Aveiro is also present here, with ambient MMHg concentrations being higher in summer, which reinforces the hypothesis of higher temperatures promoting favorable conditions for the production of MMHg (Canário 2007a). In ROS and ALC, doesn't seem to exist any significant difference between vegetated sediments and non-vegetated sediments. In the case of ALC, the highest concentration of ambient MMHg was found in a non-vegetated core, representing 4.6% of the ambient THg. In the case of ROS, ambient MMHg concentrations are similar in colonized and non-vegetated sediments in both seasons and the highest MMHg concentration was recorded in a vegetated sediment colonized by *S. Fruticosa* representing 30.4% of the ambient THg (SF1 – Summer core).

Comparing plant species, both seem to present ambient MMHg concentrations in similar ranges in both seasons within each saltmarsh. However, a very high ambient MMHg concentration was found in the sediments of a *S. fruticosa* colonized core collected in ROS (SF1 – Summer Core). Once again, the highest concentration seen was in vegetated sediment, but the unique high value may also indicate that along seasonal variation, there is also spatial variation, that can be a reflex of different structures of the microbial community coupled with geochemical properties of sediments (Monteiro et al., 2016).

Because data is shorter for MMHg, relation with depth is more difficult to determine. In the non-vegetated sediments, values generally appear do decrease with depth, showing higher concentrations of MMHg near the surface. In vegetated sediments, don't seem to exist any specific relation with higher values being recorded closest to the surface and also deeper, in some cases between 15 to 20 cm in depth (SF1 – Summer core) (Figure 34). This maybe a sign of the influence of vegetation. Due to the existence of roots, Hg may be methylated in the rizhosphere at different depths, namely because of their heterogenous distribution that can promote better conditions for microbial communities and consequently for methylation (Canário et al., 2010). Higher values of MMHg near the surface in sediments from Tagus estuary is consistent with the data reported by Monteiro et al (2016).

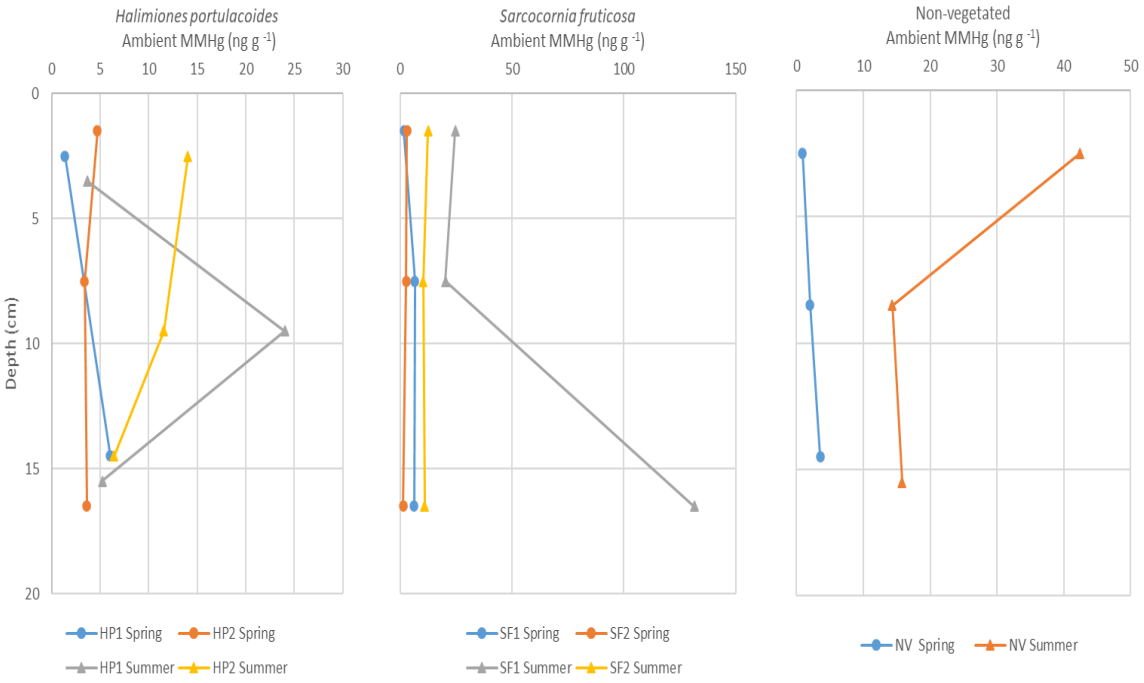

Figure 34 - Vertical profiles of ambient MMHg (ng g^{-1}) from sediments collected in Rosário (ROS) saltmarsh, Tagus estuary

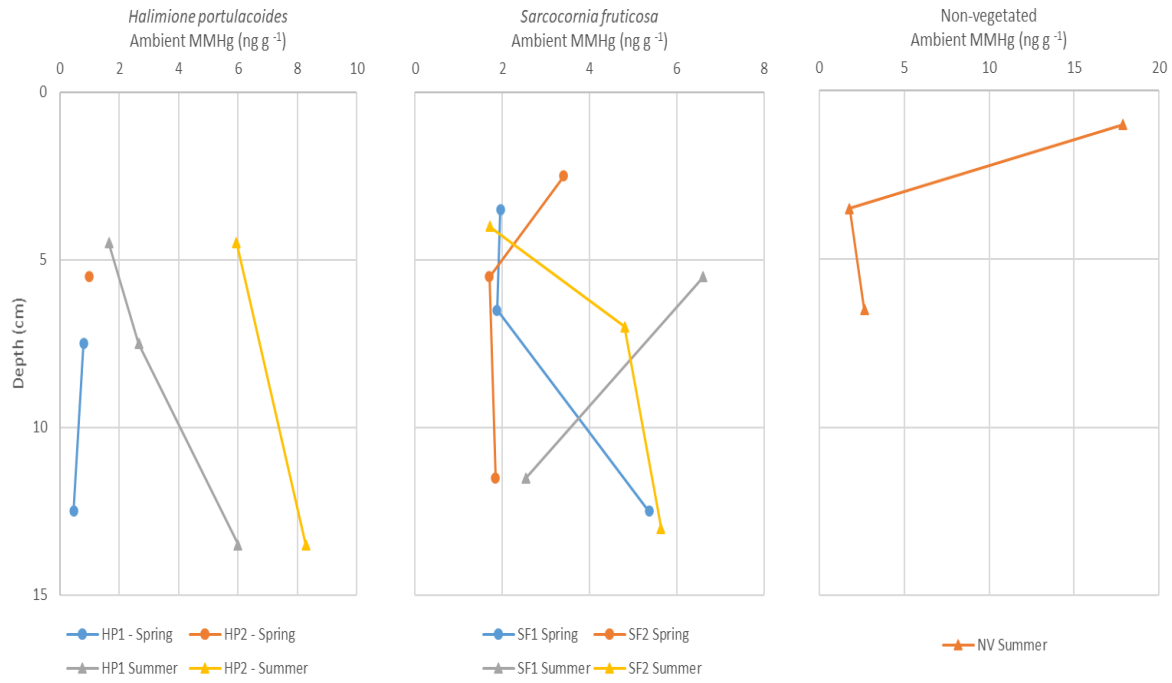

Figure 35 - Vertical profiles of ambient MMHg (ng g^{-1}) from sediments collected in Alcochete (ALC) saltmarsh, Tagus estuary

3. Mercury Methylation and Monomethylmercury Demethylation Rates

The employed method in this experiment allows both methylation and demethylation rates to be directly comparable because they were measured during the same amount of time (5 hours) and in the same amount of sediment. It's not known what the relevant Hg species are in the environment, so it's assumed that the added isotopic Hg behaves in the same way that the Hg species naturally available. In the case of MMHg, because it's subjected to rapid demethylation, not having time to "age", it's thought that the obtained rates should be good estimates for what happens in nature (Cesário et al., 2017). The same behavior is not observed in Hg conversion, exactly because the assumption that the added Hg behaves like the naturally available one, may not be entirely true. However, given the available data, this method is currently the better one to determine the ratio of methylation and demethylation rates. To better understand MMHg concentrations in the environment, a method that allows both rates to be comparable is essential, because the pool sizes of MMHg are controlled by the balance between both Hg methylation and MMHg demethylation (Cesário et al. 2017).

