UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia, Campus Gambelas

Centro de Ciências do Mar & Centro de Investigação Marinho e Ambiental

COMPARING *ZOSTERA* AND *SPARTINA* ENVIRONMENTS IN RELATION TO CARBON BURIAL: A SEDIMENTARY AND GEOCHEMICAL APPROACH FROM RIA FORMOSA.

Natalia Duque Núñez, a4870

Master's thesis

Master integrated in marine biology

Work performed under the guidance of:

Dra. Cristina Veiga – Pires and Dr. Rui Santos.

FARO

2015

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia, Campus Gambelas

Centro de Ciências do Mar & Centro de Investigação Marinho e Ambiental

COMPARING *ZOSTERA* AND *SPARTINA* ENVIRONMENTS IN RELATION TO CARBON BURIAL: A SEDIMENTARY AND GEOCHEMICAL APPROACH FROM RIA FORMOSA.

Natalia Duque Núñez, a4870

Master's thesis
Master integrated in marine biology

Work performed under the guidance of:

Dra. Cristina Veiga – Pires and Dr. Rui Santos.

FARO

2015

COMPARING *ZOSTERA* AND *SPARTINA* ENVIRONMENTS IN RELATION TO CARBON BURIAL: A SEDIMENTARY AND GEOCHEMICAL APPROACH FROM RIA FORMOSA

“The important thing is not stop questioning. Curiosity has its own reason for existing”

Albert Einstein (1879-1955).

ACKNOWLEDGEMENT

Firstly would like to thank Professor Rui Santos for this support, coordination and positive comments, as well as his teaching in some of the courses he lectured and Professor Cristina Veiga-Pires, whose dedication to this study as well as her support, orientation, advices and friendship have been one of the keys for the success of this study. Thank you both for giving me this opportunity to wave great work I would also like to thank Paulo Santana for his support and help he has provided during the laboratory analyses. I would to mention to Isabel Barrote, Monya M Costa, Ana Alexandre and whole team of ALGAE for the priceless help they have offered me, for letting me part of the team and the good moments we have spend at work. I would like mention personally Fernando Canovas for this orientation, dedication and help, as well as, his friendly. Also want to thank Gianmaria Califano for the sampling collection. Furthermore thank to Vera Gomes for her help during the laboratory analyses of elemental composition.

In general thank you so much to all! I still have many things to learn from you. Thank to increase my interest in the science!

With respect to my "Familia gaditana",i cannot forget to thank Emilio, Pablo, Isma, Vito, Susi, Canto, Sara, Helena, Mikel, Borja, Miguelito , Dani, Dani rastas and Laura "chochi". Thank to all of you and those that i am not mentioning for yours friendship and for all the incredible moments and adventures that we have spend together, I would love to travel back in time!!! Part of this family went with me on the adventure of two years in Faro, and I have to thank to Peter, Laura, Sofi and Claudia , as well as Jorge and David for the great moment, friendship, support and help, thank for making me feel at home anywhere. Now where are we going?

Especially, Cucus, thanks for always being my inexhaustible battery and trust me, and Lau thanks to be my wings in this very special years two years, my sisters, I feel lucky! Also i want to thank to my family Angola, Nelson and Domingas. Not to forget my abulense team Bea, Javi, Chory, Mireya, Sandra, Marta, Laura and Puy, whose huge friendship is not affect by the distant. I also want to thank Fuen and Marta for supporting me unconditionally.

Finally, I would to mention to a very special person to me, thank for all the moments and experiences that we have live together and for you help, support and love. Thank for all Javier Gamero! Asqueroso!

I do not to finish without highlighting the two people most important of my life my mother and my sister. It would not have been possible without them. Mom Thanks for helping me, always take care and support me in all my decisions and always lead me to my happiness unconditionally. Ainhoilla, simply, Thank you for existing! We are the best team of three! I love you so much!

I cannot feel more grateful to have had you support Thank you so much everyone!

For my mum. THANK!

RESUMO

Os sumidouros de carbono são reservatórios naturais ou artificiais, nos quais o carbono pode ser acumulado durante um determinado período de tempo. Mangais, sapais, salinas e pradarias marinhas são habitats que têm um papel importante no balanço de carbono dos oceanos e, assim, influenciam o ciclo oceânico. Eles representam um *hotspot* mundial para armazenamento de carbono orgânico (OC). Estes habitats compartilham uma parcela excessiva no sequestro de C em relação aos habitats terrestres. Este OC pode ser encontrado na biomassa viva especialmente enterrada nos sedimentos. A acumulação de OC em sedimentos marinhos fornece armazenamento de C a longo prazo. Esta acumulação OC é influenciada por alguns parâmetros ambientais, tais como, por exemplo, a distância ao continente e/ou o tamanho de grão e pH, assim como o tipo de ambientes de marés.

Devido à falta de dados de deposição de carbono na área de estudo e também para destacar a importância destes ecossistemas no sequestro de carbono, neste estudo pretendeu-se avaliar "sumidouros de C" em relação a estes parâmetros ambientais tais como, por exemplo, a hidrodinâmica marinha relativamente à distancia ao continente, ou o sedimento, relativamente ao tamanho do grão nas diferentes estações de amostragem. Adicionalmente, dois diferentes ecossistemas intertidais da Ria Formosa, *Zostera noltii* vs *Spartina marítima*, foram avaliados. Esta abordagem multidisciplinar e integrada inclui análises biológicas, geológicas e químicas, para melhor compreender os processos que conduzem à acumulação de carbono, conservação ou degradação.

Para tal, foi realizada uma amostragem ao longo de um canal principal e um canal secundário da Ria Formosa, em quatro estações diferentes, sendo que em cada estação os dois ecossistemas foram amostrados. No laboratório, analisámos as características sedimentológicas, onde foi determinado o tamanho das partículas por difração laser e por uso de peneiros, com o obejctivo de estimar o nível relativo de energia presente no ambiente onde o sedimento foi transportado e depositado. A cor dos sedimentos foi analisada em todo o espectro de luz visível por reflectância difusa, permitindo-nos adquirir uma aproximação da composição do sedimento. Também foi estudada a composição mineralógica por difração de raios-X. Por outro lado, foram analisadas as características geoquímicas, o que incluiu a determinação da matéria orgânica e carbonato perdidos por

combustão, análise de composição elementar (OC, IC, IN e ON) através de um sistema de combustão elementar e o raio de C/N foi calculado, para ter uma ideia aproximada da origem ou fonte da matéria orgânica. Também foi determinada a concentração de pigmentos, onde por um lado foram analisadas as concentrações de clorofila e carotenóides, usando uma extração simples com acetona e medidas as concentrações através do espectrofotómetro. Seguidamente, através de cromatografia HPLC, foram analisados os pigmentos específicos. Os resultados destas últimas análises foram, no entanto, não representativos, uma vez que os valores obtidos apresentavam artefactos de degradação, não tendo sido considerados.

O processamento de dados foi realizado utilizando o software estatístico R. Todas as propriedades físicas e bioquímicas de sedimentos foram avaliadas para cada estação e para cada tipo de habitat, avaliando a sua variabilidade. Um estudo ANOVA de dois fatores, sendo um de eles 'estação' e o outro 'tipo de comunidade biológica', foi aplicado a cada variável, de modo a saber se houve ou não diferenças significativas dependentes de cada fator individualmente ou devido ao efeito da interferência de ambos. Nos casos em que se verificaram diferenças, foi usado um post-hoc para determinar a origem da diferença, neste caso usamos o teste de Tukey. O software Gradistat, foi utilizado para calcular o cálculo estatístico do tamanho de grão.

Nos resultados em relação às características sedimentológicas, o tamanho da partícula reflete um gradiente em que o tamanho do grão nas amostras diminui à medida que nos afastamos do canal principal e nos aproximamos das estações do canal secundário. Este gradiente é mais marcado no caso da *Z. noltii* do que no da *S. maritima*. Esta diferença na intensidade do gradiente entre ambos ambientes pode ser devido às diferenças de hidrodinamismo entre os dois meios, uma vez que a *Z. noltii* está mais exposta que a *S. maritma* devido à sua posição no intertidal. Relativamente aos resultados de cor, foi observada a possível presença de várias formas de Fe e goetita devido aos tons do sedimento, apresentando um aumento em ambos os valores ou aumentado o conteúdo dessas substâncias da estação 1 para a 4 para ambos os ambientes. No composição mineral verificaram-se diferenças entre o teor de quartzo e polissilicatos entre as estações, aumentando o conteúdo de polissilicatos e redução do teor de quartzo nas estações mais

protegidas. Também a presença de pirita e siderita poderia explicar os altos valores de matéria orgânica, ao proporcionar um possível ambiente redutor.

Um grande conteúdo em carbonatos foi encontrado na estação 4, podendo explicar-se devido à possível presença de foraminíferos. Em relação ao sequestro dos carbonos, é influenciado por praticamente todas as variáveis estudadas, já que influenciam as características do solo, favorecendo ou desfavorecendo a acumulação de carbono. Como por exemplo a presença de determinados compostos minerais ou substâncias que foram determinadas na análise da composição mineral e cor, que favorecem a agregação de matéria orgânica, ou outras resultando em condições reduzidas permitindo que ocorra uma maior acumulação no sedimento. Nem todas as variáveis mostram o mesmo padrão ou tendência relativamente às estações ou ao tipo de comunidade biológica. Para todas as variáveis estudadas neste trabalho, apenas algumas delas não apresentaram variações em ambos os fatores estudados. A melhoria destas representam diferenças entre estação e entre ambientes e mais da metade respondem à interação deles. Atendendo ao objetivo principal deste estudo, foram encontradas diferenças significativas entre os dois ambientes, mostrando a *S. maritima* aproximadamente o dobro do conteúdo de carbono do que a *Z. noltii*. Esta variabilidade foi relacionada com o tamanho do grão, observando-se uma relação positiva entre a concentração de carbono orgânico e a presença de sedimentos mais finos. Todos os fatores encontram-se influenciados pela composição do solo e hidrodinâmica. Finalmente, quando foi calculada a taxa da acumulação do carbono, *S. maritima* acumula dobro do que a *Z. noltii,* com resultais de valores de 131.8 g $OC.m^{-2}.year^{-1}$, 83.9 g $OC.m^{-2}.year^{-1,}$ respectivamente. Estas diferenças foram relatadas pela influência de todas os parâmetros analisados em este estudo.

Chaves palavras: *Zostera noltii, Spartina maritima,* Ria Formosa, sumideiro de carbono, carbono orgânico, taxa da acumulação do carbono.

ABSTRACT

Carbon sinks are natural or artificial reservoirs in which carbon can be accumulated for a certain length of time. Mangroves, salt marshes and seagrasses beds are habitats that have an important role on the carbon budget of the oceans and thus influence the oceanic cycle. In this study we aimed is to evaluate C storage capacity of two different intertidal environments, *Zostera noltii* vs *Spartina maritima* from Ria Formosa, as well as to evaluate the influence of hydrodynamics and sediment grain size in the C storage. This multidisciplinary and integrated approach includes biological, geological and chemical analyses in order to better understand the processes leading to Carbon accumulation in sediments. For such a purpose, we analyzed and measured the granulometry, color and mineral composition of the sediment, as well as the organic matter, calcium carbonate contents and the elemental composition. The results obtained reflect that the carbon sequestration (organic carbon content), is related to practically all the studied variables, Furthermore, there are significant differences between both biological communities. *S. maritima* shows nearly twice the organic carbon content than *Z. noltii*. On the other hand, the distance to the main navigation channel, a proxy to hydrodynamics, affected all parameters, strongly affected C accumulation, with higher variability in *Z. noltii* than *S. mariima*. C accumulation and sediment grain size were related to this gradient, as expected, where both parameters increased from the first station, close to the main channel, to last station the most remote. The carbon accumulation rate for *S. maritima* environment was twice as high as those for *Z. noltii* environment, 131.8 g $OC.m^{-2}.year^{-1}$, 83.9 g $OC.m^{-2}.year^{-1,}$ respectively, these differences were related to the influence to all the parameters analyzed in this study.

Keys words: *Zostera noltii, Spartina maritima,* Ria Formosa, carbon sink, organic carbon, carbon accumulation rate.

INDEX

INDEX FIGURES **Pag.**

SYMBOLS, ABBREVIATIONS AND ACRONYM

Organic matter: OM.

Calcium carbonate: $CaCO_3$.

Carbon dioxide: CO_2.

Carbon: C.

Organic, inorganic and total carbon: OC, IC and TC.

Organic, inorganic and total Nitrogen: ON, IN and TN.

Hydrogen potential: pH.

Degrees Celsius: ºC.

Sodium Polyphosphate PRS: $(NaPO_3)_n$

Hydrogen peroxide: H_2O_2.

Diffuse reflectance spectrophotometry: DRS.

Visible spectrum: VIS spectrum

X-ray diffraction: XRD.

Angle: θ.

Wavelengths: λ.

Weight total: P total.

Samples weight before to liofilizate: P1.

Samples weight after to liofilizate: P2.

Vegetation weight: P3.

Samples weight before to burn: P4.

Samples weight after to burn at 450ºC: P5.

Samples weight after to burn at 950ºC: P6.

Carbon, Hydrogen, Nitrogen, Sulfur and oxygen: CHNS-O.

Thermal conductivity detector: TCD detector.

Hydrogen molecular or dihydrogen: H_2.

Nitrogen molecular or dinitrogen: N_2.

Oxygen diatomic: O_2.

Carbon monoxide: CO.

Carbon tetrahydride (Methane): CH_4.

Hydrogen hydroxide (Water): H_2O

Sulfur oxide: SO x.

Ion of "x" element: X-.

Nitrogen oxide: NO_x.

Carbon, Hydrogen and Nitrogen: CHN.

A revolution per minute: rpm.

Chlorophyll derivatives: CD.

Total carotenoids: TC.

Analysis Of Variance: ANOVA.

Particle size distribution classification: PSDC.

Station 1- 4 in the environment of *Z. noltii* replicates" a- d": Zn1a…Zn4d.

Station 1- 4 in the environment of *S. maritima* replicates" a- d": Sp1a…..Sp4d.

1. Introduction

1.1. Carbon cycle in marine systems.

The carbon cycle has two important parts. One part is the biological pump, i.e. redistribution of biologically active elements such as carbon, nitrogen and silica in the ocean waters and the second part is the removal of these by deposition and burial in sediments. The formation of organic matter (OM) and calcium carbonate ($CaCO_3$) allows part of the carbon that is fixed by plankton to sink toward the deep ocean and is stored and buried for long periods of time before returning to the atmosphere (Sanchez et al., 2013).

This accumulation and sequestration of carbon is known as biological carbon sink, whenever CO_2 emission is lower than its inputs (Diaz., 2014). The ocean is considered an important carbon sink, which is referred commonly as Blue carbon (Nellemann *et al*., 2009). The publication of the Blue carbon report by Nellemann and co-authors (2009) showed the importance of the marine ecosystems in carbon sequestration. Coastal Marine ecosystems such as Mangroves, saltmarshes and seagrass beds particularly show an elevated carbon sequestration with relation to their global area (Laffley and Grimsditch, 2009). Recent data estimates that these ecosystems are responsible for capturing up to 70% of the organic carbon from the marine environment (Nellemann *et al*., 2009). These ecosystems have a great capacity to sequester and store important amounts of carbon in their sediments. About 20% of the global carbon sequestration is retained by these ecosystems despite occupying only 0.1% of the ocean surface (Duarte et al., 2013).

Seagrass meadows bury carbon at a rate 35 times greater than the terrestrial ecosystems e.g. the tropical forests (McLeod *et al*., 2011). Furthermore, there is a difference between the accumulation time of carbon in the terrestrial forests when compared to seagrass meadows, ranging from decades to thousands of years respectively (Macreadie *et al*., 2014). These ecosystems are adapted to marine life and can be found both permanently or temporarily submerged (Duarte et al., 2013). They provide many ecosystem services. For example, tidal salt marshes protect coastal fisheries of physical energy of the sea and provide shelter for juvenile fish. They provide valuable habitat for a lot of plants, birds and others animals, whose can serve as food resources. They also

provide services and supplies to recreational hunters and fisher, many of which can give indirect economic benefits. They also offer protection from storm surges (Chmura., 2013).

With respect to the accumulation of carbon in seagrass meadows, it is due to breaches, stems and leaves (above-ground biomass), roots (below-ground biomass), and litter and dead wood (non-living biomass) (Grimsdith et al., 2013), as well as to sedimentary organic and inorganic constituents such as bacteria, microalgae, macro algae, detritus and carbonates respectively (Macreadie *et al*., 2014). This carbon is stored over millennia in the sediments. In addition, like the sea level rise, their sediments continue to accrete vertically, and hence do not become saturated with organic carbon; unlike terrestrial ecosystems that reach soil carbon equilibrium with time (centuries or millions of years) (Grimsdith et al., 2013).

Another explanation of the high carbon storage capacity of these environments is their capacity of acting as particulate traps. They develop large canopies that affect the flow of water existing above them, which in turn can modify their environmental abiotic conditions. One consequence of this develop of canopies is the accumulation of sediment and decreasing the resuspension of particles in the background. The formation of these canopies favors effectiveness of benthic subtracts, increasing the deposition surface of the sediment and probability of contact. Another characteristic that increases carbon accumulation is the presence of epiphytes on the leaves, because they increase the roughness of the leaves (Duarte et al., 2013). Thus, the coastal marine sediments are extremely rich and contain the majority of blue carbon (Grimsdith et al., 2013).

To understand this capacity to sequester and store of carbon in the sediment of theses ecosystems, it is important to define carbon stock in an ecosystem, as the amount of carbon stored, which is altered by having accumulation or release carbon in that ecosystem (Macreadie *et al*., 2014). For example, the accumulation of carbon as carbohydrates in the rhizomes can be used by the plant for respiration, or released in the sediments, where the secondary microbial production is supported. The carbon structure of the leaves is decomposed by bacteria and recycled by seawater. These two forms of carbon accumulation have a short residence time and thus cannot be considered part of the carbon stock. However, as the roots and rhizomes carry out their growth in the sediments under

anoxic conditions, they have a low nutritional value for the bacteria (Macreadie *et al*., 2014). This low interest is due to the low concentration of nutrients (nitrogen and phosphorus) in their tissues (Duarte et al., 2013), so their decomposition is very slow and thus they accumulate in the sediments along large periods of time and can hence be considered as part of the carbon stock (Macreadie *et al*., 2014). Some authors have defined that 80% of the production of seagrass is not consumed by herbivorous and the rate of decomposition of this detritus is very low (Duarte et al., 2013). In terms of environmental factors affecting C capture and storage by seagrass, Macreadie and co-authors (2012) proposed some effects such as that of altered physical-chemical states on remineralization rates and the role of bioturbation fauna in facilitating mechanical flux of buried C out of sediment (role of microbial communities, also known as early diagenesis).

In summary, the high carbon storage capacity of seagrass has been explained because of its high primary production, its high capacity to filter out particles from water column and its high sediment retention capability. All this combines with a slow rate of decomposition in a seagrass environment low oxygen sediment and the lack of fires underwater (Fourqurean *et al*., 2012).

On the other hand, there is also an important variation in the carbon capture and storage between species. Carbon sequestration will vary according to their different rates of primary production, their ability to trap allochthones carbon through their canopies, the recalcitrance of the OC in their organs and in accordance with the characteristics of its preservation in sediments. In addition, the sp-habitat interactions influence carbon sequestration such as latitudinal, habitat ranges of temperature variations, sediment type, respiration in the sediment and remineralization (Labery et al., 2013). For example, the biochemical composition of the cell wall and other exterior cellular components among the seagrass meadows are probably different when looking at a high detailed level. These differences may require changes in microbial enzymatic processes of different dissimilative bacteria or fungi (Zak *et al*., 2006). In fact, one sediment profile can result from both aerobic and anaerobic processes. Different suites of microbes and microbial digestive systems may occur depending on the ecology of habitats, with differences between tropical and temperate habitats (Hoppe *et al*., 2002). Therefore, the integration of

different environmental, chemical and biological factors is of fundamental importance in order to decipher their influence on the carbon storage capacity of seagrass. Understanding the causes for the high capacity of seagrass to capture and store carbon is fundamental to manage these ecosystems. Therefore, the seagrass may be even more important to the oceanic C cycle than previously considered (Fourqurean *et al*., 2012).

During recent times, close to 30% of seagrass area worldwide has been destroyed (Waycott *et al*., 2009), which is a major loss of C sink. Increasing the concern of large C leaks to the atmosphere, and thus accelerating climate warming. Current global rates of global loss of these ecosystems are calculated to be 0.7–2% per year (Grimsdith et al., 2013), among the highest rates of any ecosystem on the planet. This loss of seagrass C stocks, like terrestrial environments, is also due to anthropogenic disturbances either directly or indirectly. Indeed, sediments can be dredged, but the most common cause of seagrass disappearance is the degradation of water quality (Fourqurean *et al*., 2012). Thus, when these ecosystems are degraded or destroyed, large emissions of carbon dioxide are produced. This source of carbon dioxide is caused by the oxidation of carbon in biomass and organic content of sediment. This process occurs in the decade after the disturbance, but continues with the continuous oxidation of carbon that has been accumulated in the sediments for millennia (Grimsdith et al., 2013). Such a loss also means a decrease in its services to the ecosystem, such as symbiotic habitat and nutritional base for fish, shellfish and others animals. Another important function being lost with the loss of these ecosystems is the stabilization of bottom sediments, cleaning of the water to the sediments and nutrients suspended in water (Touchette and Burkholder, 2000).

In addition, the most pervasive threat to the remaining area of salt marsh is probably accelerated sea level rise. Implications of this threat are increasing duration of tidal flooding, limiting vegetation production at the lower elevations along the seaward edge of the marsh and the possible disappearance of these marshes due to landward lateral accretion (Chmura., 2013).

According to previous studies the vast majority of database on organic carbon (OC) in seagrass are referred to North America, Western Europe and Australia. In South America and Africa there is remarkably fewer data, as well as in the tropical Indo-Pacific

or southern Europe and thus, given the large spatial extent of seagrass, few data is effectively known (Fourqurean *et al*., 2012).

Because the collected data attributed to the capture of OC in seagrass is very limited, it tends to base almost all the estimates in OC content of oceanic sediments from the Mediterranean *Posidonea oceanica* meadows. The *P. oceanica* however is unusual in its ability to capture C because it can extend several meters below the sediment and persist for thousands of years, which is a massive storage of C. From what it is known so far, there is no other marine grass that has these characteristics. Nellemann and co-authors (2009) recognized that an upper estimate of Blue Carbon sink might exist, which could be due to uncertainties in accumulation rates of different seagrass ecosystems (Lavery *et al*., 2013).

1.2. Characterization of the Ria Formosa.

The studied zone is located in a shallow mesotidal lagoon system with a surface area of close 84 km^2 on the south coast of Portugal (Guimarães et al., 2012). It has important natural biogeochemical cycles regulated by tidal exchanges at the seawater boundaries and at the sediment interface (Newton *et al*., 2003) and producing highly productive tidal environment (Friend *et al*., 2003). This area can be thus classified as rich ecosystem thanks to its lagoon system and barrier islands and its physic-chemical, biological and geological characteristics.

