INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

XTREMEVEGGIES
HALOPHYTE CULTIVATION USING SALINE WATER AND AMENDED UNDERUSED SOILS AND SEDIMENTS

ANA LÚCIA TEIXEIRA CORTINHAS

SCIENTIFIC ADVISORS:
Ph.D Maria Manuela Silva Nunes Reis Abreu
Ph.D Ana Cristina Riepenhausen Delaunay Caperta
Ph.D Luísa Margarida Batista Custódio

THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN AGRICULTURE ENGINEERING

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Abstract

The estuarine ecosystems are among the most productive, but also among the most threatened ecosystems. This PhD project arise as a response to two questions raised by society, which reflect two environmental problems in an estuarine context: the deposition of aquaculture sediments around the ponds, contributing to the degradation of a saltmarsh area in the Guadiana estuary; and an abandoned and degraded saltmarsh area, mainly due to the industrial activity, in the Tagus estuary. This thesis develops an answer to these two environmental issues based on Sustainable Development Goals: waste reutilisation to a circular economy, halt of biodiversity loss, develop agricultural practices to protect freshwater and contribute to the recovery of degraded areas. Sediments proprieties of aquaculture ponds in the Guadiana estuary and Fluvisol properties from the Tagus estuary were improved using organic and inorganic wastes from local activities: agriculture (substrate used in the strawberry crop, pruning wastes) and/or industries (distilleries, breweries), allowing reutilisation of these wastes that would be disposable. Contributing to the species and estuarine ecosystems conservation, the Technosols constructed with the sediments/Fluvisol permitted the cultivation of native plants, which contribute to the estuaries' stabilisation, some of them endemics, threatened and/or have an economic potential: *A. macrostachyum, L. algarvense, L. daveaui,* e *S. vera*. Since these species are halophytes, high NaCl concentrations tolerant, they also were able to grow and develop under irrigation with water from the respective estuary (Guadiana or Tagus), being this methodology a freshwater protection cultivation technique. Altogether these measures may constitute the first step in the recovery of these degraded salt marsh areas in the Guadiana and Tagus estuaries.

Keywords: Aquaculture sediments, Estuarine water, Fluvisol, Halophytes, Wastes

Resumo

Os ecossistemas estuarinos encontram-se entre os ecossistemas mais produtivos, mas também entre os mais ameaçados. Esta dissertação surge como resposta a duas questões apresentadas pela sociedade, que refletem dois problemas ambientais em contexto estuarino: a deposição de sedimentos de aquacultura na área adjacente aos tanques, contribuindo para a degradação de uma área de sapal no estuário do Guadiana; e uma área de sapal abandonada e degradada, sobretudo devido à antiga atividade industrial ali instalada, no estuário do Tejo. Esta tese desenvolve uma resposta a estas duas problemáticas ambientais, baseada nos objetivos de desenvolvimento sustentável: reutilização de resíduos para uma economia circular, travar a perda de biodiversidade, desenvolver práticas agrícolas de proteção da água doce e contribuir para a recuperação de áreas degradadas. As propriedades dos sedimentos, provenientes dos tanques de aquacultura no estuário do Guadiana, e do Fluvissolo, proveniente do estuário do Tejo, foram melhoradas utilizando resíduos orgânicos e/ou inorgânicos, provenientes de atividades locais: agrícolas (substrato de cultura de morangueiro, podas de biomassa) e/ou industriais (destilarias, cervejarias), permitindo também a reutilização destes resíduos que de outra forma seriam descartados. Contribuindo para a conservação das espécies e dos ecossistemas estuarinos, os Tecnossolos construídos com os sedimentos/Fluvissolo e os resíduos orgânicos/inorgânicos, possibilitaram o cultivo de plantas nativas que contribuem para a estabilização dos estuários, e que são endémicas, ameaçadas e/ou têm um potencial económico: *Arthocnemum macrostachyum* (Moric.) Moris, *Limonium algarvense* Erben, *Limonium daveaui* Erben, e *Suaeda vera* Forssk. ex J. F. Gmel. Sendo estas espécies halófitas, tolerantes a elevada concentração de NaCl, as mesmas foram capazes de se desenvolver regadas com água proveniente do respetivo estuário (Guadiana ou Tejo), contribuindo assim para a proteção da água doce. No seu conjunto, estas medidas podem constituir um primeiro passo na recuperação destas áreas de sapal degradadas, no estuário do Guadiana e do Tejo.

Keywords: Água estuarina, Fluvisol, Halófitas, Resíduos, Sedimentos de Aquacultura.

Resumo alargado

A escassez de água doce é um problema ambiental bastante atual. Em 2022, Portugal atravessou o ano mais quente e seco desde que há registo, à semelhança do que aconteceu por toda a Europa. Por outro lado, a área de solo degradado, devido à salinização, tem vindo a aumentar, estando os ecossistemas estuarinos entre os habitats mais ameaçados. Na Europa, mais de metade dos resíduos produzidos vão para aterro ou incineração e 6% tem destino desconhecido, terminando muitas vezes em rios e estuários. Os Tecnossolos, caracterizados por conterem uma quantidade significativa de artefactos (IUSS Working Group, 2015), podem também ser construídos com o objetivo de recuperação de sedimentos/solos degradados e valorização de resíduos (Macías, 2004). Quando aplicados como estratégia de recuperação de solos/sedimentos salinos devem ser acompanhados pelo cultivo de plantas adaptadas a estes ambientes, como por exemplo, espécies com tolerância a concentrações de NaCl elevadas (halófitos; Flowers et al., 2008). Esta dissertação apresenta pela primeira vez uma estimativa do número de halófitas presentes em Portugal, cerca de 163 espécies, sendo que a ordem Caryophyllales é a mais representada em Portugal, à semelhança do que acontece mundialmente. Entre as espécies pertencentes a esta ordem estão *Arthocnemum macrostachyum* (Moric.) Moris, *Limonium daveaui* Erben, *Limonium algarvense* Erben e *Suaeda vera* Forssk. ex J.F. Gmel. Estas espécies constituem casos de estudo interessantes devido ao seu papel na estabilização dos ecossistemas, estatuto de conservação, e/ou à sua potencial aplicação em diferentes indústrias, como a alimentar e farmacêutica.

O projeto de investigação apresentado nesta dissertação de doutoramento visa responder a dois problemas colocados pela sociedade. Num dos desafios societais, uma empresa de aquacultura, situada no estuário do Guadiana, pretende uma valorização ambiental e económica para os sedimentos que necessita retirar do fundo dos seus tanques por serem prejudiciais à qualidade da água e sobrevivência dos peixes. Para além disso, estes sedimentos têm criado problemas ambientais ao serem depositados no sapal em redor dos tanques de aquacultura, não permitindo o normal desenvolvimento das espécies vegetais nativas do estuário e contribuindo para a sua degradação. O outro desafio, a que se deseja responder com este projeto, diz respeito a uma área de sapal no estuário do Tejo pertencente à Câmara Municipal de Vila Franca de Xira e que o município pretende reabilitar. Esta área encontra-se abandonada e degradada devido a forte atividade antrópica, nomeadamente antigas indústrias ali instaladas. No intuito de encontrar respostas para os problemas apresentados, foram colhidos sedimentos de aquacultura (SED), água do estuário do Guadiana (AG), solo

(Fluvisol, FLU) da área de sapal degradada, água proveniente do estuário do Tejo (AT) e sementes das quatro espécies em estudo acima mencionadas. Nas águas estuarinas, foi analisado: pH, condutividade elétrica (CE), $Cl^{–}$, $HCO_3^{–}$, $CO_3^{2–}$, Na^{+}, Ca^{2+} e Mg^{2+} e calculada a razão da adsorção de sódio (RAS). Estas águas mostraram ser fortemente salinas com pH neutro e uma elevada concentração em Cl^{-}, Na^{+}, seguido de Mg^{2+}, assim como o valor de RAS, podendo ser prejudiciais para culturas glicófitas, mas toleradas pelas plantas halófitas. Para melhorar as propriedades dos SED de forma a permitir o cultivo de *L. algarvense*, foi produzido um Tecnossolo utilizando estes SED e uma mistura de resíduos orgânicos resultantes de produção de licor/aguardente de (alfarroba, *Ceratonia siliqua* L. (CW); medronho, *Arbutus unedo* L. (AW)), substrato utilizado na cultura do morango (AgW), resíduos de café (CoW) e cinzas de biomassa (BA). Foram testadas duas concentrações da mistura de resíduos, 180 g/kg de sedimento (Tec180) e 360 g/kg de sedimento (Tec360), respetivamente. Para melhorar as características de FLU possibilitando o cultivo de *A. macrostachyum*, *L. daveaui* e *S. vera* foi produzido um Tecnossolo (TEC) com FLU e resíduos orgânicos e inorgânicos, como areia, podas de biomassa, gravilha de calcário, lamas de ETAR e lamas de kieselguhr provenientes da Sociedade Central de Cervejas e Bebidas, S.A.. O SED/FLU, os resíduos, Tec180, Tec360 e TEC foram caracterizados para pH, CE, K extraível ($K_{extraível}$) e P extraível ($P_{extraível}$) N total (N_{total}), N-NO_3^{-}, $NH4^{+}$, C orgânico (C_{org}). Para o FLU foi também determinada a capacidade de troca catiónica (CTC), a granulometria e a concentração de micronutrientes, esta última também determinada nas lamas e no TEC.

No primeiro caso de estudo, os SED revelaram ser ácidos com elevada CE possuindo baixa concentração em $C_{org,}$ $P_{extraível}$ e $K_{extraível}$ e elevada concentração em S. A acumulação dos sedimentos no fundo dos tanques, contribui para um aumento da população bacteriana e um maior consumo de oxigénio podendo levar à formação de sulfuretos e diminuição do valor do pH. Contudo, as características dos resíduos aplicados ao SED, como o pH alcalino das BA, a elevada concentração de C_{org} de AW, CoW e de CW, e os altos valores de $P_{extraível}$ de AgW e CoW bem com de $K_{extraível}$ em CW, permitiram melhorar as propriedades dos sedimentos. Esta mistura de resíduos orgânicos revelou-se adequada para melhorar a fertilidade e a estrutura dos SED. Em seguida, foi avaliado o cultivo de *L. algarvense* em SED, Tec180 e Tec360, regado com AG e/ou água desionizada. As plantas cultivadas nos SED e regadas com AG morreram. O alto teor de argila e limo deste substrato, aliado à elevada concentração de Na em AG, terá contribuído para a dispersão das argilas e compactação dos sedimentos resultando em condições físicas adversas para o crescimento das plantas. Na mudança de regime de rega, apenas uma planta cultivada em Tec180 não sucumbiu enquanto todas as transplantadas para Tec360 sobreviveram, possivelmente por este último possuir um maior teor de C_{org} contribuindo para a diminuição da oxidação dos sulfuretos e melhoria das propriedades físicas do SED. As plantas Tec360 regadas com água desionizada tiveram desenvolvimento maior do que aquelas regadas com AG, porém estas últimas também apresentaram um bom crescimento vegetativo e reprodutivo.

No segundo caso de estudo, o FLU apresentou textura franco-argilo-limosa, sendo salino-sódico e possuindo baixas concentrações em C_{org}, N_{total} e $P_{extraível}$. Com exceção de Zn, o TEC apresentou um aumento significativo na concentração para todos os nutrientes determinados, especialmente em $P_{extraível}$, Fe e Cu. Este aumento de fertilidade deveu-se sobretudo às lamas de ETAR e de kieselguhr. Os resíduos de biomassa também aplicados como fonte de C e nutrientes contribuíram igualmente para melhorar a agregação/estrutura do FLU, e a adição de areia e gravilha melhoraram a sua textura e a permeabilidade. No final do ensaio, ambos os substratos apresentaram uma maior CE, maior concentração de $K_{extraível}$ e menor concentração de Fe e Cu, do que no início dos ensaios. Antes destes ensaios de cultivo foram realizados testes de germinação de *L.* d*aveaui* e das suculentas, *A. macrostachyum* e *S. vera,* em papel filtro, TEC ou FLU, humedecidos com água desionizada, respetivamente. No caso das suculentas, foram realizados pré-tratamentos para quebra de dormência das sementes com uma solução de 3% de H_2O_2 ou 98% de H_2SO_4. Todas as espécies obtiveram a maior percentagem de germinação no papel de filtro. As sementes de *A. macrostachyum* apresentaram uma maior taxa de germinação quando previamente tratadas com a solução de H_2SO_4, enquanto *S. vera* obteve um melhor resultado em sementes tratadas com solução de H_2O_2. As plântulas das três espécies resultantes da germinação no papel de filtro foram cultivadas em FLU ou TEC. No caso de *L. daveaui*, foi avaliado o seu desenvolvimento sob rega com AT, enquanto para suculentas, o seu cultivo foi também testado sob rega com soluções aquosas com diferentes concentrações de NaCl (mmol): 0 (água desionizada) 200 ou 400. As espécies *L. daveaui, A. macrostachyum* e *S. vera* apresentaram um maior desenvolvimento vegetativo e reprodutivo quando cultivados em TEC, por comparação ao cultivo em FLU, e regadas com água desionizada ou com AT.

Os resultados apresentados nesta dissertação mostram que é possível a valorização dos sedimentos de aquacultura e do Fluvisol salino-sódico, através da produção de Tecnossolos com a reutilização de resíduos e cultivo das espécies *A. macrostachyum, L. algarvense, L. daveaui,* e *S. vera,* sob rega com água estuarina. Para além da sua importância nas comunidades vegetais de ecossistemas estuarinos, as mesmas têm potencial económico. O cultivo destas halófitas pode constituir uma fonte de rendimento alternativa não dependente de água doce, como também esta solução pode ser o primeiro passo na reabilitação de sapais. Assim, os resultados desta tese enquadram-se nas seguintes metas globais para 2030, estabelecidas pela Assembleia Geral das Nações Unidas (United Nations, 2022b): "água limpa e saneamento", através da promoção de práticas agrícolas para proteção da água doce; "produção e consumo sustentáveis", promovendo a reutilização de resíduos; "vida terrestre", através da conservação da biodiversidade e da recuperação de habitats degradados.

General Introduction

Coastal zones are those located at the interface between land and sea and include beaches, dunes, cliffs, coastal lagoons, and estuaries (Carneiro et al., 2007). Such zones offer multiple ecosystem services, being very important to the economy of countries (Carneiro et al., 2007; Barbier et al., 2011). A high percentage of the world's population depends on these areas, living about 29% of the world's population closer than 50 km away from the coast (Burke et al., 2001) and 11% in low elevation coastal areas, under 10 m of elevation above sea level (Haasnoot et al., 2021). In Portugal, 75% of population lives along coastalines (Carneiro et al., 2007; Pereira and Coelho, 2013), which 3.2% lives in the low elevation locations (Haasnoot et al., 2021). However, people overlook the ecosystems services offered by coastal ecosystems that are frequently used as wastes deposit (Duarte et al., 2013; Peres et al., 2016). In Europe, 55.8% of total of wastes are landfilled or incinerated, only 37.9% are recycled, and 6.3 % have an unknown destination, many times ending up in the rivers (Eurostat, 2018). Anthropic activities and climate change are the main causes of coastal areas' degradation (Carneiro et al., 2007, Haasnoot et al., 2021). The sea level is rising between 4 to 15 mm per year (Oppenheimer et al., 2019), and currently, more than 2.3 billion people face freshwater shortage (United Nations, 2022a). Climate change aggravates soil salinisation due to freshwater scarcity and sea level rise, causing saltwater intrusion into coastal areas (FAO, 2021). Further, over 85% of the planet's wetlands are degraded or even lost, putting survival of 40% of the world's plant and animal species at risk (United Nations, 2022b).

The ecosystems and their living organisms represent the planet's biodiversity (CBD, 1992; Pascual et al., 2021). Biodiversity provides several ecosystem services (*e. g.,* air, soil, and water purification) and goods (*e. g.,* food, medicines, water, wood, shelter), which are valued 1.5 times the word gross domestic product (OECD 2019; United Nations, 2022b). Biodiversity losses result in a world lost around USD 10 to 31 trillion per year OECD (2019). Halt biodiversity loss, reverse land degradation, enhance farming practices to protect freshwater, promote sustainable agriculture, and reduce waste by promoting the circular economy, are part of the Sustainable Development Goals (United Nations, 2022b). Scientific research aims to answer society's issues. Therefore, this thesis intends to valorise underused resources unsuitable to traditional agriculture, such as saline sediments/saltmarsh soils to cultivate endemic/add-value salt tolerant plant species. The main goal of this thesis is to contribute to solving two societal problems. First, an aquaculture company has the following question: how to value the sediments removed from aquaculture ponds in the Guadiana estuary, since the company must pay for the sediments' transport and treatments. Another question is how to avoid this cost as the company has been disposing the aquaculture sediments on the saltmarsh land around aquaculture ponds and saltmarsh' native species no longer grow in this area? Second, the Vila Franca de Xira city council ask support to develop a landscape recovery project for an abandoned and degraded saltmarsh area, in the Tagus estuary, by creating a cultivation and leisure area for the population.