3.1 Hg methylation rates K_M

Table 7 shows the range of methylation rates K_M (day^{-1}) calculated for sediments from the saltmarshes of Ria de Aveiro.

Table 7 - Range of methylation rates K_M (day^{-1}) for sediments collected in Laranjo (LAR) and Chegado (CHE) saltmarshes, Ria de Aveiro, colonized by Halimione portulacoides (HP1 and HP2), Juncus maritimus (JM1 and JM2) and non-vegetated ones (NV)

	Methylation rates K_M (day^{-1})			
	Laranjo (LAR)		Chegado (CHE)	
Sediment Cores	Spring	Summer	Spring	Summer
HP1	0.0042 – 0.0052	0.0381 – 0.1717	0.0031 – 0.0494	0.0086 – 0.1241
HP2	0.0031 – 0.0067	0.0653 – 0.1019	0.0089 – 0.0217	0.0284 – 0.4521
JM1	0.0099 – 0.0168	0.0305 – 0.3120	0.0110 – 0.0347	0.0240 – 0.1640
JM2	0.0045 – 0.0298	0.0770 – 0.2834	0.0482 – 0.0918	0.0157 – 0.0570
NV	0.0054 – 0.0104	0.0517 – 0.1220	0.0087 – 0.0181	0.0014 – 0.1260

Figures 36 and 37 illustrate the methylation rates K_M obtained in the sediments collected in LAR and CHE saltmarshes, respectively. The depths represented are the ones were the spike solution was injected. It's possible to see a trend, with values in summer being significantly higher than those found in the spring.

In LAR, methylation rates had a big increase from spring to summer, in both vegetated and non-vegetated sediments, with a particularly big difference in the sediments colonized by *H. portulacoides.* The highest value recorded in summer (0.1717 day^{-1}) was 25 times higher than the highest value recorded in spring (0.0067 day^{-1}). In sediments colonized by *J. maritimus*, as well as non-vegetated sediments, the highest K_M in summer (JM: 0.3120 day^{-1}; NV: 0.1220 day^{-1}) was approximately 11 times higher than the highest K_M recorded in the spring (JM: 0.0298 day^{-1}; NV: 0.0104 day^{-1}). In CHE, the highest K_M were also observed in summer. However, the discrepancy between seasons wasn't so big. In sediments colonized by *H. portulacoides*, the highest K_M in summer (0.4521 day^{-1}) was 9 times higher than the highest K_M in spring (0.0494 day^{-1}), but in *J. maritimus* was only approximately 2 times higher in summer (0.1640 day^{-1} in summer and 0.0918 day^{-1} in spring) and in non-vegetated sediments was 7 times higher in the warmer season (0.1260 day^{-1} in summer and 0.0181 day^{-1} in spring).

These findings corroborate what was previously mentioned – in several works, that - summer conditions enhance methylation of Hg (Canário et al., 2010; Monteiro et al., 2016). It seems clear that the higher values of ambient MMHg found in summer can be explain by the higher methylation rates. As mentioned before, the enhance of microbial activity is probably the main factor contributing to the raise in methylation capacity. Although abiotic methylation mechanisms are known, they are of minor importance (Ullrich et al., 2001). But even playing a smaller part, the increase of temperature and the presence of humic matter as a methyl donor may also contributed to higher methylation rates (Nagase et al., 1982). The increase in microbial activity is thought to be related with the increase of temperatures, in fact optimal methylation conditions within a cell of a specific type of SRB were reported to be 35ºC and pH 6.5 (Ullrich et al., 2001). Once pH doesn't seem to vary much between seasons in both saltmarshes of Ria de Aveiro, the temperature may be a critical factor. Also, in summer, primary production is maximal which enhances nutrient availability for microbes and greater abundance of organic matter rich in Hg (Canário et al., 2007a). In the case of vegetated sediments, warmer temperatures and more sunlight exposure may favor plant growth and its development below ground. The developing root system in the rhizosphere can create an improve microenvironment where bacterial growth is favored. The optimal conditions for methylation mentioned earlier were also reported to be oxygen sensitive (Ullrich et al., 2001) and changes in the rhizosphere due to O_2 release by plant roots (Sundby et al., 2003) may also contribute to the development of the microbial community.

Comparing methylation rates between the two different types of plants, it's possible to see that the sediments colonized by *J. maritimus* seem to have higher methylation capacity. In LAR saltmarsh, the average values for methylation rates were higher in sediments colonized by *J. maritimus* in both seasons (HP – Spring: 0.0048 day^{-1}; JM - Spring: 0.016 day^{-1}; HP – Summer: 0.0910 day^{-1} and JM – Summer: 0.175 day^{-1}). However, in CHE saltmarsh, the same trend is noticeable in spring (HP: K_M=0.0181 day^{-1} and JM: K_M= 0.0438 day^{-1}), but in summer, sediments colonized by *H. portulacoides* presented a higher average value (HP: K_M=0.1251 day^{-1} and JM: K_M=0.0592 day^{-1}). It's also seen in both saltmarshes that the difference between seasons is more relevant in colonized sediments by *H. portulacoides*. This may be an indication, that this halophyte specie has a bigger development between seasons. In fact, the only data in Ria de Aveiro for belowground biomass that allows comparisons between spring and summer, was obtained in CHE saltmarsh, and shows a decrease in the percentage of belowground biomass in *J. maritimus* colonized sediments from spring to summer (Figure 17). This may explain the smaller differences in K_M between spring and summer in *J. maritimus* samples of CHE saltmarsh.

Evaluating variation with depth, it´s possible to see that in non-vegetated sediments of both saltmarshes, methylation rates tend do decrease with depth. Closer to the surface, microbial communities appear to have better conditions to thrive, but in vegetated samples variation with depth doesn't present the same trend. Higher K_M values may be found at deeper depths which is also a favoring argument for the role of plant roots in Hg methylation. In fact, looking at the results, it's possible to see that the higher K_M are coincident with the highest values of ambient MMHg (JM1 – Summer, JM2 – Summer of LAR and HP2 – Summer of CHE) (see Figures 32 and 33). In fact, this data may indicate that those layers of sediments were probably places of well-developed microbial communities and once both are vegetated samples, the presence of plant roots once again may be a decisive factor.

Another important factor to consider is that K_M from LAR are comparable with those obtained in CHE, although existing a big difference in the degree of contamination between both saltmarshes. This is an indication that ambient THg concentration may not be a decisive factor because not all the Hg found in sediments may be available for methylation.