This lagoon system named Ria Formosa is separated from the sea by a 55 km long barrier islands chain which is characterized by the presence of coastal landscapes such as beaches, coastlines of sand barriers, marshes and dunes. Furthermore, Ria Formosa habitats have a varying stability from cohesive to non-cohesive sediments (Friend *et al*., 2003), as it is composed of sand, mud and muddy sands.

The main habitat of Ria Formosa consists of an intertidal zone of about 30 km long and an elevation of 0.4-0.5m above the mean sea level. Andrade (1990) distinguishes three types of intertidal flat, according to distinctive characteristics, such as elevation, sediment type and position within the lagoon system:

- Back-barrier flats located behind the spits: Due to their weak hydrodynamic regime, they consist of fine sediment, and are located at a higher elevation than the other types;
- Flood-delta flats corresponding to the innermost part of tidal inlets: They are essentially sand deposits, often covered by bed forms generated during the flood tide;
- Creek-edge flats as the most common type across the lagoon: They are located along main channels and secondary creeks furthest away from the inlets.

In the lagoon, a distinctive sediment zonation is found moving across the profile from the lower to the upper intertidal environment, with a fining upward trend of the sediments (Friend *et al*., 2003). Saltmarshes are found at ever increasing density as one moves away from the inlets.

The dynamics of the lagoon is dominated by the exchange of water at six entry points linking the lagoon to ocean and coastal ecosystems.

From a more general point of view, Ria Formosa has an average depth of 2 m and a tidal range that varies from a maximum of 3.7 m in spring to a minimum of 0.4 m. The salinity values are in a range between 35.5 and 36.9 throughout the year, except during periods of intense rain and thus salinity may be less than 15. The water temperature varies from 12 ° C to 27 ° C (winter to summer, respectively) (Friend *et al*., 2003).

The aquatic vegetation of the Ria Formosa is distributed spatially in relation to the elevation of the tide. Saltmarsh species such as *Spartina marítima* and *Sarcocornia pernneisse* are found in the upper intertidal area that is only flooded during high tides. *Zostera noltii* seagrass beds, green (*Enteromorpha* spp, *Ulva* spp) and brown (*Fucus versiculosus*) macroalgal mats are found in the lower intertidal flats (Friend *et al*., 2003).

1.3. Objective of the present study.

Since until now, there have been no studies on carbon accumulation in this environments in the Ria Formosa coastal lagoon system, Southern Portugal, the present study aims to fulfil this lack of information and thus to study the importance of the C stocks in two different environments.

Accordingly, the main objective of the present study is to evaluate C and N storage capacity of two different intertidal environments, *Z. noltii* vs *S .maritima* from Ria Formosa, as well as to evaluate the influence of environmental parameters such as the sediment grain size, which is a proxy of hydrodynamics.

In order to reach this main objective, the following questions will be assessed:

- Is there a difference in C sequestration between *S. maritima* and *Z. noltii* habitats?
- What is the relation between OC content and the sediment granulometry?
- What is the relation between OC content and the sediment color?
- What is the relation between the ON and the OC?
- Is there a relationship between the concentrations of pigments and C sink?

This is thus a multidisciplinary and integrated approach that includes biological, geological and chemical analyses in order to better understand the processes leading to carbon accumulation, conservation or degradation.

2. Materials and methods.

2.1. Sampling sediment in the Ria Formosa

Figure 2.1: Sampling sediments in the Ria Formosa in a *Z. noltii* zone (left) and transportation between stations (right).

The sampling was carried out in the main channel and in a tributary channel of Ria Formosa Lagoon system (Fig. 2.1), in four different stations according to a transect along the tributary channel. Stations coordinates are the following (Fig. 2.2):

- Station 1 (36º58’56.65’’N ; 7º53’16.56’’W)
- Station 2 (36º59’20.51’’N ; 7º52’33.08’’W)
- Station 3 (36º59’56.88’’N ; 7º52’45.08’’W)
- Station 4 (37º00’08.22’’N ; 7º52’53.72’’W)

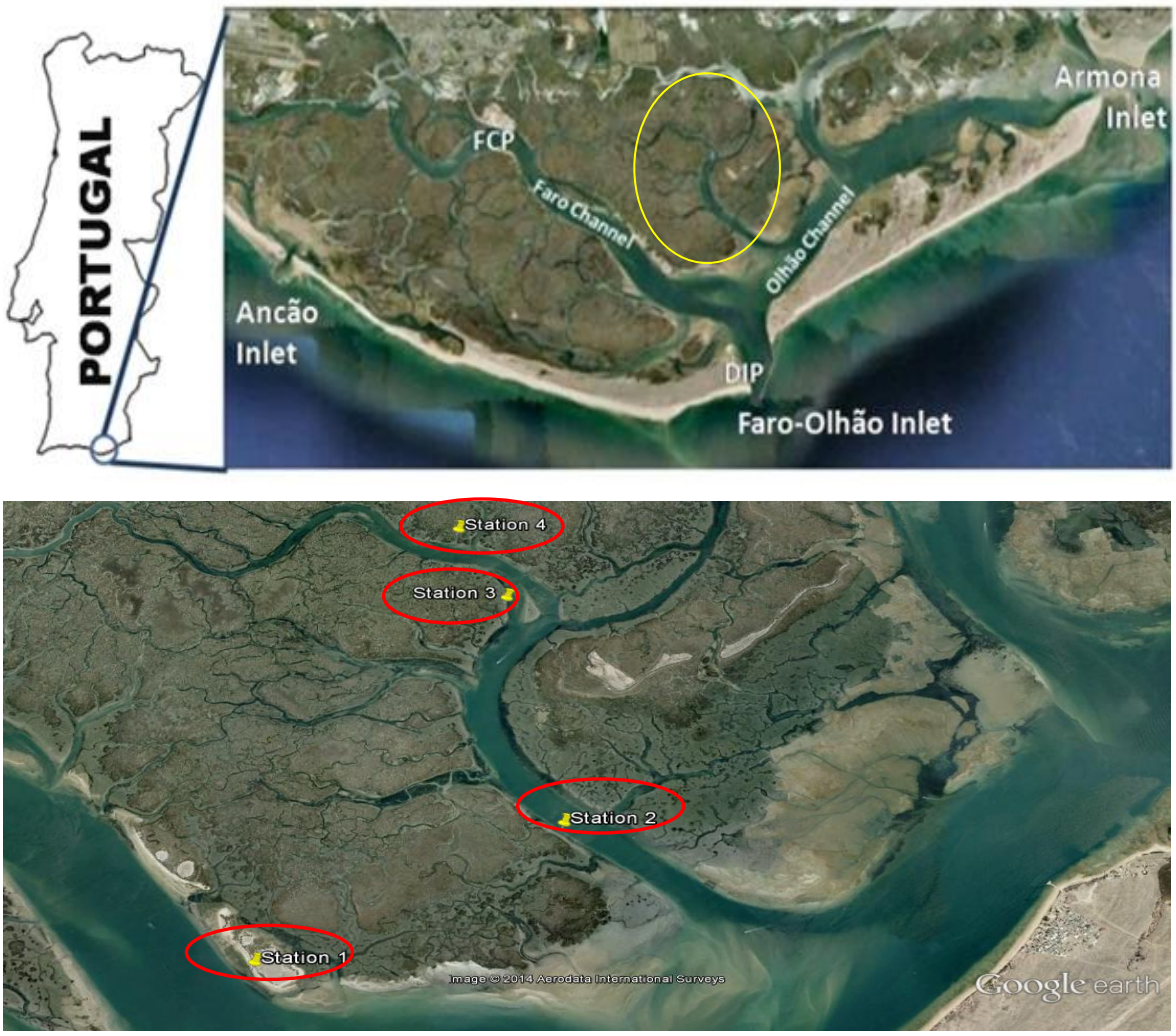


Figure 2.2: Sample location of the Ria Formosa lagoon on the south coast of Portugal (upper image). The Stations were numbered Station 1 to Station 4. Image obtained from Google Earth (lower image).

At each station, two different environments, namely with *Z. noltii* or *S. maritima*, were sampled in order to assess the variability in organic carbon (C_{org}) stock in the respective sediments. Four replicates of each sample were collected using 2.5 cm diameter syringes pushed 8 times into the top 5 cm of the sediment (Fig. 2.3).

Figure 2.3: Photos of the sampling method with syringes showing one replicate sampling with several holes

Station 4 was sampled before *S. maritima* sample from station 3 was collected due fast tidal inundation at station 4.

A calibrated portable pH meter was used to measure in situ pH (NBS scale) at each sampling station. Temperature and salinity were also measured alongside with pH readings (Table 1 in the Annex 1). No measurements of Station 3 for *S. maritima* because the tide was already high

Around 25 ml of each sample replicate was sub-sampled onboard the boat into 50 ml falcon previously prepared for liquid nitrogen freezing. These subsamples were then put into the -80ºC freezer at University arrival. The other samples were maintained in the fridge at 6 °C.

2.2. Laboratory work.

The laboratory analyses required for a basic assessment of C stocks consisted into two distinct analyses:

- Sedimentological Analyses.
- Geochemical Analyses.

Figure 2.4 illustrates the processes carried out in the laboratory for each sample.

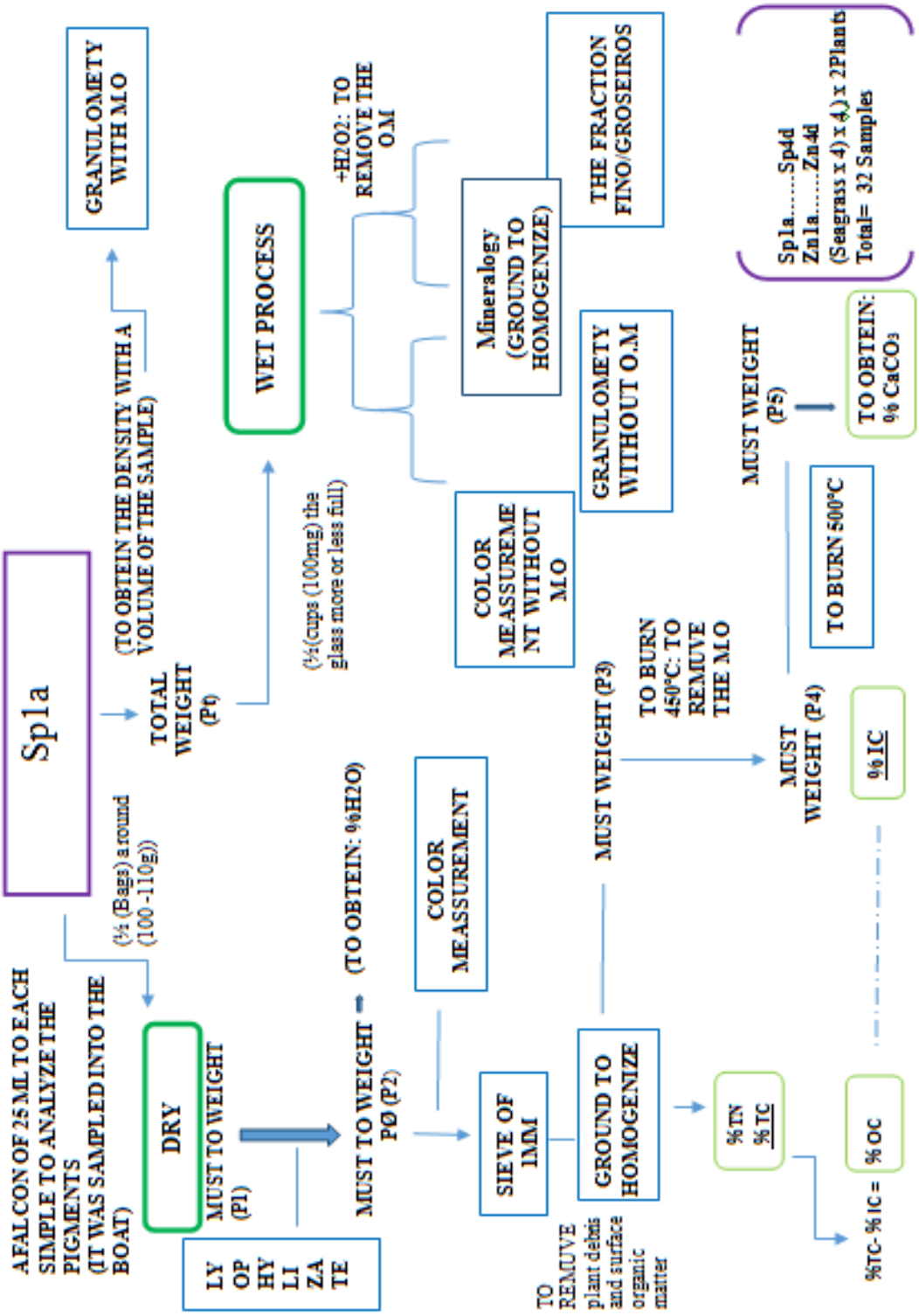


Figure 2.4: Process scheme followed for each of the sample. The example is with Sp1a sample that is the "a" replicate of the sample retrieved at station 1 from *S. maritima* environment.

2.2.1. Sediment Analysis.

In this analysis the granulometry, the color determination and the mineral composition of the sediment were measured, with the following procedures:

2.2.1.a. Sediment granulometry by laser counting and sieving.

Fine particle grain size analysis was performed using a diffraction laser particle-size analyzer, namely through a Mastersize 2000 model APA of 2000 of ©*Malvern Instruments Ltd*., showed in the figure 2.5.

Figure 2.5: Picture showing the Mastersize 2000 model APA of 2000 of ©*Malvern Instruments Ltd.*

This technique is based on the assumption that a particle diameter is equivalent to a sphere that gives the same diffraction as the particle does. This method sees the particle as a two-dimensional object and gives its grain size as a function of the cross-sectional area of that particle (Konert et al. 1997).

The Malvern equipment measures the angle variation intensity of light scattered as a laser beam passes through the sample dispersed in water. Hence large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles. So to calculate the particles size, the angular scattering intensity data is analyzed.

The device has 52 detectors located at different positions. This set of detectors generates a distribution of light intensity. Each detector element emits a signal, which is amplified and digitalized, to an electronic measuring system. This is transferred to a

computer that is appropriate to their analysis using the appropriate software (Gázquez., 2011) in this case the Mastersizer Micro software.

To perform the granulometry we have to prepare 20 L of helix water with an additional 20 g of Sodium Polyphosphate PRS $(NaPO_3)_n$ to disperse the grains. The solution was shacked for 15 min to allow dilution.

A day before performing the granulometry, all samples were prepared in precipitating glasses of 250 ml, by mixing a small amount of sample (a small spoon) with a the solution described above. The roots and plant debris were removed with tweezers as far as possible, because in the Malvern method these elements can induce errors.

Two analyses were made, one in which the samples still contained their organic matter content and the other after its removal using peroxide (H_2O_2). This last process is slow, needing several days for a complete oxidation of the organic matter.

This analysis allowed estimating the relative energy level present in the environment when the sediment was transported and deposited.

On the other hand, fine to coarse grain ratio was done using a sieve sequence of 1 mm and 63 μm mesh. This allowed assessing the proportion of sand present in each sample (Fig. 2.6).

Figure 2.6: Sediment granulometry by the sieving process for evaluating the coarse grain ratio.

2.2.1.b. Diffuse Reflectance Spectroscopy for color determination.

Visible light diffused reflectance spectra was determined with a spectrophotometer Colortron^{TM}. This spectrophotometer is a device that measures the color at the surface of

solids and transfers it to the ColorshopTM program. This technique can use various color scales such as CIE Lab, CIE RGB, CIE XYZ, among other scales.

In this study of color we used the CIE L * a * b * system (Commission International de l'Eclairage) designed in 1976. Where: L* axis is the lightness, the minimum value being 0 (black) and maximum 100 (white); a* axis from red (positive values) to green (negative values) or zero (neutral); b * axis from yellow (positive values) to blue (negative values) or zero (neutral).

Diffuse reflectance spectrophotometry (DRS) is a rapid and nondestructive technique, which is based on the percent reflectance of a sample relative to white light (e.g. Font et al., 2013.)

The device is designed to scan the light reflected from the sample surface, using a white standard such as barium sulfate. With the characteristic spectral signature of some minerals of geological materials in this wavelength range may be qualified (Balsam and Deaton, 1991) and quantified (Balsan and Deaton, 1996) or even identified (Balsam et al., 1998) namely for materials from the VIS spectrum (metal oxides, hydroxides and sulfides).

Two analysis were made, one in which the samples still contained organic matter and the other after removal of organic matter using H_2O_2. Color fluctuates in function of grain size, the sediment composition and humidity, hence three measurements were done on each sample in order to account for the sample heterogeneity.

2.2.1.c. Mineral identification.

The mineralogy of each sample was determined using a X- Ray Diffractometer "X´pert" **(Fig**. 2.7) as described in Pozo et al. (2010). This will allow determining the origin of the mineral fraction (detrital vs antigenic).

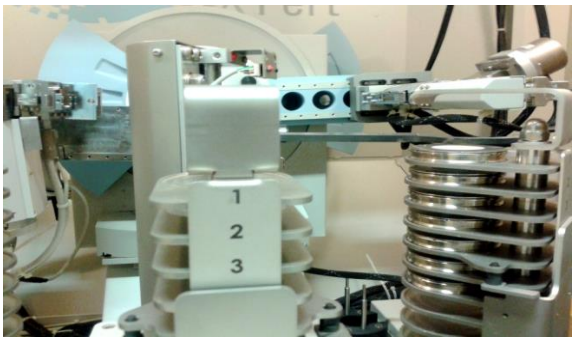


Figure 2.7: Full X- Ray Diffractometer "X´pert" (left) and its autosampler (right).

X-ray diffraction (XRD) is the primary, non-destructive tool for identifying and quantifying the mineralogy of crystalline compounds. Every mineral or compound has a characteristic X-ray diffraction pattern whose 'fingerprint' can be matched against a database. The diffraction traces produced by individual constituents and highly complex mixtures can be interpreted by modern computer-controlled diffraction systems.

Monochromatic X-rays are projected onto a crystalline material at a characteristic angle (θ). Diffraction occurs when the distance traveled by the rays reflected from successive planes differs by an integer (n) of wavelengths (λ) (British geological Survey, 2015).

Around 2 g of sediment were placed in two containers grinding with 6 ml of alcohol. The mixture was ground for 10 min. Once ground, the content was poured into a petri plate. The petri allowed to dry the powder in an oven. Sample was then homogenized in an agate mortar and finally, in order to start the measurement, the sample was placed on own supports for analyzing RX. (**Fig. 2.8**)

Figure 2.8: The figure shows the process carried out for each sample for mineralogical analysis as described in the text, namely: the grounding equipment, the two containers and resulting wet fine sediment, the drying petri dishes in the oven and the agate mortar.

2.2.2. Geochemical analysis:

At first, each sample was weighed (P total) and its density (g ml^{-1}) was calculated as the weight divided by the total sampled sediment volume.

Samples were weighted before (P1) and after lyophylization (P2), which was realized using Savant lyophilizer Module. The process consisted in lyophilizing the samples during 42 h to remove the water. The percentage of water was then calculated based on the weight difference.

After the lyophilization, the samples were passed through a sieve of 1mm in order to assess the vegetables percentage (P3). Dried and sieved samples were then homogenized using a ball mill (FRITSCH, planetary micro mil, pulverisette 7) (Fig. 2.9). Samples were split into two parts, or subsamples in order to analyze their Elemental composition, and to determinate their organic matter and carbonate contents

Figure 2.9: Homogenization process using a ball mill (FRITSCH, planetary micro mil, pulverisette 7): Agate containers with balls (left), ball mill equipment (center), resulting powders (right).

2.2.2.a. Determination of Organic matter and carbonate contents by Loss of Ignition.

First, about 1.5g of dried and homogenized sample was weighted to know the exact weight (P4) before burning. Then samples were burnt to ash in a furnace at 450° C for 2 hours, and the burnt samples were then weighted (P5) again to obtain the percentage of organic matter by differences of weights. In the next step, samples were burnt at 950ºC for

2 hours and then weighted (P6) again to obtain the percentage of carbonates by weight differences.

2.2.2.b. Determination of Elemental composition: OC, IC, ON, IN and C/N.

The measurement of carbon and nitrogen contents in sediment was done following procedures described by Campbell and co-authors (2014), using a Costech 410 Elemental Analyzer (Fig. 2.10).

Figure 2.10: The Costech 410 Elemental Analyzer.

The Elemental Combustion System is based on an automatic analytical unit whose operation, from sampling to signal detection, is microprocessor controlled. Helium carrier gas circulates within the analytical circuit which consists of a combustion reactor for Carbon, Hydrogen, Nitrogen and Sulfur (CHNS) or a pyrolysis reactor for Oxygen. The carrier gas brings the products of combustion or pyrolysis to a gas chromatographic separation column and TCD detector for CHNS-O analysis (Costech, Analytical Tecnologies. 2014).

For this analysis, the sample was weighted into a tin capsule and introduced into oxygen-rich environment through an auto-sampler, and then the combustion process occurs. After the entire sample combustion, the gases (N_2, CO_2 and H_2) are irreversibly adsorbed, and each component is determined in thermal conductivity detector.

The reactions (**Formula 2.1)** involved in combustion process are based on the following reaction:

$R\text{-}N + O_2 \longrightarrow N_2 + NO_x + O_2 + CO + CO_2 + CH_4 + X^- + SO\,x + H_2O$ **(Formula 2.1)**

At the end of the analysis, results of the sample composition are given in CHN total proportion between 0.01% and 100% of dry weight.

Once the total CHN obtained, samples witch were burnt to ash in a furnace at 450° C, (samples without organic matter) had to be analyzed using the Costech 410 Elemental Analyzer the samples in order to obtain the CHN inorganic content. Hence the organic carbon (OC) was calculated (expressed in units of % dry weight) as: OC = TC – IC **(Formula 2.2)**

After analyzing the elemental composition, to have an idea or first approximation of the source of the material composing the sediment was calculated C / N ratio.

2.2.2.c. Determination of Pigment contents.

During the sedimentary pigment analysis the sediment was been protected against direct light and excessive warning to avoid the degradation of the pigments. Samples were freeze dried at -80ºC. Three tests were done in order to know the sediment concentration that would be necessary to have for significate pigment concentration. 150 mg, 0.5g, 1.5g, 3g and 2g was analyzed. In the case of 3 g there was too much sediment, so the extraction was impossible to do. Finally 150mg of sediment were used to do the analysis. The extraction was done and expressed as Sanger and Gorham (1972) modified as Lami and Buchaca (2002).