To achieve the main goal, the specific goals were addressed as follows:

- Improvement of knowledge on Portuguese halophyte species from estuarine saltmarshes and their potential uses.
- Cultivation and evaluation of saltmarshes' native species with a conservation status and/or economical interest, using resources unsuitable to traditional agriculture.
- Evaluation of Technosols' efficiency to improve aquaculture sediments/saltmarsh soils properties.
- Determination of germination data on target halophyte species in different substrata and with/without different seed break dormancy treatments.
- Assessment of physiological status, leaf anatomy, and production data of plants cultivated in different substrata and irrigated with distinct saline water regimes, in greenhouse conditions.

This PhD thesis is divided in six chapters. Chapter I reviews the literature on the main concepts addressed in this thesis. Chapter II to Chapter V, express results obtained during development of this PhD project (internationally refereed papers published/under revision in scientific journals) (Figure 1). Chapter II presents a review on halophytes' uses in Portugal, which allow selection of the most suitable species to study on Chapter IV and Chapter V, based on four criteria: saltmarsh native species, economic potential, conservation status, and less-studied species. Chapter III approaches the cultivation of *Limonium algarvense* Erben, an endemic species with food and pharmaceutical potential, in Technosols produced with aquaculture sediments and two different rates of local amendments application, irrigated with estuarine water from Guadiana estuary. Based on Chapter III results, Chapter IV assess the potential of a tailored soil constructed with a saltmarsh soil and a mixture of local low-value inorganic and organic wastes for use in reintroduction of *Limonium daveaui* Erben in native habitats. In this study, species' growth and development was evaluated using a Technosol irrigated with water from the Tagus estuary. In Chapter V, a comparison on the influence of two dormancy-breaking treatments in seed germination of *Arhrocnemum macrostachyum* (Moric.) Moris and *Suaeda vera* Forssk. ex J. F. Gmel, two halophytes with edible tips and edible seed oil, is performed. These species' growth in tailored soil irrigated with water from Tagus River, and saline solutions with different NaCl concentrations is shown. Chapter VI presents the general discussion and conclusion of PhD study as well as future research topics.

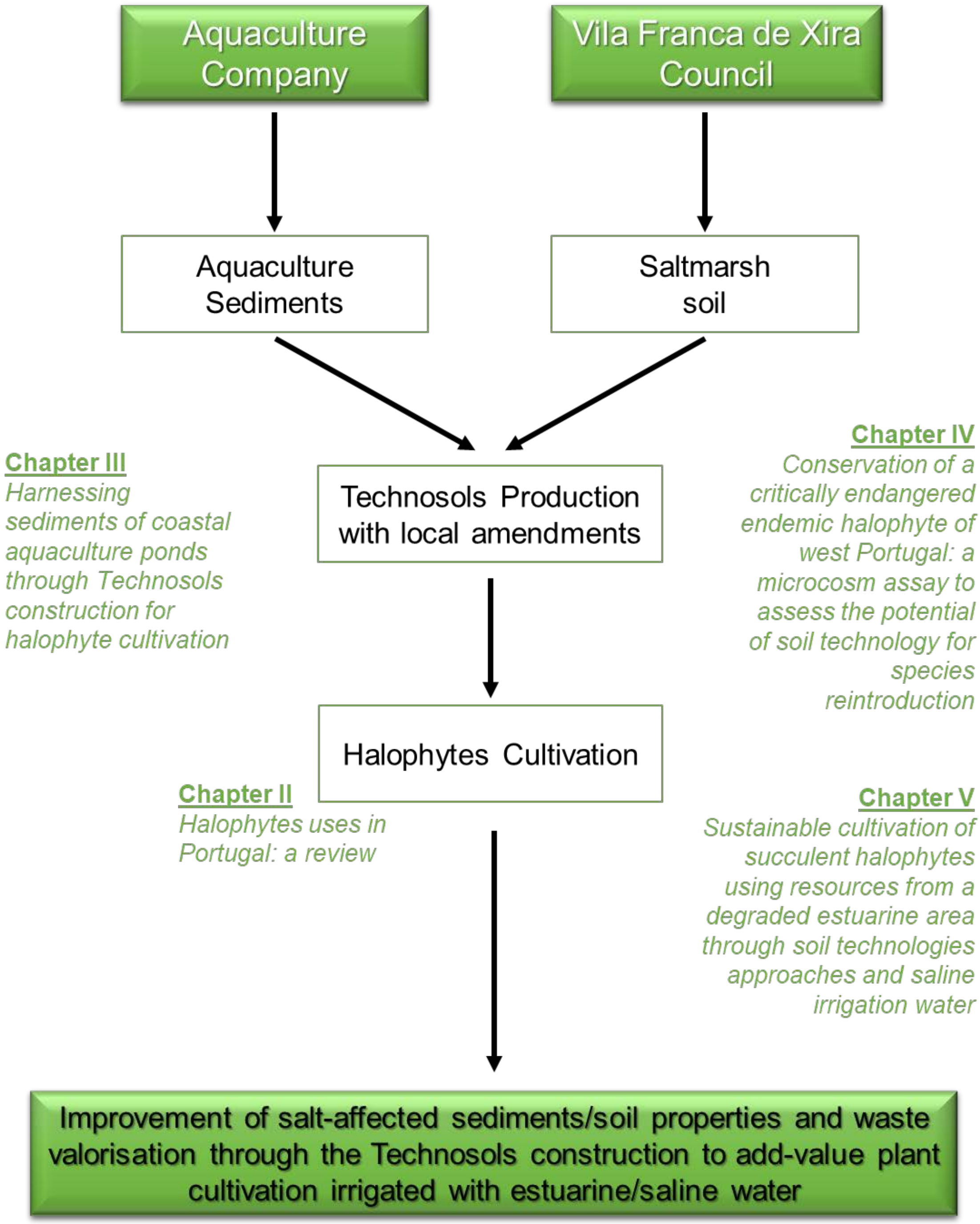

Figure 1. General scheme of PhD study

Chapter I. *Literature review*

Estuary

Estuary can be defined in a physical, geomorphological and/or biological perspective (*e. g.*, Perillo, 1995; Potter et al., 2010). This thesis adopts the European Commission (2013), an ecological definition followed by 27 countries: "*Downstream part of a river valley, subject to the tide and extending from the limit of brackish waters (…) The mixing of freshwater and sea water and the reduced current flows in the shelter of the estuary lead to deposition of fine sediments, often forming extensive intertidal sand and mud flats (..)".* Estuaries are commonly denominated as 'estuarine ecosystems' since they are an ecological unity constituted by different habitat types such as salt meadows, salt steppes and saltmarshes (Alfa, 2004; Europe Commission, 2013). Habitats Directive (92/43/EEC) protects these ecosystems integrated in coastal areas, providing numerous ecosystem services. The estuaries conservation is the cheapest, safest, and easiest way to prevent some climate change's impact, namely against sea level rise (Duarte et al., 2013).

The Tagus and Guadiana estuaries

The Tagus estuary (38°50' N, 8°60' W), western Portugal, with 32 000 ha is the largest estuary of Portugal and one of the largest of western Europe (Carina et al., 2020; Freire et al., 2021). It connects with Atlantic Ocean and the upstream estuary limit reaches Vila Franca de Xira city (Fortunato, 1997; Dias and Valentim, 2011). Due to its biological and ecological characteristics, the estuary and its surrounding areas are protected by three different conservation status: national, community and international (DL n. 565/76, RCM n.115-A/2008, Ramsar, 1992). The Tagus estuary is a nursery for numerous fish marine species, a habitat of several wintering aquatic birds and a habitat of botanical species (DL n. 565/76, RCM n.115-A/2008). In the last category, endemic species critically endangered such as *Limonium daveaui* Erben are found (Caperta and Carapeto, 2020). To preserve estuary functions, habitats and species, the estuary and its surrounding areas, are classified as a Nature Reserve (14 416.14 ha) under national legislation (DL n. 565/76), are part of List of Wetlands of International Importance (14 563 ha; 7PT001; Ramsar, 1992), and classified as Special Protection Area for wild birds (44 772 ha; PTZPE0010; RCM n.115-A/2008) and as a Site of Community Importance for the Mediterranean biogeographical region (44 609 ha; PTCON0009; RCM n.115-A/2008) under EC law (Figure I.1). The Fluvial beach "Praia do Sobralinho", one of the study areas in this thesis, is at the limit of special protection area (SPA) (Figure I.1).

The Guadiana estuary with 2200 ha lies at southeastern Portugal (37º12'N, 7º26'W) being a natural border between Portugal (right bank) and Spain (left bank) (Faria et al., 2006; Mills et al., 2020). The estuarine area is delimited by Mértola (Portugal) and the Gulf of Cádiz (Spain), in the Atlantic Ocean (Garel et al., 2009; Guimarães et al., 2011). The Guadiana estuary has a high biodiversity of birds, fishes, and plant species such as endemic-Moroccan *Limonium algarvense* Erben (Almeida, 2016; Caperta et al., 2017). Further, the activities developed in this area such as fishing, saltpans exploitation and aquaculture contribute to the regional economic development (DL n. 162/75). The Guadiana estuary is integrated in the Sapal de Castro Marim e Vila Real de Santo António Nature Reserve (2 307.99 ha, DL n. 162/75), which is present in the List of Wetlands of International Importance too (2 235 ha, 7PT010; Ramsar, 1993). Moreover, the estuarine area is covered by the Special Protection Area for wild birds Sapal de Castro Marim (2 147 ha; PTZPE0018; RCM n.115-A/2008) and by the Site of Community Importance for the Mediterranean biogeographical region of Ria Formosa-Castro Marim (17 520 ha; PTCON0013; RCM n.115-A/2008) (Figure I.2).

Figure I.1. Map of the different protected areas in Tagus estuary (adapted from ICNF, 2022)

Figure I.2. Map of the different protected areas in Guadiana estuary (adapted from ICNF, 2022)

Saltmarshes

Saltmarshes are alluvial areas resulting from the marine and/or fluvial sediments deposition such as clay, silt, and sand (Almeida, 2016). These areas, in the interface between land and estuarine water, are influenced by tides (Moreira da Silva et al., 2015), and in the case of European estuaries by twice-daily tides (Elliott and McLusky, 2002). Saltmarshes are dominated by halophyte species (salt-tolerant), which contribute to saltmarsh's stabilisation like *A. macrostachyum, L. algarvense, L. daveaui* and *S. vera*, among others. The roots of these plant species retain sediments, while the aboveground biomass reduces the tidal currents' velocity and turbulence contributing to erosion control (Almeida, 2016; Sarika and Zikos, 2020). The saltmarshes are one of the most productive ecosystems due to their high primary and secondary productivities (Barbier et al., 2011). They also provide ecosystem services such as food, shelter and nursery for numerous species and filtering and detoxification services supported by suspension feeders and submerged vegetation (Barbier et al., 2011; Duarte et al., 2021). In addition, saltmarshes play an important role in climate change mitigation, being considered one of the most effective ecosystems in the capture and storage of carbon (Sousa et al., 2017) and offer coastal protection against flooding and erosion by the seawater level rise caused by climate change (Duarte et al.,2013; Almeida et al., 2016). Further, saltmarshes are favourable areas for developing activities such as saltpans exploitation, agriculture, fishing, shellfish catch, nautical and tourism activities (Newton et al., 2020). In the last two decades, the development of aquaculture activity occupying the abandoned saltpans has also increased, taking advantage of the salt industry crisis (Almeida et al., 2014; Rocha et al., 2022).

Saltmarsh soils

Most saltmarshes soils (Figure I.3) are classified as Fluvisols, since saltmarshes are alluvial areas resulting from marine and/or fluvial sediments deposition (Almeida, 2016; IUSS Working Group, 2015). These soils have naturally a high salts concentration and therefore recognised as salt-affected soils (FAO, 2021). Depending on pH, electrical conductivity, and exchangeable sodium percentage (ESP), these soils can be saline, saline-sodic, or sodic (US Salinity Laboratory, 1954). The saline soils present a pH normally lower than 8.5, electrical conductivity higher than 4 dS/m, and exchangeable sodium percentage less than 15; while sodic soils present pH above 8.5 achieving 10 or superior in some cases, electrical conductivity lower than 4 dS/m, and ESP higher than 15 (US Salinity Laboratory, 1954; Brady and Weil, 2008).

The saline-sodic soils are a result of the two combined pedogenetic processes, salinisation, and alkalisation, being an intermediate condition between saline soils and sodic soils: pH lower than 8.5, electrical conductivity higher than 4 dS/m and ESP higher than 15 (US Salinity Laboratory, 1954; Brady and Weil, 2008). In saltmarshes, these two processes can be influenced by natural causes, such as deposition of marine sediments brought from seawater and/or marine salts carried by the wind as well as the tides' influence (Gonçalves et al., 2015; Mendes, 2015). Other causes could be of anthropic origin since the estuaries are frequently a sink of saline industrial wastes (Gonçalves et al., 2015; Mendes, 2015). The saline-sodic soils also present a relatively high salinity and Na^+ is dominant in the cation exchange capacity complex (CEC) (de Varennes, 2003; Brady and Weil, 2008). The relatively high concentration of soluble salts is associated to a low osmotic potential in the soil solution, reducing the plants' capacity to absorb water (Alexandre et al., 2018). The high levels of Na^+ in CEC are harmful to most soils' properties, namely fine texture soils, which have a high rate of clay (Alexandre et al., 2018), resulting in the separation of clay mineral layers leading to structureless soil (Castanheira et al., 2020) (Figure I.3). The absence of porosity is detrimental to soil water-holding capacity, permeability, aeration, and plants' roots' development (Castanheira et al., 2020). The saline-sodic soils having pH values between 7 - 8.5

(Gonçalves et al., 2015) resulting in P, Zn, C, Fe, and Mn nutrients deficiency (Meena et al., 2019; Brady and Weil, 2008). Further, these soils usually present low rates of N and organic carbon (C_{org}) (Wong et al., 2010; Meena et al., 2019). The high salt concentration has also a toxic effect on microbial development and activities, which play an essential role in the aggregates' stability and in organic matter mineralisation, a fundamental process in providing essential nutrients in assimilable forms by plants (*e. g.,* NH_4, H_2PO_4) (Abbas et al., 2021). Therefore, soil enzyme activities have been utilised as an indicator of soil quality, namely cellulases, phosphatases, urease, and dehydrogenase (Roldán et al., 2005; Singh, 2016). Some authors reported that these enzymes' activity can be inhibited by pedogenetic processes such as salinisation and alkalisation (Li et al., 2014; Singh, 2016). However, some bacteria are well-adapted to these salty environments by supplying nutrients and protecting plants (*e. g*., A. *macrostachyum*) against phytopathogens thereby contributing to seed germination enhancement and halophytes growth (Navarro-Torre et al., 2017).

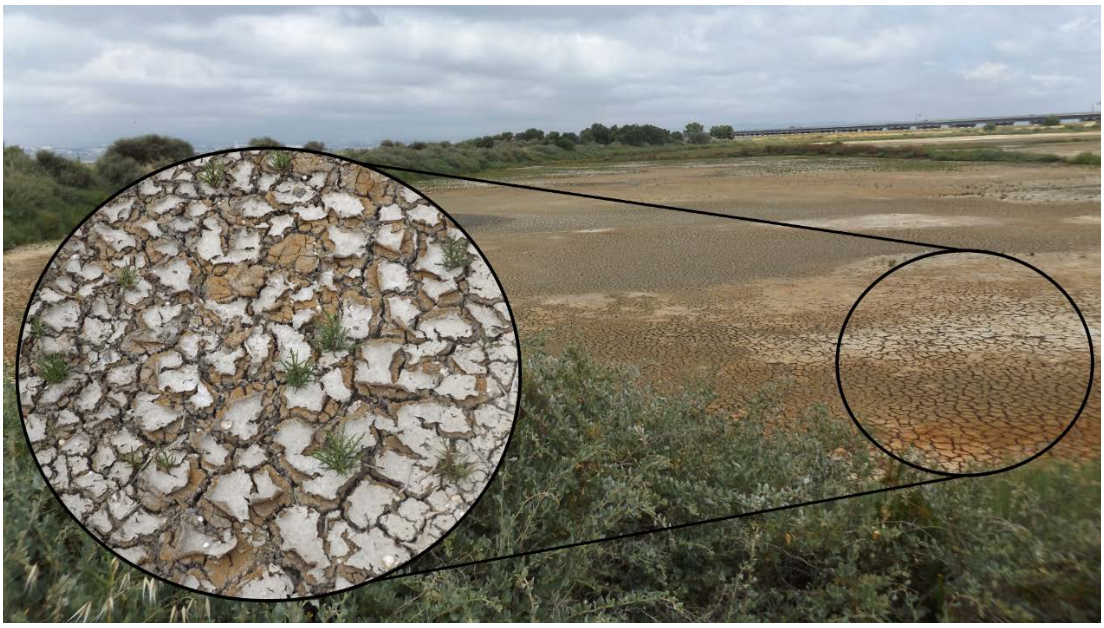

Figure I.3. Tagus estuary at low tide. Detail of dry soil presenting a white salt crust and cracks on the soil surface due to clays dispersion and/or high amount of sodium.