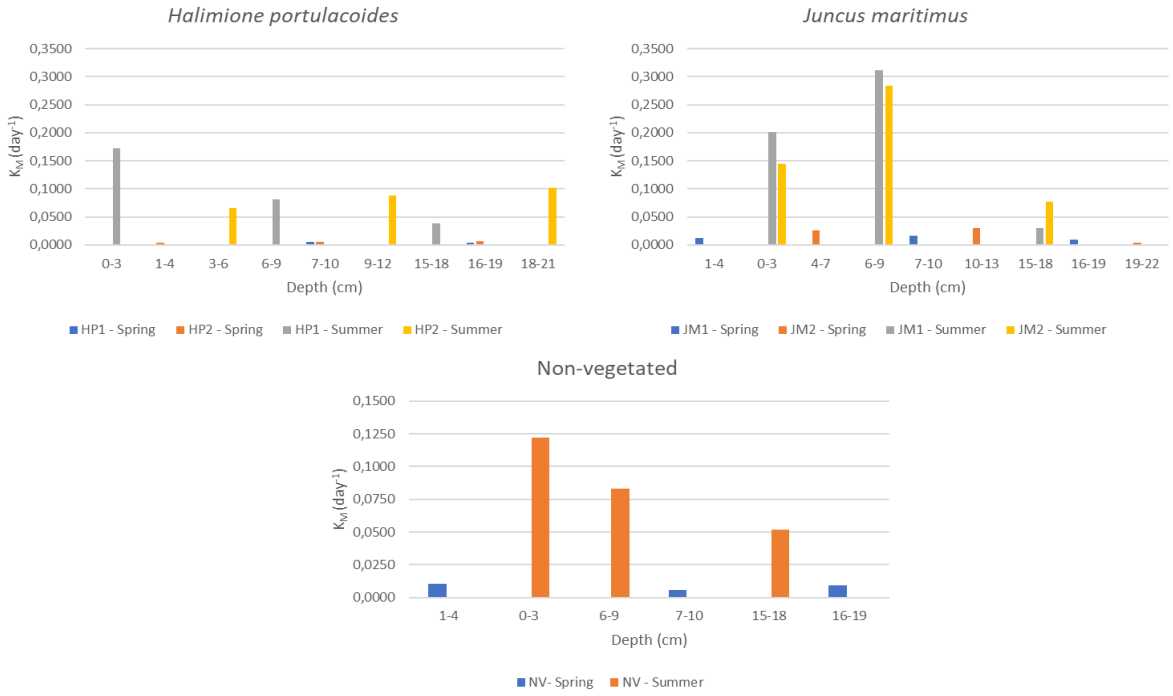

Figure 36 - Methylation Rates K_M (day^{-1}) obtained from Halimione portulacoides and Juncus maritimus colonized sediments and non-vegetated ones in Laranjo (LAR), Ria de Aveiro

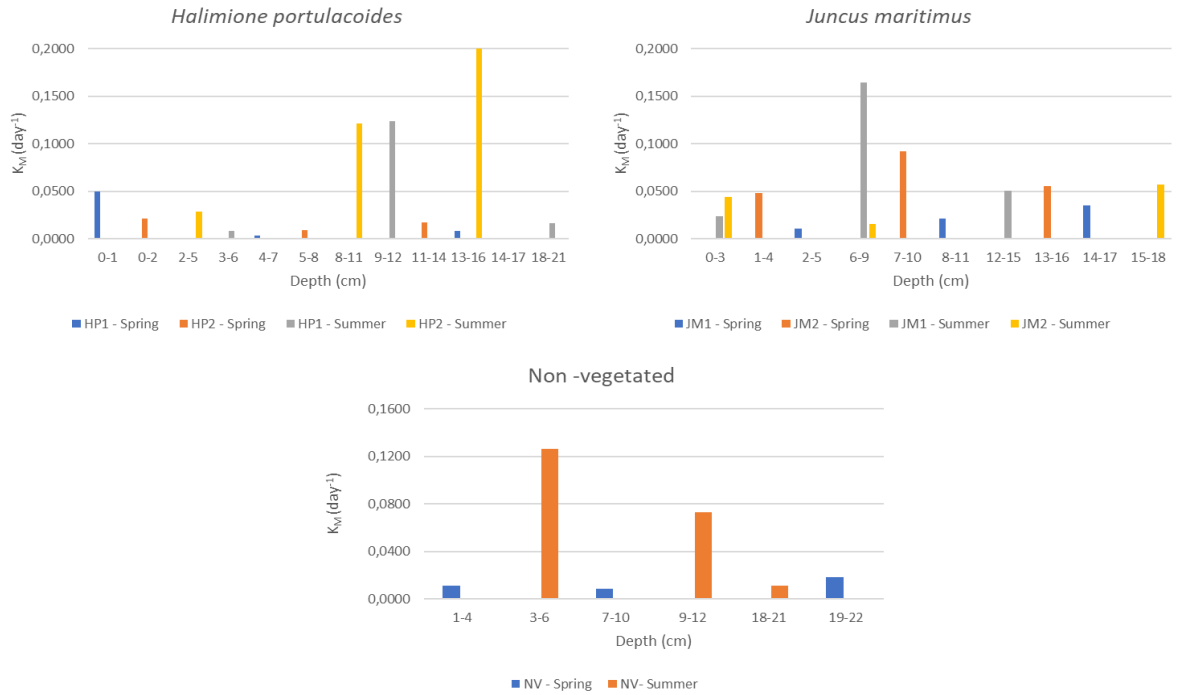

Figure 37 - Methylation Rates K_M (day^{-1}) obtained from Halimione portulacoides and Juncus maritimus colonized sediments and non-vegetated ones in Chegado (CHE), Ria de Aveiro

In the following table are presented the range of methylation rate constant K_M calculated in the sediments collected from the saltmarshes of Tagus estuary (Table 8).

Table 8 - Range of methylation rates K_M (day^{-1}) for sediments collected in Rosário (ROS) and Alcochete (ALC) saltmarshes, Tagus estuary, colonized by Halimione portulacoides (HP1 and HP2), Sarcocornia fruticosa (SF1 and SF2) and non-vegetated ones (NV)

	Methylation rates K_M (day^{-1})			
	Rosário (ROS)		Alcochete (ALC)	
Sediment cores	Spring	Summer	Spring	Summer
HP1	0.0224 – 0.0711	0.0372 – 0.1192	0.0231	0.0093 – 0.0204
HP2	0.0065 – 0.0525	0.0185 – 0.0837	0.0301	0.0041 – 0.0153
SF1	0.0178 – 0.0698	0.0306 – 0.0829	0.0071 – 0.0474	0.0141 – 0.0234
SF2	0.0138 – 0.0281	0.0102 – 0.0328	0.0107 – 0.0169	0.0072 – 0.0370
NV	0.0018 – 0.1518	0.0319 – 0.0611	x	0.0264 – 0.1354

The range of K_M values is very similar in both saltmarshes (0.0018 – 0.1518 day^{-1} in ROS and 0.0041 – 0.1354 day^{-1} in ALC) which, once again, may indicate that methylation isn't dependent on the degree of Hg contamination, but rather on its bioavailability and on the microbial community present in the sediments.

Comparing the obtained values in terms of seasonal differences, it's noticeable that the difference between spring and summer is not so evident in Tagus estuary as it was in Ria de Aveiro. Looking at the results from ROS, in the colonized sediments it was summer samples that presented the higher rates of methylation (HP – 0.1192 day^{-1}, SF – 0.0829 day^{-1}), however the difference between seasons greatly decreases, with K_M values from spring being comparable with those from summer. In ALC the trend is reversed and the higher K_M values in the colonized sediments are obtained in the spring (HP – 0.301 day^{-1}, SF – 0.0474 day^{-1}), except for the SF2 – Summer core. In non-vegetated sediments, samples from ROS showed the highest K_M in spring, but in

ALC isn't possible to make a comparison, although a very high K_M was found in summer. The reason why there are no K_M values for the non-vegetated core from Alcochete is because MMHg concentrations were not measured. When measuring ambient THg, wasn't found the presence of the spike solution and due to limited measuring capacity, it was chosen not to measure MMHg concentrations.

Once again, when performing an analysis between methylation rates and depth there are similarities between the layers with the highest K_M and the highest concentrations of ambient MMHg. This is more noticeable in the sediments colonized by *S. fruticosa* of both saltmarshes (Figures 34 and 35). On the other hand, what was observed in Ria de Aveiro for non-vegetated sediments, doesn't appear to happen in Tagus estuary. Higher methylation rates were not found consistently closer to the surface. The higher methylation rates were found approximately between 5 to 10 cm depth. In ROS, NV – Spring (0.1518 day^{-1}) and in ALC, NV – Summer (0.1354 day^{-1}).