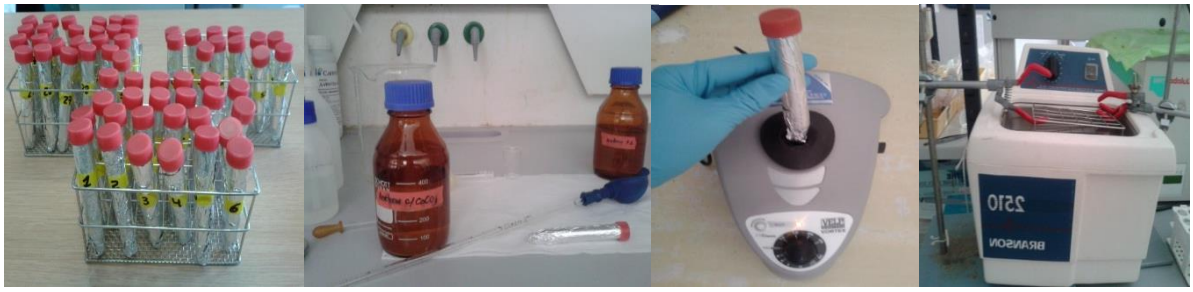


Figure 2.11: Pigment extraction process: samples protected against direct light (first image), acetone addiction (second), vortex (three) and sonication process (fourth).

Pigment extraction was been carried out from 150 mg of dry sediment sonicated with 2 ml of 100% acetone for 40 seconds and then the sample was centrifuged around 10 minutes at 3000rpm. Supernatant was taken, and the process was repeated the same form one more time. Finally, the 4 ml was filtered with a filter of 0.22 µm pore diameter to determinate the chlorophyll derivatives (CD) and the total carotenoids (TC) (Fig. 2.11).

The filtered solution was measured immediately in a spectrophotometer UV / Visible, Beckman Coulter, Du´650 (Fig. 2.12).

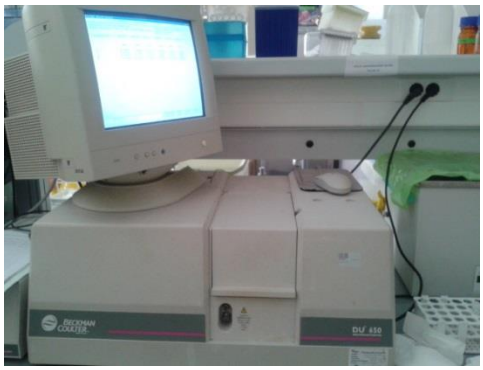


Figure 2.12: Spectrophotometer UV / Visible, Beckman Coulter, Du´650.

The spectrophotometer uses the properties of light and its interaction with other substances, to determine the nature of the same. In general, light from special lamp characteristics is guided through a device that selects and separates light of a particular wavelength and passes through a sample. The intensity of light leaving the sample is captured and compared to the intensity of light that penetrated the sample and from this the transmittance of the sample is calculated. It allows to determine the concentration of a substance (in this case the determinate the chlorophyll derivatives (CD) and the total carotenoids (TC)) in a solution, allowing the realization of quantitative analysis.

On the other hand specific pigments were determined by ion pairing, reverse-phase HPLC in Centre of Marine Science (CCMAR) by the Marine Plant Ecology group, University of Gambelas, Faro.

2.3. Statistic Analysis.

Data processing was focused on characterizing and understanding the C concentration present in the surface sediment, with respect to the geochemical and sedimentological differences and physical properties of the four studied stations in both environments (*Z. noltii* and *S. maritima*). The data processing was performed through the statistical software R. This software is an open source language and programming. It seeks to explain correlations and dependencies of a physical or natural phenomenon occurring randomly or conditionally, i.e., it is a language and environment for statistical and graphical analysis.

An exploratory data analysis was performed from graphical representation of boxplot, biplot and correlations of variables to better understand the trend of the data. Two way ANOVAs with station and type of environment as factors were also performed for each variable to see whether or not there are significant effects of each independent factor and of their interaction. In the case of significant difference, a post hoc analysis was applied to determine where the difference was localize, in this case we used Tukey test.

In addition, it is important to note that Gradistat program (Blot and Pye., 2001) was also used to do the calculation of grain size statistics based on Folk and Ward (1957) including: mean, mode(s), sorting (standard deviation), skewness, kurtosis, and a range of cumulative percentile values (the grain size at which a specified percentage of the grains are coarser)

3. Results

3.1. Sediment Analysis:

3.1.3. Sediment granulometry by laser counting and sieving.

The grain size results are presented in Table 2.I in Annex 2. The textural composition of the samples varies between 5 and 25 % wv of clay (Fig. 3.1), 1 and 16 % wv of silt and 7 and 88 % wv of sand. The grain size of samples after removing the organic matter range 17.80 – 126.70 µm, with an average of 56 µm whereas the grain size of samples with organic matter range 21.34 – 1007.30 µm, with an average of 69.47 µm. The coarser sediment is found in station 1 for *Z. noltii* environment, whereas the finest sediment is from station 4 also in *Z. noltii* environment. Thus a progressive grain size diminution from station 1 to station 4 was observed, as well as a higher variability in *Z. noltii* than *S. maritima*. It is important to note that this gradient is mainly observed in the granulometry made on samples after removing the organic matter.

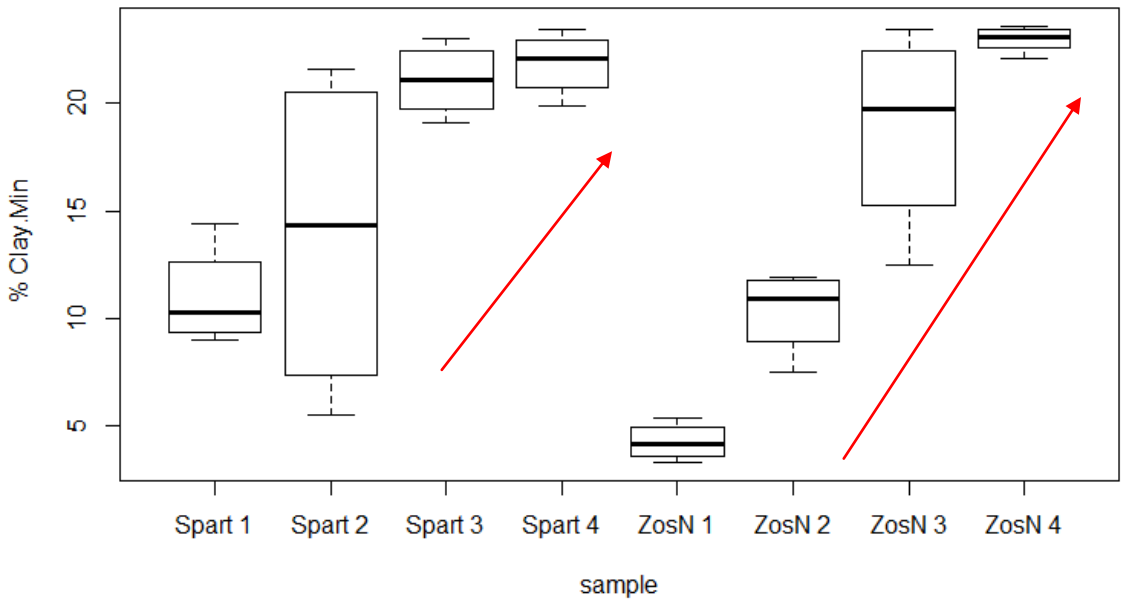


Figure 3.1: Percentage of clay size particles with no organic matter (% dw, Clay Mineral) in function of sampling station for each species.

In a similar manner as seen for the Clay content from the sediment without organic matter ("Mineral Clay"), the fine/coarse ratio (Fig. 3.2) shows the same observed trend from station 1 to 4 with ratios varying from 1 to 6, but this time in both environments.

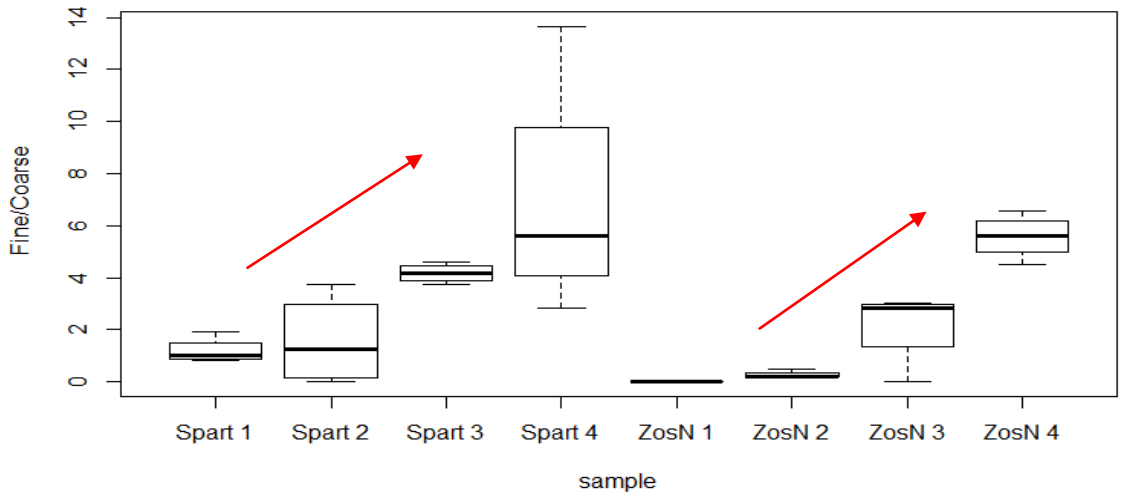


Figure 3.2: Percentage Fine/Coarse in function of sampling station for each species.

This similarity in trends can be verified in the high correlations observed between the two variables, being higher for *Z .noltii* than *S. maritima* with a value of 88% of correlation (Fig. 3.3), compared with a value of 44 % for *S. maritima* (Fig. 3.4).

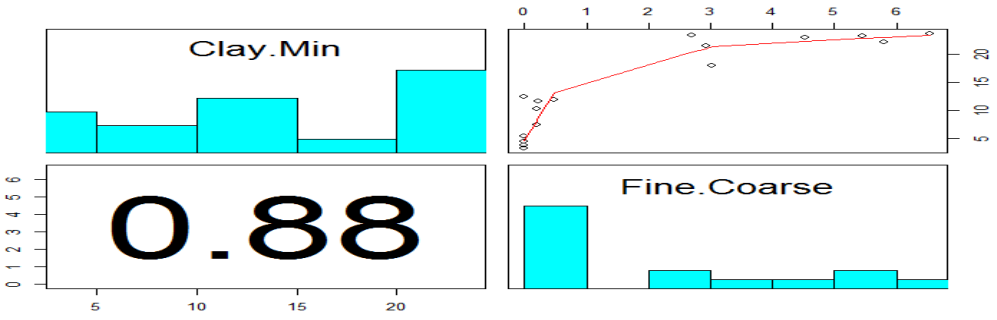


Figure 3.3: Rplot correlations between the clay content from the sediment without organic matter ("Mineral Clay") and fine/coarse ratio in *Z. noltii.*

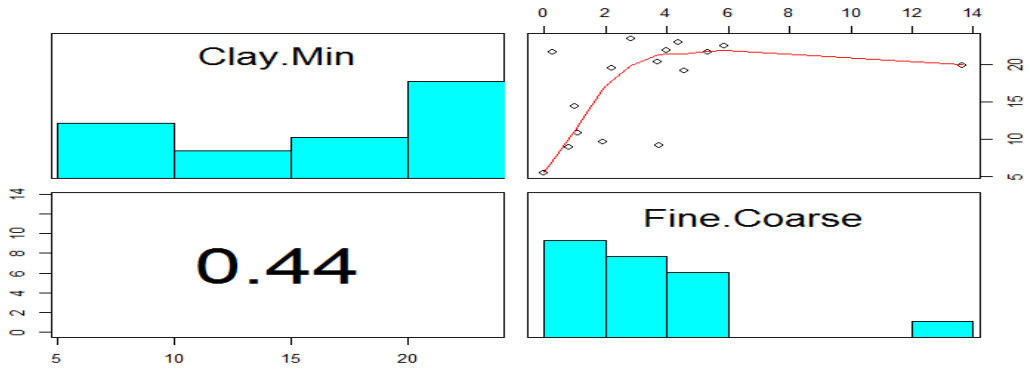
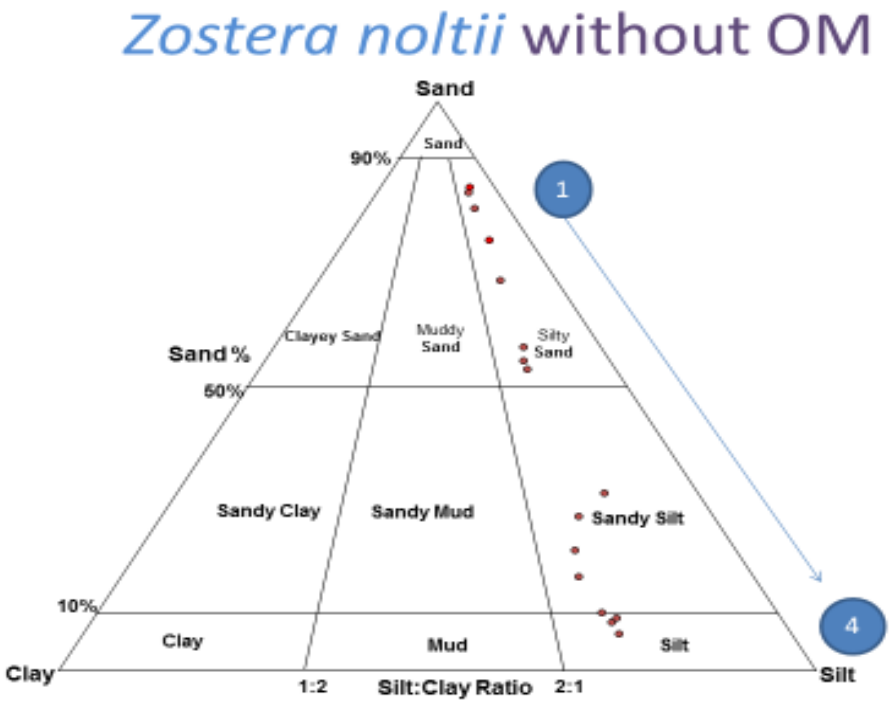


Figure 3.4: Rplot correlations between the Clay content from the sediment without organic matter ("Mineral Clay") and fine/coarse ratio in *S. maritima*.

When observing the `particle size distribution classification' (PSDC) of sediment type based on the proportions of sand, silt and clay, which are plotted as a ternary diagram in Fig. 3.5, the classification correspond with the progressive grainsize diminution in *Z. noltii*, environment from station 1, with a high percentage of sand, to station 4, with a higher percentage of silt Fig. 3.5 (A), whereas *S. maritima* environment shows a smaller gradient, being able to group the stations 1 and 2 on one hand and 3 and 4 on the other (Fig. 3.5 (B).

Nevertheless, both environments are characterized by silty sand in station 1 and silt in station 4, even though *S. maritima* environments show slightly coarser sediment due to a higher content of silt in relation to the clay content.

(A)



(B)

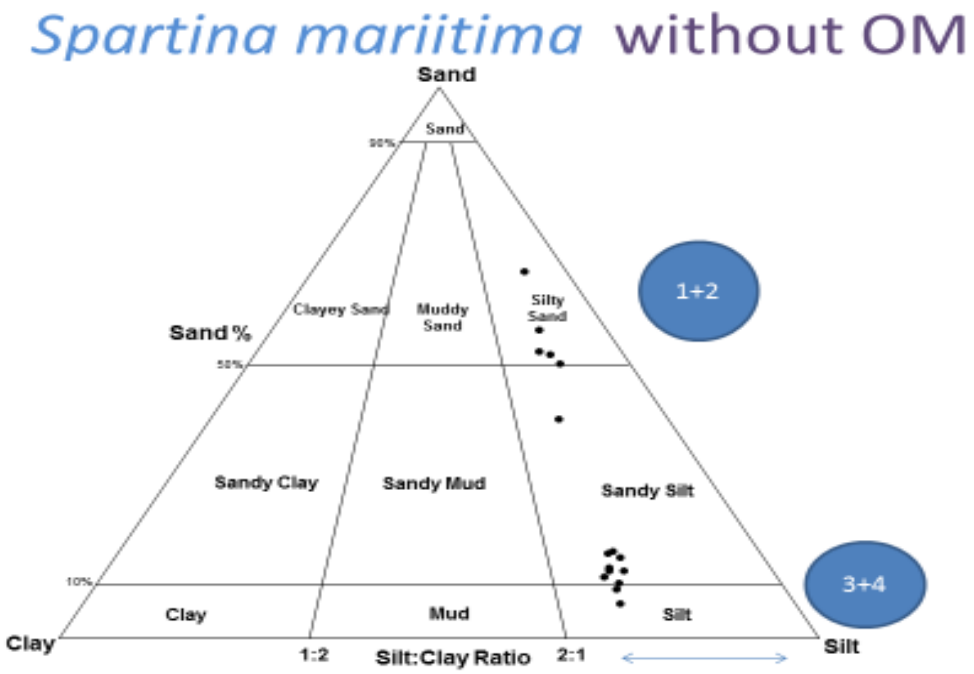


Figure 3.5: Grain size ternary diagram for sediment classification (Folk 1954) showing the classification schemes for *Z. noltii* (A) and *S. maritima* (B) based on the relative percentages of sand, silt and clay. The numbers inside of the blue circle indicate the stations.

3.1.2. Diffuse Reflectance Spectroscopy for color determination.

Color results obtained in each of the treatments are summarized in Table 2.V in the Annex 2. L* measured in the samples with organic matter (L*) has a range of variation between 47.08 and 66.40, whereas L* measured in samples after removing the organic matter (L*.no.OM) varies between 0 and 20.50. Similarly, a* varies from -2 to 3.06 and a*.no.OM between 0 and 47.08 whereas b* ranges from 16.28 to 33.70 and b* no.OM from 0 to 99.72 (see table2.V in annex 2).

These results clearly show differences between samples containing organic matter and samples after removal of organic matter.

The results of the analyzed samples that contained organic matter, show that the a* color present significant difference (ANOVA, $p<0.001$) between the *Z. noltii* environment and *S. maritima* environment. The highest values are found in *Z. noltii* environment and the smallest values in *S. maritima* (Fig.3.6). In the case of *S. maritima* environments values are below 0, thus colors with a green component, whereas for *Z. noltii* environments values are above 0, corresponding to a red component.

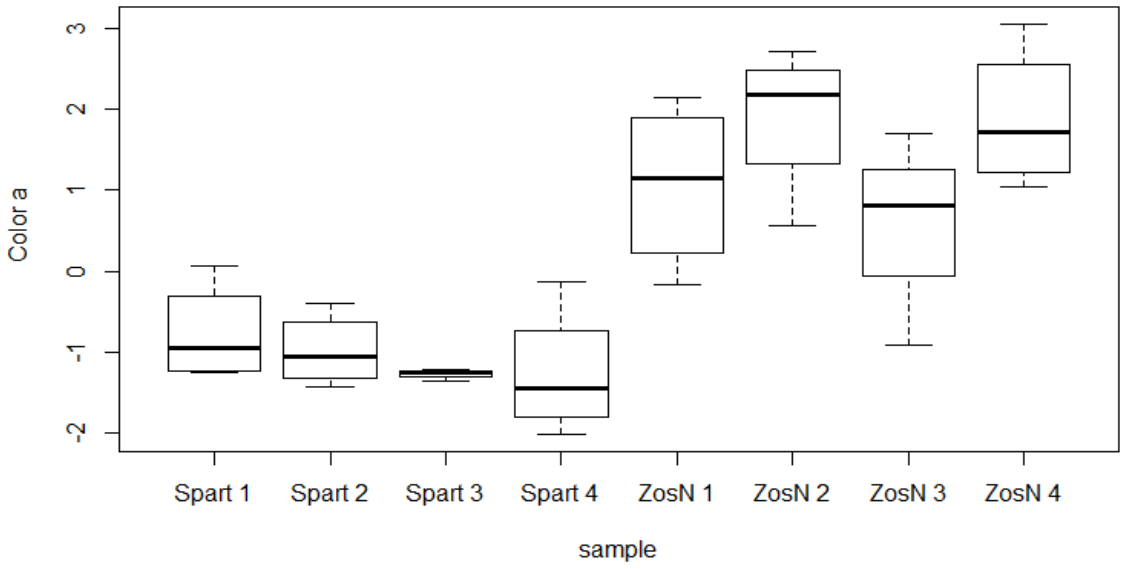


Figure 3.6: a* color for samples with organic matter in function of sampling station for each species.

In the other hand b *(ANOVA, p >0.05) and L* (ANOVA, p>0.05) colors do not provide information for samples with organic matter (not shown here), nor does L* in samples after organic matter removal, showing no significant differences (ANOVA, p >0.05) by environments, i.e. there are no significant changes in these color components.

When observing the results of the analyzed samples after removal of the organic matter, both a*no.OM (ANOVA, p<0.001) (Fig.3.7) and b*no.OM (ANOVA, p<0.001) (Fig.3.8) colors show differences in function of the sampling station for each specie and between species. High values for both colors are registered in station 1 for *S. maritima* environment, whereas station 4 is characterized by smaller values in *S. maritima* environment, showing thus a progressive diminution of the values of both colors from station 1 to station 4.

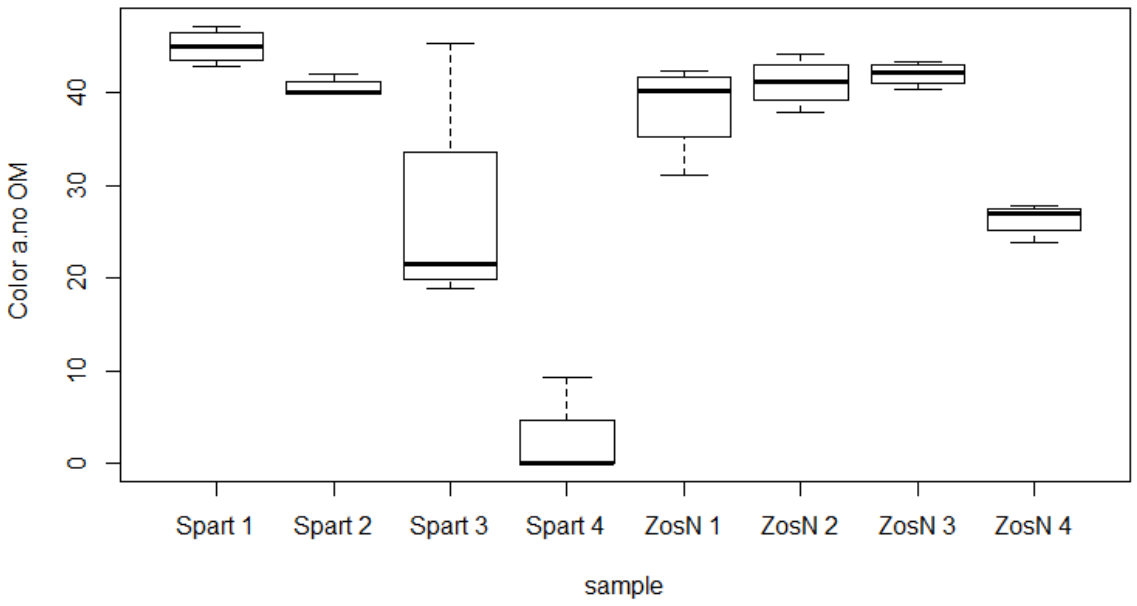


Figure 3.7: a* color without organic matter in function of sampling station for each species.