Aquaculture sediments

The global aquaculture food production is increasing, and the world is producing 87.5 million tonnes of aquaculture food, valued in USD 265 billion (FAO, 2022a). Europe generates 3.2 million tonnes (FAO, 2022a) among which Portugal produces 14,552 tonnes, the 16th main producer in European Union (FAO, 2022b; Rocha et al., 2022).

There are several aquaculture methods, and the earthen coastal ponds are one of the most common due to their low labour and easy management (Mirzoyan et al., 2010; European Commission, 2019; Figure I.4) Most earthen coastal ponds are converted from abandoned saltpans and are constructed with saltmarsh soil (Almeida et al.,2014; Rocha et al., 2022). The aquaculture sediments are also composed of uneaten feed and fish excretions that gradually accumulate at the bottom of ponds (Burducea et al., 2022). These sediments reduce the living space available for fish and become harmful to the pond ecosystem (Burducea et al., 2022). The fishes excrete 2/3 of feed consumed through gills or from faeces, as non-faecal or faecal losses, mostly in ammonia (NH_3) form and a residual quantity in urea (NH_2CONH_2) form (Gichana et al., 2018). Ammonia is a product of amino acid metabolism highly toxic to the organism and highly soluble in the water (Ip and Chew, 2010). However, in the aquatic environment there also is a rapid interconversion between NH_3 and NH_4^+ (Randall and Wright, 1987). This last one is less toxic than NH_3, but the high NH_4^+ concentration in the aquatic environment constrains the NH_3 diffusion through fish' gills increasing the NH_3 concentration in fish's blood being toxic to them (Gichana et al.,2018). According to Datta (2012), 89% of N and 68% of P of fish feed are accumulated in the bottom sediments. The uneaten feed and excretions deposited in the bottom of ponds also increase the bacteria population and metabolic activity (Datta, 2012). These phenomena result in high oxygen consumption, gases release such as carbon dioxide, ammonia, methanethiol (CH_3SH) and hydrogen sulfide (H_2S), and a decrease in pH value of sediments (Datta, 2012; Mandario et al., 2019). The increase of these gases and the excess of N and P contribute to phytoplankton bloom leading to eutrophication (Granada et al., 2015). The decrease in oxygen (anoxic conditions) due to senescence and disintegration of phytoplankton can cause a decrease in water quality, and consequently, fish mortality (Granada et al., 2015). Therefore, it is necessary to remove bottom sediments between fish crops to improve both water and sediments quality, to maintain an economical fish production (Burducea et al., 2022). However, aquaculture sediments have a high organic matter content and/or salt burden or even a potentially hazardous elements' content (Mirzoyan et al., 2010), and the disposal of these sediments on vacant land sometimes covering fertile soils can lead to degradation of soil properties and contamination of groundwater (Dróżdż et al., 2020). Nonetheless, in general, aquaculture sediments are disposed on the land adjacent to aquaculture ponds, in the salt marshes (Wu et al., 2014).

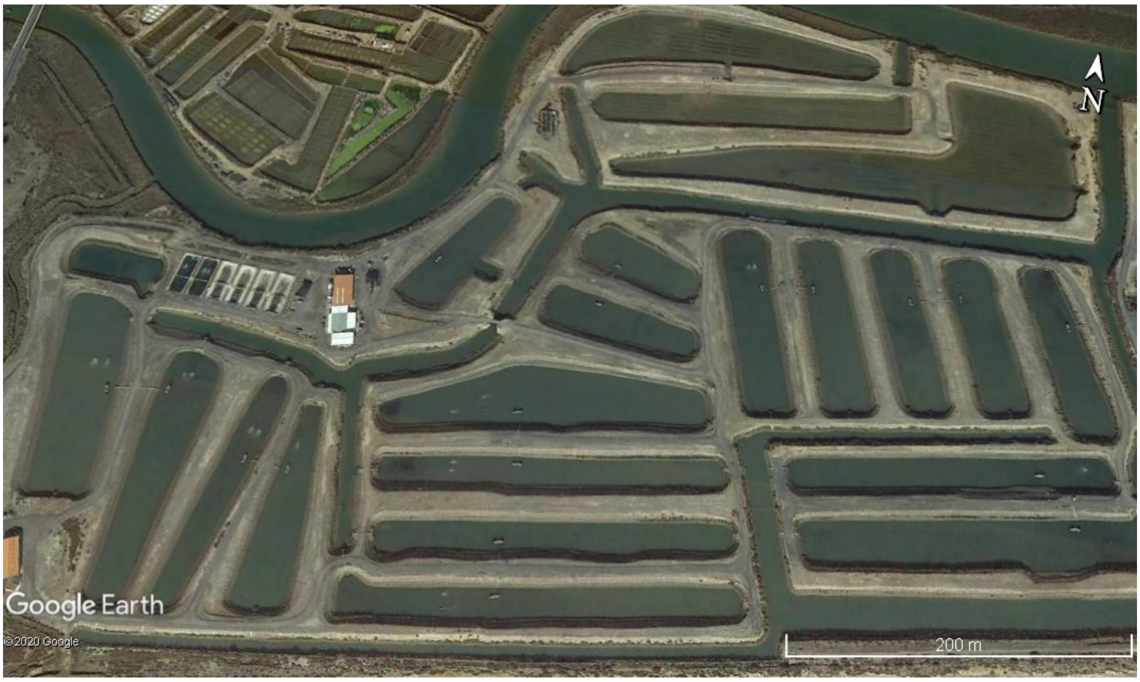

Figure I.4. Earthen coastal ponds converted from saltpans abandoned, in Guadiana estuary.

Tailored Soils and Amendments

Soil traditionally has been considered a natural formation resulting from different factors (IUSS Working Group, 1998, 2015; Hartemink, 2016). However, Rossiter (2005) explains that even uninhabited areas have been influenced by anthropic activity (*e. g.,* dust carried by the wind to these places during dust storms caused by over-grazing or even industrial dust) and proposes Technosols as a new group of the World Reference Base for Soil Resources. Technosols are characterised by containing a significant amount of artefacts (liquids or solids substances created or modified, or brought from a depth to the surface by human activity; IUSS Working Group, 2015). They were created unintentionally across the world as a by-product of human activity (Rossiter, 2005), but they can also be designed (Macías, 2004). Macías (2004) formulated for the first time these tailored-made soils developed from wastes, elucidating their importance in soil conservation such as degraded/sediments' recovery, contaminated, or incipient soils. Since then, various studies have demonstrated Technosols' efficacy in dredged fluvial/marine sediments management (Figure I.5.) (Macía et al., 2014; Mattei et al., 2016; Fourvel et al., 2019); mining soils and tailings (Macías-Garcia et al., 2009; Asensio et al., 2013; Santos et al., 2014; 2016a; Moreno-Barriga et al., 2017; Arán et al., 2021); and industrial soils (Hafeez et al., 2012; Villenave et al., 2018). Technosols are an eco-engineering technique that presents advantages compared to common amendments strategies, which need frequently application (Arán et al., 2021). Different wastes can be used such as organic substances, metabolizable material, and inorganic substances that help to stabilize organic matter, allowing a higher lasting of C and nutrients in the biogeochemical cycles, reducing their lost overtime (Macías et al., 2007).

Furthermore, this low-cost technique is easy to apply *in situ* over large areas (Arán et al., 2021). In addition to their importance in soil conservation, Technosols also play an important role in waste valorisation (Macías, 2004). According to the last data available, Portugal produced 11.43 million tonnes of non-municipal wastes (agricultural, industrial wastes, etc.) in 2019 (APA, 2022) and in 2020, 5.279 million tonnes of municipal wastes were generated (APA, 2021). Research has reported these wastes' potential as low-cost amendments in Technosols production (Macías, 2004; Asensio et al., 2013; Villenave et al., 2018). Instead of their deposit in landfills, there is an economical valorisation of wastes, contributing to a circular economy, which is a common goal of the Strategic Portuguese Plan for Non-Urban Waste 2030 (APA, 2022) and EU Soil Strategy for 2030 (European Commission, 2021) (Figure I.5.).

The Technosol constructed must fulfil the healthy soil functions defined by EU Soil Strategy for 2030 (Abreu and Magalhães, 2009; European Commission, 2021). Besides, the amendments' availability near the application local, *i. e.*, the ecological footprint of wastes' transport to the *in-situ* site, must also be considered. Amendments and their application rate should base on their properties and on degraded soils/tailings' traits that need to be improved (Abreu and Magalhães, 2009). Often these traits are pH, organic matter content, texture and structure, water retention, CEC, microbial activity, and fertility. Studies on salt-affected soils/sediments have used different organic and inorganic amendments to improve these characteristics. As inorganic materials, gravel, sand, gypsum, sulfur, sulfuric acid, phosphoric acid, and rubble are commonly used (Brady and Weil, 2008). The gravel and sand are utilised to improve soil texture and permeability (Su et al., 2022) whereas SO_4^{2-} from gypsum (Ca_2SO_4), reacts with Na^+ present in the soil producing Na_2SO_4 that can be leached, thus replacing Na^+ to Ca^{2+} in the exchange complex (Hanay et al., 2004; Sheik et al., 2022). When soils present sodium carbonate (Na_2CO_3) or hydrogencarbonate ($NaHCO_3$), elemental sulfur or sulfuric acid is generally used allowing carbonates dissolution and pH decrease (Sadiq et al., 2007; Day et al., 2019). To reclaim a calcareous saline-sodic soil, phosphoric acid can be more effective and cheaper than gypsum, sulfur or sulfuric acid (Gharaibeh et al., 2010). Moreover, a rubble barrier can be utilised as an effective tool to cut off salts' capillary rise from groundwater to the rooting zone, allowing a better plant development (Chávez-García and Siebe, 2019).

The most common organic materials used are municipal solid waste compost, sludge, biochar and pruning residues. Municipal solid waste compost application increases organic matter content and promotes aggregates formation and stability as well as permeability (Hannay et al., 2004). Besides, it provides essential nutrients (N, P and K) and stimulates microbial biomass and enzymatic activities (Lakhdar et al., 2009). However, the Municipal solid waste compost application can lead to a nitrification process and possible groundwater contamination (Lakhdar et al., 2009; Chávez-García and Siebe, 2019). Other studies describe the sewage sludge as a potential amendment, reporting a pH decrease in salt-affect soils and an increase of C, N and P contents (Lakhdar et al., 2010; Shan et al., 2021). The sewage sludge valorisation in agriculture takes part in the Strategic Portuguese Plan for Non-Urban Waste 2030 goals (APA, 2022). Biochar contributes to soil organic matter increases and improves soil porosity and water holding capacity since it is a porous material (Saifullah et al., 2018; Chávez-García and Siebe, 2019). Residual biomass of pruning are wastes commonly used and present similar properties to the organic amendments mentioned previously, such as nutrients and C supply and soil texture/aggregation improvement (Garcia-Franco et al., 2021). These wastes are more effective when incorporated into the soil than used as mulch (Garcia-Franco et al., 2021).

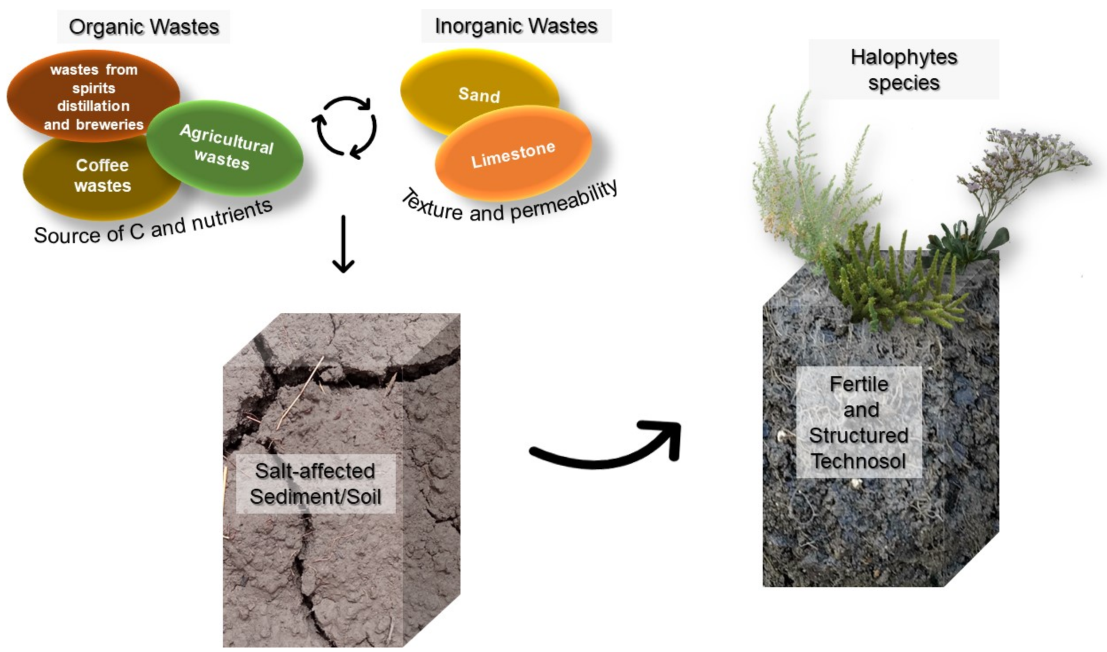

Figure I.5. Scheme of a Technosol produced with non-municipal and municipal wastes contributing a circular economy.

Saline Agriculture

In the world, there is only 2.5% of freshwater of which 68.7% found in glaciers and icecaps (USGS, 2018). The remaining 31.3% (0.78% of Earth's water) is groundwater and surface water (USGS, 2018), and 70% of this is used in agriculture (FAO, 2017). In July this year, according to the European Drought Observatory (Toreti et al., 2022), 47% of the EU territory was under warning drought conditions (soil moisture deficit), and 17% was under alert drought conditions (vegetation stress following soil moisture and vegetation deficit). In the same month, the Portuguese Institute for Sea and Atmosphere (IPMA, 2022) reported that the entire Portuguese territory was under extreme drought (55.2%) and severe drought (44.8%) conditions.

Moreover, FAO (2021) reports that more than 833 million hectares are salt-affected soils. In Portugal, the area of salt-affected soils is estimated as 150.000 hectares (Gonçalves et al., 2015). Therefore, it is necessary to look for other income sources for farmers besides traditional agriculture, which requires freshwater and non-salt-affected soils. Saline agriculture can be an economical alternative to freshwater scarcity and land salinisation increasing. Saline agriculture is "*Profitable and improved agricultural practices using saline land and saline irrigation water with the purpose to achieve better production through a sustainable and integrated use of genetic resources (plants, animals, fish, insects, and microorganisms) avoiding expensive soil recovery measures"* (Ladeiro, 2012). The first studies on halophytes and in saline agriculture emerged in Europe due to interest in plant distribution's geographical drivers, like salinity (Schimper, 1898; Tansley, 1911 both cited by Epstein, 1985), and in the United States of America (USA) (Hilgard, 1906 cited by Epstein, 1985) due to interest in growing crops in arid and semi-arid lands. Later, in the USA, Hayward and Wadleigh (1949) reviewed salt-affected soils' effect on plants' growth and addressed salt sensitive crops (glycophytes) and salt tolerant (halophyte) species. Further, in Israel, Boyko and Boyko (1959) showed the potential of seawater irrigation in the add-value halophytes' cultivation in a semi-arid coastal region. These studies' results triggered interest of the worldwide scientific community in saline agriculture as a new topic, and two research lines arose: improvement of traditional salt-sensitive crops salt tolerance (Yeo and Flowers, 1989; Flowers, 2004; Roy et al., 2014; Kotula et al., 2020); and halophyte species cultivation (O'Leary, 1989; Glenn et al.,1998; Ventura and Sagi, 2013; Lombardi et al., 2022). The former research line is based on crossing salt-tolerant relatives with salt-sensitive crops (Yeo and Flowers, 1989), and the latter one, approached in this thesis, aims to cultivate wild halophyte species (Glenn et al., 1998). To convert wild halophyte species into crops it is necessary to choose native species since they are well adapted to environment and can be economically profitable species besides creating cultivation protocols (Ventura et al., 2015). Thus, studies on different halophyte species under saline conditions (soil and water irrigation) reported seeds germination rate (Muñoz-Rodríguez et al., 2017; Debez et al., 2018, Lombardi et al., 2019), evaluated growth parameters (Ventura et al., 2014; Hsouna et al.,2020; Castiglione et al., 2021), and analysed their physiological status (Rangani et al., 2016; Barhoumi, 2019; Palchetti et al., 2021). Since halophytes have several potential economic uses (Duarte and Caçador, 2021) research have also tested other plant characteristics such as chemical composition (Pereira et al., 2017a; Lopes et al., 2021; Sousa et al., 2022), animals' digestibility and palatability (Attia-Ismail, 2016; Tlili et al., 2020), translocation coefficient and biological absorption coefficient for potentially hazardous elements (Abreu et al., 2012; Santos et al., 2017a).