It was also observed that in Tagus estuary the presence of plants doesn't appear to be enhancing methylation, because the highest methylation rates obtained in ROS (0.1518 day^{-1}) and ALC (0.1354 day^{-1}) were for non-vegetated sediments. It was expected that colonized sediments were enhancing methylation, so these values present themselves as unexpected. However, explanations can be proposed. The first is that the non-vegetated core in fact contained roots from a nearby plant. As reported before, when sampling, obtaining a truly non-vegetated core was a challenge. Another possible explanation is that the plant rhizosphere had contributed to the formation of cinnabar (HgS), that can be formed when SRB reduce sulfate to sulfide (Patty et al., 2009). HgS precipitates and can immobilize Hg in the sediment. This biostabilization could leave Hg less available to be methylated, reducing methylation rates in vegetated sediments.

Comparing plant species, *H. portulacoides* seems to better enhance the methylation of Hg. In ROS, it was in a sediment colonized by *H. portulacoides* that was found the highest value in vegetated sediments (0.0837 day^{-1}). Also, the average K_M in sediments colonized by *H. portulacoides* (Spring – 0.041 day^{-1} and Summer – 0.055 day^{-1}) were higher than the average K_M in sediments colonized by *S. fruticosa* (Spring – 0.032 day^{-1} and Summer – 0.036 day^{-1}). In ALC, the same trend is observed in spring (HP – 0.027 day^{-1} and SF – 0.019 day^{-1}) but in summer the trend is reversed (HP – 0.012 day^{-1} and SF – 0.020 day^{-1}) and the highest value recorded in a vegetated sediments was in one colonized by *S. fruticosa* (0.0474 day^{-1}).

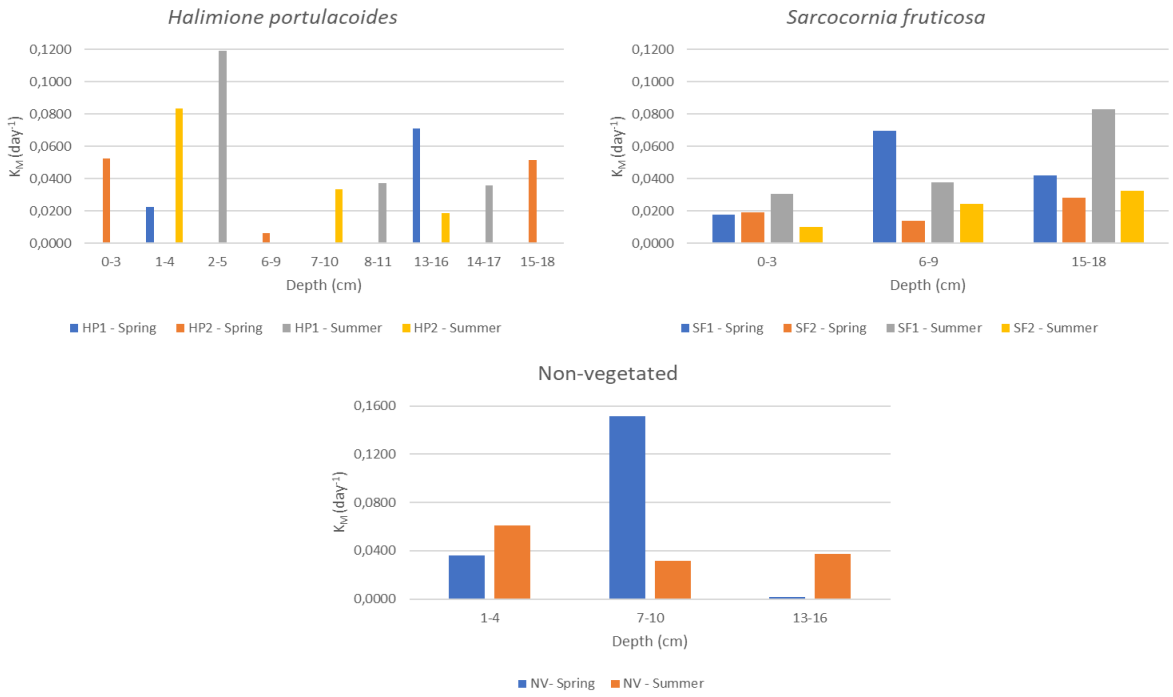

Figure 38 - Methylation Rates K_M (day^{-1}) obtained from Halimione portulacoides and Sarcocornia Fruticosa colonized sediments and non-vegetated ones in Rosário (ROS),Tagus estuary

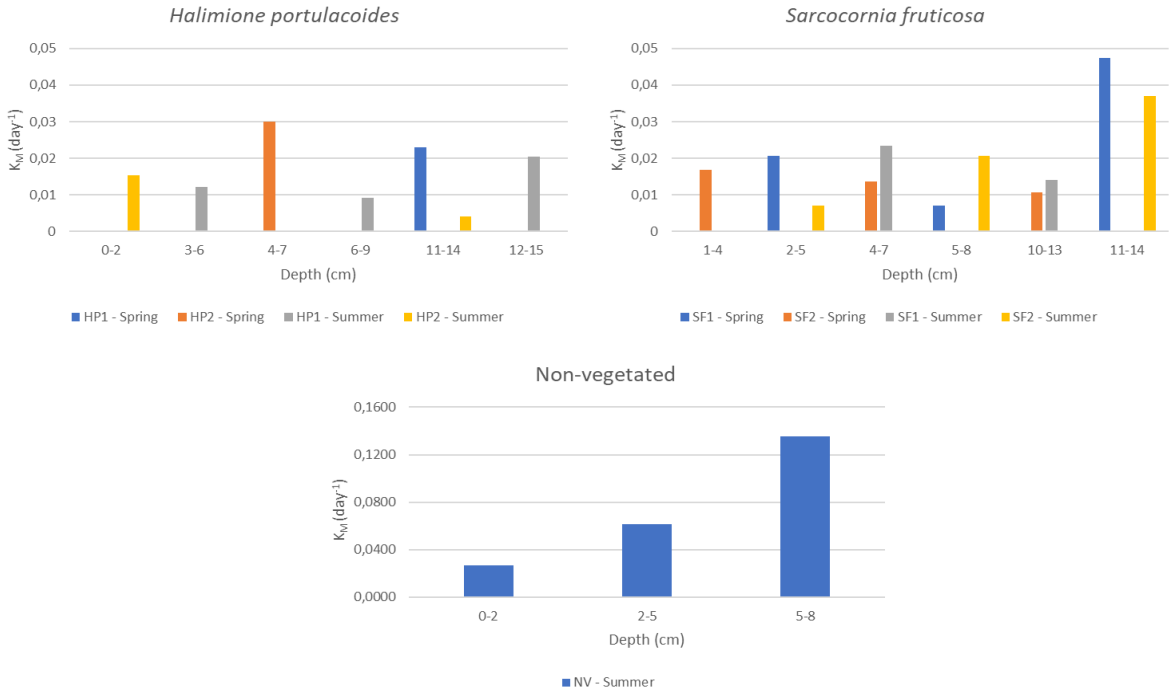

Figure 39 - Methylation Rates K_M (day^{-1}) obtained from Halimione portulacoides and Sarcocornia fruticosa colonized sediments and non-vegetated ones in Alcochete (ALC), Tagus estuary

3.2 MMHg demethylation rates K_D

To evaluate the pool size of MMHg is necessary to understand the demethylation process, because it's also depending on it that the concentration of MMHg and of bioavailable Hg is increased or diminished. Once again, the demethylation rates (K_D) were only obtained for the spiked layers of sediment. Due to technical problems, in this study was only possible to obtain the K_D for some sediment cores from spring season. Despite having smaller amount of data, it's still possible to interpret them and deduce some possibilities.