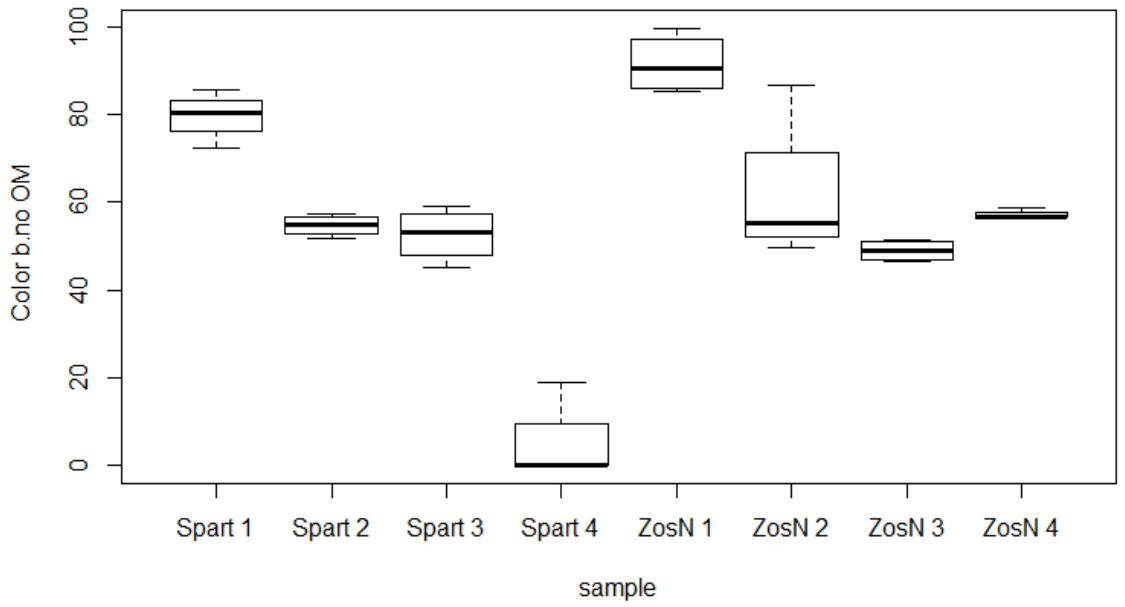


Figure 3.8: b* color without organic matter in function of sampling station for each species.

3.1.3. Mineral identification.

With respect to the analysis of mineral composition, no significant differences in the sediments are found neither between types of environment or between stations (Fig 3.9). All the samples had a similar mineral composition (Table 3.1). However, some samples have small amounts (< 5%) of plagioclase, feldspars or siderite. Furthermore, it is important to note that the biggest difference in the mineral composition is in the percentages between quartz and clay minerals. The quartz percentage is higher in the station nearer to the main channel than the more remote station, the opposite occurs for clay minerals, their percentage increased from station 1 to station 4. With respect to the difference between biological communities, generally the mineralogical composition for *Z. noltii* is more variable and complex than for *S. maritima*.

Table 3.1: Mineral composition **(%)** for each station in function of sampling station for each species.

	Sp1	Sp2	Sp3	Sp4	Zn1	Zn2	Zn3	Zn4
Mineral	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Pyrite			2.4					
Goetite								
Hematite								
Magnesite								
Siderite		4.2	4.2	3.2		3.4	7.3	8.4
Dolomite								
Ankerite								
Calcite					1.4		2.6	2.1
Plagioclase	3.7	2.4	5	3.3			3.5	3.7
Feldspar K	3.4	2.8	3.8		3.5	3	2.2	
Quartz	80	82	59	62	95	85	69	48
Aragonite								
Anhydrite						1.6		
Cristobalite								
Phylosilicates	15	10	33	32		19	14	37
Gypsum							2.8	2.8

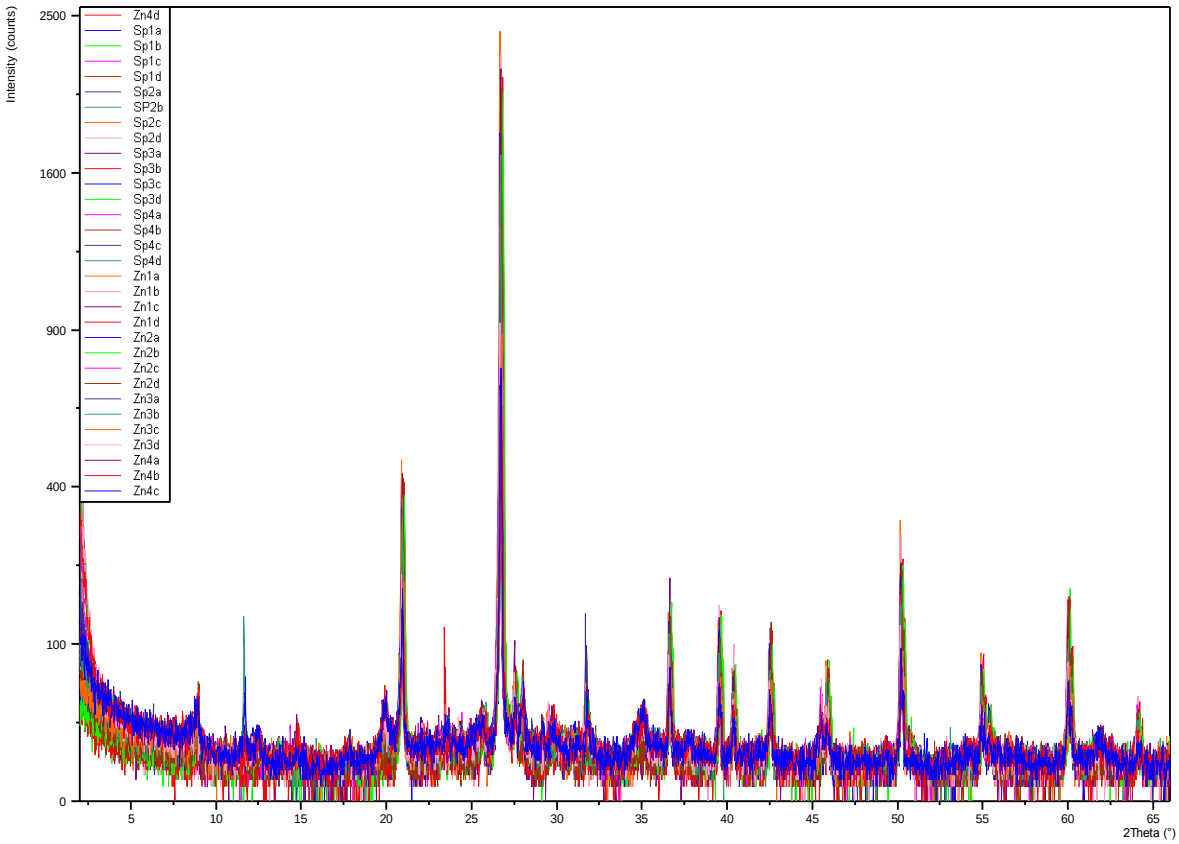


Figure 3.9: Pattern of X-ray diffractometer for all the samples.

3.2. Geochemical Analysis.

3.2.1. Determination of Water, Organic matter and carbonate contents by Lost of Ignition.

The results of water, organic matter and carbonate contents are presented in Table 3.I in Annex 3. The water contents have an average value of 46.12 % ww, with a minimum of 22.68 % ww for station 1 in the *Z. noltii* (Zn1c) environment and a maximum of 57.80 % ww for the station 4 in environment of *Z. noltii* (Zn4d).

The existence of a difference in water content is observed between the two environments (*S. maritima* and *Z. noltii*) (ANOVA, $p<0.001$), *S. maritima* presents an average off 11% ww more than *Z. noltii*. In both environments, there are differences between stations (ANOVA, $p<0.001$), there is an increase in water content throughout stations. Furthermore, it is possible to note also stabilization in the stations more distant from the main channel. For each environment, the smallest percentage of water content is found in station 1, whereas the highest percentage is from station 4 in *Z. noltii* environment, showing thus a progressive increase from station 1 to station 4 (Fig.3.10).

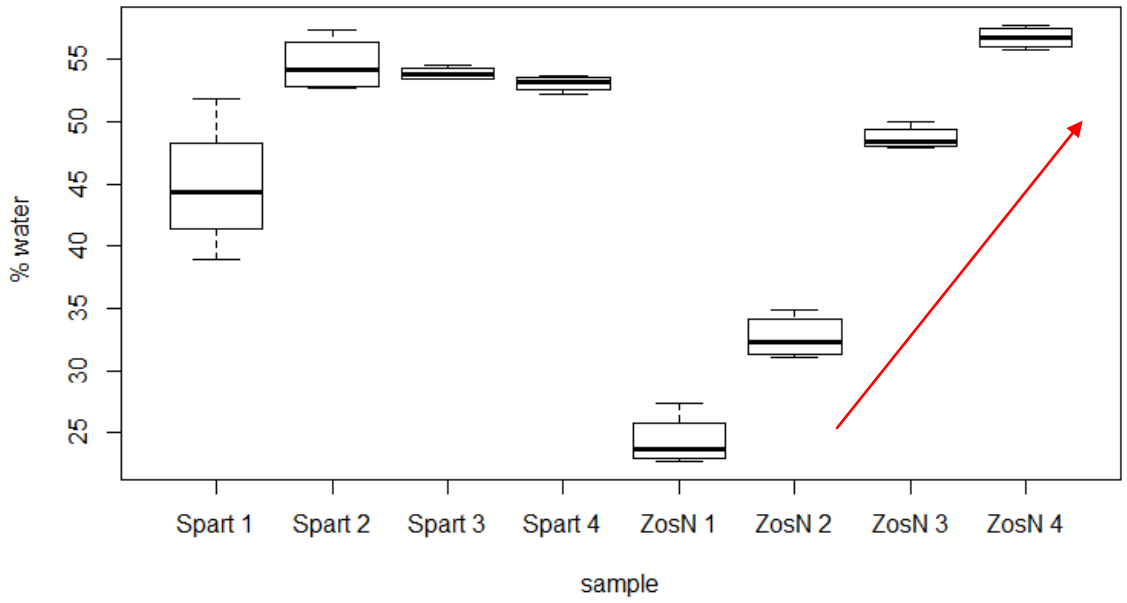


Figure 3.10: Water content (% ww) in function of sampling station for each species.

Regarding organic matter content, data show an average of 7.9 % dw, with a minimum and a maximum of 1.85 % dw and 11.92 % dw for stations 1 from *Z. noltii* (Zn1c) and 2 from *S. maritima* (Sp2a) respectively.

When observing Figure 3.11, it is possible to observe differences (ANOVA, $p<0.001$) in the content of organic matter between both environments. Hence, *S. maritima* environments present higher average of 9.87 % dw than for *Z.noltii* with an average of 5.93 % dw of organic matter. In *S. maritima*, the percentage of organic matter does not have significant variations between stations, but it is important to note the existence of

differences between station 1 and the other ones. In *Z. noltii* environments, a progressive gradient is observed again with the percentage of organic matter increasing from station 1 to station 4.

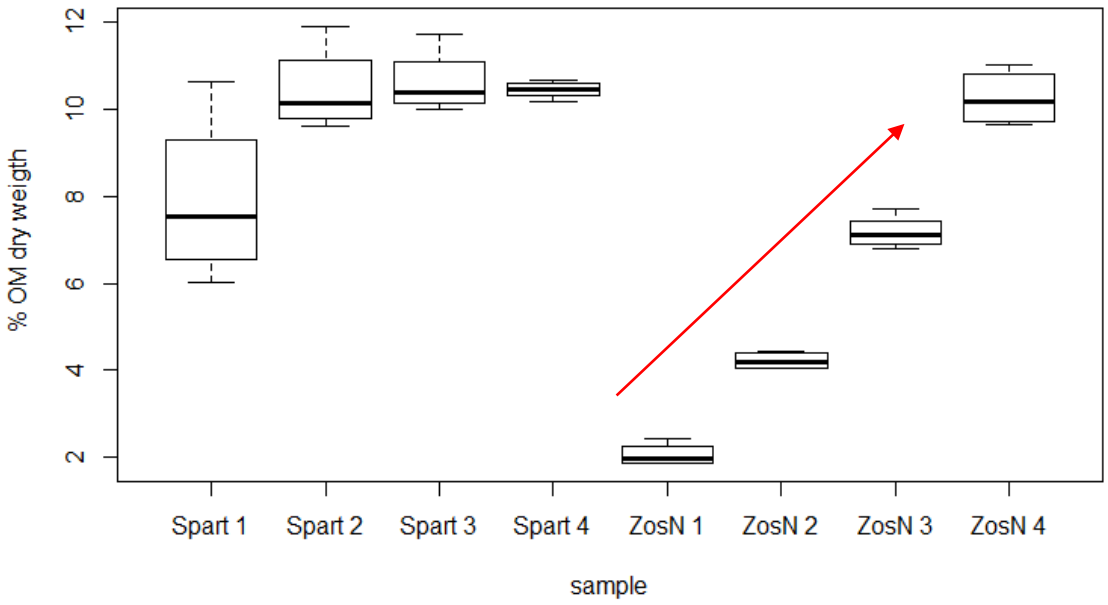


Figure 3.11: Organic matter content (% dw) in function of sampling station for each species.

In relation to carbonate content, its average value is 2.54 % dw, with a minimum of 0.62 % dw for station 3 in *S. maritima* (Sp3c) and, a maximum of 4.08 % dw for station 4 in *Z. noltii* (Zn4d).

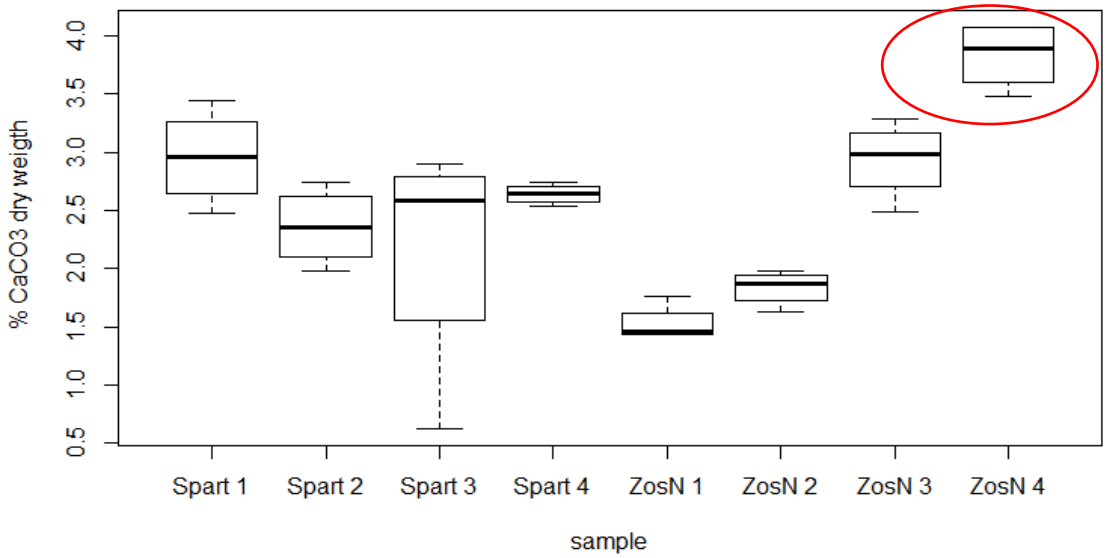


Figure 3.12: Carbonate content (% dw) in function of sampling station for each species.

As it can be observed in Figure 3.12, carbonates contents show significant differences (ANOVA, $p<0.01$) in Station 4 of *Z. noltii* environment with respect to other stations, whereas there is no significant difference (ANOVA, $p>0.05$) between the environments.

3.2.2. Determination of Elemental composition: OC, IC, ON, IN and C/N.

The Elemental composition results are presented in Table 3.II in Annex 3. This composition varies between 0.29 and 3.23 % dw, with an average value of 1.75 % dw of OC (organic carbon), 0.03 and 3.23 % dw, with an average value of 0.20 % dw of ON (organic nitrogen), 0.01 and 1.11 % dw with an average value of 0.53 % dw of IC (inorganic carbon) and -0 and 0.13 % dw, with an average value of 0.05 % dw of IN (inorganic nitrogen).

The highest organic carbon content is found in station 4 for *Z. noltii* environment (Zn4b), whereas the smallest percentage is from station 1 also in *Z. noltii* environment (Zn1b), showing thus a progressive increase of the organic carbon content from station 1 to station 4 as well as a higher variability in *Z. noltii* environments (Fig. 3.13). It is important to note that this same gradient is also observed in the result of the organic nitrogen content

(Fig. 3.15), where the maximum value is found in station 4 for *S. maritima* and the minimum value is found in station 1 for *Z. noltii* (Zn1b and Zn1c). It is possible to observe differences (ANOVA, $p<0.001$) for both, organic carbon and organic nitrogen contents with respect to the different environments. The organic carbon content was almost double for *S. maritima* than *Z. noltii*, 2.25% dw and 1.24% dw, respectively. In the other hand, the organic nitrogen content was also double for *S. maritima* than *Z. noltii*, 0.27 % dw and 0.14 % dw.

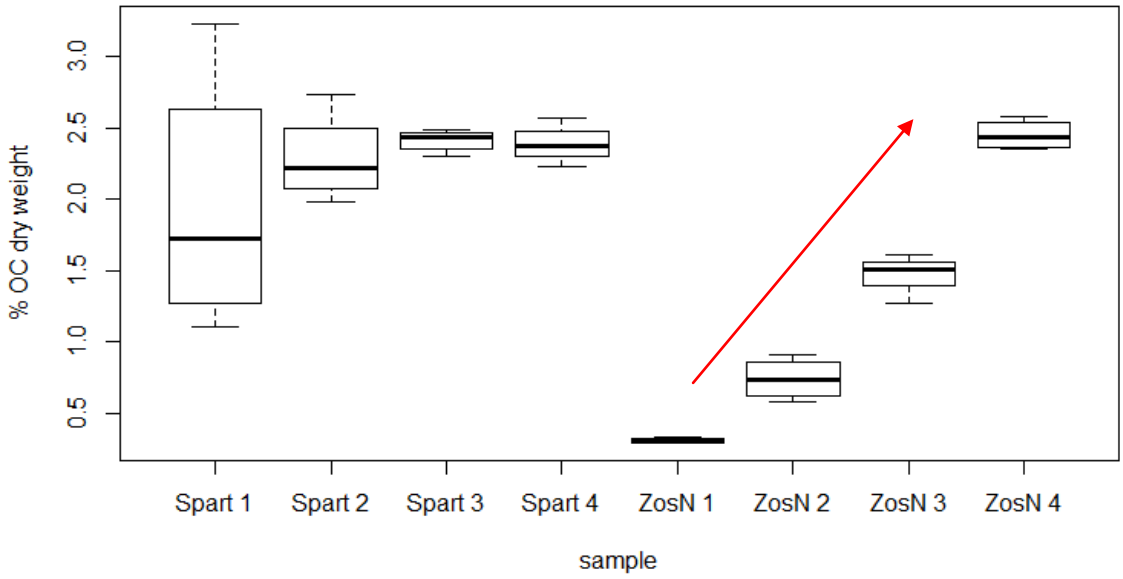


Figure 3.13: Organic Carbon content (OC % dw) in function of sampling station for each species.

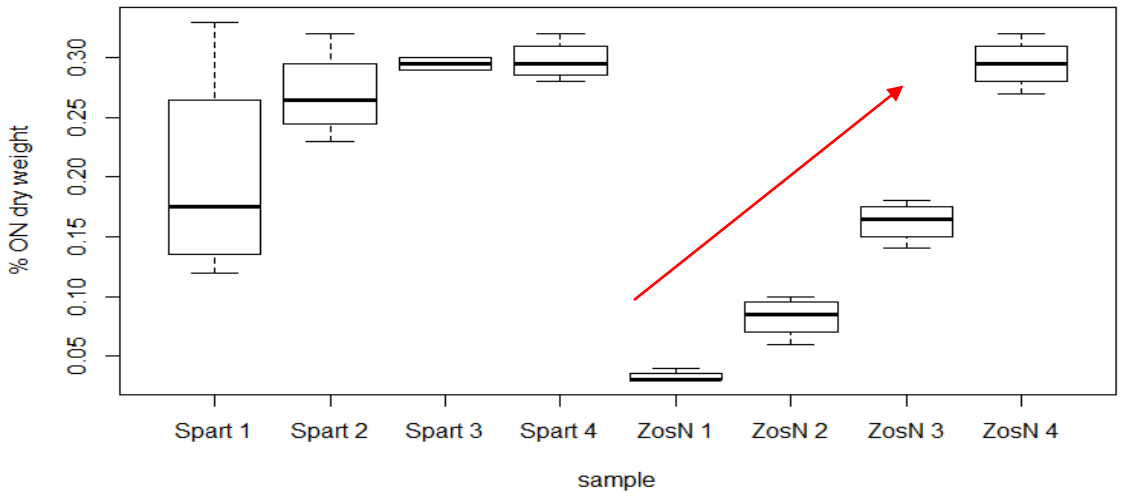


Figure 3. 14: organic nitrogen content (ON % dw) in function of sampling for each species.

This similarity in trends can be verified in the high correlations observed between the two variables, with a value of 94 % in *S. maritima* (Fig. 3.15) and a value of 100 % for the correlation in *Z. noltii* (Fig. 3.16).

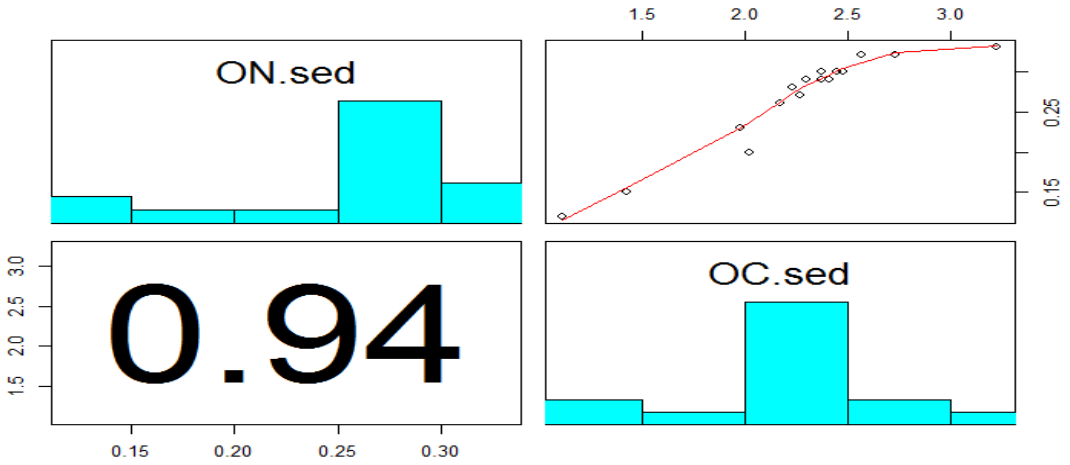


Figure 3.15: Rplot correlations between organic carbon (% dw OC) and organic nitrogen (% dw ON) to *S.maritima* environments.