Halophytes

Halophytes represent 1% of the world flora and thus they are quite rare amongst flowering plants (Flowers et al., 2008; Flowers and Colmer, 2015). According to the world database of halophytes (eHALOPH; http://www.sussex.ac.uk/affiliates/halophytes) there are 1216 species. Halophytes grow in different habitats such as salt deserts, mangroves, coastal dunes, and saltmarshes (Cortinhas et al., 2019). In Europe, these species occur mainly in saltmarshes covering around 451.652 ha and the Mediterranean area is rich in endemic halophytes (Duarte and Caçador, 2021). Among them, the Iberian-Moroccan endemism *L. algarvense* (Caperta et al., 2017) and the Lusitanian endemic *L. daveaui,* are threatened due to estuary degradation (*e. g.*, anthropic pressures) and invasive species (*e. g.*, *Carpobrotus edulis* (L.) N.E.Br.) (Almeida, 2016; Caperta and Carapeto, 2020). These species and *A. macrostachyum* and *S. vera* are characteristic species of the coastal halophilous *Limonium* associations that occur in saltmarshes of the western Iberian Peninsula, some of which are included in the *Salicornitea fruticosae* (=*Sarcocornetea fruticose*) class (Costa et al., 2014; Salazar-Mendías and Lendínez, 2020). These communities and the respective habitat type in which they thrive are under the Habitats Directive protection (European Comission, 2013).

Most halophytes can survive under 200 mmol NaCl or approximately 20 dS/m EC) (Flowers and Colmer, 2008), and plant' growth can be stimulated within a salinity range of 15–25 dS/m EC (Rozema and Schats, 2013). The osmotic stress can lead to seed germination delay since high salinity reduces the entry of water into the seed (imbibition). (Muñoz-Rodríguez et al., 2017). Several species just germinate after the decrease of salinity conditions as for example, after rainfall episodes (Krauss and Ball 2013); however, seeds can remain viable after long periods under salinity conditions (Keiffer and Ungar, 1995). Moreover, halophytes present different mechanisms that allow them living in highly saline environments. These include accumulation of the Na^+ and Cl^- in the root parenchyma (Flowers et al., 2018); excretion of salt through salt bladders or salt glands (Caperta et al., 2020); accumulation of ions in leaf or in stem cells vacuoles to reduce the cell water potential allowing water uptake and contributing to succulence (leaf succulent or stem succulence), as in *S. vera* and *A. macrostachyum*, respectively (Khan et al., 2005; Song and Wang, 2014).

Since ancient times halophytes properties have been recognised (Boyko and Boyko, 1959). Aronson (1985) reviewed halophytes uses, reporting fodder, land reclamation, landscaping and fuel as major uses, and food products and medicines as minor uses. However, across these last 37 years, an extensive research on halophytes uses has been realised, and the current scenario is different (Glenn et al., 1991; El-Shami, 1993; Brown et al., 1999; Caçador et al., 2000; Kefu et al., 2002; Almeida et al., 2006; Weber et al., 2007; Ksouri et al., 2008; Oliveira et al., 2009; El Shaer, 2010; Barros et al., 2013; Panta, 2014; Santos et al., 2017a; Rodrigues et al., 2019; Hussain et al., 2020; Giordano et al., 2021). Even the comparison between the current data on halophytes' uses (eHALOPH, 2022) and the available data from four years ago (eHALOPH, 2018) shows already differences in the major uses of halophytes. Nowadays, the halophytes' applications as fodder, forage, grazing, and bioremediation purposes are still among the most common uses but food and medicine potential of such species are no longer minor uses (eHALOPH, 2022). Halophytes synthesize antioxidative enzymes and non-enzymatic molecules such as phenolic compounds (Song and Wang, 2014) to tackle the reactive oxygen species' toxicity induced by salt stress (Ventura et al., 2014). Phenolic compounds have antioxidant and antimicrobial activities that are attractive to the food, cosmetics, pharmaceutical and nutraceutical industries (Lopes et al., 2021; Hulkko et al., 2022). For example*, A. macrostachyum, L. algarvense* and *S. vera* have high phenolic compounds (phenolic acids, flavonoids, and tannins) content and they also have a nutritional profile rich in polyunsaturated acids, making these species very attractive for the nutraceutical industry (Custódio et al., 2012; Rodrigues et al., 2015; Vizetto-Duarte et al., 2019; Duarte et al., 2022). The last decade presented a high number of research articles on compounds synthesised by halophytes and most studies are based in plant material collected in natural habitats (Cabral et al., 2013; Alves-Silva et al., 2013; Ventura et al., 2014; Rodrigues et al., 2016; Oliveira et al., 2017; Souid et al., 2019; Sánchez-Gavilán et al., 2021, Sousa et al., 2022). To contribute to reduce future overexploitation of halophyte species and to sustainable compounds' extraction it is necessary to focus on halophytes cultivation to conserve these rare species.

Chapter II. *Halophytes uses in Portugal: a review*

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Abstract

Halophytes represent circa 1% of the world flora and are able to grow in environments with over 200 mmol NaCl concentration. Halophyte species are often herbaceous plants rich in proteins, fibre, amino acids, and vitamin C, and the oilseed of some species, like *Arthocnemum macrostachyum*, is rich in polyunsaturated fatty acids. That type of fatty acid is essential to the human diet, being required for biological processes and the human body cannot synthesize them. Pharmaceutical uses include the prevention of variety of diseases like cancer, cardiovascular diseases, and chronic inflammation, due to several biological activities, as for example antioxidant and anti-inflammatory properties. The halophyte *Crithmum maritimum* was used by sailors in ancient times to prevent scurvy; recent studies demonstrate this species is rich in phenolic compounds and is a potential source of antioxidants. *Limonium algarvense* is another halophyte with a strong antioxidant activity. Infusions and decoctions of flowers from that species have similar antioxidant properties as green tea and can be used as a nutraceutical to alleviate symptoms related with Alzheimer's disease. Halophytes are also promising phytostabilisation species, reducing the bioavailability of hazardous elements in contaminated sediments. For example, *Halimione portulacoides* has the capacity to immobilise metal such as cobalt, copper and lead. Other studies report that cultivation of *Salicornia sp*. can rehabilitate abandoned and saline areas. In this review, the ancient and present-day uses of halophytes in Portugal are presented and discussed, focusing on their traditional uses and biological activities. The review concludes by suggesting future requirements and perspectives for further exploitation of these species within the context of sustainability and climate change.

Keywords: Halophytes Uses, Portugal, Food, Pharmaceutical, Neutraceutical, Phytostabilisation

Introduction

The term “halophyte” derives from the ancient Greek words: halas (salt) and phyton (plant), *i. e.*, salt tolerant plants (Sruthi et al., 2017). Aronson (1989) listed 1560 halophyte species that are able to survive the most part of their life cycle under a minimum of 80 mM NaCl concentration, and with the same definition Menzel and Lieth described 2600 species (Flowers et al., 2010). Later, Flowers and Colmer (2008) defined halophytes as species able to complete their life cycle in at least 200 mM NaCl concentrations. Thus, currently there are 1395 species records in the most recent world database of halophytes (Santos et al., 2015).

Halophyte species are widely distributed across the orders and respective families of flowering plants, suggesting a polyphyletic origin (Flowers et al., 1977). A recent review using information from fossils and phylogenies revealed that most orders with halophyte families have less than 1% of salt-tolerant plants, supporting the hypothesis that each order evolved salt tolerance independently and that there are multiple origins of halophytes (Flowers et al., 2010).

Based on the eHALOPH database (http://www.sussex.ac.uk/affiliates/halophytes), the total number of families and genera of halophytes was estimated and the order to which each family belongs was identified (Table II.1). Halophytes are distributed across 38 orders, 116 families, 521 genera and 1395 species. Caryophyllales and Lamiales are the largest orders, which together contain 26% of halophyte families. The Amaranthaceae (Caryophyllales) covering 63 genera and 353 species is the largest family representing 25% of halophyte species (eHALOPH, 2018). This family together with Poaceae (Poales), Leguminosae (Fabales), Asteraceae (Asterales) and Plumbaginaceae (Caryophyllales) contain 50% of halophyte plants (eHALOPH, 2018).

Table II.1. Orders and respective families containing halophyte species, based on the eHALOPH database (sorted in descending order of number of families).

Ordem	Family	N.º of families
Caryophyllales	Anacampserotaceae, Amaranthaceae, Aizoaceae, Basellaceae, Cactaceae, Caryophyllaceae, Didiereaceae, Frankeniaceae, Gisekiaceae, Halophytaceae, Molluginaceae, Nyctaginaceae, Plumbaginaceae, Polygonaceae, Portulacaceae, Sarcobataceae, Stegnospermataceae, Talinaceae, Tamaricaceae	19
Lamiales	Acanthaceae, Avicenniaceae, Bignoniaceae, Lamiaceae, Myoporaceae, Oleaceae, Orobanchaceae, Plantaginaceae, Phrymaceae, Scrophulariaceae, Verbenaceae	11

Alismatales	Alismataceae, Cymodoceaceae, Hydrocharitaceae, Juncaginaceae, Posidoniaceae, Potamogetonaceae, Ruppiaceae, Zosteraceae	8
Malpighiales	Chrysobalanaceae, Clusiaceae, Euphorbiaceae, Linaceae, Phyllanthaceae, Putranjivaceae, Salicaceae	7
Brassicales	Bataceae, Brassicaceae, Capparaceae , Cleomaceae, Resedaceae, Salvadoraceae	6
Sapindales	Anacardiaceae, Meliaceae, Nitrariaceae, Rutaceae, Sapindaceae, Simaroubaceae	6
Ericales	Ericaceae, Lecythidaceae, Primulaceae, Sapotaceae, Tetrameristaceae	5
Poales	Cyperaceae, Juncaceae, Poaceae, Restionaceae Typhaceae,	5
Gentianales	Apocynaceae, Gentianaceae, Loganiaceae, Rubiaceae	4
Myrtales	Combretaceae, Lythraceae, Myrtaceae, Onagraceae	4
Asparagales	Amaryllidaceae, Asparagaceae, Xanthorrhoeaceae	3
Fabales	Casuarinaceae, Leguminosae, Surianaceae	3
Malvales	Malvaceae, Sterculiaceae, Thymelaeaceae	3
Rosales	Elaeagnaceae, Moraceae, Rhamnaceae	3
Apiales	Apiaceae, Araliaceae	2
Asterales	Asteraceae, Goodeniaceae	2
Magnoliales	Annonaceae, Myristicaceae	2
Polypodiales	Blechnaceae, Pteridaceae	2
Solanales	Convolvulaceae, Solanaceae	2
Arecales	Arecaceae	1
Boraginales	Boraginaceae	1
Commelinales	Pontederiaceae	1
Cornales	Loasaceae	1
Cucurbitales	Cucurbitaceae	1
Cycadales	Zamiaceae	1
Dilleniales	Dilleniaceae	1
Celastrales	Celastraceae	1
Geraniales	Geraniaceae	1
Liliales	Liliaceae	1
Najadales	Najadaceae	1
Nymphaeales	Nymphaeaceae	1
Pandanales	Pandanaceae	1
Picramniales	Picramniaceae	1
Pinales	Podocarpaceae	1
Piperales	Saururaceae	1
Rhizophoraceae	Rhizophoraceae	1
Saxifragales	Cynomoriaceae	1
Zygophyllales	Zygophyllaceae	1

By comparing data from the databases eHALOPH and Flora-on (2014: www.flora-on.pt), 163 halophyte species in Portugal, distributed in 18 orders, 40 familes and 99 genera were identified (Table II.2). These data comprise 12% of species, 50% of orders, 34% of families and 19% of genera of world records. In Portugal also the order with the highest number of species is the Caryophyllales (54), followed by the Poales (43), representing 60% of halophytes. The most common familes are the Poaceae (16%), Amaranthaceae (14%), Leguminosae (8%) and Plumbaginaceae (7%).

Since ancient times, halophyte species have been used in human consumption as for example *Salicornia sp.* (Chevalier, 1922), in popular medicine to prevent diseases as *Crithmum maritimum* (Pereira et al., 2017a), or even in the glass industry such as *Salsola soda* (Figueiredo, 1825). Nowadays, studies reveal that halophytes have also a high potential as fodder crops (El Shaer, 2010), ornamental plants used in landscapes (Cassaniti et al., 2012), as sources of biofuel (Song and Wang, 2015), along with pharmaceutical and nutraceuticals properties (Rodrigues et al., 2016; Rocha et al., 2017). They also play an important role in nutrient removal from saline aquaculture wastewater (Brown et al., 1999, Waller et al., 2015, Custódio et al., 2017), in the recycling of hyper-saline drainage water for later use in agriculture (Grattan et al., 2008), and in the phytostabilisation of contaminated soils (Serafim et al., 2013; Santos et al., 2017a). The purpose of this review is to compile all the information relative to traditional and present -day uses of halophytes in Portugal, including the Portuguese colonial period.

Table II.2. Number of Portuguese halophyte species by family and respective order (sorted by order with more species).

Order	Family	Genus	N.º of species
Caryophyllales	Aizoaceae	Carpobrotus, Drosanthemum, Mesembryanthemum, Tetragonia	5
	Amaranthaceae	Arthrocnemum, Atriplex, Bassia, Beta, Chenopodium, Halopeplis, Salicornia, Salsola Sarcocornia Suaeda	20
	Caryophyllaceae	Honckenya, Sagina, Spergularia	5
	Frankeniaceae	Frankenia	2
	Plumbaginaceae	Armeria, Limoniastrum, Limonium	11
	Polygonaceae	Polygonum, Rumex	3
	Portulacaceae	Portulaca	1
	Tamaricaceae	Tamarix	4
Poales	Cyperaceae	Bolboschoenus, Carex, Cyperus, Eleocharis, Isolepis, Schoenoplectus, Scirpoides	10
	Juncaceae	Juncus	4
	Juncaginaceae	Triglochin	1
	Poaceae	Agrostis, Arundo, Crypsis, Cynodon, Elymus Festuca, Hainardia, Hordeum, Imperata, Lygeum, Parapholis, Paspalum, Phragmites, Polypogon, Setaria, Spartina, Sporobolus, Stenotaphrum Vulpia	25
	Typhaceae	Typha	3
Fabales	Leguminosae	Acacia, Lotus, Medicago, Melilotus, Trifolium	13

Alismatales	Cymodoceaceae Hydrocharitaceae Potamogetonaceae Ruppiaceae Zosteraceae	Cymodocea Najas Potamogeton, Zannichellia Ruppia Zostera	1 1 4 2 2
Apiales	Apiaceae Araliaceae	Ammi, Apium, Crithmum, Daucus, Eryngium, Oenanthe Hydrocotyle	7 1
Brassicales	Brassicaceae	Brassica, Cakile, Cochlearia, Lepidium, Lobularia, Raphanus	7
Lamiales	Oleaceae Orobanchaceae Plantaginaceae Scrophulariaceae	Phillyrea Cistanche Plantago Limosella	1 1 4 1
Asterales	Asteraceae	Otanthus, Achillea, Cotula, Dittrichia	4
Solanales	Convolvulaceae Solanaceae	Calystegia, Cressa Lycium	3 1
Boraginales	Boraginaceae	Heliotropium	1
Ericales	Primulaceae	Anagallis	1
Gentianales	Gentianaceae	Centaurium	1
Malpighiales	Linaceae	Linum	1
Malvales	Malvaceae	Modiola	1
Myrtales	Myrtaceae	Eucalyptus	1
Najadales	Najadaceae	Triglochin	1
Saxifragales	Cynomoriaceae	Cynomorium	1
Zygophyllales	Zygophyllaceae	Tribulus	1

Ecological and Physiological Adaptations of Halophytes to Salt Tolerance

Halophytes have different life forms, as they can be annual, aquatic, chaemaephyte, fern, geophyte, hemicryptophyte, perennial herbaceous, nano-chamaephyte, parasite, perennial grass, seagrass, shrub, succulent, tree and vine (Santos et al., 2015). The values obtained based on the information available in eHALOPH database indicate that most of halophytes are herbaceous perennials (22%), trees (14%) and shrubs (13%). In Portugal, the predominant halophytes are also herbaceous perennials (37%) like *Limonium algarvense* and *C. maritimum*, followed by annuals such as *Plantago coronopus* and *Salicornia ramosissima* (21%).