Analyzing the obtained values, the most significant conclusion is that demethylation rates are significantly higher than methylation rates, sometimes 1, 2 or 3 orders of magnitude higher. These results show the importance of the demethylation process. If MMHg can be demethylated so fast, it means that this process is essential to assure that concentrations of this toxic compound do not raise to higher levels. It also shows the high capacity of the microbial community present in these estuarine environments in the demethylation process. Although demethylation may also occur by photolytic decomposition (Ullrich et al., 2001) in this study that was not consider, because only the top layer of sediments is exposed to radiation and the abiotic process is more relevant in surface waters.

The results don't allow to compare seasonal differences in K_D values and are scarce for evaluating if there is significant difference between K_D values in colonized or non-vegetated sediments, but even so it doesn't seem to exist a difference. Comparing methylation with demethylation rates, it is also possibly to say that high K_M values don't necessarily mean high K_D values. If so, values from ROS and ALC saltmarshes would be significantly lower than those recorded in LAR and CHE, because observed K_M values in Ria de Aveiro were higher than those in Tagus estuary. However, K_D values form both estuaries are comparable. Also, high MMHg concentrations don't appear to influence the K_D values, exactly for the same reason. Ria de Aveiro showed significant higher concentrations of ambient MMHg concentrations when compared to Tagus estuary, but didn't show significantly higher K_D values.

Not having K_D results from summer it's not possible to corroborate the possibility mentioned earlier, in which the increase of MMHg in summer months may be not only the result of the increase methylation but also related with the decrease of demethylation. Further studies will be needed to evaluate that possibility.

Table 9 - Range in demethylation rates K_D (day^{-1}) in sediments from in Laranjo (LAR) and Chegado (CHE) saltmarshes, Ria de Aveiro, during the spring season.

	Demethylation rates K_D (day^{-1})	
	Laranjo (LAR)	Chegado (CHE)
Sediment Cores	Spring	
HP1	5 – 9.6	3.5 – 4.3
HP2	9.1 – 17	1.0 – 4.1
JM1	9.7	2 – 8.7
JM2	5.6 – 13.4	-
NV	5.4 – 7.2	8.6 – 15.2

Table 10 - Range in demethylation rates K_D (day^{-1}) in sediments from Rosário (ROS) and Alcochete (ALC) saltmarshes, Tagus estuary, during the spring season.

	Demethylation rates K_D (day^{-1})	
	Rosário (ROS)	*Alcochete (ALC)*
Sediment cores	Spring	
HP1	6.2 – 11.7	5.2 – 7.8
HP2	12.1 – 25.6	9
SF1	-	4.3-12.9
SF2	7.3 – 13.7	7.8 – 13.6
NV	8 – 20.8	-

3.3 Correlation analysis

To try to see if results obtained (ambient THg, MMHg and Methyation rates K_M) established a correlation between each other or with a specific parameter, several correlations were plotted.

3.3.1 Ambient THg vs Belowground biomass

In this study it was found a linear correlation between the amount of belowground biomass and the concentration of ambient THg in two species of plants (*H. portulacoides*, r=0.782, p<0.01(Figure 40); *J. maritimus*, r=0.826, p<0.01 (Figure 41)). This highlights that the presence of roots in the rhizosphere plays an important role in the accumulation of Hg. However, the lack of relationship between belowground biomass and ambient MMHg concentrations indicate that Hg methylation in the rhizosphere is probably more influenced by other factors, such as: presence of microbial community or the availability of Hg to be methylated. Also, *H. portulacoides* and *J. maritimus* appear to have a higher capacity to retain Hg in sediments around their roots than *S. fruticosa* because no correlation was found between *S. Fruticosa* belowground biomass and ambient THg or ambient MMHg. This is an important result because it shows that different plants deal with Hg contamination in different ways. In this study, it wasn't analyzed the concentration of Hg in plant tissue, but these results may also indicate that these two plants may be more able to uptake Hg. Similar to what was found in previous studies, Canário et al (2010) observed that elevated levels of Hg in colonized sediments can be interpreted as the result of Hg uptake by roots and subsequent retention in the buried litter, as well as incorporation in the abundant organic matter retained in sediments.

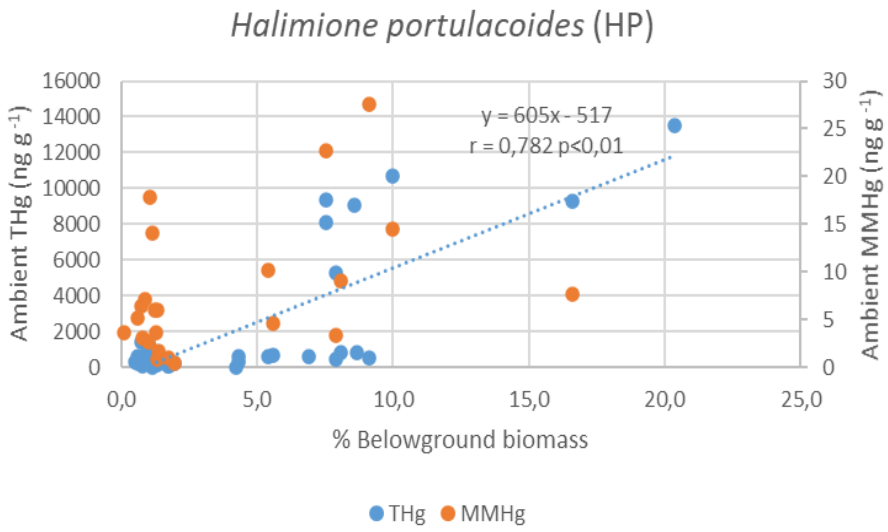

Figure 40 - Correlation between ambient THg and MMHg (ng g^{-1}) with % of belowground biomass in sediments colonized by H. Portulacoides collected in all four saltmarshes (regression line refers to THg vs. belowground biomass).

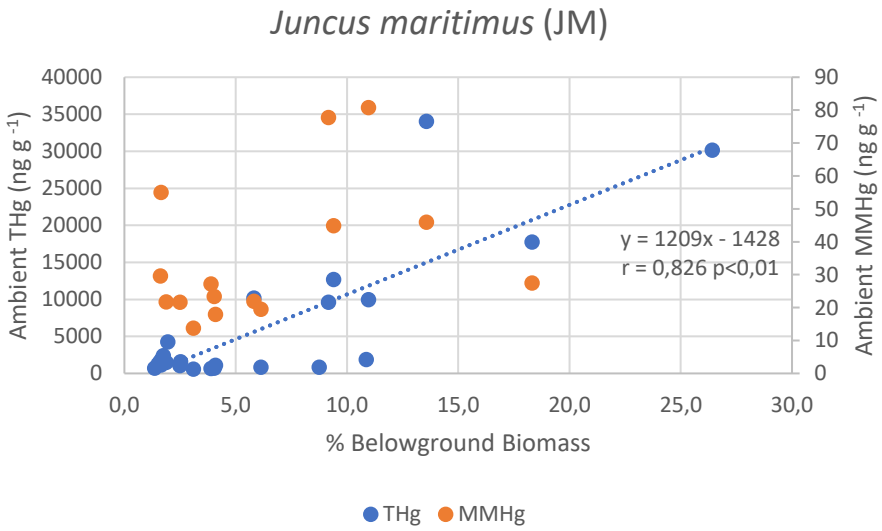

Figure 41 - Correlation between ambient THg and MMHg (ng g^{-1}) with % of belowground biomass in sediments colonized by H. Portulacoides collected in Laranjo (LAR) and Chegado (CHE) saltmarshes, Ria de Aveiro (regression line refers to THg vs. belowground biomass).