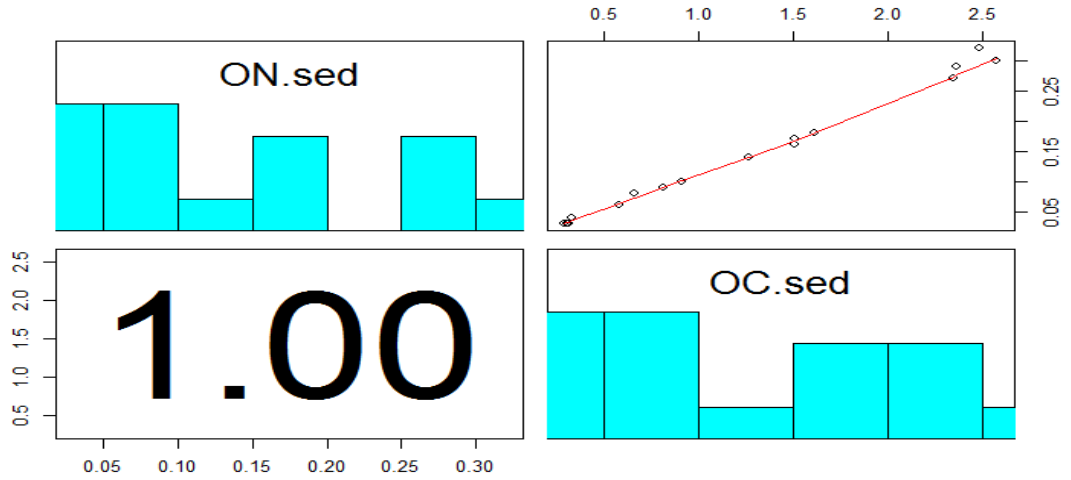


Figure 3.16: Rplot correlations between organic carbon (% dw OC) and organic nitrogen (% dw ON) to *Z.noltii* environments.

In the other hand, the inorganic nitrogen contents show significant differences (ANOVA, $p<0.001$) between both environments and smaller differences between stations (ANOVA, $p<0.01$). It is possible to observe higher values in *S. maritima* than *Z. noltii* environments (Fig. 3.17).

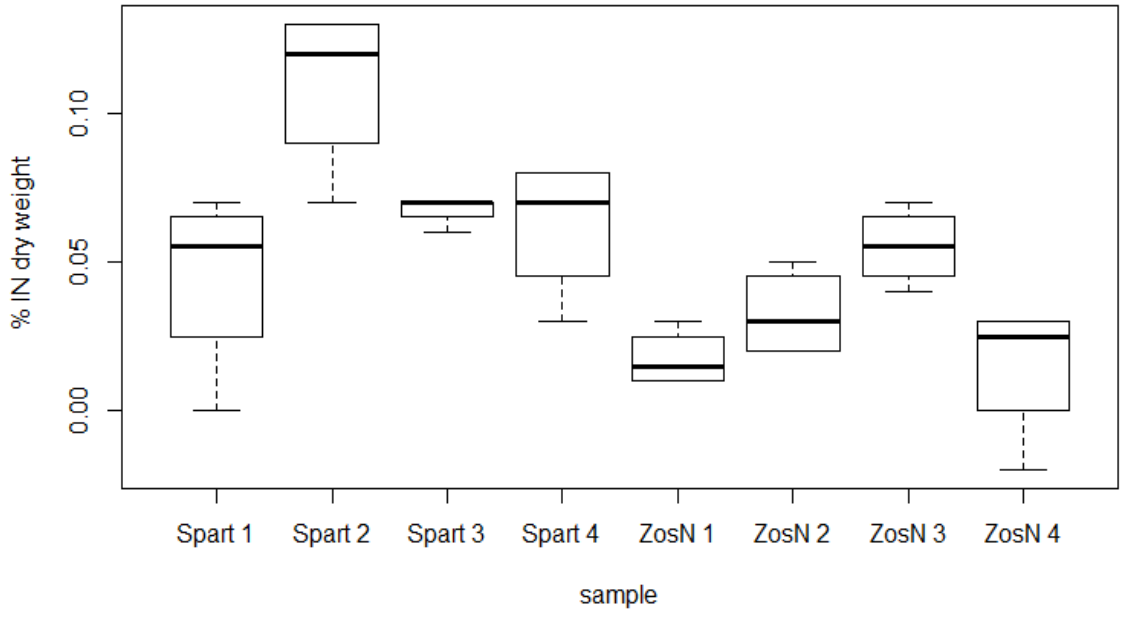


Figure 3.17: Inorganic Nitrogen content (IN % dw) in function of sampling station for each species.

In the case of inorganic carbon content, as it can be observed in Figure 3.18, there are significant differences (ANOVA, $p<0.01$) in Station 4 of *Z. noltii* environment with respect to other stations.

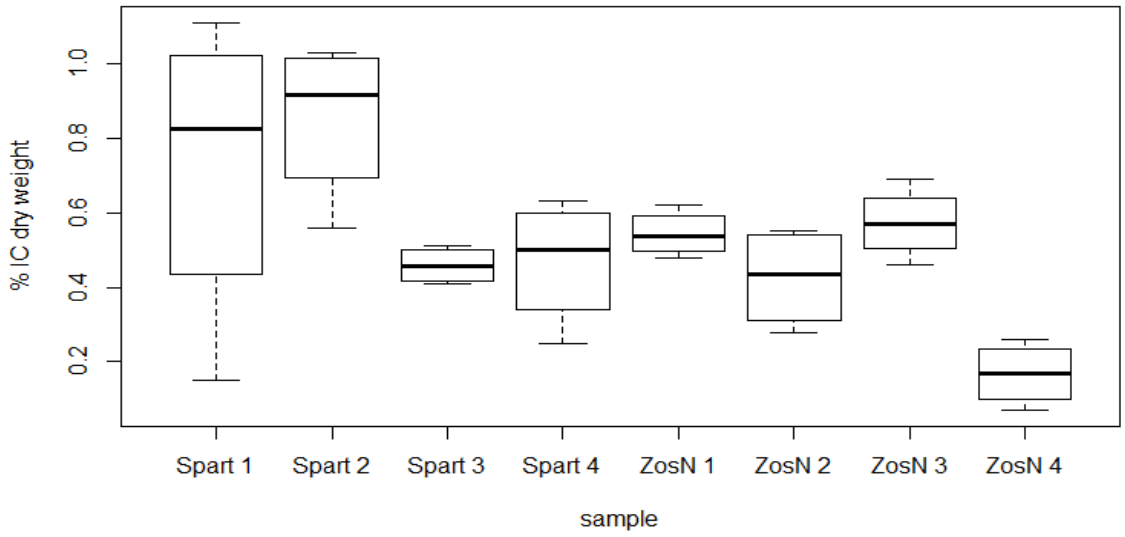


Figure 3.18: Inorganic Carbon content (IC % dw) in function of sampling station for each species.

The C/N ratio values ranged from 7.78 to 10.33, with an average value of 8.75. No significant differences were obtained between both biological communities (ANOVA, $p<0.05$), the C/N ratio for *Z .noltii* has a similar value as for *S. maritima*, 8.99 and 8.58, respectively. However, it is important to note significant differences between stations (ANOVA, $p<0.001$); hence, it is possible to appreciate a progressive diminution from station 1 to station 4 in both environments (Fig. 3.19).

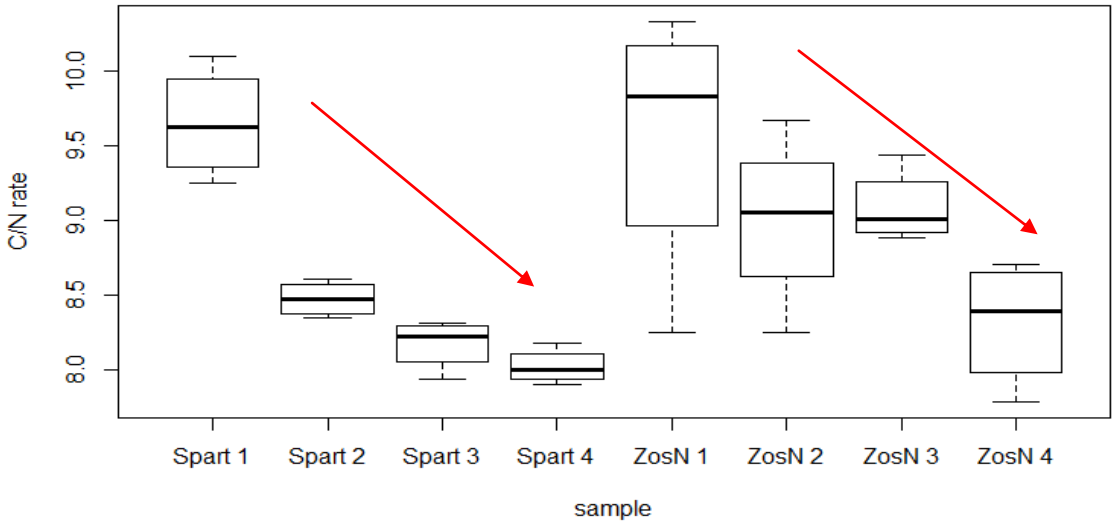


Figure 3.19: C/N rate in function of sampling station for each species.

3.2.3. Determination of Pigment contents.

Regarding the results of the analysis of the determination of pigments they were considered not representative because of an apparent degradation of the pigments therefore not be taken into account in this work (Annex 3).

3.2. Result Summary

In order to facilitate a whole perception of results, a summary is presented in table 3.2. From all the variables presented in this work, just a few of them did not vary with the two studied factors, namely the station and the type of environments. The great majority of variables present differences between stations as well as between environment types and more than half of these variables also respond to the interaction between the two considered factors.

Table 3.2: Summary result (with/without differences to each variable) with respect both factors (Distance to the continent/ Hydrodynamics, i.e., station vs type of environment/biological community)**.** Where > or < means bigger or smaller differences between both habitats and + or - , increased gradient or decreased.

Variable	Without differences	Type (*Zostera* vs *Spartina*)	Station (distance to the sea)	Interaction both factors
Clay ToT (% wv)		<	+	X
Clay Min (% wv)		<	+	
Fine/Coarse		<	+	
Vegetation (% dw)	X			
O.M (% dw)		<	+	X
Water (%ww)		<	+	X
CaCO3 (% dw)			+	X
Density (g/ ml)		<	-	X
a* no.OM		<	-	X
b* no. OM		<	-	X
L* no.OM	X			
a*		>		
b*	X			
L*	X			
O.C (% dw)		>	+	X
I.C (% dw)		<	-	
O.N (% dw)		>	+	X
I.N (% dw)		<	+	X
C/N			-	

4. Discussion

4.1. Sediment characterization

4.1.1. Sediment Analysis.

Our results of sediment granulometry ranged between 3.3 – 23.6 % wv (wet volume) of clay in mineral samples (i.e. after organic matter removal), 1.6-15.8 % wv of silt and 86.8 -7.3 % wv of sand varying slightly with respect data previously described by Brotas et al., 1990 with a range 0.2 – 9.4 % of clay (ɵ < 2 µm), 2.4 – 76.6 % of silt (2 µm < ɵ < 60 µm) and 97.3 – 14.0 % of sand (ɵ > 60 µm) for samples of Ria Formosa. The difference with the present study, namely coarser sediments in general, can be explained by the difference in the location of the sampling stations. In our study, stations have a progression from the main channel, with high currents, continuing along a secondary channel, with weaker currents, while in the other case the location of the sampling stations are randomly chosen. Values reported for other coastal systems with presence of both ecosystems (Mondego estuary, Portugal, Couto et al., 2013) are 50% of sand between 100 - 50 microns (fine sand) in the case of *S. maritima* and 70% for *Z. noltii*. Silt and clay content values are only referred as being greater than 20%, in a similar manner as seen for our results where higher sand proportions were obtained in *Z. noltii* rather than in *S. maritima*.

Granulometry results show a progressive diminution from station 1 to station 4 (Fig. 3.1) as well as a higher variability in *Z. noltii* environments in relation to *S. maritima* environments. This gradient can be explained by decreasing energy environment, i.e., as a decreasing of current speed (Fig. 4.1), as you move away from the main channel into the secondary channel, causing deposition of smaller particles (Zaimes and Emanuel, 1902). The mode grain size from station 1 to station 4 was 0.126, 0.105., 0.062 and 0.016mm, respectively, reflecting as the mean current velocity decreased from station 1 to station 4, in a range close of 10 cm. sec^{-1} to station 1 and 2, and around 1.0cm. $sec^{-1}$to the station 3 and 4 (Fig. 4.1). Hence, the smaller particles correspond with smaller settling-deposition velocity. Whit respect to the habitats, no differences in the mode grain size were found. The average values were 0.07 to *S. maritima* and 0.08 to *Z. noltii*.

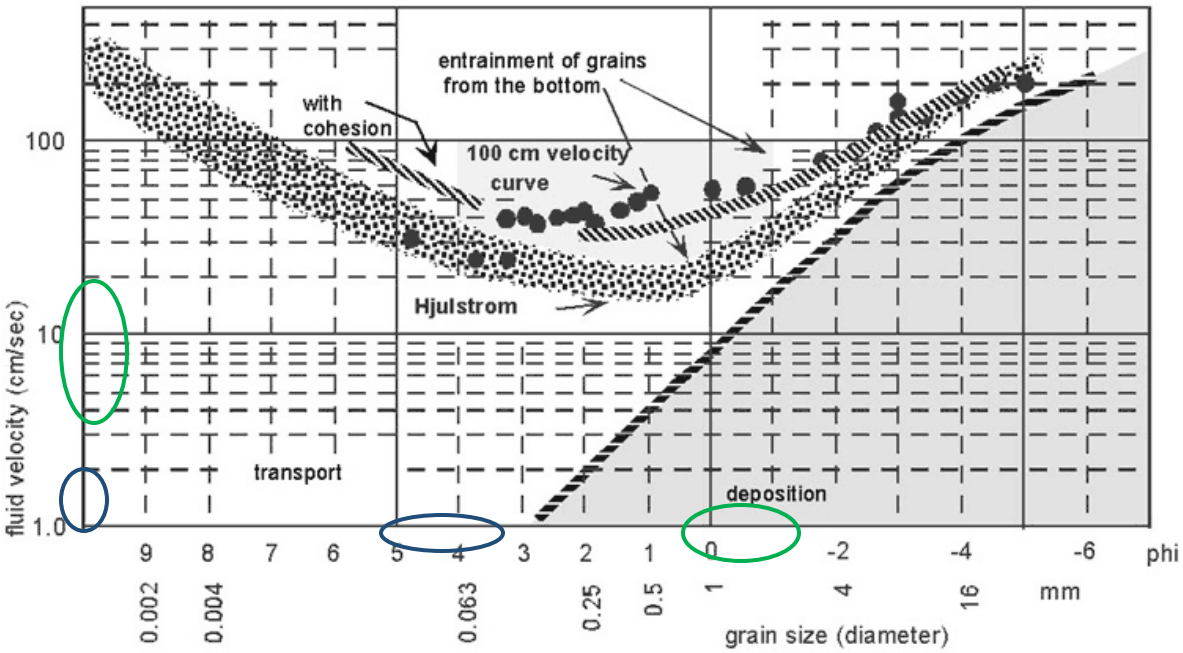


Figure 4.1: Hjulström Diagram of transport and deposition for particles for different sizes, regarding the average current speed (cm/sec) (Friedman et al., 1992) Blue circle: Approximation of current speed to stations 3 and 4; Green: Approximation of current speed to stations 1and 2.

The existence of different gradient with respect to the grain size between the two environments may be due to the different position with respect to the sea level of both ecosystems. *Z. noltii* environments have a greater exposure to marine hydrodynamics than *S. maritima*, which is higher in the intertidal and therefore shows a smaller gradient. It is important to note that this difference in gradient for both environments was observed in most of the studied parameters studied (see for example Fig. 3.10, 3.11, 3.12).

Color is one of the most useful parameter to characterize and distinguish the soil or sediment composition allowing to obtain much information about the materials that compose it. (Dominguez et al., 2011). The results obtained in the color analysis of the sediment of our study area, show differences for a *color (a * color With organic matter) between both environments (Fig. 3.6), with values below zero for the *S. maritima*, turning green, with an average of -1.072 and values above zero in the *Z. noltii*, with an average of 1.36, being more reddish. This red coloration may be due to the presence of minerals that act as coloring agents, such as oxides and hydroxides of Fe and Al or minerals with Iron

(Dominguez et al., 2011). The possible presence of different forms of Fe either in crystals, such as, such as pyrite and siderite, or as free element can be the cause of this difference between the two environments and mostly associated with a reducing environment. Hence, *Z. noltii* could be more reductant environment than *S. maritima*. Furthermore differences were also found regarding a * color without organic matter (a *no.OM colors) and b * color without organic matter (b * no.OM) in *S. maritima* environment where a gradient was observed in both colors (Fig 3.7 and 3.8). Values decreased from station 1 to station 4, becoming less reddish and yellow, which can be interpreted with the decrease of various forms of Fe (reddish) and hydrated goethite (yellow-brownish) (Dominguez et al., 2011). With respect to the different habitat, both colors showed bigger values in *Z. noltii* than *S. maritima*. Respect to L*color, with and without organic matter in samples, showing no significant differences between environments, so there are no significant changes in these color components. This means that the reflectance of the samples was similar for all of them, probably reflecting the same origin of the organic matter and of the mineral particles in all the samples.

With respect to mineral composition, no differences in sediments sample are found neither between types of environment or between stations. However, some samples had small amounts (< 5%) of plagioclase, feldspars or siderite. Furthermore, the main difference in the mineral composition was found in the percentages of quartz and clay minerals (phyllosilicates) (Table 3.9). The clay mineral proportions for the most remote station from the main channel were coincident with the grain size. Hence, the major accumulation of organic matter in these stations could be explained based on the morphologic and chemical characteristic of clay mineral. This could be due to the strongly absorption of organic matter by clay minerals. This interaction between the clay and some kinds of compounds can create relatively stable aggregates, and consequently, the moisture and aeration properties of the sediment are influenced. In addition, for this interaction result a protective effect of the organic compound from biological degradation. Due to this effect of protection for degradation, the organic matter of the sediments is normally positively correlated with the clay mineral content (Norman, 1964), with smaller grain size, as well as, the effect of hydrodynamics Whereas the differences in the proportions of quartz between stations, could be explained with a relation with the granulometry. Some

authors reported that the quartz has a positive correlation with the percentage of sand (AE de Limnoligía, 1984). The proportions of quartz could increase with the sand content due to the fact that sands are mostly composed by quartz, hence in the stations exposed to higher energy, which are associated with the deposition of sand, (Macreadie et al., 2012). At the same time the presence of pyrite and siderite, may be associated to the existence of reduction conditions (Lemos et al., 2007), which would allow for the permanence of the organic material for a longer period of time in the sediment.

4.1.2. Geochemical Analysis.

The average values of the water content was 46.12 ± 11.30 % ww (wet weight), with *S. maritima* environment having close 11% more water than *Z. noltii*, with values of 51.6 and 40.6 % ww, respectively ours values are quite similar to the analysis performed in the Ria Formosa, Ramalhete, by Friend and co-authors (2003), where the values obtained were approximately 30 ± 16 % wv and 32 ± 18 % wv for their two campaigns, respectively. No significant differences in the water content, for either transects or habitats, were detected between the two campaigns. Furthermore, for both campaigns the highest mean water contents occurred within *S. maritima* ecosystems, which a mean value of 48 % wv. The water content of the sediment is directly related to grain size and is a consequence of the density of the sediment (Fig. 2.I and Fig. 2.II in the Annex 2). According to Halmilton (1974) there are empirical relation between mean grain size and density and also between grain size and the porosity (Syvitski, 2007). An increase of the water content was observed as the proportion of fine grain size decreases and the consequent decrease in density (Fig. 3.I in the Annex 3). This result shows in turn that the choice of the stations location was pertinent for increasing confinement and thus reducing hydrodynamics along the stations profile.

Regarding the organic matter content, values were between1.86 and –11.92% dw, which are in accordance with the study of Cabaço and co-authors (2010), where it showed values with an average of 2 % dw. It is important to note that percentage of organic matter was, approximately a 40%, higher in *S. maritima* than *Z. noltii*. This difference could be due to the variation in the hydrodynamic levels to which the biology communities are

exposed. *S. maritima* environment is less influenced by the hydrodynamics, so it could be possible that more organic matter deposition occur than in *Z. noltii* environment. At the same time, an increasing gradient was observed in the organic matter content from the first stations, close to the main channel, to last stations the most remote (Fig. 3.11), gradient that is in agreement with the grainsize variation. According to other studies, (Fabiano y Danovaro, 1994) the accumulation of organic matter in small grain sizes is favored because dissolved oxygen penetration is smaller and there is thus no oxidation of the organic matter. Therefore in sandy sediments which have a larger grain size than the silt and clay, penetration of oxygen is facilitated, leading to higher rate of organic matter oxidation. In addition the sediments formed by silt or clay may favor the formation of hypoxic or anoxic bottom layers due to lack of dissolved oxygen.

Seagrass and saltmarsh are biological communities that support a lot of calcified organisms due to the accumulation of particulate inorganic carbon by the presence of epiphytic and benthic invertebrates. Sedimentation and deposition of carbonates is associated with particle sedimentation and photosynthetic capacity of these ecosystems, which provides an optimal pH environment for deposition (Mazarrasa et al., 2015). Carbonate content values in this study were in the range 3 - 14% dw (mean of 2.54 ± 0.77), similar to values reported for Ria Formosa with contents < 5% (Andarde et al., 2004). The carbonate content obtained in ours samples showed significant differences in Station 4 for *Z. noltii* with respect to other stations in both environments (Fig. 3.12). By linking the carbonate content with the grain size, it is possible to observe that increased carbonate content is proportional to the finer sediment (Fig. 2.III in the Annex 2), which could be explained by the possible presence of foraminifera. Several studies such as Mechler and Grady (1984) have reported fine sediment preference of certain foraminifera such as *A.beccarii var. Sobrina* and *A. beccarii var.tepida*.

The organic nitrogen content of the sediment of *S. maritima* and *Z. noltii* communities has an average value of 0.20 ± 0.10% dw, being similar to the reported value by Peralta et al. (2000) in the Ria Formosa, of 0.16 ± 0.01% dw in the central channel and 0.26 ± 0.04% dw in high intertidal zone. This lower sediment nitrogen content could result in a higher rhizome elongation, and consequent greater root development, with the

objective to increase nutrient uptake from the sediment (Peralta et al., 2000). It is important to note that *S. maritima* environment contained approximately the double of organic nitrogen than *Z. noltii*. This can be due to greater degradation in the *Z. noltii* sediment or also to greater removal caused by the greater hydrodynamics, at which it is exposed.