Depending on their habitat, halophytes can be classified as hydrohalophyte, chasmophyte, psammophile, xerophyte and xerohalophyte (Santos et al., 2015). The hydrohalophytes are the most common (40%), growing in tidal marshes, mangroves, coastal lagoons and/or saltmarshes. Usually the chasmophytes live in cliff-dwellings close to the sea whereas the psammophiles thrive in sandy soils. Xerophytes and xerohalophytes can live in inland salt deserts, as they are plants adapted to extreme drought (Santos et al., 2015). In Portugal, most halophytes are present in saltmarshes or sandy soils along the coastline. According to the values calculated from the eHALOPH database, they are mostly hydrohalophyte (33%) like *Arthocnemum macrostachyum* and psammophiles (22%) such as *Polygonum maritimum*.

Halophytes are plants that can grow and reproduce in environments with high levels of salinity due to their adaptative mechanisms, namely Na^+ and Cl^- compartmentation, their ability to maintain Ca^{2+} and K^+ uptake, succulence, synthesis of particular compounds and the presence of salt glands (Flowers et al., 2010; Song and Wang, 2015). The compartmentation of ions in the vacuoles is needed for osmotic balance; however, any excess of ions can also cause damage in the plant tissue (Flowers et al., 2015). The transport of Na^+ ions to cell vacuoles is realised by a variety of transporters, proteins that contribute to keeping Na^+ away from the metabolic machinery in the cytoplasm (Flowers et al., 2018). *Salicornia europaea* and *Suaeda maritima* subsp. *salsa* species reveal an increase of Na^+/H^+ antiporters with an increase of NaCl concentrations (Song and Wang 2014). Several stresses including salinity lead to an increase of Ca^{2+} concentration in the cytosol, and this cation also contributes to maintaining high-affinity K^+ uptake (Song and Wang, 2014). Some plants, such as *Suaeda fruticosa*, in high Na^+ concentrations increase the uptake of Ca^{2+} and K^+ nutrients to maintain the Na^+ homeostasis (Diray-Arce et al., 2015). The accumulation of Na^+ and Cl^- ions in the vacuoles reduces the cell water potential, contributing to water uptake under saline conditions and leading to an increase of stem and leaf succulence. Succulence is related to an increase in cell size and to a high water content per surface area unit (Song and Wang, 2014). Another mechanism that contributes to osmotic adjustment is the accumulation of non-toxic compounds in the cytoplasm, such as glycinebetaine and proline (*e. g.*, *Suaeda maritima*) (Song and Wang, 2014). These osmoprotectans protect cellular membranes and enzymatic activity (Diray-Arce et al., 2015). Salt stress induces the production of reactive oxygen species (ROS), which can disrupt the integrity of cellular membranes and the enzymatic activity (Ventura et al., 2014). To avoid ROS toxicity, halophytes produce antioxidative enzymes (*e. g.*, catalase and superoxide dismutase), and non-enzymatic molecules such ascorbic acid, glutathione, flavonoids, and polyphenolic compounds (Song and Wang, 2014; Ventura et al., 2014). Some families of halophytes, like Amaranthaceae and Plumbaginaceae, developed salt glands in the leaves, and these structures allow plants to remove excess salt, thus avoiding damage (Flowers et al., 2010).

Uses of Halophytes in Portugal

According to the species and their economic uses listed in eHALOPH, we have estimated that there is at least one use for 44% of halophytes. Several different uses (*e. g.*, food, ornamental and timber) were identified, and the most common were salt-tolerant ornamental species with *ca.* 30% of halophyte species, followed by fuel with 24%, and fodder with 14%. In Portugal, only 26% of the halophytes were recorded as having traditional and/or potential uses. Concerning halophyte species used during the Portuguese colonial period, only nine species were identified (Table II.3), most of them shrub or tree hydrohalophytes, by comparison to the remaining species that were mostly perennial herbaceous hydrohalophytes. In total 43 species with five different types of use were identified: pharmaceutical, food, nutraceutical, phytostabilisation and rehabilitation of soils and other habitats. The majority of species showed more than one use and the most common use was pharmaceutical (23 species), followed by food (18 species), and phytostabilisation and rehabilitation of contaminated soils (seven species).

Pharmaceutical

Since ancient times halophytes have been used in Portugal, in popular medicine; for example, to treat cutaneous problems as swellings, wounds or infections in which poultices made of leaves from *Arundo donax* were used (Carapeto, 2016) or lotions prepared from *Atriplex halimus* (Figueiredo, 1825). In Angola, to treat those problems poultices made of fruits from *Solanum incanum* were also utilised. The juice of these fruits was also used as enemas, and the water in which roots of this plant was cooked was applied as an oral antiseptic (Santos, 1989). *Tamarix gallica* leaves were also utilised in toothache treatment (Ficalho, 1884; Camejo-Rodrigues, 2006). Recent studies demonstrate that some species used in folk medicine in former times have pharmaceutical proprieties such as *C. maritimum* used by sailors to prevent scurvy. In fact, C. maritimum has a high polyphenolic content and a strong antioxidant potential (Ventura, 2014; Pereira et al., 2017a). *Chenopodium ambriosiodes* leaf infusions, which was earlier used as anthelmintic and as a treatment for stomach pain (Espírito Santo, 1969), are rich in terpenic hydrocarbons and have high antioxidant activity (Costa, 1975; Barros et al., 2013). An infusion of *Plantago coronopus* leaves' was commonly applied in the past as an oral antiseptic and to relieve sore throat pain (Camejo-Rodrigues, 2006; Neves et al., 2009); it is currently recognised as a good source of bioactive molecules with a potential anti-inflammatory activity (Rodrigues et al., 2014a; Pereira et al., 2017b). The compounds synthetised by halophytes as a response to salt stress, such as flavonoids, polyphenols and polysaccharides, have antioxidant, anti-inflammatory, and antitumoral properties that can be used in the prevention of some diseases like diabetes, cancer and neurological disorders (Ksouri et al., 2008; Vinholes et al., 2011; Rocha et al., 2017). For example, the leaves and roots of *P. maritimum*, rich in bioactive polyphenols (Camejo-Rodrigues, 2003), can be used for alleviating symptoms associated with diabetes, Alzheimer's disease and other neurodegenerative disorders

(Rodrigues et al., 2014b; Rodrigues et al., 2017a). Juncunol, the biocompound extracted from species such as *Juncus acutus* L. (Figure II.1 A) and *J. maritimus*, is able to inhibit enzymes related with the onset of neurological disorders leading to an improvement of cognitive functions. Juncunol also has chemotherapeutic proprieties (Rodrigues et al., 2017b). Inula crithmoides (Figure II.1 B) has a high hydrocarbon content (Costa et al., 1975) and is a valuable source of metabolites with activity against *Leishmania infatum* Nicolle, a protozoan parasite and causative agent responsible for the propagation of infantile visceral leishmaniasis disease, which affects around 13 million people (Oliveira et al., 2017). There are several halophyte species in Portugal with a pharmaceutical potential, which are shown in Table II.3.

Food

In Portugal, the leaves of some halophytes such as *Chenopodium murale*, *Rumex crispus* (Bicho, 2015), *Portulaca oleracea* (Carvalho and Morales, 2010), and *Tetragonia tetragonoides* (Valagão n.d.) are traditionally used in soups and salads. Another species of great importance is *Brassica nigra* whose seeds are used to obtain black mustard (Costa, 1975) (Table II.3). However, the most common Portuguese crops, as in the whole world, are glycophytes (salt-sensitive species). Nevertheless, the increasing soil salinisation due to excessive irrigation of agricultural land with low quality water, vegetation clearance and poor drainage of suitable soils (Flowers and Yeo, 1995), and the scarcity of freshwater (Rozema and Flowers, 2008) is leading to an insufficiency of agricultural land needed to feed the population (Beltran and Manzur, 2005; Diray-Arce et al., 2015). In Europe, mainly in the Mediterranean countries, 1 to 3 million hectares are affected by soil salinisation (Ladeiro, 2012). In Portugal, it is estimated that 150,000 ha are salt-affected, 50,000 ha due to anthropic causes (Gonçalves et al., 2015). These facts have increased the interest in the cultivation of halophytes and in their potential in European diets. Some studies demonstrated that the cultivation of *S. ramosissima* and *Salicornia patula* by seedlings transplanted from greenhouses shows a higher survival rate than the direct sowing in marshes, mainly for *S. patula* (Salazar et al., 2013; Santos et al., 2017b). The presence of shade can promote fresh biomass of these species (Salazar et al., 2013; Santos et al., 2017b). *Salicornia ramosissima*, *S. patula, A. macrostachyum* (Figure II.1C) and *Sarcocornia perennis* have a nutritional profile suitable for human consumption that is rich in nutrients, vitamins, proteins, and polyunsaturated fatty acids essential for the human diet (Custódio et al., 2012; Barreira et al., 2017). These species can also be considered fibre-rich vegetables, mainly the latter two species, because of their high fibre content. Currently, it is possible to find these species in high-rank restaurants with a gourmet cuisine as well as in some supermarkets: young fleshly tips are commonly used in salads, soups, and main dishes as a fresh vegetable or as a seasoning substituting salt (Barreira et al., 2017). The infusions and decoctions of *C. maritimum* (Figure II.1D), *Carpobrotus edulis* and *L. algarvense* leaves have benefits for human consumption due to its strong antioxidant activity, neuroprotective proprieties and high polyphenolic and nutrients content (Custódio et al., 2012, Rodrigues et al., 2016, Pereira et al., 2017a). The infusions and decoctions of *L. algarvense* flowers have antioxidant proprieties similar to green tea (*Camelia sinensis*) (Rodrigues et al., 2016).

Nutraceutical

The term 'nutraceutical' derives from the words nutrition and pharmaceutical, meaning functional food (food rich in compounds such as vitamins, proteins, phenols among others) that contribute to the prevention or treatment of some diseases and disorders (Kalra, 2003). *Arthrocnemum macrostachyum*, *L. algarvense* and C. edulis are species suitable to human consumption, which can be considered functional food (see 'Food' section) and also have an important role in the prevention of certain diseases (Table II.3). *Arthrocnemum macrostachyum* and *L. algarvense* (Figure II.1E) can alleviate symptoms related to Alzheimer's disease and other neurodegenerative disorders (Custódio et al., 2012; Rodrigues et al., 2014b; Barreira et al., 2017). The specie *C. edulis* (Figure II.1F) reduce the risk of cardiovascular and coronary heart diseases (Custódio et al., 2012; Rocha et al., 2017) and its high content of oleanolic acid and uvaol have been shown effective against mouse lymphoma cells reducing their replication and thus revealing a potential as a chemotherapeutic agent (Martins et al., 2010). Another species with potential nutraceutical is the Spergularia rubra whose the infusions, commonly consumed because of their diuretic proprieties, are rich in organic acids and phenolic compounds that can suppress hyperglycemia (Vinholes et al., 2011), and also have a strong anti-inflammatory propriety against leishmaniasis disease propagation (Oliveira et al., 2017).

Phytostabilisation and Rehabilitation of Soils/Sediments

As previously mentioned, in Portugal most halophytes grow in saltmarshes or in sandy soils along the coast. The estuarine environments are some of the most dynamic and productive ecosystems in the world, which provide several ecosystem services (*e. g.*, coastal protection, erosion control, food and raw materials) (Barbier et al., 2011; Peres et al., 2016). However, they are also one of the most threatened. In Portugal, these habitats are heavily affected by human activities such as agriculture, industry, aquaculture, navigation (Serafim et al., 2013) and their frequent use as a deposit of the wastes of these activities (Reboreda and Caçador, 2007). The accumulation of potentially hazardous elements in the environment harms the services that saltmarshes offer, can become a risk to animal and human health and can also cause the abandonment of these areas (Reboreda and Caçador, 2007). Consequently, degradation of soil (*e. g.*, compactation, contamination, hydrological conditions) and reduction in biodiversity (fauna and flora) is observed (Peres et al., 2016; Santos et al.,2017b).

Halophytic plants have the capacity to reduce the bioavailability of toxic elements in sediments, thus protecting themselves from metal(loid) toxicity (Sousa et al., 2008), through mechanisms such as: the release of root exudates contributing to precipitation and/or chelation of elements (Caçador et al., 2000; Santos et al., 2017a); the accumulation of oxy-hydroxides of iron or manganese in the surface of the roots constituting plaques that work as a sink for potentially hazardous elements (Almeida et al., 2006; Marques et al., 2011); the compartmentation of metals in the vacuoles and cell wall (Sousa et al., 2008); the synthesis of organic compounds like phytochelatins (Almeida et al., 2006); and excretion by salt glands (Santos et al., 2017a). For these reasons halophytes play an important role in the phytostabilisation and rehabilitation of saltmarshes (Table II.3). For example, *Halimione portulacoides* (Figure II.1G) has the capacity to immobilise potentially hazardous elements such as cadmium, cobalt, copper and lead (Caçador et al., 2000; Sousa et al., 2008). This species seems able to immobilise those metals in the rhizosphere through the co-precipitation with iron oxides in the iron plaques (Caçador et al., 2000) or accumulating the potentially hazardous elements in the roots by compartmentation of metals in the cell wall and vacuoles, avoiding high metal concentration in the cytoplasm so protecting the plant from the toxic substances (Sousa et al., 2008). *Juncus maritimus* shows the same mechanisms to reduce the mobility of copper and mercury in the sediments, *i. e.*, through iron plaques in the rhizosediment interface and accumulation in the roots, with a consequent low translocation factor of metals from roots to the leaves (Almeida et al., 2006; Marques et al., 2011). Another species with potential in the phytostabilisation of contaminated soils/sediments is *Tamarix africana* with a strong capacity to take up potentially hazardous elements such as aluminium, arsenic, chromium, copper, iron, lead and zinc, as well as sodium from soils/sediments and to accumulate them in its roots (Santos et al., 2017a). This species also presents a low translocation rate of those elements to the leaves, together with salt glands that, in addition to salt excretion, allow the excretion of toxic elements (Santos et al., 2017a). Studies demonstrated that *S. ramosissima* (Figure II.1H) stores cadmium in its roots with a low translocation of this element to the aerial part (Pedro et al., 2016). The sustainable cultivation of this species as well as *S. patula* allows the rehabilitation of abandoned estuarine areas contributing to an improvement of soils/sediments properties and also to the increase of biodiversity and the recover of several ecosystem services offered by the estuarine environments (Santos et al., 2017b).

Other Uses

In addition to the uses already mentioned, halophytes show other usages that have been recorded less frequently, like applications in perfumes, soaps, candles (Costa et al., 1975), textiles (Ficalho, 1884) or even in the extraction of sodium carbonate (Figueiredo, 1825) (Table II.3). In Angola, *Pelargonium capitatum* leaves were commonly used in the manufacturing of perfumes and soaps, and in Mozambique and Timor, essential oils were extracted from the resin of *Calophyllum inophyllum* and candles made from the fat of fruits (Costa et al., 1975). In Cape Verde, the long trichomes present in the fruits of *Calotropis procera* were earlier used to fill mattresses and, mixed with cotton, to create strong textiles (Ficalho, 1884). The *S. soda* species was very important in former times since sodium carbonate, a very important compound for the manufacture of glass and currently synthetised in the laboratory, was obtained from the ashes of this plant (Figueiredo, 1825).

Figure II.1. (A) *Juncus acutus* L. and (B) *Inula crithmoides* L., halophyte species with pharmaceutical potential; (C) *Arthocnemum macrostachyum* (Moric.) Moris and (D) *Crithmum maritimum* L., species with potential in food industry; (E) *Limonium algarvense* Erben and (F) *Carpobrotus edulis* N.E.Br., halophytes with application in nutraceutical industry; and (G) *Halimione portulacoides* L. and (H) *Salicornia ramosissima* J. Woods, promising phytostabilisation species. Photos by Ana Cortinhas and Ana D. Caperta

Table II.3. Traditional and potential uses of halophytes in Portugal, including during the Portuguese colonial period.