3.3.2 Ambient MMHg vs THg

Finding correlations between the amount of ambient MMHg and THg can provide an insight on the bioavailability of Hg in saltmarshes. Hg concentrations not always can be linked with the amount of MMHg present in the environment because its transformation is subjected to bioavailable forms. Inorganic Hg can be heavily present in the environment, but if it's subjected to immobilization, may not be available for methylation. Biogeochemical factors can have a strong influence, as well as plant presence.

In the four saltmarshes of this study, it was only a found positive and relevant correlation between ambient THg and MMHg in ALC vegetated sediments. In the saltmarsh of Tagus estuary, vegetated sediments showed a positive correlation ($r=0.656$, $p<0.05$).

This data suggests that the existing MMHg in ALC saltmarsh is related with the presence of bioavailable mercury. However, this is a low contamination area in comparison with the rest of the study sites and showed fewer concentration of MMHg. This appears to indicate that despite having less THg concentration, the proportion of bioavailable amount could be higher than in the rest of the locations.

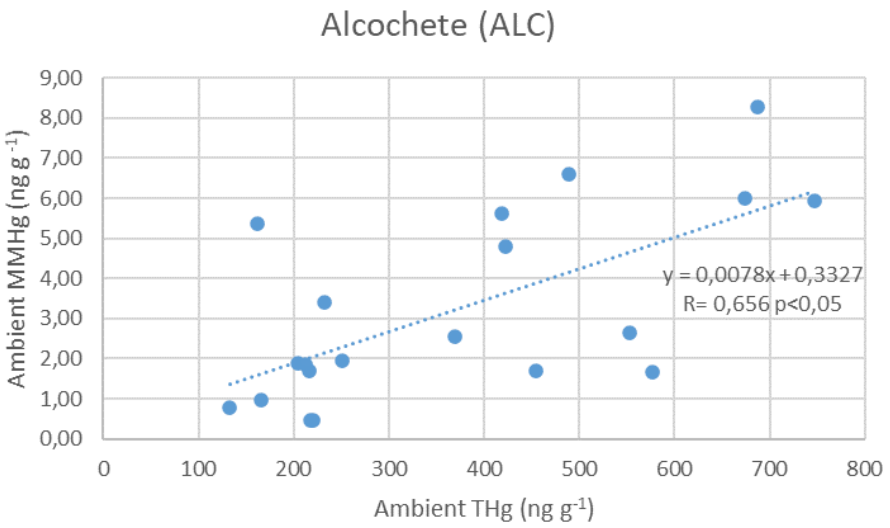

Figure 42 - Correlation between ambient MMHg (ng g^{-1}) and Ambient THg (ng g^{-1}) in vegetated sediments collected in Alcochete (ALC) saltmarsh, Tagus estuary

3.3.3 Ambient MMHg vs Methylation Rates

Analyzing the ambient THg and the obtained methylation rates K_M it wasn't found any correlation between both. This corroborates what was previously mentioned, that the amount of Hg present in the sediments is not a good indicator for the methylation capacity. Ambient THg obtained doesn't allows to know what species of Hg are present in the environment and their ability to be methylated. In fact, this lack of relationship suggests that methylation rates and MMHg content in these saltmarshes sediments are mainly dependent on the environmental and microbiological factors affecting methylation process rather than the availability of Hg (Canário et al., 2010).

However, were found strong and positive correlations between the concentrations of ambient MMHg and the methylation rates in the vegetated sediments of Laranjo. In the most contaminated saltmarsh of this study, ambient MMHg concentrations correlate linearly with methylation rates (LAR, $r=0.771$, $p<0.01$) how it can be seen in figures 43.

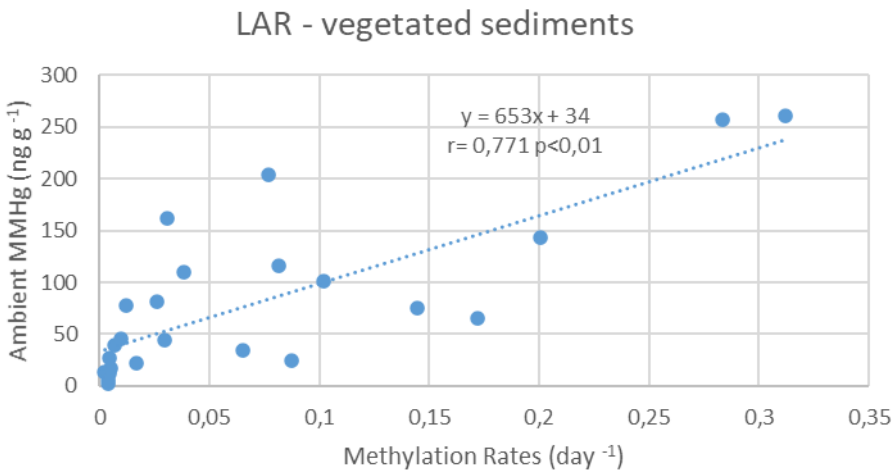

Figure 43 - Correlation between ambient MMHg (ng g^{-1}) and methylation rates K_M (day^{-1}) in vegetated sediments collected in Laranjo (Lar) saltmarsh, Ria de Aveiro.

Once again, the fact that this strong correlation had only been found in vegetated sediments can be an indication of the effect on plant roots in the methylation of Hg. Also, the fact that correlation was only found in the more contaminated site may be a sign that roots influence on the methylation of Hg in sediments may occur only with higher contamination levels (Mendes, 2012).

3.4 Comparison of present study with other published ones

Having obtained the results, it's valuable to compare them with others found in similar studies. In the last years, the effect of Hg in coastline ecosystems has been evaluated around the world to determine levels of contamination and to evaluate possible impacts in the environment. Methylation results may help to provide an insight on how the cycle of Hg is influencing its mobility and speciation and obviously its transformation into MMHg.

The following table presents a comparison of total mercury (THg) and monomethylmercury (MMHg) concentrations and also the methylation rates (K_M) found in the four saltmarshes of this study with other national and international estuarine/coastal systems.

Table 11 - Comparison between results obtained in the present study and similar studies in estuarine/coastal environments for total Hg (THg) concentrations, monomethylmercury (MMHg) concentrations and methylation rates (K_M).

Estuarine/Coastal System		*THg (ng g^{-1})*	*MMHg (ng g^{-1})*	*K_M (day^{-1})*	*Reference*
Ria de Aveiro, Portugal	Laranjo	69 - 58525	6.3 – 260.5	0.0031 – 0.3120	Present study
Tagus Estuary, Portugal	Chegado	47 - 4263	3 – 334.3	0.0014 – 0.4521	
	Rosário	5 - 2363	0.9 – 131.5	0.0065 – 0.1518	
	Alcochete	7 - 747	0.79 – 17.86	0.0071 – 0.1354	
Tagus Estuary, Portugal	Alcochete	148 - 469	0.73 – 0.70	0.0150– 0.0410	
	Rosário	818 - 1590	2.6 – 8.6	0.00290 – 0.0440	Cesário et al. (2017)
Guadiana Estuary, Portugal		301 - 404	0.69 – 1.2	0.0220 – 0.0890	
Adour River Estuary, France		244	2.37	0.0021 – 0.0361	Rodriguez Martín-Doimeadios et al. (2004)
San Pablo bay, Usa		350	5.4	0.014	Marvin-DiPasquale et al. 2003
Baltimore Harbor, Usa		553.5-635.5	0.388-1.08	0.011 – 0.092	Kim et al. (2006)

Looking at the results, it's possible to see that the obtained values can be compared with all of them. Compare methylation rates between different studies isn't a simple process. The conditions in which the experiment was done play a big role in the outcome of the study. Different procedures can have a great influence in methylation and demethylation rates because exist various factors to consider. Incubation time, isotope tracer added to the sample and sediment depth are probable different in all the studies presented. Knowing that methylation is mainly mediated by bacteria, it also needs to be considered all the abiotic factors that influence its activity. Redox conditions, temperature, organic matter, pH (Ullrich et al., 2001) and many other factors that make a comparison between methylation rates obtained in different sites and with different procedures to be something very limiting.