On the other hand, organic carbon contents in our study were slightly lower, with values of 1.75 ± 0.85 % dw compared. to 2.0 ± 0.5% dw and 2.9 ± 0.4%, dw in Peralta et al. (2000). Also our values of organic carbon were in the range of, or even similar, to values for other coastal systems as the ones reported by Couto et al. (2013) with both environments such as Mondego estuary, Portugal, with 2.2 ± 0.2 % dw. In our study, the fact that *S. maritima* environment contained approximately twice more organic carbon than *Z. noltii*, could be explained due to a higher carbon oxidation or degradation in sediments, as well as a greater remove or less deposition in *Z. noltii* environment due to higher local hydrodynamics (action of currents and sediment washing).

In addition, our data showed a progressive increase of organic content, carbon and nitrogen, from station 1 to station 4 as well as a higher variability in *Z. noltii* environments (Fig. 3.13). This gradient could be explained by the presence of an inverse relationship between the size of the sediment particles and organic matter content (Fig. 2.IV and Fig. 2.V in the Annex 2). The deposition of OM (and all particles) increases with decreasing hydrodynamics. Then the OM may better stabilized in sediments because they get attached to the fine sediment fractions. Hence, the material with higher silt-clay fractions has higher concentrations of organic compounds (Silva et al., 1998). Furthermore, according to Duinker, (1980), the silt-clay particles can better be covered with a layer of organic material, because these tend to have negative charges associated with their structure. As eventually this component of the sediment sink to the ocean floor, remove a significant amount of organic material from seawater. This author also indicates that sands are always composed by quartz, which have no charge, hence not attracting organic matter and are therefore associated with lower amounts of organic material. Moreover, environments associated with the deposition of sand are higher energy environments, so the currents produced mixed sand and carbon oxidation occur, resulting in lower amounts of this latter component (Macreadie et al., 2012).

In relation to inorganic content, for inorganic carbon values no marked differences between environments or between stations, with the exception of station 4 in *Z. noltii*, which presents the lowest values (Fig. 3.18) and cannot be relate with the high values of carbonates associate with the finest sediments explained above. Brambai and co- authors (1991) indicated that the presence of inorganic carbon also might be due to the presence of calcite, in particular Mg-calcite, which would be of biogenic origin. With respect to inorganic nitrogen values were slightly higher in *S. maritima* environment than in *Z. noltii* environment (Fig. 3.17). This difference in the content of inorganic nitrogen may be due to increased presence of bacteria in the sediment, responsible for the remineralization of organic nitrogen to form their inorganic form.

In the other hand, C/N organic ratio results ranged from 7.8 to 10.3 with an average of 8.8 with no significant differences between biological communities with similar values as 8.58 to *S. maritima* and 8.99 to *Z. noltii*, whereas important differences were observed between stations. Indeed, there is a progressive diminution C/N from station 1 to station 4 in both environments (Fig. 3.19). This gradient could be due to differences in the hydrodynamics at each station or a progressive difference in the origin of the organic matter. The trend observed could indicate that the sediments for the more exposed stations to hydrodynamics, could suffer greater degradation of nitrogen, hence, a greater C/N ratio was observed. Conversely, the stations that are less exposed to hydrodynamics could suffer less nitrogen degradation and therefore would have less C/N ratio. Similar results with respect to values reported for Peralta et al. (2009), in Ria Formosa, were 14.2 ± 1.3 for the main channel and 12.7 ± 2.6 for a higher intertidal channel. Higher ratios of 6.63 would be possible to explain as the presence of variables amounts of organic nitrogen poorest into terrigenous or edaphic material. When the C/N ratio exceeds 10, it may be because there is an increase in mineralization, causing a decrease of organic nitrogen. This means that the N component of OM during decomposition decreases much faster than the C component (Silva and Ortíz., 2002) , thus the C:N tends to be lower in the sediment than in live plants.

4.2. Carbon sequestration rate

The carbon accumulation rate was calculated using unpublished data of sedimentary rates of 0.5 cm.year^{-1} for *S. maritima* environment and 0.9 cm year^{-1} for *Z. noltii* (Veiga-Pires and Santos, personal communication).The carbon accumulation estimates for ours samples ranged from 37.30 to 182.0 g OC.m^{-2}.year^{-1}, with an average of 120.0 ± 37.30 g OC.m^{-2}.year^{-1}. More specifically, the carbon accumulation rate for *S. maritima* environment was twice as high as those for *Z. noltii* environment, with values of 131.8 g OC.m^{-2}.year^{-1}and 83.9 g OC.m^{-2}.year$^{-1,}$ respectively. In addition, it is possible to appreciate a progressive increase from station 1 to station 4 in both environments as well as a higher variability in *Z. noltii* (Fig 4.2).

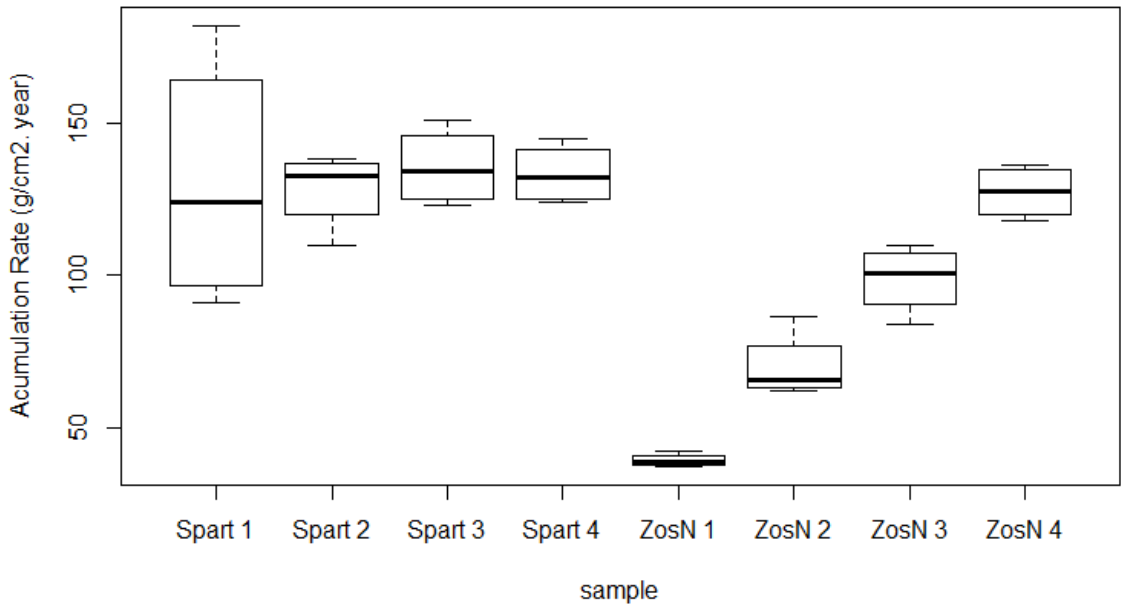


Figure 4.2: Carbon accumulation rate (g. m^{-2}. year^{-1}) in function of sampling station for each species.

This difference could be due to the difference between the sediment rates for both environments, as well as the difference in sediment density, because the carbon accumulation rate is highly dependent of the sediment rate and organic volumetric content (Lavery et al., 2013).

Due to the lack of published data regarding rates of carbon accumulation in the Ria Formosa, our results were compared to values described by Duarte et al. (2005), which it searched the published literature for estimates of carbon burial in vegetated ecosystems to obtain estimates of average carbon burial of these habitats. Ours values were in the range of, or even similar, to values of this publication, where the estimates of carbon accumulation rate were 139.0, 151.0 and 83.0 g C m^{-2} yr^{-1} to mangroves, salt marsh and seagrass, respectively. Hence, these results agreed with our data because the mangroves and salt marsh were identified as important environment for carbon burial, with rates twice bigger than seagrass meadows.

5. Conclusions

In order to reach the main objective of this study, the questions raised in the beginning were answered

- Is there a difference in C sequestration between *S. maritima* and *Z .noltii* habitats?

Yes, significant differences were found between both ecosystems. *S.maritima* environment showed nearly twice the organic carbon content than *Z. noltii* environments. Which is reflected in theirs accumulation rate the same form, because the salt marsh were identified as important environment for carbon burial, with rates twice bigger than seagrass meadows.

- What is the relation between OC content and the sediment granulometry?

It was observed an inverse relationship between the size of the sediment particles and organic carbon content. Hence, gramulometry results show a progressive grainsize diminution from station 1 to station 4, so inversely a progressive increase of the organic carbon content was observed along stations.

- What is the relation between OC content and the sediment color?

The possible presence of different forms of Fe either in crystals, such as pyrite and siderite, or as free element can be cause of the difference in the organic carbon contents between the two environments and mostly associated with a reducing environment. Furthermore, the reflectance of the samples was similar for all of them, probably reflecting the same origin of the organic matter and of the mineral particles in all the samples.

- What is the relation between the ON and the OC?

It was observed a bigger correlation between ON and OC, both organic contents show the same performance. *S. maritima* environment showed approximately twice more organic carbon and nitrogen contents than *Z. noltii*. Also, our data showed a progressive increase of organic content from station 1 to station 4 as well as a higher in *Z. noltii* environment. Which could be explained by the existence of an inverse association between the size of the sediment particles and organic contents or by the differences in the level of energy, which are exposed.

- Is there a relationship between the concentrations of pigments and C sink?

With respect to the concentration of pigment, we fail to answer this question due to the possible degradation of the pigments. This result were considered not representative therefore not be taken into account in this work.

6. References

Andrade C.F. 1990. Estudo da susceptibilidade ao galgamento da Ria Formosa. *Geolis.* 4, 69:76.

Andrade C., Freitas M.C., Moreno J., Craveiro S.C. 2004. Stratigraphical evidence of Late Holocene barrier breaching and extreme storms in lagoonal sediments of Ria Formosa, Algarve, Portugal. *Marine Geology.* 210, 339:62.

Balsam W.L., Deaton B.C. 1996. Determining the composition of late Quaternary marine sediments from NUV, VIS, and NIR diffuse reflectance spectra. *Marine Geology.* 134, 31:55.

Balsam W.L., Deaton B.C. 1991. Sediment dispersal in the Atlantic Ocean: Evaluation by visible light spectra. *Reviews in Aquatic Science.* 4, 411:47.

Balsam W.L., Deaton B.C., Damuth J.E., 1998. The effects of water content on diffuse reflectance spectrophotometry studies of deep-sea sediment cores. *Marine Geology.* 149, 177:89.

British geological survey. Natural environment research council, [On line]. [Date of consultation: 20 July 2015]. Available in: (http://www.bgs.ac.uk/scienceFacilities/laboratories/mpb/xrd_tech.html).

Brambati A., Fontolan G., Simeoni U. 1991. Carta sediemntologica dello Stretto di Magallano. Programa nazionale di Ricerche in Antartide, Universitá degli Studi di Trieste, Italia.

Brotas V., Amorim-Ferreira A., Vale C., Catarino F. 1990. Oxygen profiles in intertidal sediments of Ria Formosa (S. Portugal). *Hydrobiologia.* 207, 123:9.

Campbell D.I., Smith J., Goodrich J.P., Wall A.M., Schipper L.A. 2014. Year-round growing conditions explains large CO2 sink strength in a New Zealand raised peat bog. *Agricultural and Forest Meteorology*. *192-193*, 59:68.

Cabaço S., Ferreira O., Santos R. 2010. Population dynamics of the sea grass Cymodocea nodosa in Ria Formosa lagoon following inlet artificial relocation. *Estuarine, Coastal and Shelf Science*. 87, 507-16.

Costech, Analytical Tecnologies, [On line]. [Date of consultation: 10 July 2015]. Available in: (http://www.costechanalytical.com/products/ecs4010.aspx).

Couto T., Duarte B., Caçador I., Batea A., Marques J.C. 2013. Salt marsh plants carbon storage in a temperate Atlantic estuary illustrated by a stable isotopic analysis based approach. *Ecological Indicators.* 32, 305:11.

Chmura G.L. 2013. What do we need to assess the sustainability of the tidal salt marsh carbon sink? *Ocean &Coastal Management.* 83, 25:31.

Copertino M. S. 2011. World View, Add coastal vegetation to the climate critical list. *Nature*. *473,* 255.

Deevy, E. 1973. Sulfur, nitrogen and carbon in the biosphere. In G.M. Woodwell & E.V. Peacan (eds.). Carbon and the biosphere. The U.S. Army Environmental Command, 182:90.

Díaz A.E. 2012. Estudio del valor socioeconómico de las praderas de *Posidonia oceánica* de Andalucía como sumideros de carbono y oportunidades de financiar su conservación a través de fondos para la mitigación del cambio climático. Junta de Andalucía. Conserjería de medio ambiente y ordenación del territorio.

Domínguez S.J.M., Román G.A.D., Pietro G. F. 2011. Evaluación de color en suelos del Cerro de Denganthza, municipio de Francisco I. Madero, Hidalgo. Acta Universitaria 21. (Universidad de Guanajuato).

Duarte C.M., Middelburg J.J. Caraco N. 2005. Major role of marine vegetation on the oceanic carbón cycle. *Biogeosciences.* 2, 1:8.

Duarte C.M., Kennedy H., Marbà N., Hendriks I. 2013. Assessing the capacity of seagrass meadows for carbon burial: Current limitations and future strategies. *Ocean & Coastal Management.* 83, 32:8.

Duinker J.C. 1980. Suspended matter in estuaries: Absorption and desorption process. In Olausson E, Cato I (eds) Chemistry and Biogeochemistry of Estuaries. Wiley, New York, 121:53.

Fabiano M. and Danovaro R. 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): Relationship with bacteria and microphytobenthic biomass. *Hydrobiologia.* 277, 71:84.

Folk R.L. 1954. The distinction between grain size and mineral composition in sedimentary-rock nomenclature. *Journal of Geology*. 62, 344:59.

Font E., Veiga-Pires C., Pozo M., Nave S., Costas S., Riz Muñoz F., Abad M., Simões Duarte S., Rodríguez- Vida J. 2013. Benchmarks and sediment source(s) of the 1755 Lisbon tsunami deposit at Boca do Rio Estuary. *Marine Geology.* 343, 1:14.

Fourqurean J.W., Duarte C.M., Kennedy H., Marbà N., Holmer M., Mateo M.A., Apostolaki E.T., Kendrick G.A., Krause-Jensen D., McGlathery K.J., Serrano O. 2012. Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience Advance*. 5, 505:9.

Friedman, G. M., Sanders, J. E., and Kopaska-Merkel, D. C. 1992. In ‘‘Principles of Sedimentary Deposits.’’ p. 717, Macmillan Publishing Company, New York.

Friend P.L., Ciavola P., Cappucci S., Santos R. 2003. Bio-dependent bed parameters as a proxy tool for sediment stability in mixed habitat intertidal areas. *Continental Shelf Research.* 23, 1899:917.

Gázquez G.M.J. 2011. Caracterización y valorización de residuos generados en la industria de producción de dióxido de titanio. Huelva. Universidad de Huelva. Departamento de Fisica Aplicada, *Grupo FRYMA*. (Tesis doctoral).

Grimsditch G., Alder J., Nakamura T., Kenchington R., Tamelander J. 2013. The blue carbon special edition- Introduction and overview. *Ocean & Coastal Management*. 83, 1:4.

Guimarães M.H.M.E., Cunha A.H., Nzinga R.L., Marques J.F. 2012. The Distribution of seagrass (*Zostera noltii*) in the Ria Formosa lagoon and the implications of clam farming on its conservation. *Journal for Nature Conservation* 20, 30:40.

Hoppe H.G., Arnosti C., Herndl G.J. 2002. Ecological significance of bacterial enzymes in the marine environment. Enzymes in the Environment: Activity, Ecology, and Applications. *Marcel Dekker New York*. 73:108.

Kornert M., Vandenberghe J. 1997. Comparison of laser grain size analysis with pipette and sieve analysis: a solution for the underestimation of the clay fraction. *Sedimentology*. 44, 523:35.

Laffoley D., Grimsditch G. 2009. The management of natural coastal carbon sinks. *International Union for Conservation of Nature*. 53.

Lami A., Buchaca T. 2009. Sedimentary Pigment Analysis. C, N, S Elemental Analysis. European Mountain Lake Ecosystems: Regionalisation, diaGnostics & socio-economic Evaluation.

Lavery P.S., Mateo M.A., Serrano O., Rozaimi M. 2013. Variability in the Carbon Storage of Seagrass Habitats and Its Implications for Global Estimates of Blue Carbon Ecosystem Service. *Plos One.* 8, e73748.

Limnología, A. E. 1984. *LIMNETICA Revista de la Asociacion Espanola de Limnologia*. Edicions Universitat Barcelona.

Macreadie P.I., Baird M.E., Trevathan-Tackett S.M., Larkum A.W.D., Ralph, P.J. 2014. Quantifying and modelling the carbon sequestration capacity of seagrass meadows - A critical assessment. *Marine pollution bulletin*. *83*, 430:9.

Macreadie P.I., Allen K., Kelaher B.P., Ralph P.J., Skilbeck C.G. 2012. Paleoreconstruction of estuarine sediments reveal human-induced weaking of coastal carbon sinks. *Global Change Biology*. 18, 891:901.

Malvern, [On line]. [Date of consultation: 10 July 2015]. Available in: (http://www.malvern.com/en/products/technology/laser-diffraction/)

Mazarrasa I., Marbà N., Lovelock C.E., Serrano O., Lavery P.S., Fourequrean J.W., Kennedy H., Mateo M.A., Krause-Jensen D., Steven A.D.L., Duarte C.M. 2015. *Biogeosciences Discuss.* 12, 4107:38.

Mechler L.S., Grady J.R. 1984. Recent Foraminifera of St. Anderw Bay, Florida: Abtract. *American Association of Petroleum Geologists.* 68, 1216:1216.

Mcleod E., Chmura G.L., Bouillon S., Salm R., Björk M., Duarte C.M., Lovelock C.E., Schlesinger WH., Silliman B.R. 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. *Frontiers in Ecology and the Environment.* 9, 552:60.

Nellemann C., Corcoran E., Duarte C.M., Valdés L., De Young C., Fonseca L., Grimsditch G. 2009. Blue Carbon. A Rapid Response Assessment. *United Nations Environment Programme*. 80.

Newton A., Icely J., Falcao M., Nobre A,. Nunes J., Ferreira J., Vale C. 2003. Evaluation of eutrophication in the Ria Formosa coastal lagoon, Portugal. *Continental Shelf Research.* 23, 1945:61.

Norman A.G. 1963. Advances in Agronomy. In Academic Press inc.(London) LTd. Berkeley Square House (eds). Library of Congress catalog Card Number: 50-5598. The United States of America. Elservier.

Peralta G., Pérez-Lloréns J.L., Hernández I., Brun F., Vergara J.J., Bartual A., Gálvez, J.A., García C.M., 2000. Morphological and physiological differences between two morphotypes of *Zostera noltii* Hornem. from the south-western Iberian Peninsula. *Helgoland Marine Research.* 54, 80:6.

Pergent G., Bazairi H., Bianchi C.N., Boudouresque C.F., Buia M.C., Clabaut P et Verlaque M. 2012. Les herbiers de Magnoliophytes marines de Méditerranée – Résilience et contribution à l'atténuation des changements climatiques. *International Union for Conservation of Nature (*IUCN), Gland, Switzerland and Málaga, Spain. 80.

Pozo M., Ruiz F., Carretero M.I., Rodríguez V.J., Cáceres L.M., Abad M., González-Regalado M.L. 2010. *Sedimentary Geology*. 225, 1:18.

Redfield A.C., Ketchum B.H., Richard F.A. 1963. The influence of organism on the composition of sea-water. *The Sea.* 2, 26:77.

Rullkötter J. 2000. Organic matter: the driving force for early diagenesis. Schulz HD, Zabel M (eds.), *Marine Geochemistry*, 129:172.

Sanchez A., González-Yajimovich O., Balart E., López-Ortiz B.E., Aguíñiga-García S., Ortiz-Hernández M.C. 2013. Acumulación de carbon orgánico total y carbonato de calico en la zona de oxígeno mínimo del Pacífico nororiental mexicano. *Revista Mexicana de Ciencias Geológicas.* 30, 222:32.

Sanger J.E., Gorham E. 1972. Stratigraphy of fossil pigments as a guide to the postglacial history of kirchner marsh, Minnesota. *Limnology and oceanography.* 17.

Silva N., Ortiz P. 2002. C y N, su distribución y estequiometría, en sedimentos superficiales de la región sur de la zona de fiordos y canales australes de Chile, 52º-56ºS (Crucero Cimar Fiordo 3). *Ciencia. Tecnologia. Mar*. 25, 89:108.

Silva N., Maturana J., Sepúlveda J.I., Ahumada R. 1998. Materia orgánica, C y N, su distribución estequiometria, en sedimentos superficiales de la región norte de los fiordos y canales australes de Chile (Crucero Cimar-Fiordo 1). *Ciencia. Tecnologia. Mar*. 21, 49:74.

Syvitski, J. P. 2007. Principles, methods and application of particle size analysis. Cambridge University Press.

Touchette B.W., Burkholder J.M. 2000. Overview of the physiological ecology of carbon metabolism in seagrasses. *Journal of Experimental Marine Biology & Ecology*. 250, 169:205.

Waycott M., Duarte C.M., Carruthers T.J.B., Orth R.J., Dennison W.C., Olyarnik S., Calladine A., Fourqurean J.W., Heck K.L., Hughes A.R., Kendrick G.A., Kenworthy W.J., Short F.T., Williams S.L. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciencie.* 106, 12377:81.

Zak D.R., Blackwood C.B., Waldrop M.P. 2006. A molecular dawn for biogeochemistry. *Trends Ecology Evolution journal.* 21, 288:95.

ANNEXES

Annex 1: Physical properties

Table 1: Physical properties for each station according to a transect along the tributary channel.

Station	Type	Temperature (ºC)	Salinity (psu)	pH (NBS scale)	time
1	Main Channel	19.30	36.35	7.34	9:00
1	Zostera	18.43	28.18	7.64	
1	Spartina	18.82	28.12	7.40	
2	Main Channel	19.92	36.80	8.27	10:24
2	Zostera	19.48	21.43	7.74	
2	Spartina	19.28	36.86	7.67	
3	Main Channel	20.55	38.81	7.74	11:15
3	Zostera	20.15	30.38	7.60	
3	Spartina				13:00
4	Main Channel	19.98	36.64	8.19	11:42
4	Zostera	20.62	34.81	7.87	
4	Spartina	22.39	35.79	8.25	

Annex 2: Sedimentological analysis

Table 2.I: Results of grain size for all samples obtained from Sediment granulometry by laser counting and sieving.

Table 2.II: *Two-way* analysis of variance (*ANOVA*) to Clay ToT variable.