Genus	Species	Author	Country	Traditional/Popular uses	Plant Part	Biological Properties/Activity	Potencial uses	References
Aloe	*vera*	(L.) Burm.f.	Portugal	—	—	Antifungal and Antioxidant activity	Food and phamarceutical	Silva et al. (2013); Vieira et al. (2016)
Ammi	*visnaga*	(L.) Lam.	Portugal	Aromatic/condiment	Seeds	Coumarins	—	Costa (1975)
Apium	*graveolens*	L.	Portugal	—	Stems, leaves and flowers	Antioxidant and antimicrobial activities	Food and phamarceutical	Alves et al. (2013)
Arthrocnemum	*macrostachyum*	(Moric.) Moris	Portugal	—	Shoots and leaves	Polyunsaturated fatty acids content	Food, nutraceutical and pharmaceutical	Custodio et al. (2012); Barreira et al. (2017)
Arundo	*donax*	L.	Portugal	Cataplasms to treat swellings	Whole plant	—	—	Carapeto (2006)
Atriplex	*halimus*	L.	Portugal	Lotions to use in cutaneous wounds treatment	—	—	—	Figueiredo (1825)
Brassica	*nigra*	(L.) K.Koch	Portugal, Mozambique and Cape Verde	Black mustard used in food	Seeds	—	—	Costa (1975)
Calophyllum	*inophyllum*	L.	Mozambique and Timor	Essentials oils and candles	Resin and fruits	—	—	Costa (1975)
Calotropis	*procera*	(Aiton) Dryand.	Cape Verde	Fill mattresses and create textiles	Fruits	—	—	Ficalho (1984)

Carpobrotus	*edulis*	(L.) N.E.Br.	Portugal	—	Leaves	Neuroprotective properties and antioxidant activity	Food, nutraceutical and phamarceutical	Martins et al. (2010); Rocha et al. (2017);
Chenopodium	*ambrosioides*	L.	Portugal, Mozambique, Cape Verde, S. Tomé and Príncipe and Timor	Infusions to stomach ache treatment and as vermifuge	Leaves	Antioxidant activity	Phamarceutical	Espírito Santo (1969); Costa (1975); Barros et al. (2013)
Chenopodium	*murale*	L.	Portugal	Soaps	Leaves	—	—	Bicho (2015)
Cocos	*nucifera*	L.	Angola, Mozambique, S. Tomé and Príncipe	Edible fruit	Fruit	—	—	Costa (1975)
Cotula	*coronopifolia*	L.	Portugal	Infusion to treat bladder problems	Flowers	—	—	Carapeto (2006)
Crithmum	*maritimum*	L.	Portugal	To prevent the scurvy	Leaves and flowers	Polyphenolic and ureides compounds; antioxidant activity	Food and phamarceutical	Pereira, et al. (2017a)
Frankenia	*laevis*	L.	Portugal	—	Leaves and flowers	Phenolic componds; antioxidant activity	Pharmaceutical and cosmetics	Lopes et al. (2016)
Frankenia	*pulverulenta*	L.	Portugal	—	Leaves and flowers	Flavonoids and phenolic componds; antioxidant activity	Pharmaceutical and cosmetics	Lopes et al. (2016)
Halimione	*portulacoides*	L.	Portugal	—	Whole plant	Phenolic compounds, mostly sulfated flavonoids; anti-inflammatory activity	Pharmaceutical; Phytostabilisation of contaminated soils	Caçador et al. (2000) Sousa et al. (2008)

								Rodrigues et al. (2014a)
Inula	*crithmoides*	L.	Portugal	—	Leaves	Phenolic and flavonoids compounds; anti-inflammatory properties	Pharmaceutical	Oliveira et al. (2017)
Juncus	*acutus*	L.	Portugal	—	Leaves and roots	Phenolic compounds; antioxidant activity	Pharmaceutical and phytostabilisation of contaminated soils	Rodrigues et al (2014a, 2017b)
Juncus	*maritimus*	Lam.	Portugal	—	Leaves and roots	Polyunsaturated fatty acids and phenolic compounds; antioxidant activity	Pharmaceutical and phytostabilisation of contaminated soils	Rodrigues,et al (2014a); Marques et al. (2011); Almeida et al. 2006
Limonium	*algarvense*	Erben	Portugal	—	Flowers	Phenolic compounds content; antioxidant and anti-inflammatory properties	Food, nutraceutical and phamaceutical	Rodrigues et al (2014b); Rodrigues et al. (2016);
Melilotus	*indicus*	(L.) All.	Portugal	Perfums	—	Coumarins	—	Costa (1975)
Otanthus	*maritimus*	(L.)	Portugal	—	Leaves and flowers	Chrysanthenone; antifungal and anti-inflammatory activity	Pharmaceutical	Cabral et al. (2013)
Pelargonium	*capitatum*	(L.) L'Hér.	Angola	Perfums and soaps	Leaves	—	—	Costa (1975)
Plantago	*coronopus*	L.	Portugal	Emollient and dermatologic protector; the infusion as oral antiseptic throat	Leaves and flowers.	Anti-inflammatory and antiseptic	Pharmaceutical	Rodrigues,et al (2014a); Pereira et al (2017b);

				wash and to sore throat treatment				Camejo-Rodrigues (2006); Neves et al. (2009)
Plantago	*lanceolata*	L.	Portugal	Cough; asthma and lungs problems	Leaves and flowers	Analgesic; Anti-inflammatory; Astringent; Depurative and antiseptic.	Pharmaceutical	Neves et al. (2009)
Plantago	*major*	L.	Portugal	Infusions to voice problems, cataplasms to treat eye inflammation and furuncles	Leaves	Anti-haemorrhagic; Anti-inflammatory; and diuretic	—	Camejo-Rodrigues (2003); Neves et al. (2009)
Polygonum	*maritimum*	L.	Portugal	—	Leaves and roots	Bioactive polyphenols	Food and pharmaceutical	Rodrigues et al (2014b); Rodrigues et al. (2017a)
Portulaca	*oleracea*	L.	Portugal	Salads and soups	Leaves	Omega-3 and omega-6 fatty acids, organic acids and phenolic compounds; antioxidant activity	—	Carvalho and Morales (2010)
Rumex	*crispus*	L.	Portugal	Soups	Basal Leaves	—	—	Bicho 2015
Salicornia	*patula*	Duval-Jouve	Portugal	—	Roots	—	Rehabilitation of soils;	Santos et al. (2017b)
Salicornia	*ramosissima*	J.Woods	Portugal	—	Whole plant	Lipophilic phenolics compounds;	Food insustry; phytostabilisation	Carapeto (2006);

						flavonoid;antioxidante and anti-inflammatory	and rehabilitation of soils;	Pedro et al. (2016); Barreira et al. (2017); Santos et al. (2017b)
Salsola	*soda*	L.	Portugal	Soda (Sodium carbonate)	Ashes of plant	—	—	Figueiredo (1825)
Sarcocornia	*fruticosa*	(L.) A.J.Scott	Portugal	—	Whole plant	—	Food	Carapeto (2006)
Sarcocornia	*perennis*	(Mill.) A.J.Scott	Portugal	—	—	—	Food	Barreira et al. (2017)
Solanum	*incanum*	L.	Angola	Cataplasm to treat the cutaneous wounds or infections resulting of surgeries and the fruit juice as clyster for kids.	Roots and fruits	—	—	Santos (1989)
Spartina	*maritima*	(Curtis) Fernald	Portugal	—	Roots	—	Phytostabilisation of contaminated soils	Moreira da Silva (2015)
Spergularia	*rubra*	(L.) J.Presl & C.Presl	Portugal	—	Leaves and flowers	—	Pharmaceutical and food	Vinholes et al. (2011); Oliveira et al. (2017)
Tamarix	*africana*	Poir.	Portugal	—	Roots	—	Phytostabilisation of contaminated soils	Santos et al. (2017a)

Tamarix	*gallica*	L.	Portugal and Cape Verde	Treatment in the toothache and as oral antiseptic	Bark and leaves	—	—	Ficalho (1884); Camejo-Rodrigues (2006)
Terminalia	*catappa*	L.	Cabo Verde, India, S. Tomé and Príncipe	Edible seeds	Seeds	—	—	Ficalho (1884)
Tetragonia	*tetragonoides*	(Pall.) Kuntze	Portugal	Soups	Leaves	—	Food	Valagão (2008)

Conclusion

As far as the authors know, this is the first time that the number of halophytes in Portugal has been estimated. There are 163 species distributed among various orders and families of angiosperms. The most represented order (Caryophyllales) and family (Amaranthaceae) in Portugal is the same as found across the whole world.

Although there is little information of the uses of halophytes in Portugal, a few studies have revealed that they have potential value in several areas, such as pharmaceuticals, nutraceuticals, food, and in the phytostabilisation and rehabilitation of contaminated soils/sediments of estuarine areas. However, based on the world database of halophytes, the most common uses of halophytes are salt-tolerant ornamentals, fuel and fodder, but in Portugal these uses are not yet exploited. Future research should focus on the potential of the sustainable cultivation of halophytes in Portugal. The cultivation of halophytes can be a great economic benefit due to the ecosystem services and goods that these species provide.

Chapter III. *Harnessing sediments of coastal aquaculture ponds through Technosols construction for halophyte cultivation*

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Abstract

The Mediterranean aquaculture has been developed mostly in estuarine environment in inactive coastal salt production areas. This study aims to utilise Technosols made with aquaculture sediments for *Limonium algarvense* Erben cultivation. This species that has nutraceutical potential thrives in halophilic environments in the southwest of the Iberian Peninsula and in Morocco. A microcosm assay was set up with plants grown in bottom sediments (C^{+}), commercial substrate (C^{-}), and Technosols with amendments mixture application at 180 g/kg (Tec180) or at 360 g/kg (Tec360). These plants were irrigated with saline (assay 1) and/or with deionised water (assay 2). The bottom pond sediments, coffee wastes and the estuarine water were evaluated for diverse physicochemical parameters. Plant growth was characterised through a combined methodology using morphometric, SEM and physiological analysis. The Technosols were constructed with bottom sediments and a mixture of organic wastes used as amendments. Results revealed that the bottom sediments had low pH 3.2, C_{org} and extractable P and K contents, and high electroconductivity (EC) and N-NH_4 concentration. The estuarine water had a neutral pH, high EC and high Cl^{-}, HCO_3^{-}, Na^{+}, Mg^{2+} and Ca^{2+} but low N-NO_3^{-} content. The Technosols showed a significant increase of pH, C_{org}, K and P and a decrease in N-NH_4 and EC in comparison with sediments. Principal component analysis separated the different experiments in three groups: C^{-}, A1 and A2 assays. The C^{-} was highly correlated with C_{org}, P, K, N-NO_3 parameters and total ascorbate. The A1 assay showed a strong association with Na, Ca and EC parameters, whereas the A2 assay presented a strongly correlation with plant growth. Plants from Technosols had greater development when irrigated with deionised water than under salty irrigation as opposed to plants cultivated in unamend sediments. In conclusion, these results support that highly saline sediments could be valorised through Technosols construction to cultivate plants with saline water, with potential application in the agro-food and pharmaceutical industry.

Keywords: Tailored soil, Estuarine Water, *Limonium algarvense*, SEM analysis, Soil Technology, Wastes

Introduction

Mediterranean aquaculture includes a wide range of production activities of marine species using a variety of technologies, from extensive mollusc or fish production to highly intensive raceways or netcage fish farming (Grigorakis and Rigos, 2011; Rosa et al., 2012). In the Mediterranean region, aquaculture has expanded over the past decade, particularly in the estuarine environment and some of the inland production systems use abandoned or inactive coastal salt production areas (solar saltworks, salterns or *Salinas*), which have been converted to semi-intensive to intensive aquaculture (Rosa et al., 2012).

In the Mediterranean region, aquaculture installations converted from salt ponds appear to be one of the major human-driven stressors on habitat, local flora, and fauna, especially in protected areas (Simard et al., 2008; Claudet and Fraschetti, 2010;). Accumulation of sediments in these ponds can damage previous bottom sediments quality and can negatively impact water quality (Munsiri et al., 1996; Thunjai et al., 2004). Thus, a general practice is to remove sediment at intervals of several years or when sediment removal becomes a necessity to improve bottom sediments quality (Thunjai et al., 2004). However, disposing of these sediments on vacant land sometimes covering fertile soils can lead to degradation of soil properties, turbid runoff and other ecological hazards mainly because these sediments present high salt burden or even potential hazardous elements content (Aljerf and AlMasri, 2018).

A management alternative could be sediment valorisation by using them in building Technosols, which are mainly characterised by containing significant amounts of anthropogenic material, and whose properties and pedogenesis are dominated by their technical origin (IUSS Working Group, 2015). In the Mediterranean region, Technosols can be utilised for rehabilitation of mine wastes (Abreu and Magalhães, 2009; Rodríguez-Vila et al., 2015; Santos et al., 2016a), cultivation of non-food crops to alleviate environmental and health risks induced by pollutants (*e. g.*, *Miscanthus* spp., a biomass crop) (Nsanganwimana et al., 2014), growing plants with odoriferous/fragrance interest (*e. g., Cistus ladanifer* L.; Santos et al., 2016a) as well as for the management of marine dredged materials (Macía et al., 2014). Nonetheless, very little is known on the valorisation of disposed sediments from aquaculture ponds, salt or coastal marshes with the aim of plants cultivation (Manley et al., 2006).

An environmentally friendly approach for the utilisation of sediments from aquaculture could be the cultivation of halophytes, *i. e.*, salt-tolerant plants that can grow and reproduce at salinity levels up to 200 mM NaCl (Flowers and Colmer, 2008), using soil and water unsuitable for conventional crops. Halophytes can have different uses such as crops to produce fodder, food and biofuels, cultivated as ornamentals, and utilised for remediation, landscaping, and pharmaceuticals (Ventura et al., 2015). In Portugal, since ancient times, halophyte species have been used in human consumption and in popular medicine to prevent diseases (Cortinhas et al., 2019). Recent studies revealed that they have potential value in pharmaceuticals, nutraceuticals, food, and in phytostabilisation and rehabilitation of contaminated soils/sediments of estuarine areas (Oliveira et al. 2017; Santos et al. 2016b).

Halophytes *Limonium* spp. (Plumbaginaceae, sea lavenders) are found in coastal areas all over the world (Kubitzki, 1993) and produce metabolites with diverse bioactivities (Lee et al., 2011; Saidana et al., 2013). *Limonium algarvense* Erben is an Iberian-Moroccan endemism (Caperta et al., 2017) valued source of antioxidants with potential applications in the agro-food industry (Rodrigues et al., 2015). Its flowers' infusions and decoctions have similar or higher antioxidant and anti-inflammatory properties than green tea (Rodrigues et al., 2015). Recently, it was demonstrated that the production of such compounds is strongly increased with saline water irrigation (Rodrigues et al., 2019).

The main objective of this study was to test the utilisation of Technosols made with bottom sediments accumulated in aquaculture ponds and disposed on their walls (*marachas* and slopes) and adjacent landscape to grow and develop valued marine halophyte *L. algarvense*. This work provides an evaluation of plant growth using tailored soils (Technosols) made with a mixture of amendments at different rates for improving its properties under distinct saline water irrigation regimes.

Materials and Methods

Study area and species

The Castro Marim Nature Reserve (PTCON0013; 37º12'N, 7º26'W; ICNF, 2012), in Algarve, in the south-eastern part of Portugal, is a 1222 ha coastal saltmarsh area near the mouth of the Guadiana River. In these saltmarshes, plant communities are dominated by the chamaephytes *Myriolimon diffusum* (Pourr.) Lledó, Erben & Crespo (= *Limonium diffusum* (Pourr.) O. Kunze) and *Limonium algarvense* (*Myriolimo diffusi-Limonietum algarvensis*) occurring in sandy well drained soils, inundated only during the highest tides, under the thermomediterranean dry bioclimate (Costa et al., 2014). Other plant communities found are the *Polygono equisetiformis-Limoniastretum monopetali* and the *Cistancho phelypaeae-Suaedetum verae* associations (Costa et al., 2014). Salinas occupy about 30% of the surface, some of them converted to aquaculture ponds. These saltpans are key habitats for feeding and breeding of many shorebirds (Dias et al., 2014). Nonetheless, a continuing decline in these halophilic communities is found in the last years in these landscapes facilitated by the abandonment of artisanal *Salinas* and invasion with exotic invasive species (Almeida et al., 2014; Chefaoui and Chozas, 2019).