With this in mind, it's possible to see that all the methylation rates of the compared studies fit in the range of methylation rates obtained in this study. However, in the obtained results, the highest values are significantly higher than those found in other sites, inclusively in comparison with values from other study that was conducted in the same locations of Tagus estuary. Comparing the results with those found by Cesário et al., (2017) it's observed that highest methylation rates found in this study are approximately 3 times higher than the ones found by the authors. This could be explained by the different conditions in both studies. Vegetated cores were from another plant and they were only collected in spring. Comparing with the other sites, the results can mean that the locations selected for the present study may present better conditions for methylation or that the higher values obtained may not be entirely true. Another thing to consider, is that none of other locations presents the same level of contamination. Both estuaries selected for this study, were highly contaminated sites and, despite comparison between methylation rates cannot be a good assessment, seems to indicate that there is a possibility that higher contamination may, somehow, influence methylation rates.

3.5 Factors affecting methylation

Here will be presented the factors that may have influenced Hg methylation, based on the results obtained in this study.

- Seasonal Variation

The variation between spring and summer was very different when comparing the saltmarshes of Ria de Aveiro and Tagus Estuary. In fact, methylation rates in spring were comparable and relatively similar in all four saltmarshes, but summer methylation rates varied greatly between estuaries. In LAR and CHE it was noticed a significant difference in methylation rates between spring and summer, with summer presenting higher values. This shows that methylation rates were favored by summer conditions. In ROS and ALC, variation was almost non existing and in some cases methylation rates in spring exceed the ones in summer. However, MMHg concentrations in both saltmarshes of Tagus estuary were higher in summer, which indicates that the methylation was also favored by summer conditions.

- Presence of saltmarsh plants

Once again, differences between estuaries were found when drawing conclusions about the effect of plants roots in sediments. In Ria de Aveiro, sediments colonized by halophyte plants not only presented the highest values of MMHg, but also higher methylation rates. As reported by several authors (Canário et al., 2010; Windham-Myers et al., 2013; Cesáio et al., 2017), the presence of roots in the rhizosphere appears to have an influenced not only in the accumulation of Hg (indicated by the correlation in figures 40 and 41), but also in its methylation. The difference in methylation rates was even more significant in summer, when the two factors enhanced methylation process together. In the saltmarshes of Tagus estuary, plant presence didn't seem to enhance methylation in comparison with non-vegetated sediments. This could mean that plant influence is dependent on the degree of contamination because saltmarshes of Ria de Aveiro presented much higher values of THg when compared with saltmarshes from Tagus estuary.

- Plant Specie

In this study were used three species of halophyte plants: *Sarcocornia fruticosa*, *Halimione portulacoides* and *Juncus maritimus*. With the results obtained, it appears that *J. maritimus* and *H. portulacoides* promoted a rhizosphere environment more adequate for Hg accumulation and methylation when compared with *S. fruticosa*. The highest value of ambient THg concentration and the higher average of methylation rates were found in sediments colonized by *J.maritimus* and the highest MMHg concentration and highest methylation rate was found in sediments colonized by *H. portulacoides.* Because one of the plants in study was present in samples from both estuaries it's also possible to compare the methylation rates in sediments colonized by *H. portulacoides* in different saltmarshes. In fact, when comparing the results, it's possible to see that in all four saltmarshes the K_M values are comparable in spring, but in summer the raise in methylation rates is much more significant in both saltmarshes from Ria de Aveiro. Possible explanations could be related with the type of sediment, the degree of Hg contamination or with biogeochemical factors. For example, the amount of organic matter was higher in sediments of Ria de Aveiro, as well the contamination degree, and the constituents of the sediments were probably very different. The difference in sediments composition may affect the plant capacity to develop its roots belowground and the affinity of MMHg adsorption onto particles (Monteiro et al., 2016). Depending on the type of materials that constitute sediments, as observed for other metals or caused by grain-size effect of the sediment particles (Monteiro et al., 2016), MMHg may have less affinity with the sediment and become less stable.

- Depth Variation

In all the collected sediment cores existed a variation with depth. In the case of Hg concentration, some explanations were already proposed, with highly contaminated places presenting higher levels of ambient THg at bigger depths due to sedimentation. However, higher MMHg

concentrations weren't specifically coincident with the depths of high Hg contamination. This may indicate that exists preferential layers of retention and optimal zones for methylation. In the cases of vegetated sediments, these layers are probably related with root presence and their availability to provide conditions for microbial community. In the case of non-vegetated sediments, the layers may be more related with the availability of inorganic mercury.

- Humidity

Although water content isn't on its own a factor with the ability to increase or decrease methylation or demethylation rates and influence Hg speciation, it may have an impact in other ways. In the saltmarshes of LAR and CHE the percentage of humidity in sediments was significantly higher in comparison with the water content in sediments of ROS and ALC. This means that the sediments of Ria the Aveiro are in presence with significant higher pore waters contents. Dissolved organic matter (DOM) which is ubiquitous in soil, water and sediment environments is relevant for the cycling of metals and the most significant process between DOM and Hg is its effect on its chemical speciation and bioavailability for methylation by microorganisms (Aiken et al., 2012). Higher water content in sediments may reflect higher DOM, which can enable the bioavailability Hg and as consequence produce higher methylation rates. Also, the presence water can enhance the solubility of Hg species making them more easily transported for the aquatic environment.

VII. Conclusions

First, this study allowed to verify the degree of contamination that these four saltmarshes are subjected. In these ecosystems of great importance in the estuarine/coastal environments was once again proved the ability to accumulate Hg and to provide conditions for its methylation.

The results obtained in this study showed that Hg methylation and possible MMHg demethylation rates were affected by seasonal changes and by the presence of saltmarsh plants. The results were not consistent in all four saltmarshes but appear to be similar within the same estuary, which indicates that the difference may reside in biogeochemical Hg sources and other hydrological factors that differ from one to another.

In Ria de Aveiro, sediments colonized by halophyte plants were more able to convert Hg into MMHg, especially under summer conditions. In Tagus estuary, the influence of plants wasn't so easy to determine. In the case of ROS, although the range of methylation rates were similar for vegetated and non-vegetated sediments, the percentage of ambient MMHg relative to THg was in some cases more than 2, 3 or even 6 times higher for colonized sediments than for non-vegetated sediments. In fact, one vegetated sediment from ROS presented the higher percentage of ambient MMHg (30.4%) found in this study. Only in ALC, non-vegetated sediments appear to present higher methylation rates and more MMHg concentration than vegetated ones. However, is important to consider that this may be site specific and a consequence of a complex interaction of a variety of environmental variables, as well as lack of data, once in ALC it was only possible to obtain methylation rates for one non-vegetated core.

Seasonal changes were also more evident in Ria de Aveiro. In LAR and CHE, methylation rates increased significantly with the change of season, but in ROS and ALC saltmarshes from Tagus estuary didn't happen the same. However, ambient MMHg concentrations were also found to be higher in summer which indicates an increase of the methylation capacity with warmer weather.

Hintelmann & Wilken, (1995) stated that THg concentrations may not always be a reliable measure of the supply of available inorganic Hg for methylation processes because bioavailability is dependent of innumerous environmental variables. In this study several facts seem to suggest the same, however due to the differences recorded between Ria de Aveiro and Tagus Estuary and the difference in contamination between both locations, it remains the possibility that the influence of plants and seasonal changes may also be dependent on the contamination degree of the sites. Also, the difference trend observed in ALC in comparison with all other sites, may be a consequence of what was previously mentioned, once it is the less contaminated one.