Table 2.III: *Two-way* analysis of variance (*ANOVA*) to Clay Min variable.

Table 2.IV: *Two-way* analysis of variance (*ANOVA*) to Fine/coarse variable.

Table 2.V: Color results obtained in each of the treatments. (Commission International de l'Eclairage).

Table 2.VI: *Two-way* analysis of variance (*ANOVA*) to a*.noOM variable.

Table 2.VII: *Two-way* analysis of variance (*ANOVA*) to b*.noOM variable.

Table 2.VIII: *Two-way* analysis of variance (*ANOVA*) to L*.noOM variable.

Table 2.IX: *Two-way* analysis of variance (*ANOVA*) to a* variable.

Table 2.X: *Two-way* analysis of variance (*ANOVA*) to b* variable.

Table 2.XI: *Two-way* analysis of variance (*ANOVA*) to L* variable.

Figure 2.I: Rplot of Correlation of Density (g. ml $^{-1}$), Content of Water (% ww) and clay in mineral samples (i.e. after organic matter removal) (% wv) fron *Z.noltii* enviroments.

Figure 2.II: Rplot of Correlation of Density (g. ml $^{-1}$), Content of Water (% ww) and clay in mineral samples (i.e. after organic matter removal) (% wv) fron *S. maritima* enviroments.

Figure 2.III: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and carbonate content (% dw) from *Z.noltii* environments.

Figure 2.IV: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and organic carbon content (% dw) from *Z.noltii* environments.

Figure 2.V: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and organic carbon content (% dw) from *S.maritima* environments.

Table 2.I: Results of grain size for all samples obtained from Sediment granulometry by laser counting and sieving.

Sample	Station	Type	Mean ToT	Mean Min	Mediana ToT	Mediana Min	Mode ToT	Mode Min	Clay ToT (%wv)	Clay Min (%wv)	Silt ToT(%wv)	Silt Min (%wv)	Sand ToT (%wv)	Sand Min (% wv)	Fine/Coarse
Zn1a	1	ZosN	101.40	111.80	100.30	110.40	122.00	130.60	3.70	5.40	5.90	2.90	67.50	77.40	0.000
Zn1b	1	ZosN	97.75	122.10	100.30	121.10	152.20	130.60	3.40	4.50	5.80	2.20	73.30	83.10	0.000
Zn1c	1	ZosN	110.30	126.70	122.00	125.00	177.30	130.60	3.70	3.90	5.80	1.90	68.50	85.90	0.000
Zn1d	1	ZosN	109.90	125.60	123.30	122.00	71.53	130.60	3.20	3.30	5.00	1.60	72.00	86.80	0.000
Sp1a	1	Spart	58.08	79.72	35.33	71.53	112.10	130.60	4.80	10.90	13.30	6.90	35.70	53.70	1.091
Sp1b	1	Spart	52.13	84.64	26.82	78.50	17.94	130.60	5.60	9.00	15.80	5.80	30.70	57.60	0.798
Sp1c	1	Spart	51.37	61.55	29.09	38.37	70.91	112.10	5.20	14.40	15.00	8.10	30.40	41.30	0.979
Sp1d	1	Spart	54.69	75.66	31.76	68.37	82.61	112.10	5.00	9.70	14.30	6.80	33.00	53.00	1.937
Zn2a	2	ZosN	61.65	104.20	34.02	104.90	130.60	130.60	7.60	7.50	12.70	4.20	39.50	70.30	0.198
Zn2b	2	ZosN	64.03	87.00	38.05	82.89	130.60	130.60	6.80	10.30	12.50	6.10	41.10	58.50	0.210
Zn2c	2	ZosN	64.52	83.53	35.53	78.04	152.20	130.60	7.20	11.60	12.70	6.30	40.80	56.00	0.234
Zn2d	2	ZosN	54.06	82.38	25.56	75.99	112.10	130.60	7.70	11.90	14.20	6.80	33.90	54.40	0.482
Sp2a	2	Spart	53.38	25.57	31.99	9.40	70.91	52.25	5.00	21.60	13.80	12.20	32.00	13.90	0.277
Sp2b	2	Spart	59.69	95.85	38.99	91.65	82.61	112.10	4.30	5.50	12.20	3.50	36.80	68.40	0.000
Sp2c	2	Spart	53.25	71.57	31.59	64.86	70.91	96.24	5.20	9.20	14.00	5.60	32.00	51.40	3.745
Sp2d	2	Spart	55.94	29.06	36.60	10.52	70.91	60.87	4.40	19.50	12.50	12.60	34.10	16.90	2.218
Zn3a	3	ZosN	54.69	33.96	33.59	12.51	82.61	70.91	6.90	21.50	13.00	9.00	35.50	22.30	2.940
Zn3b	3	ZosN	51.02	47.33	28.79	35.64	82.61	70.91	7.40	12.50	13.20	9.10	32.60	32.50	0.000
Zn3c	3	ZosN	48.84	28.55	25.10	8.63	82.61	70.91	8.00	23.40	3.80	11.10	30.20	17.50	2.699
Zn3d	3	ZosN	50.00	41.49	47.87	23.02	60.87	70.91	6.10	18.00	13.70	9.00	32.40	28.40	3.031
Sp3a	3	Spart	48.70	28.40	25.84	11.58	70.91	60.87	6.20	20.30	15.20	11.60	28.70	16.60	3.722
Sp3b	3	Spart	52.38	23.25	30.32	8.53	70.91	52.25	5.60	23.00	14.10	12.60	23.20	12.10	4.375
Sp3c	3	Spart	53.16	24.98	31.10	9.15	52.25	52.25	5.50	21.90	13.80	13.00	31.70	13.40	4.000
Sp3d	3	Spart	40.97	28.49	25.82	12.24	44.85	52.25	6.10	19.10	14.90	11.60	31.50	15.80	4.588
Zn4a	4	ZosN	48.30	20.19	21.34	6.96	13.22	5.29	7.20	23.00	16.00	14.60	27.90	9.50	4.521
Zn4b	4	ZosN	57.07	21.38	26.28	7.42	177.30	5.29	6.60	22.10	14.70	14.80	33.60	10.20	5.812
Zn4c	4	ZosN	55.52	18.01	25.40	6.27	152.20	5.29	6.80	23.20	15.00	15.80	32.70	7.50	5.448
Zn4d	4	ZosN	57.42	22.20	28.51	7.20	152.20	5.29	6.10	23.60	14.50	13.80	34.10	11.20	6.552
Sp4a	4	Spart	63.95	17.80	55.73	7.01	96.24	6.16	4.70	23.40	10.50	15.10	45.90	7.30	2.836
Sp4b	4	Spart	44.15	25.59	22.64	11.04	17.94	52.25	6.80	19.90	16.00	12.50	25.40	13.40	13.663
Sp4c	4	Spart	44.00	21.23	23.05	8.21	38.50	44.85	6.50	22.50	16.00	13.50	24.90	9.90	5.854
Sp4d	4	Spart	45.42	22.17	23.39	8.00	20.90	6.16	6.70	21.60	15.80	14.80	26.00	11.00	5.360

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	3.645	3.645	11.65	0.00228	**
Station	3	24.575	8.192	26.18	9.65E-08	***
Type: Station	3	18.945	6.315	20.18	9.48E-07	***
Residuals	24	7.51	0.313			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.III: *Two-way* analysis of variance (*ANOVA*) to Clay Min variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	65.6	65.6	5.234	0.0313	*
Station	3	1126.5	375.5	29.981	2.76E-08	***
Type: Station	3	63.6	21.2	1.693	0.1951	
Residuals	24	300.6	12.5			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.IV: *Two-way* analysis of variance (*ANOVA*) to Fine/coarse variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	16.99	16.99	4.811	0.0382	*
Station	3	163.47	54.49	15.4333	8.36E-06	***
Type: Station	3	0.82	0.27	0.077	0.9718	
Residuals	24	84.73				

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.V: Color results obtained in each of the treatments. (Commission International de l'Eclairage).

Sample	Station	Type	L*.no.OM	a*.no.OM	b*.no.OM	L*	a*	b*
Zn1a	1	ZosN	5.513	31.120	99.723	66.397	-0.177	16.277
Zn1b	1	ZosN	7.263	39.320	85.450	50.923	0.630	19.167
Zn1c	1	ZosN	7.750	41.170	94.540	61.233	2.143	33.703
Zn1d	1	ZosN	8.437	42.273	86.443	55.457	1.663	27.080
Sp1a	1	Spart	9.680	44.060	80.637	65.780	-1.213	21.447
Sp1b	1	Spart	10.480	42.830	79.883	62.217	0.070	28.880
Sp1c	1	Spart	10.713	47.183	85.480	65.843	-0.687	23.283
Sp1d	1	Spart	11.797	45.837	72.463	66.020	-1.253	26.707
Zn2a	2	ZosN	10.570	44.127	86.750	57.237	0.557	21.560
Zn2b	2	ZosN	5.033	37.847	54.117	59.560	2.710	27.120
Zn2c	2	ZosN	5.783	40.600	56.023	56.483	2.270	27.277
Zn2d	2	ZosN	6.567	41.880	49.773	59.623	2.097	30.283
Sp2a	2	Spart	6.293	42.063	55.947	48.583	-0.400	20.930
Sp2b	2	Spart	6.373	39.863	51.830	60.300	-1.240	25.493
Sp2c	2	Spart	5.667	40.257	53.793	58.590	-0.863	23.410
Sp2d	2	Spart	4.617	39.873	57.237	61.277	-1.427	22.717
Zn3a	3	ZosN	6.533	41.653	50.630	57.693	1.693	33.693
Zn3b	3	ZosN	8.913	43.260	47.323	59.767	-0.923	25.100
Zn3c	3	ZosN	6.297	40.330	51.377	53.777	0.813	25.613
Zn3d	3	ZosN	8.223	42.607	46.533	59.617	0.823	29.877
Sp3a	3	Spart	9.600	45.260	45.267	54.950	-1.353	22.200
Sp3b	3	Spart	0.047	20.983	55.533	51.637	-1.227	20.283
Sp3c	3	Spart	20.497	18.860	50.750	58.913	-1.270	23.250
Sp3d	3	Spart	0.320	22.003	59.200	62.923	-1.230	24.823
Zn4a	4	ZosN	0.620	23.790	58.707	57.403	2.053	29.143
Zn4b	4	ZosN	1.943	27.213	56.653	61.903	1.377	30.050
Zn4c	4	ZosN	1.610	26.567	56.590	55.330	1.047	26.137
Zn4d	4	ZosN	2.070	27.830	56.940	57.843	3.060	28.817
Sp4a	4	Spart	0.817	9.297	18.967	48.677	-0.137	19.923
Sp4b	4	Spart	0.000	0.000	0.000	47.083	-1.573	18.573
Sp4c	4	Spart	0.000	0.000	0.000	53.597	-2.023	20.293
Sp4d	4	Spart	0.000	0.000	0.000	52.077	-1.327	19.540

Table 2.VI: *Two-way* analysis of variance (*ANOVA*) to a*.noOM variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	555	554.6	20.43	0.000141	***
Station	3	3898	1299.2	47.87	2.79E-10	***
Type: Station	3	1147	382.2	14.08	1.68E-05	***
Residuals	24	651	27.1			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.VII: *Two-way* analysis of variance (*ANOVA*) to b*.noOM variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	2288	2288	36.57	3.03E-06	***
Station	3	12253	4084	65.28	1.09E-11	***
Type: Station	3	3629	1210	19.34	1.36E-06	***
Residuals	24	1502				

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.VIII: *Two-way* analysis of variance (*ANOVA*) to L*.noOM variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0.44	0.44	0.034	0.855304	
Station	3	299.57	99.86	7.625	0.000949	***
Type: Station	3	29.88	9.96	0.761	0.527236	
Residuals	24	314.29	13.1			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.IX: *Two-way* analysis of variance (*ANOVA*) to a* variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	47.51	47.51	73.151	9.48E-09	***
Station	3	2.86	0.95	1.469	0.248	
Type: Station	3	2.79	0.93	1.431	0.258	
Residuals	24	15.59	0.65			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.X: *Two-way* analysis of variance (*ANOVA*) to b* variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	149.4	149.4	10.537	0.00343	**
Station	3	10	3.33	0.235	0.87114	
Type: Station	3	106.8	35.61	2.512	0.08266	
Residuals	24	340.3	14.18			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

Table 2.XI: *Two-way* analysis of variance (*ANOVA*) to L* variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	4.3	4.34	0.259	0.6153	
Station	3	226.5	75.5	4.514	0.012	*
Type: Station	3	202.6	67.52	4.037	0.0186	*
Residuals	24	401.4	16.73			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	` .´ 0.1	`´ 1

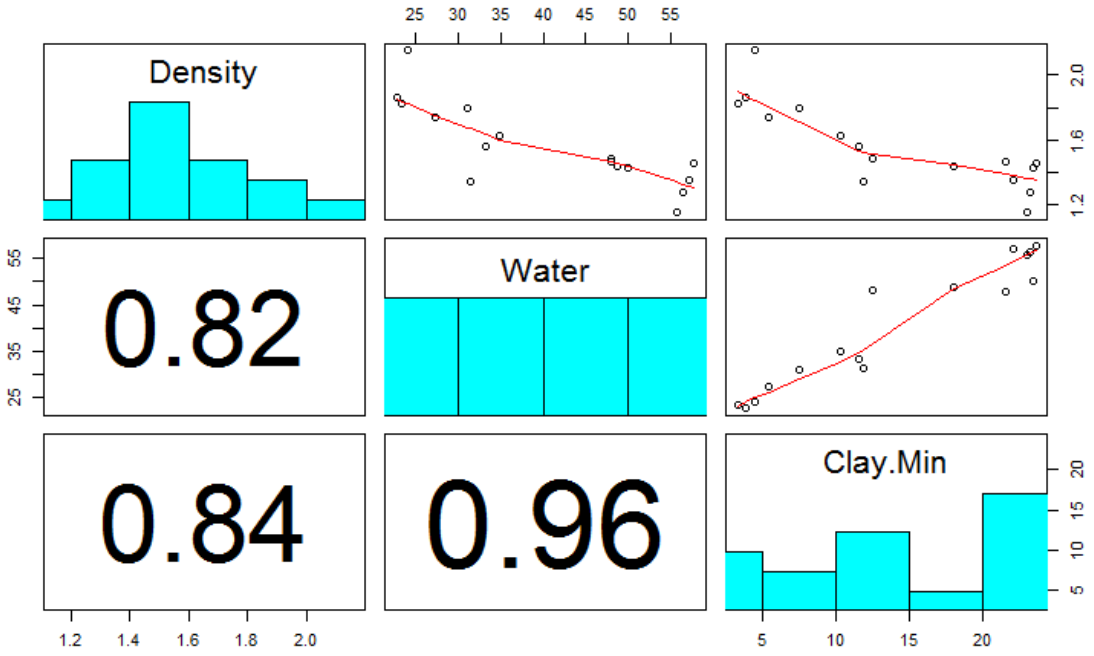


Figure 2.I: Rplot of Correlation of Density (g. ml $^{-1}$), Content of Water (% ww) and clay in mineral samples (i.e. after organic matter removal) (% wv) fron *Z.noltii* enviroments.

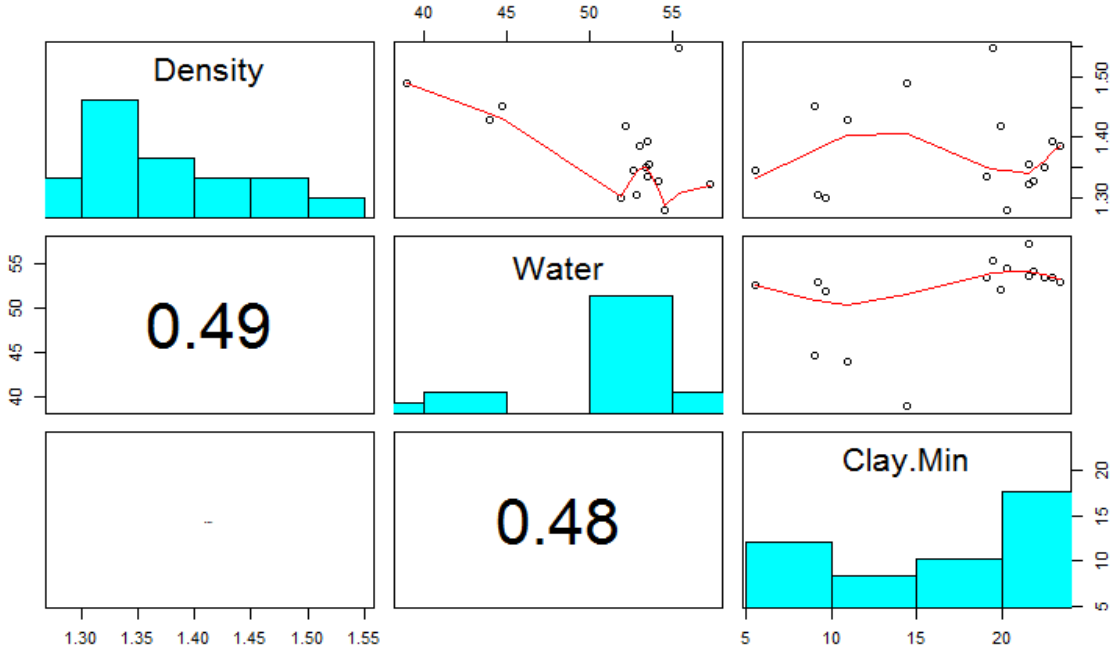
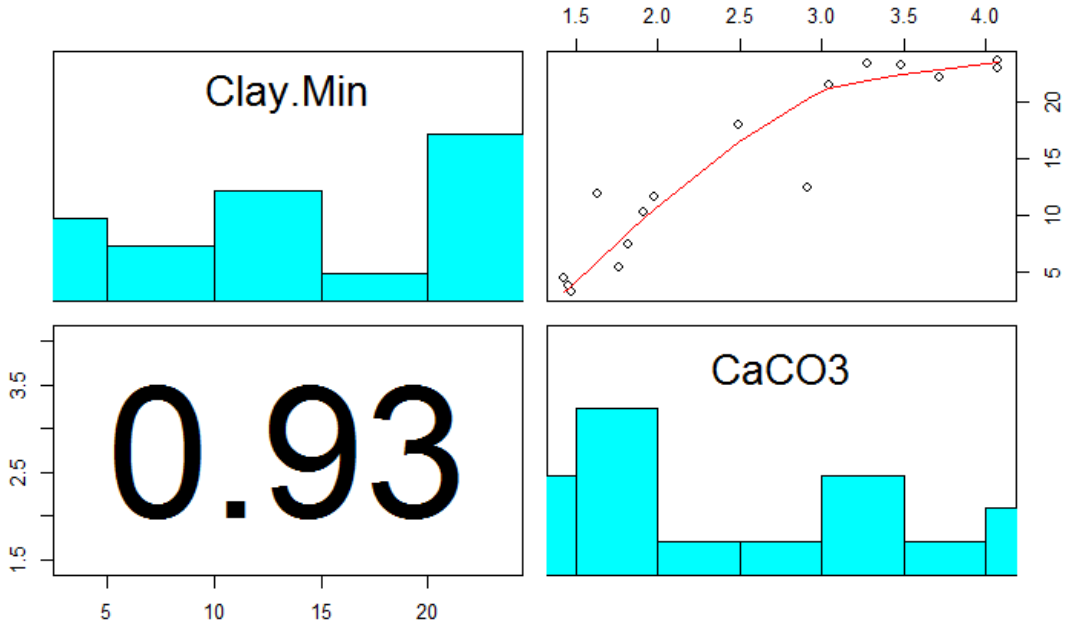


Figure 2.II: Rplot of Correlation of Density (g. ml $^{-1}$), Content of Water (% ww) and clay in mineral samples (i.e. after organic matter removal) (% wv) fron *S. maritima*



enviroments.

Figure 2.III: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and carbonate content (% dw) from *Z.noltii* environments.

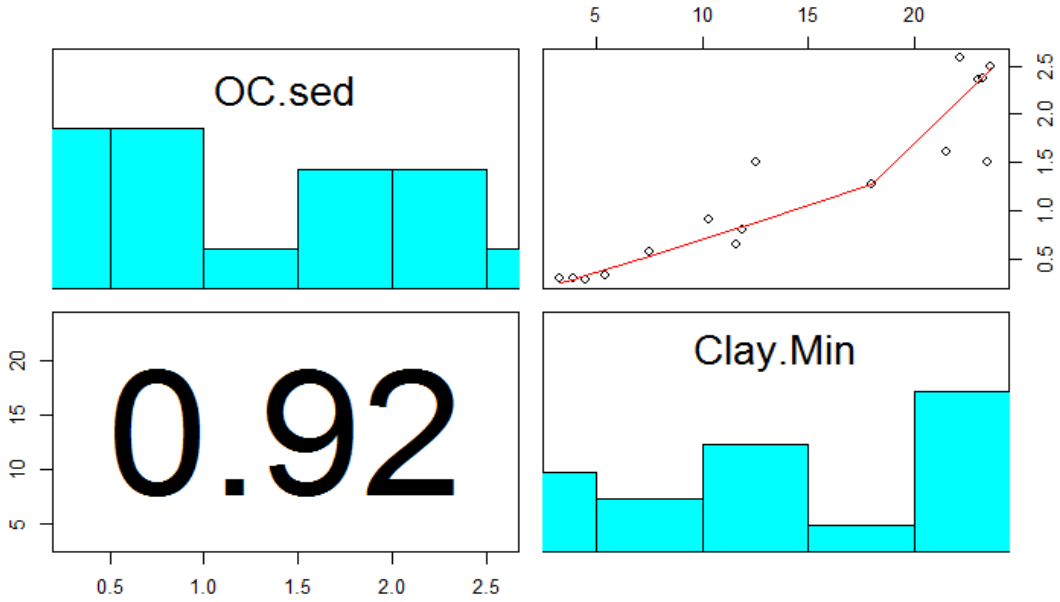


Figure 2.IV: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and organic carbon content (% dw) from *Z.noltii* environments.