Experimental growth conditions

The initial sediments were collected in the walls of aquaculture ponds converted from abandoned *Salinas* of a private company, in the protected area Reserva Natural do Sapal de Castro Marim. The walls of aquaculture ponds were built with sediments collected in the bottom of the ponds. Technosols were produced using these sediments and a mixture of organic wastes mixed manually. Mixtures of wastes from *Ceratonia siliqua* L (CW) and *Arbutus unedo* L (AW) fruit spirits distillation, substrate used in strawberry crops (AgW) and coffee wastes (CoW) were utilised as amendments at rates 2.5:3.5:2:2, respectively. A microcosm assay was set up in triplicate with four treatments using pots containing 2 kg of the following substrata: (1) commercial substrate (du Vitor, Portugal) (negative control, C^{-}); (2) unamended sediments (positive control, C^{+}); (3) Technosols composed of sediments with an amendments mixture application at 180 g/kg of sediment (Tec180) or at 360 g/kg of sediment (Tec360) (4). Biomass ashes (BA) (100 g/kg of sediment) were incorporated in the Technosols to increase pH to ≈7.5. The Technosols were incubated at 70% of the maximum water-holding capacity for 15 days before plant transplanting.

Seeds of *L. algarvense* were collected in plants grown in saltmarshes in Castro Marim (Guadiana estuary, Algarve, Portugal) and germinated in *jiffy* pots. Seedlings were transferred to pots with commercial substrate (du Vitor, Portugal) one month after germination and allowed to grow for an additional four months irrigated with deionised water.

In Assay 1 (A1; Fig III.1), plants (n = 12) were transferred to three pots for each substrate *i. e.*, C^{+}, C^{-}, Tec180 or Tec360 as above described, while other six pots of Tec180 and Tec360 remained bare. Except to C^{-} pots and bare pots, all other pots were irrigated with estuarine water (Table III.1), which was collected from a channel located in the estuary of the river Guadiana that brought estuarine water into the aquaculture ponds.

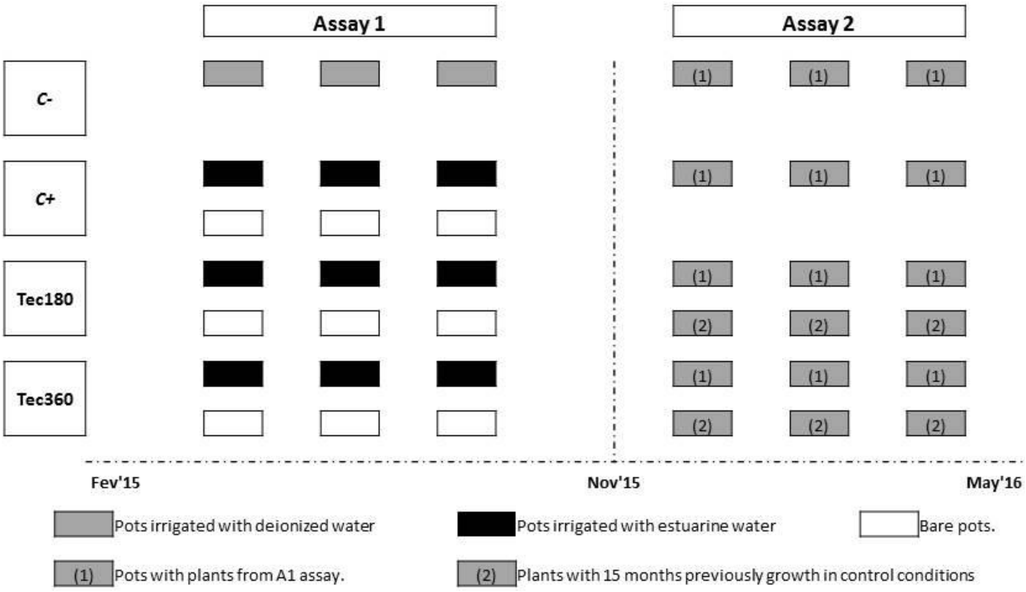


Figure III.1. Schematic representation of the microcosm set-up subdivided in two assays (A1 and A2) with four treatments: C^+ (sediment), C^- (commercial substrate), and Technosol with amendments mixture application at 180 g/kg of sediment (Tec180) or at 360 g/kg of sediment (Tec360).

The Assay 1 was performed in February 2015/November 2015 in a greenhouse, and all pots (with and without plants) were kept at 70% of the maximum water-holding capacity. The Assay 2 (A2; Figure III.1) was implemented in December 2015/May 2016 in the same greenhouse using the same pots with plants of assay A1. Plants (n = 6) of the same age of Assay 1 (fifteen months old) previously grown in control conditions (commercial substrate and deionised water) were transferred to each of the bare pots containing Tec180 and Tec360 Technosols. Plants were not transferred to C^+ bare pots, as plants grown in C^+ in Assay 1 succumbed, or to C^-, because since the beginning of A2 all plants were only irrigated with deionised water and the plants cultivated in C^- were already irrigated with deionised water in Assay 1.

Table III.1. Chemical characteristics of estuarine water collected in a channel located in the estuary of the river Guadiana. Electrical conductivity is given in mS/cm and anions and cations concentrations in mg/L. SAR - sodium adsorption ratio ([Na]/([Ca] + [Mg])$^{1/2}$; Ayers and Westcot, 1985; Lesch and Suarez, 2009)

Chemical characteristics of estuarine estuarine water	
pH	7.2
Electrical conductivity	17.22
Cl^-	8118.1
NO_3^-	<0.5
N-NO_3^-	<0.1
N-NH_4^+	<0.1
HCO_3^-	139.8
CO_3^{2-}	<0.3
Na^+	3474.8
Ca^{2+}	229.4
Mg^{2+}	564.8
SAR	28.09

Experimental monitoring and analytical methods

The estuarine water was also analysed for: pH (potentiometry), EC (conductivimetry), Cl^- (Mohr, 1945), HCO_3^- (titration method using HCl solution methyl orange as indicator), CO_3^{2-}(titration method using phenolphthalein as indicator), NO_3^-, N-NO_3^-, N-NH_4^+ (molecular absorption spectrometry) and Na, Ca and Mg (atomic absorption spectrometry). The sodium adsorption ratio (SAR) was calculated ([Na]/([Ca] + [Mg])$^{1/2}$; Ayers and Westcot,1985; Lesch and Suarez, 2009).

The mixtures of wastes from distilleries (*C. siliqua* and *A. unedo*) and substrate used in strawberry crops were previous analysed (Santos et al., 2014; Santos et al., 2016a; Table III.2). The unamended sediment and coffee wastes as well as composite samples of the substrata (unamended sediments, commercial substrate, and Tec180 and Tec360 Technosols) from each pot were analysed in the present study.

The initial sediment, commercial substrate, and composite soil samples (Technosols) were homogenised and sieved (< 2 mm). The composite samples of materials from each pot were collected (0–15 cm of depth) and analysed at the beginning of A1 assay (after 15 days of incubation and before plant transplantation – T0), at the beginning of A2 assay (before every pots started to be irrigated with deionised water – T1), and at the end of experiment (T2). The first analysis (T0) showed the initial conditions of the experiment (A1); the second analysis (T1) assessed the influence of estuarine water irrigation conditions (A1) as well as the beginning of A2; and the last analysis (T2) allowed determination of the effects of deionised water in plant growth, in plants previously irrigated with estuarine water, and plants only irrigated with deionised water.

The initial sediment, commercial substrate and Technosol samples (fraction <2 mm) were characterised following methods in Póvoas and Barral (1992) for: pH and electric conductivity (EC) in a water suspension (1:2.5 *m/V*); extractable K and P (Egner–Riehm method); total N (Kjeldahl method); nitric and ammoniacal N (Mulvaney, 1996); and C_{org} by wet combustion. The Na, Ca and Mg concentrations in the soil solution, obtained by soil suspension (1:20 *m*/*V*) in deionised water kept in equilibrium for five days (Buján et al., 2010) were determined by atomic absorption spectrometry. Certified standard solutions, analytical replicates of the samples, blanks and laboratory standards were used as internal control of quality. The recovery rates ranged from 80–120%.

Table III.2. Chemical characteristics of organic wastes used as amendments (min–max or mean value). *AgW* Agriculture Wastes, *AW* residue from the liquor distillation of *A. unedo* fruit, *CoW* Coffee Wastes, *CW* residue from liquor distillation of *C. siliqua* fruit, and *BA* biomass ash. * - Determined in previous studies (Santos et. al., 2014, 2016a)

Chemical characteristics	*AgW**	*AW**	*CoW*	*CW**	*BA*
pH (H_2O)	6.68–7.18	4.91	5.9	6.1	8.8
Electrical conductivity (mS/cm)	0.9–3.5	1.7	1.41	0.2	12.7
Organic C (g/kg)	286.1	442.6	401.5	436.7	56.0
N (g/kg)	–	–	–	–	0.3
Total	9.7	8.9	–	11.1	–
N-NH_4	–	–	52.6 x 10^{-3}	–	–
N-NO_3	–	–	1.6 x 10^{-3}	–	–
Extractable P (g/kg)	0.34–3.15	0.18	0.97	0.1	–
Extractable K (g/kg)	0.17–6.56	3.57	4.28	9.1	–

Plants morphometric characterisation

To compare plants growth in both A1 and A2, morphometric characters such as leaf number (LN), leaf length (LL, cm), leaf width (LW, cm), and number of scapes were determined. In both assays, plants were measured in the beginning of the experiment and at the end of the vegetative growth period that is defined as the emergence of the flowering stem.

Scanning electron microscopy (SEM) analyses

Leaves from plants grown in Tec180 and Tec360 Technosols and irrigated with estuarine water were analysed by SEM. They were fixed in a 2.5% glutaraldehyde solution in 0.1 mol sodium phosphate buffer, pH 7.2, for 5 h at 4 °C as described in Hayat (1981). The material was dehydrated in a graded ethanol series (30, 50, 75 to 100% ethanol for 30 min each). Then, leaves were dried on a Critical Point Polaron BioRad E3500 and coated with a thin layer of gold on a Jeol JFC-1200. Observations were carried out at 15 kV on a JSM-5220 LV scanning electron microscope equipped with a direct image acquisition system.

The SEM observations focused on some details of the upper and lower epidermis surface such as stomatal index, $I = \{S / (E+S)\} \times 100$, according to Salisbury (1927). To calculate the salt gland index the previous formula was adopted too. All measurements and counts related with these micromorphological characteristics were done on random fields, always at comparable leaf situations and magnifications.

Indicators of plants physiological status

To evaluate the physiological status of plants from the distinct treatments the Photochemical Reflectance Index (PRI) was measured with a PlantPen model PRI 200 (Photon Systems Instruments) device, which determines the photosynthetic light use efficiency. The Normalized Difference Vegetation Index (NDVI) that is an indicator of chlorophyll content in plants and directly related to the photosynthetic capacity was measured using the PlantPen model NDVI 300 (Photon Systems Instruments). Both the PRI and NDVI indexes were determined in plants in different cultivation conditions (control and amended sediment–Technosols) and irrigation regimes. The total ascorbate (AsT) was quantified to assess the effect of plant growth conditions on the anti-oxidative system through the methodology described in Carvalho et al. (2006).

Statistical analysis

Data were analysed by three-way ANOVA (Time x Assay x Amendments) followed by the Tukey test ($p < 0.05$) used to discriminate means. A Correlation Matrix Principal Component Analysis (PCA using normalized data) at the end of experiment (T2) was performed, when all parameters (physical, chemical, physiological, and morphometric) were measured. The Pearson correlation coefficient was used to correlate the parameters analysed (r > 0.7). The tests were made using the statistical software R studio version 1.1.423 for Windows.

Results

Characterisation of initial sediments, amendments, and irrigation water

The water used for plants irrigation in A1 assay had a neutral pH, high electric conductivity, high concentrations of chlorides (8.12 g/L), hydrogenocarbonate and Na (3.47 g/L), and a low concentration of nitrates (< 0.5 mg/L) (Table III.1). The SAR (28.09) was high (Table III.1).

The initial sediments from aquaculture ponds were very acid (pH 3.12) and had low organic C (Table III.3). By opposition, they showed a high EC value and a high elemental sulfur (7.6 g/kg). The N-NO_3 content in initial sediment was lower than C^{-} but higher than amended sediment (Technosols Tec180 and Tec360, Table III.3)

The coffee wastes (*CoW*) presented acid pH, low EC (non–saline) and high concentrations of C_{org}, extractable P and K, with a higher content of N-NH_4 than N-NO_3 as in the initial sediments (Table III.2). The wastes from distilleries (*AW* and *CW*) also had acid pH, low EC and high concentration of C_{org} (Table III.2) (Santos et al., 2014; Santos et al., 2016a). The agriculture wastes (*AgW*) had the lowest concentration of C_{org}

of the wastes utilised. The *CoW* showed higher concentrations of extractable P than both *AW* and *CW* but lower than *AgW* wastes. The concentrations of extractable K in *CoW* were within the range of the other wastes used. The *CoW* had higher $N\text{-}NH_4$ but lower $N\text{-}NO_3$ concentration than the initial sediments.

The *BA* had an alkaline pH and the highest EC of all organic wastes used as amendments (Table III.2). Nonetheless, as expected it had the lowest C_{org} of all organic wastes (Table III.2).

Table III.3. **Characteristics of commercial substrate (C^{-}), unamended sediment (C^{+}) and amended sediments (Tec180 and Tec360) from A1 and A2 assays, at the beginning (T0), after ten months (T1) and at the end of the experiment (T2).** For the same parameter and reference period (T0, T1 and T2), means with different letter are significantly different ($p < 0.05$, ANOVA followed by Tukey test). The standard deviation is given in parenthesis.

Parameters	T0				T1				T2				
	A1				A1		A2		C^{-}	A1		A2	
	C^{+}	C^{-}	Tec180	Tec360	Tec180	Tec360	Tec180	Tec360		Tec180	Tec360	Tec180	Tec360
pH	3.12^{a} (0.01)	8.06^{b} (0.05)	7.58^{c} (0.08)	7.49^{c} (0.05)	7.55^{a} (0.03)	7.34^{b} (0.04)	7.29^{bc} (0.01)	6.88^{ac} (0.02)	7.78^{ab} (0.10)	7.78^{a} (0.02)	7.57^{b} (0.06)	7.52^{b} (0.06)	7.12^{c} (0.08)
E.C. (mS/cm)	4.92^{ab} (0.58)	4.48^{a} (0.23)	4.03^{b} (0.20)	3.78^{b} (0.26)	45.8^{a} (0.29)	38.7^{a} (0.04)	13.36^{a} (0.12)	13.46^{a} (0.38)	3.32^{a} (1.87)	40.66^{b} (5.02)	30.05^{c} (4.72)	11.60^{b} (2.58)	11.32^{b} (2.01)
Organic C (g/kg)	9.47^{a} (0.59)	337,6^{b} (0)	31.53^{c} (2.53)	41.07^{c} (6.35)	30.03^{a} (1.66)	42.84^{b} (4.93)	46^{ab} (11.46)	51.33^{b} (1.44)	255.8^{a} (9.78)	23.7^{b} (1.88)	41.47^{cd} (9.44)	33.43^{c} (3.49)	55.53^{d} (1.02)
N-NH_4 (g/kg)	41.10x10^{-3a} (0)	16.04x10^{-3b} (0)	7.39x10^{-3c} (0.32x10^{-3})	7.95x10^{-3d} (1.17x10^{-3})	3.91x10^{-3a} (0.54x10^{-3})	7.53x10^{-3b} (1.14x10^{-3})	11.17x10^{-3ab} (6.27x10^{-3})	7.69x10^{-3b} (0.17)	4.47x10^{-3a} (0)	2.5x10^{-3b} (0.35x10^{-3})	2.65x10^{-3bd} (0.13x10^{-3})	1.35x10^{-3c} (0.15x10^{-3})	1.89x10^{-3cd} (0.24x10^{-3})
N-NO_3 (g/kg)	8.55x10^{-3a} (0)	19.62x10^{-3b} (0)	<0.5x10^{-3c} (0)	<0.5x10^{-3c} (0)	<0.5x10^{-3c} (0)	<0.5x10^{-3c} (0)	3.97x10^{-3a} (4.43x10^{-3})	<0.5x10^{-3c} (0)	2.02x10^{-3a} (0)	0.74x10-3^{bc} (0.16x10^{-3})	0.92x10-3^{b} (0.21x10^{-3})	0.69x10^{-3b} (0.09x10^{-3})	0.51x10^{-3c} (0.15x10^{-3})
Extractable K (g/kg)	0.16^{a} (1.04X10^{-3})	3.44^{b} (0)	1.16^{c} (69.56x10^{-3})	1.47^{d} (0.18)	2.33^{a} (77.08x10^{-3})	2.72^{b} (74.66x10^{-3})	1.81^{ab} (0.14)	2.03^{ab} (0)	3.15^{a} (0)	2.17^{b} (84.65x10^{-3})	2.19^{b} (28.22x10^{-3})	1.94^{b} (0.14)	2.09^{b} (82.83x10^{-3})
Extractable P (g/kg)	62.26x10^{-3a} (5.46x10^{-3})	2.36^{b} (0)	0.32^{c} (39.65x10^{-3})	0.36^{d} (26x10^{-3})	0.13^{a} (3.96x10^{-3})	0.12^{a} (2.95x10^{-3})	0.15^{ab} (20.74x10^{-3})	0.12^{ab} (0)	0.95^{a} (0)	0.13^{b} (3.96x10^{-3})	0.14^{b} (15.58x10^{-3})	0.16^{b} (5.04x10^{-3})	0.174^{b} (14.11x10^{-3})

Characterisation of the microcosm experiment

At the beginning of the experiment (T0, A1), the amendments application improved the structure of the initial dredged sediments leading to a high increase of the effective porosity (data not shown). They also improved the organic matter content (Table III.3), and consequently, the water-holding capacity. Both Technosols showed a significant (Tec180: $p = 1.07 \times 10^{-5}$; Tec360: $p = 8.28 \times 10^{-5}$) increase (more than four units) of pH value but a not significant ($p > 0.05$) decrease of EC value when compared to C^{+} (unamended sediment) (Table III.3). The Technosols, after the plants were irrigated with estuarine water (T1, A1), did not demonstrate differences in pH, although there was a significant ($p < 1 \times 10^{-7}$) increase in EC. At the end of the experiment (T2), the pH differed significantly ($p = 0.04$) between Technosols irrigated with estuarine water (A1) or only irrigated with deionised water ($p = 1.26 \times 10^{-5}$) (A2) (Table III.3). There was also a significant ($p < 1 \times 10^{-7}$) decrease (more than the double) of EC among Technosols irrigated with salty water and Technosols only irrigated with deionised water (Table III.3). The EC values also decreased from T1 to T2 for both assays and Technosols (Table III.3). The C_{org} content was higher in Technosols than C^{+} and the Technosol Tec360 presented higher contents than Tec180 in any sampling period (Table III.3) due the highest level of wastes application used as amendments and consequently the C_{org} content present in wastes (Table III.2). In T2, there was a significant ($p = 0.04$) difference in C_{org} content among different irrigation regimes in Tec180 (Table III.3).