Evaluating the correlations found, it's possible to see that the results suggest:

- That the density and type of plant root species appears to influence the retention of Hg, but not the retention of MMHg.

- That Hg concentrations may be good predictors of MMHg concentrations but in low contaminated saltmarshes, however further studies are necessary to validate this hypothesis.

- That the good correlation obtained between methylation rates and MMHg concentrations suggest that those levels are more related with the capacity of Hg to be methylated in specific layers and not a retention process. However, this was only observed in the highest contaminated saltmarsh and once again, further studies are necessary to validate this hypothesis.

Considering the results obtained, there is a need to address the importance of managing these ecosystems in a responsible manner. Saltmarshes interact with adjacent ecosystems, namely sea water, to which they export energy-rich materials, nutrients but also pollutants. The export of

Hg or MMHg to the water column may bring serious risks for aquatic environment due to its bioaccumulation capacity. The sites chosen for this study are in very close proximity with large urban areas (Lisbon and Aveiro) and inserted in estuaries with great ecological value that also are important assets in commercial, urban and recreation activities.

Minamata Convention recently highlighted the importance on Hg contaminated sites because of the potential harm it can induce in environments and in human health and made a guidance on the management of contaminated sites. In this document, there is a list of approaches that parties should consider when addressing Hg contamination, such as:

- Site identification and characterization
- Engaging the public
- Human health and environmental assessments
- Options for managing the risks posed by contaminated sites
- Evaluation of benefits and costs
- Validation of outcomes

The realization of this study and the future work suggested ahead can be of valuable contribution in identifying and characterizing contaminated sites, in evaluating possible risks posed by them and in finding possible options for managing its risks. According to Minamata convention, is essential to further understand the biogeochemistry of Hg and its cycle because contaminated sites not only represent a risk itself but are also a source of Hg release. The 2013 Global Mercury Assessment (UNEP, 2013) estimated the release of 8–33 metric tons of Hg per year to water and 70–95 metric tons of Hg to air from contaminated sites.

VIII. Future Work

Recognizing the importance of halophyte plants and saltmarshes in the accumulation of Hg and in the formation of MMHg, becomes of extreme importance to continue to pursue the research in the fate of Hg and MMHg in these environments. Future research should focus on:

- Performing the same study and in the same saltmarshes, but in different seasons (fall and winter) to evaluate the effects of lower temperatures and to apply a truly season variation effect.

- Evaluate the effect of demethylation rates in MMHg pool size and its variation with season and plant effect. Better understanding of MMHg concentrations and their variation along time in saltmarshes is essential to study potential solutions for remediation (e.g. phytoremediation). In this study due to technical issues, demethylation rates were only possible to obtain for very few sediment samples, which didn't allow for proper comparison with methylation rates.

- Evaluate the mechanisms in the uptake of Hg and MMHg by halophyte plants and their contribution to Hg^0 reemission to the atmosphere.

- Study the toxicokinetics of different forms of Hg and their toxicodynamics to better understand their role in the biogeochemical cycle of Hg.

- Study the Hg fluxes in the sediment-water interface to evaluate the transport of Hg and MMHg species into adjacent ecosystems.

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Annex

A.

Table A.1 - Recorded *in situ* temperatures (ºC) of all the sediments collected in Laranjo (LAR) and Chegado (CHE) saltmarshes, Ria de Aveiro

Aveiro								
Laranjo								
HP			JM			NV		
	Spring	Summer		Spring	Summer		Spring	Summer
HP-1	x	22.0	JM-1	25.7	22.1	NV-1	22.7	22.8
HP-2	x	21.3	JM-2	x	21.9	NV-2	21.7	22.9
HP-3	25.8	20.9	JM-3	25.7	21.9	NV-3	20.9	21.8
HP-4	25.7	21.1	JM-4	25.9	21.7	NV-4	20.5	21.4
HP-5	25.6	21.0	JM-5	26.0	21.8	NV-5	20.6	21.3
HP-6	25.8	21.0	JM-6	25.8	21.8	NV-6	20.5	21.4
HP-7	26.0	20.9	JM-7	25.8	21.7	NV-7	20.7	21.4
HP-8	25.7	21.1	JM-8	25.8	21.8	NV-8	21.4	21.4
HP-9	25.9	21.0	JM-9	25.5	21.8	NV-9	20.3	21.3
HP-10	25.6	20.6	JM-10	25.4	21.7	NV-10	21.3	21.3
Chegado								
HP			JM			NV		
	Spring	Summer		Spring	Summer		Spring	Summer
HP-1	25.4	20.3	JM-1	25.1	21.8	NV-1	17.3	22.6
HP-2	25.1	20.4	JM-2	25.2	21.2	NV-2	15.6	22.0
HP-3	25.0	20.6	JM-3	25.0	21.3	NV-3	17.6	21.9
HP-4	25.0	20.6	JM-4	24.9	21.4	NV-4	15.4	22.0
HP-5	25.0	20.6	JM-5	24.8	21.4	NV-5	15.7	21.9
HP-6	25.2	20.6	JM-6	24.8	21.4	NV-6	17.1	22.0
HP-7	25.1	20.6	JM-7	25.3	21.5	NV-7	17.5	22.0
HP-8	25.1	20.5	JM-8	25.1	21.5	NV-8	19.1	22.0
HP-9	25.0	20.4	JM-9	24.9	21.4	NV-9	19.0	22.0
HP-10	24.9	20.3	JM-10	25.0	21.4	NV-10	19.8	22.0

Table A.2 - Recorded *in situ* temperatures (ºC) of all the sediments collected in Rosário (ROS) and Alcochete (ALC) saltmarshes, Tagus Estuary

Tejo								
Rosário								
HP			SF			NV		
	Spring	Summer		Spring	Summer		Spring	Summer
HP-1	22.9	24.2	SF-1	19.3	23.7	NV-1	22.9	29.0
HP-2	X	23.5	SF-2	21.1	22.9	NV-2	22.8	28.0
HP-3	22.6	23.3	SF-3	22.2	22.9	NV-3	22.9	27.2
HP-4	22.1	23.3	SF-4	20.3	22.9	NV-4	22.9	27.1
HP-5	23.6	23.3	SF-5	21.2	22.9	NV-5	22.7	26.6
HP-6	24.0	23.3	SF-6	21.1	22.9	NV-6	22.4	26.8
HP-7	23.4	23.3	SF-7	21.4	22.9	NV-7	22.6	26.9
HP-8	23.0	23.3	SF-8	21.9	x	NV-8	22.8	26.7
HP-9	23.6	23.3	SF-9	22.4	x	NV-9	22.8	26.8
HP-10	24.1	X	SF-10	22.1	x	NV-10	23.0	26.5
Alcochete								
HP			SF			NV		
	Spring	Summer		Spring	Summer		Spring	Summer
HP-1	24.6	30.0	SF-1	24.7	26.0	NV-1	23.4	30.1
HP-2	24.2	29.7	SF-2	25.4	25.4	NV-2	23.0	30.1
HP-3	23.6	28.5	SF-3	28.6	24.8	NV-3	22.9	29.2
HP-4	24.2	27.2	SF-4	28.5	24.2	NV-4	22.9	29.1
HP-5	24.6	26.2	SF-5	28.8	24.5	NV-5	22.9	28,9
HP-6	24.9	25.8	SF-6	28.9	24.4	NV-6	22.8	28,6
HP-7	25.5	25.8	SF-7	x	24.5	NV-7	21.1	27.5
HP-8	x	25.7	SF-8	x	24.8	NV-8	x	26.9
HP-9	x	25.7	SF-9	x	25.9	NV-9	x	26.8
HP-10	x	26.2	SF-10	x	x	NV-10	x	26.8