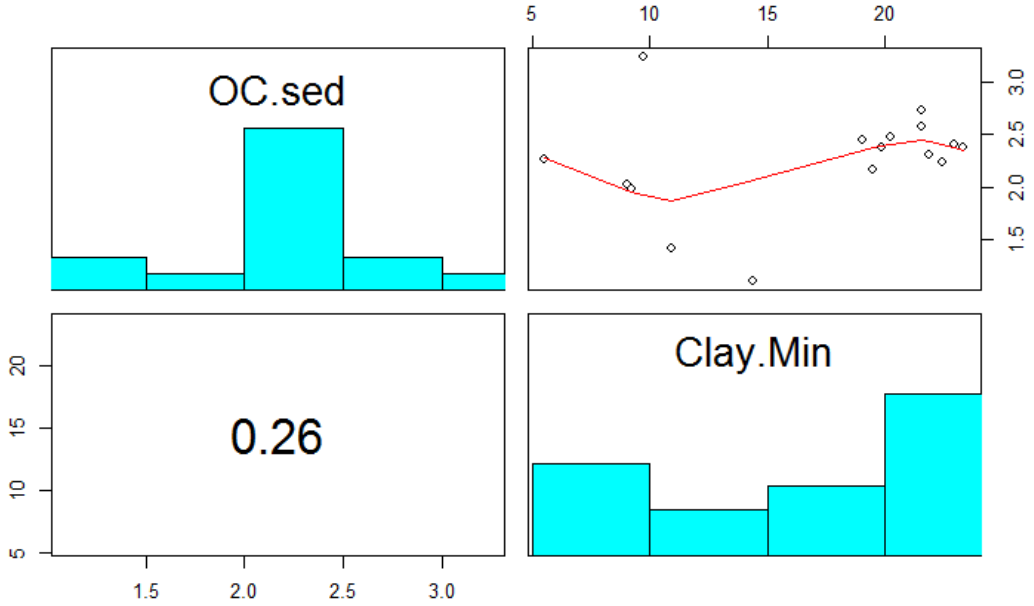


Figure 2.V: Rplot of Correlation of clay in mineral samples (i.e. after organic matter removal) (% wv) and organic carbon content (% dw) from *S.maritima* environments.

Annex 3: Geochemical analysis

Table 3.I: Results of density (g. ml^{-1}), vegetables (%), water (%), organic matter (%dw) and carbonate contents (%dw) for all samples.

Table 3.II: Results of elementar analysis: Organic Carbon (OC % dw), Inorganic Carbon (IC % dw), Total Carbon (TC % dw), Organic Nitrogen (ON % dw), Inorganic Nitrogen (IN % dw) and Total Nitrogen (TN % dw).

Table 3.III: *Two-way* analysis of variance (*ANOVA*) to Density variable.

Table 3.IV: *Two-way* analysis of variance (*ANOVA*) to carbonate variable.

Table 3.V: *Two-way* analysis of variance (*ANOVA*) to Organic matter variable.

Table 3.VI: *Two-way* analysis of variance (*ANOVA*) to vegetables percentage variable.

Table 3.VII: *Two-way* analysis of variance (*ANOVA*) to water percentage variable.

Table 3.VIII: *Two-way* analysis of variance (*ANOVA*) to inorganic nitrogen (IN).

Table 3.IV: *Two-way* analysis of variance (*ANOVA*) to inorganic carbon (IC).

Table 3.V: *Two-way* analysis of variance (*ANOVA*) to organic nitrogen (ON).

Table 3.VI: *Two-way* analysis of variance (*ANOVA*) to organic carbon (OC).

Table 3.VII: *Two-way* analysis of variance (*ANOVA*) to C/N ratio.

Figure 3.I: Density (g. ml $^{-1}$) for samples with organic matter in function of sampling station for each species.

Table 3.I: Results of density (g. ml^{-1}), vegetables (%), water (%), organic matter (%dw) and carbonate contents (%dw) for all samples.

Sample	Station	Type	Density (g ml-1)	Veg (%)	OM (% dw)	Water (% ww)	$CaCO_3$ (% dw)
Zn1a	1	ZosN	1.741	6.215	2.417	27.347	1.764
Zn1b	1	ZosN	2.158	8.222	2.096	24.086	1.430
Zn1c	1	ZosN	1.864	11.488	1.857	22.681	1.454
Zn1d	1	ZosN	1.829	12.979	1.865	23.324	1.471
Sp1a	1	Spart	1.427	9.774	7.088	43.923	3.086
Sp1b	1	Spart	1.451	13.335	7.977	44.725	2.823
Sp1c	1	Spart	1.490	13.895	6.008	38.899	2.471
Sp1d	1	Spart	1.298	7.934	10.647	51.894	3.438
Zn2a	2	ZosN	1.795	8.781	4.441	31.101	1.819
Zn2b	2	ZosN	1.621	9.002	4.356	34.919	1.913
Zn2c	2	ZosN	1.556	6.864	4.048	33.270	1.974
Zn2d	2	ZosN	1.343	15.266	4.054	31.429	1.630
Sp2a	2	Spart	1.323	9.350	11.919	57.357	2.495
Sp2b	2	Spart	1.344	20.022	9.940	52.700	2.227
Sp2c	2	Spart	1.305	3.763	9.629	52.897	1.980
Sp2d	2	Spart	1.548	7.099	10.323	55.464	2.739
Zn3a	3	ZosN	1.466	7.946	6.804	47.896	3.041
Zn3b	3	ZosN	1.486	11.014	7.018	48.068	2.916
Zn3c	3	ZosN	1.430	17.956	7.712	50.000	3.282
Zn3d	3	ZosN	1.435	17.677	7.182	48.678	2.494
Sp3a	3	Spart	1.278	9.945	10.311	54.578	2.676
Sp3b	3	Spart	1.393	5.130	10.487	53.512	2.484
Sp3c	3	Spart	1.326	10.989	11.728	54.144	0.624
Sp3d	3	Spart	1.335	2.363	10.008	53.482	2.904
Zn4a	4	ZosN	1.146	13.274	9.663	55.747	4.073
Zn4b	4	ZosN	1.346	11.047	11.039	57.143	3.719
Zn4c	4	ZosN	1.278	11.769	10.585	56.415	3.483
Zn4d	4	ZosN	1.456	9.685	9.796	57.797	4.077
Sp4a	4	Spart	1.385	11.881	10.514	53.052	2.619
Sp4b	4	Spart	1.419	18.191	10.186	52.194	2.746
Sp4c	4	Spart	1.349	3.620	10.425	53.411	2.667
Sp4d	4	Spart	1.355	9.378	10.665	53.642	2.532

Table 3.II: Results of elementar analysis: Organic Carbon (OC % dw), Inorganic Carbon (IC % dw), Total Carbon (TC % dw), Organic Nitrogen (ON % dw), Inorganic Nitrogen (IN % dw) and Total Nitrogen (TN % dw).

Sample	Station	Type	IC (%dw)	OC (%dw)	TC (%dw)	IN (%dw)	ON (% dw)	TN (% dw)	C/N
Zn1a	1	ZosN	0.62	0.33	0.95	0.03	0.04	0.0012	8.25
Zn1b	1	ZosN	0.48	0.29	0.77	0.01	0.03	0.0003	9.6666667
Zn1c	1	ZosN	0.51	0.3	0.81	0.01	0.03	0.0003	10
Zn1d	1	ZosN	0.56	0.31	0.87	0.02	0.03	0.0006	10.333333
Sp1a	1	Spart	1.11	1.42	2.53	0.07	0.15	0.0105	9.4666667
Sp1b	1	Spart	0.93	2.02	2.95	0.06	0.2	0.012	10.1
Sp1c	1	Spart	0.72	1.11	1.83	0.05	0.12	0.006	9.25
Sp1d	1	Spart	0.15	3.23	3.38	0	0.33	0	9.7878788
Zn2a	2	ZosN	0.53	0.58	1.11	0.05	0.06	0.003	9.6666667
Zn2b	2	ZosN	0.28	0.91	1.19	0.02	0.1	0.002	9.1
Zn2c	2	ZosN	0.55	0.66	1.21	0.04	0.08	0.0032	8.25
Zn2d	2	ZosN	0.34	0.81	1.15	0.02	0.09	0.0018	9
Sp2a	2	Spart	1	2.73	3.73	0.13	0.32	0.0416	8.53125
Sp2b	2	Spart	0.56	2.27	2.83	0.07	0.27	0.0189	8.4074074
Sp2c	2	Spart	0.83	1.98	2.81	0.11	0.23	0.0253	8.6086957
Sp2d	2	Spart	1.03	2.17	3.2	0.13	0.26	0.0338	8.3461538
Zn3a	3	ZosN	0.46	1.61	2.07	0.04	0.18	0.0072	8.9444444
Zn3b	3	ZosN	0.55	1.51	2.06	0.05	0.16	0.008	9.4375
Zn3c	3	ZosN	0.59	1.51	2.1	0.06	0.17	0.0102	8.8823529
Zn3d	3	ZosN	0.69	1.27	1.96	0.07	0.14	0.0098	9.0714286
Sp3a	3	Spart	0.42	2.48	2.9	0.06	0.3	0.018	8.2666667
Sp3b	3	Spart	0.51	2.41	2.92	0.07	0.29	0.0203	8.3103448
Sp3c	3	Spart	0.49	2.3	2.79	0.07	0.29	0.0203	7.9310345
Sp3d	3	Spart	0.41	2.45	2.86	0.07	0.3	0.021	8.1666667
Zn4a	4	ZosN	0.21	2.35	2.56	0.03	0.27	0.0081	8.7037037
Zn4b	4	ZosN	0.13	2.58	2.71	0.02	0.3	0.006	8.6
Zn4c	4	ZosN	0.26	2.37	2.63	0.03	0.29	0.0087	8.1724138
Zn4d	4	ZosN	0.07	2.49	2.56	-0.02	0.32	-0.0064	7.78125
Sp4a	4	Spart	0.57	2.37	2.94	0.08	0.3	0.024	7.9
Sp4b	4	Spart	0.43	2.37	2.8	0.06	0.29	0.0174	8.1724138
Sp4c	4	Spart	0.63	2.23	2.86	0.08	0.28	0.0224	7.9642857
Sp4d	4	Spart	0.25	2.57	2.82	0.03	0.32	0.0096	8.03125

Table 3.III: *Two-way* analysis of variance (*ANOVA*) to Density variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0.266	0.26663	19.706	0.000173	***
Station	3	0.4592	0.15308	11.314	8.07E-05	***
Type: Station	3	0.3145	0.10485	7.749	0.000866	***
Residuals	24	0.3247	0.01353			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.IV: *Two-way* analysis of variance (*ANOVA*) to carbonate variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0	0	0	0.991272	
Station	3	6.193	2.0645	10.21	0.000161	***
Type: Station	3	8.639	2.8798	14.24	1.55E-05	***
Residuals	24	4.855	0.2023			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.V: *Two-way* analysis of variance (*ANOVA*) to Organic matter variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	15.8	15.845	0.791	0.383	
Station	3	4.9	1.638	0.082	0.969	
Type: Station	3	75.2	25.071	1.251	0.313	
Residuals	24	480.9	20.035			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.VI: *Two-way* analysis of variance (*ANOVA*) to vegetables percentage variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	15.8	15.845	0.791	0.383	
Station	3	4.9	1.638	0.082	0.969	
Type: Station	3	75.2	25.071	1.251	0.313	
Residuals	24	480.9	20.035			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.VII: *Two-way* analysis of variance (*ANOVA*) to water percentage variable.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	967.7	967.7	178.35	1.32E-12	***
Station	3	1943.4	647.8	119.4	1.47E-14	***
Type: Station	3	917.1	305.7	56.35	5.17E-11	***
Residuals	24	130.2	5.4			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.VIII: *Two-way* analysis of variance (*ANOVA*) to inorganic nitrogen (IN).

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0.013613	0.013613	32.029	7.92E-06	***
Station	3	0.008437	0.002812	6.618	2.05E-03	**
Type: Station	3	0.004737	0.001579	3.716	2.51E-02	*
Residuals	24	0.0102	0.000425			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.IV: *Two-way* analysis of variance (*ANOVA*) to inorganic carbon (IC).

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0.323	0.322	8.904	0.00645	**
Station	3	0.5436	0.1812	5.01	7.73E-03	**
Type: Station	3	0.3257	0.1086	3.002	5.03E-02	.
Residuals	24	0.868	0.0362			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.V: *Two-way* analysis of variance (*ANOVA*) to organic nitrogen (ON).

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	0.1201	0.12005	84.493	2.48E-09	***
Station	3	0.1407	0.04691	33.018	1.11E-08	***
Type: Station	3	0.0415	0.01383	9.736	2.18E-04	***
Residuals	24	0.0341	0.00142			

Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.VI: *Two-way* analysis of variance (*ANOVA*) to organic carbon (OC).

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	8.232	8.232	62.209	4.05E-08	***
Station	3	7.407	2.469	18.658	1.83E-06	***
Type: Station	3	3.677	1.226	9.263	2.99E-04	***
Residuals	24	3.176	0.132			
Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

Table 3.VI: *Two-way* analysis of variance (*ANOVA*) to C/N ratio.

	pf	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.codes:
Type	1	1.369	1.3691	6.785	0.0155	*
Station	3	8.69	2.8967	14.355	1.45E-05	***
Type: Station	3	1.062	0.3541	1.755	1.83E-01	
Residuals	24	4.843	0.2018			
Signif.codes:	0 `***´	0.001 `**´	0.01 `*´	0.5 `.´	`.´ 0.1	`´ 1

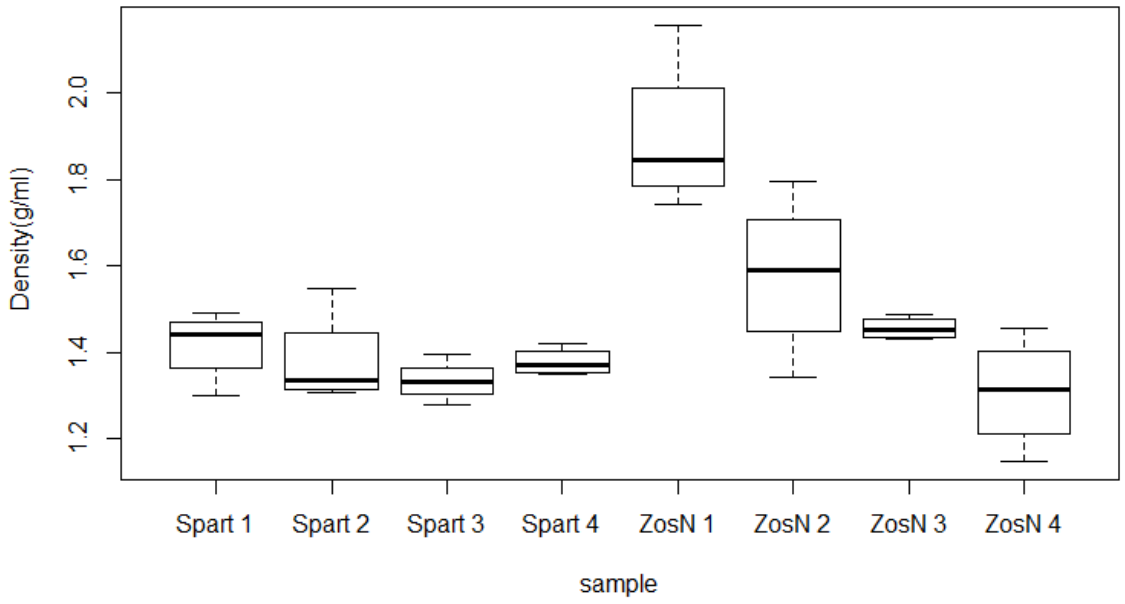


Figure 3.I: Density (g. m^{-1}.) in function of sampling station for each species

Annex 3: Pigment analysis

Table 3.I: Integration of the result of the specific pigments determined by ion pairing, reverse-phase HPLC.

Figure 3.I: Chromatogram obtained of the result of the specific pigments determined by ion pairing, reverse-phase HPLC.

Figure 3. III: Chlorophyll a (µg/g O.M) and Chlorophyll b (µg/g O.M) for samples with organic matter by sampling station for each species.

Figure 3. IV: Total Carotenes (µg/g O.M) and Total Chlorophyll (µg/g O.M) for samples with organic matter by sampling station for each species.

Table 3.I: Integration of the result of the specific pigments determined by ion pairing, reverse-phase HPLC.

	Name	Start Time (min)	RT	End Time (min)	Area	Height	% Area	Units
1		0.867	0.945	1.075	2081	512	0.65	
2		1.508	1.595	1.650	22783	6851	7.17	
3		1.650	1.742	1.833	26064	2879	8.20	
4		1.833	1.833	2.583	66030	2553	20.77	
5		5.442	5.632	5.933	71330	5778	22.44	
6		5.933	6.024	6.075	9350	1225	2.94	
7		6.075	6.123	6.383	11833	1413	3.72	
8		6.383	6.466	6.542	2224	423	0.70	
9		10.075	10.639	12.992	106247	1040	33.42	

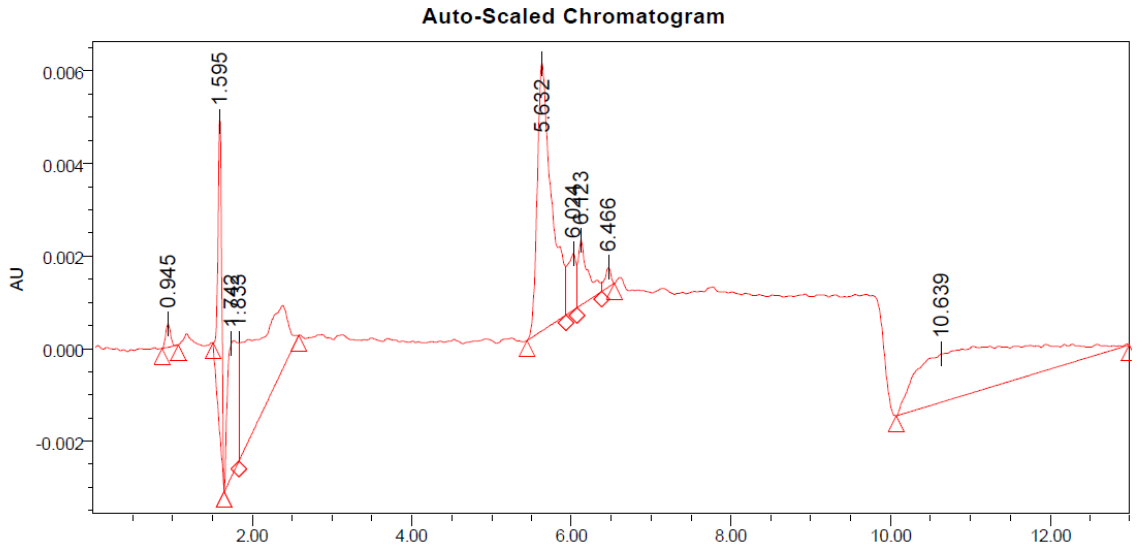


Figure 3.I: Chromatogram obtined of the result of thr spacific pigments determined by ion pairing, reverse-phase HPLC.

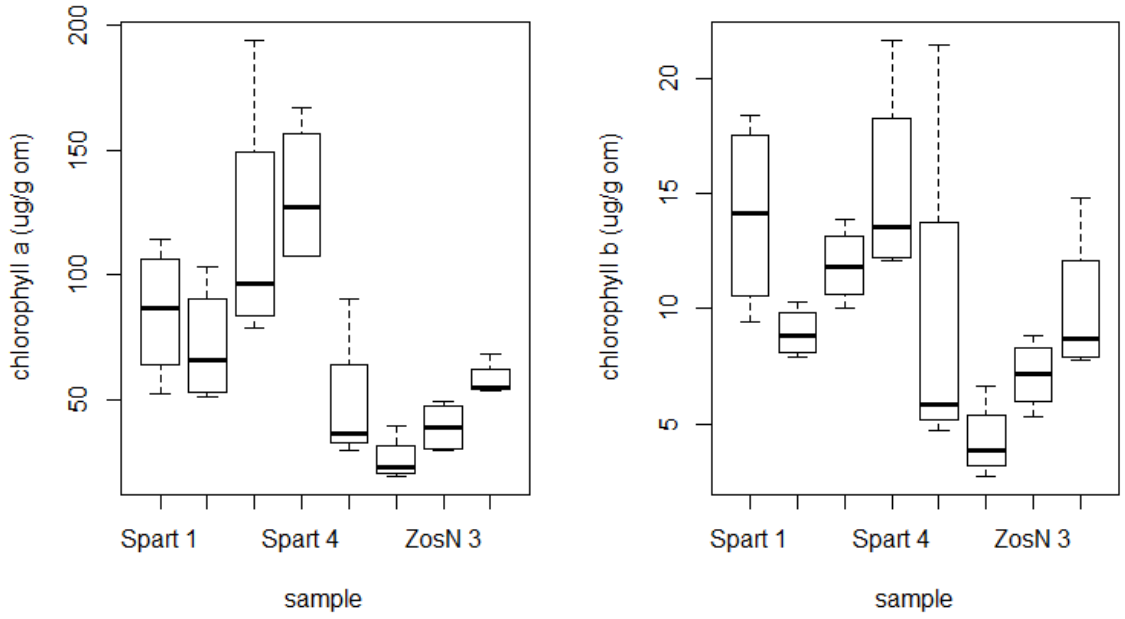


Figure 3. III: Chlorophyll a (µg/g O.M) (left) and Chlorophyll b (µg/g O.M) (right) for samples with organic matter in function of sampling station for each species.

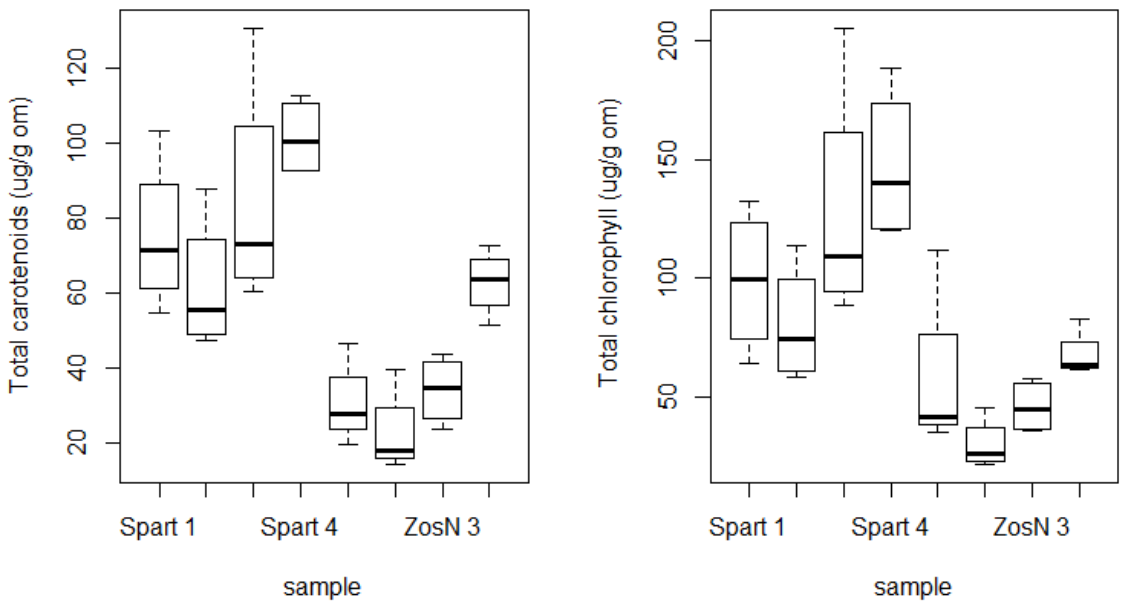


Figure 3. IV: Total Carotene (µg/g O.M) and Total Chlorophyll **(**µg/g O.M) for samples with organic matter in function of sampling station for each species.