The C^{+} had a significantly higher value in N-NH_4 ($p < 1 \times 10^{-7}$) but a lower in N-NO_3 ($p = 3 \times 10^{-7}$) content than commercial substrate. While Tec180 and Tec30 had a significant decrease in both N-NH_4 (3×10^{-3} and 7.45×10^{-3}, respectively) and N-NO_3 ($p < 1 \times 10^{-7}$) content than control substrata (Table III.3). In both assays there was a decrease in N-NH_4 content in both Technosols (Table III.3).

What regards to K and P contents, the amendments (Table III.2) improved significantly ($p < 1 \times 10^{-7}$) the initial sediment chemical composition (*C^{+}), which was poor in K and P (Table III.3). However, there were no significant differences ($p > 0.05$) between Technosols previously irrigated with estuarine water and Technosols only irrigated with deionised water (Table III.3).

Expectedly, the soils previously irrigated with salty water (A1) showed higher Na, Ca and Mg concentrations in the soil solution than soils only irrigated with deionised water (A2). The Tec180 soils showed higher values of Na and Ca in soil solution than Tec360 soils. (Table III.4). The Technosols previously irrigated with estuarine water had a high SAR, *i. e.*, they had a high Na content in relation to Ca and Mg in the soil solution in comparison with the Technosols only irrigated with deionised water (Table III.4).

Table III.4. Concentration of Na, Ca and Mg and sodium adsorption ratio (SAR ([Na]/ ([Ca] + [Mg])$^{1/2}$); Ayers and Westcot,1985; de Varennes, 2003) in the solution of commercial substrate (C^{-}) and amended sediments (Tec180 and Tec360) in Assay 1 (A1) and 2 (A2). The analysis was performed at the end of estuarine water irrigation (T1) and at the end of the experiment (T2). For the same parameter and reference period (T1 and T2), means with different letter are significantly different ($p < 0.05$, ANOVA followed by Tukey test). The standard deviation is given in parenthesis.

Parameters	T1			T2				
	C^{-}	A1		C^{-}	A1		A2	
		Tec180	Tec360		Tec180	Tec360	Tec180	Tec360
Na^{+} (mg/L)	0.91^{a} (0.77)	19.77^{b} (4.02)	19.59^{b} (2.74)	3.60^{ab} (0.87)	13.64^{a} (3.83)	8.76^{ab} (6.36)	3.91^{b} (0.98)	2.22^{b} (2.08)
Ca^{2+} (mg/L)	14.87^{a} (2.27)	8.48^{b} (0.40)	9.01a^{b} (1.90)	0.83^{a} (0.26)	8.34^{b} (0.46)	3.75^{abc} (2.08)	3.41^{c} (0.82)	2.98^{ac} (1.08)
Mg^{2+} (mg/L)	0.11^{a} (0.03)	3.46^{b} (0.19)	3.59^{b} (0.74)	0.14^{a} (0.06)	1.20^{b} (0.15)	0.86^{abc} (0.72)	0.40^{ac} (0.08)	0.41^{ac} (0.14)
SAR	0.06^{a} (0.23)	1.45^{b} (1.18)	1.39^{b} (0.77)	0.96^{ac} (0.28)	1.17^{b} (1.19)	1.06^{ab} (1.74)	0.53^{ac} (0.70)	0.31^{bc} (1.14)

Plants growth parameters

As the C^{-} plants, the Technosols plants were also able to grow and develop in the Technosols, where amendments improved the sediment structure and fertility (Figure III.2). However, other plants succumbed during saline water treatments (all C^{+} plants) and upon changes in the irrigation regime to deionised water, which caused mortality in the two Tec180 plants (Figure III.3A).

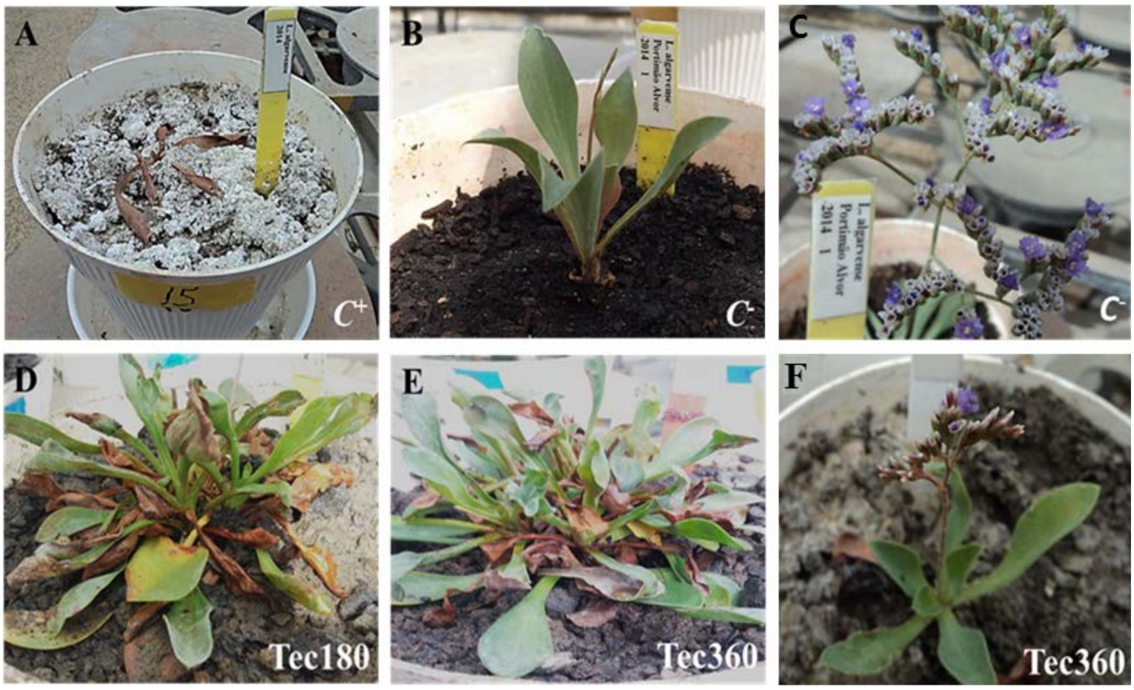
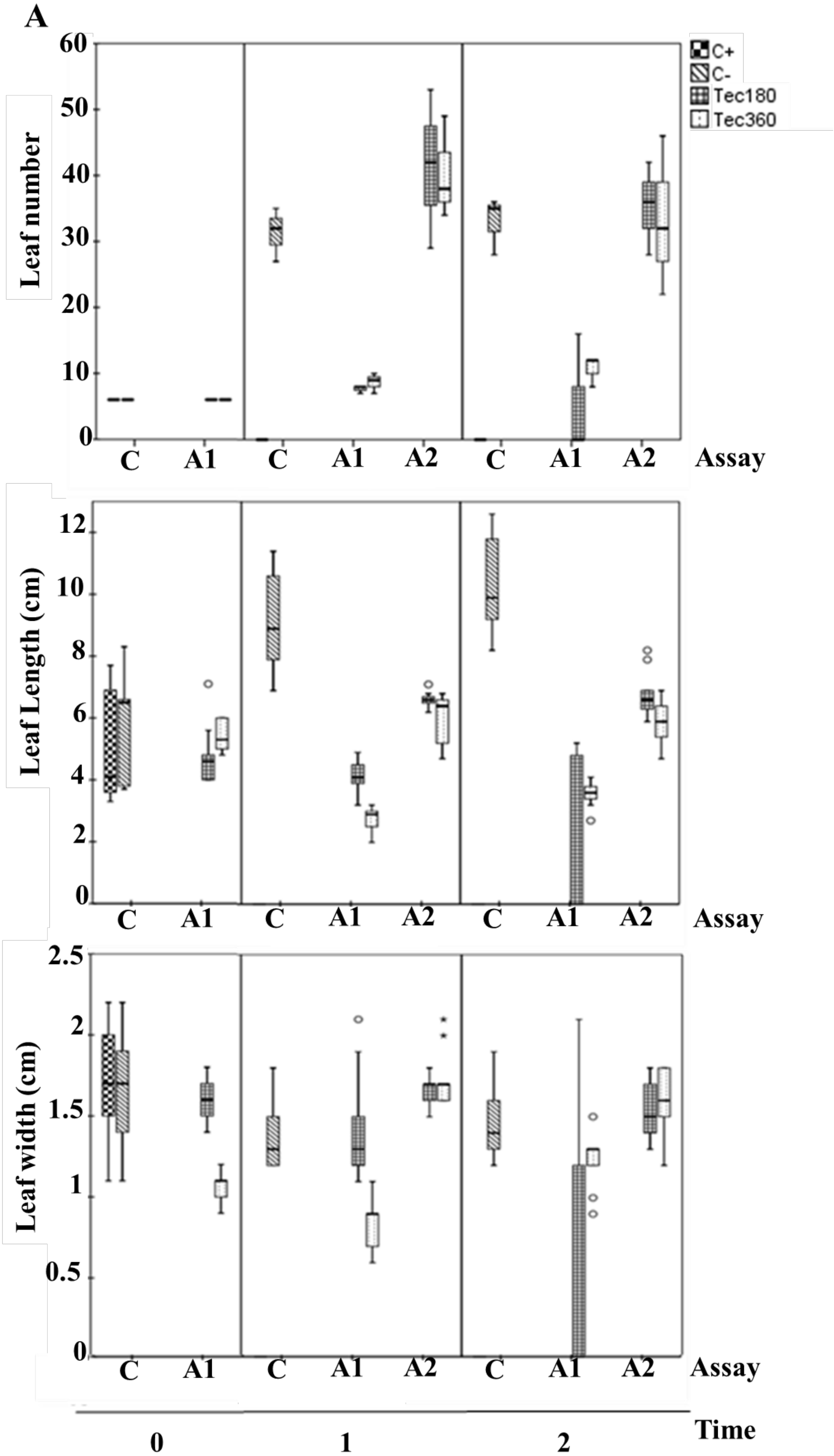


Figure III.2. *Limonium algarvense* cultivated in the initial sediments (C^+), commercial substrate ($C^–$) and Technosols. The Technosols presented an amendments mixture application at 180 g/kg of sediment (Tec180) or at 360 g/kg of sediment (Tec360). A. Plant cultivated in the initial sediment and irrigated with estuarine water (C^+); B. and C. Plants cultivated in commercial substrate ($C^–$) irrigated with deionised water, exhibiting two long scapes (C); D. Plant grown in Tec180 irrigated with estuarine water; E. and F. Plants grown in Tec360 irrigated with estuarine water, showing one scape (F).

Plants irrigated with saline water (A1) presented significantly shorter leaves than plants irrigated only with deionised water (A2). It occurred in both Technosols, in T1 (Tec180: $p < 1x10^{-7}$; Tec360: $p = 5.87 \times 10^{-5}$) and in T2 (Tec180: $p = 1.39 \times 10^{-4}$; Tec360: $p = 6.26 \times 10^{-3}$) (Figure III.3A). In the latter, the number of scapes was also significantly higher in plants irrigated with deionised water than with estuarine water (Tec180: $p = 1 \times 10^{-6}$; Tec360: $p = 8.56 \times 10^{-4}$) (Figure III.3B).


A
C+
C-
Tec180
Tec360
Leaf number
60
50
40
30
20
10
0
C
A1
C
A1
A2
C
A1
A2
Assay
Leaf Length (cm)
12
10
8
6
4
2
0
C
A1
C
A1
A2
C
A1
A2
Assay
Leaf width (cm)
2.5
2
1.5
1
0.5
0
C
A1
C
A1
A2
C
A1
A2
Assay
0
1
2
Time

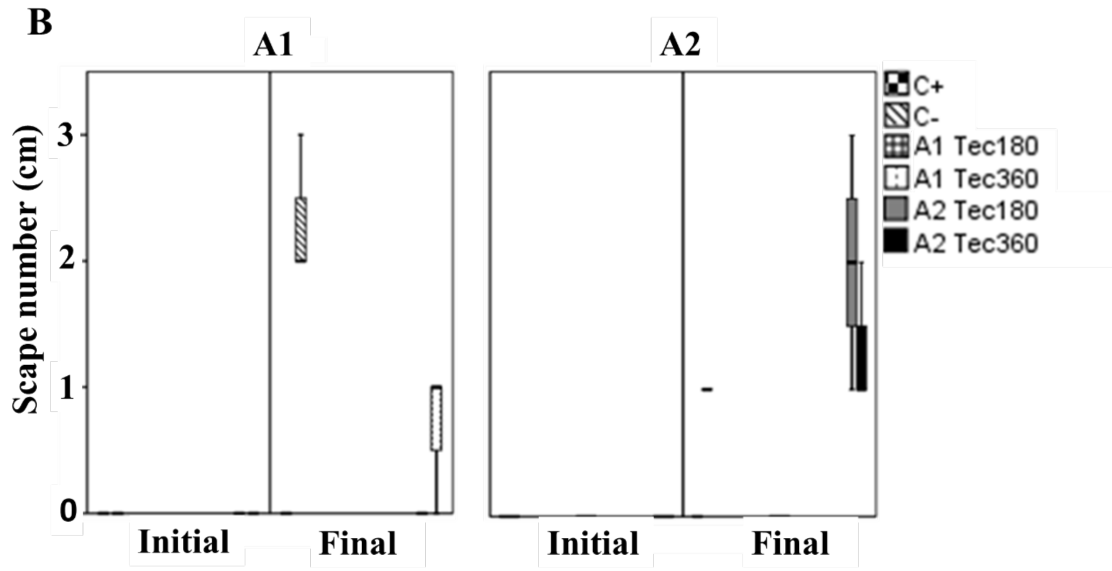


Figure III.3. Box plots of the morphometric characters obtained during *Limonium algarvense* plants growth. The box shows the twenty-fifth and seventy-fifth percentile ranges and the median; circles are outliers. 3A – Leaf number, leaf length and leaf width; 3B – Scape number.

Leaves anatomical study by SEM

Anatomical studies were only conducted in plants irrigated with estuarine water to access the influence of soil/sediment salinisation and saline water in leaf anatomy. In all samples, leaves presented polyhedral epidermal cells and a striate cuticle in both leaf surfaces (amphistomatous leaves; Figure III.4). The stomata indexes were higher in the abaxial than in adaxial leaves face, with the highest value found in C^- plants (13.9%), and the lowest value observed in Tec360 (13.3%) and Tec180 (12.9%) plants. In the adaxial leaves faces, the stomatal indexes were higher in C^- plants (13.4%) than in Tec180 and Tec360 plants. Salt glands were distributed in the whole surface of the two leaf blades (Figure III.4), being the salt gland index higher in adaxial than in abaxial leaf faces (C^- 3.6/3.1; Tec180 4.7/4.1; Tec360 4.8/3.6), respectively.

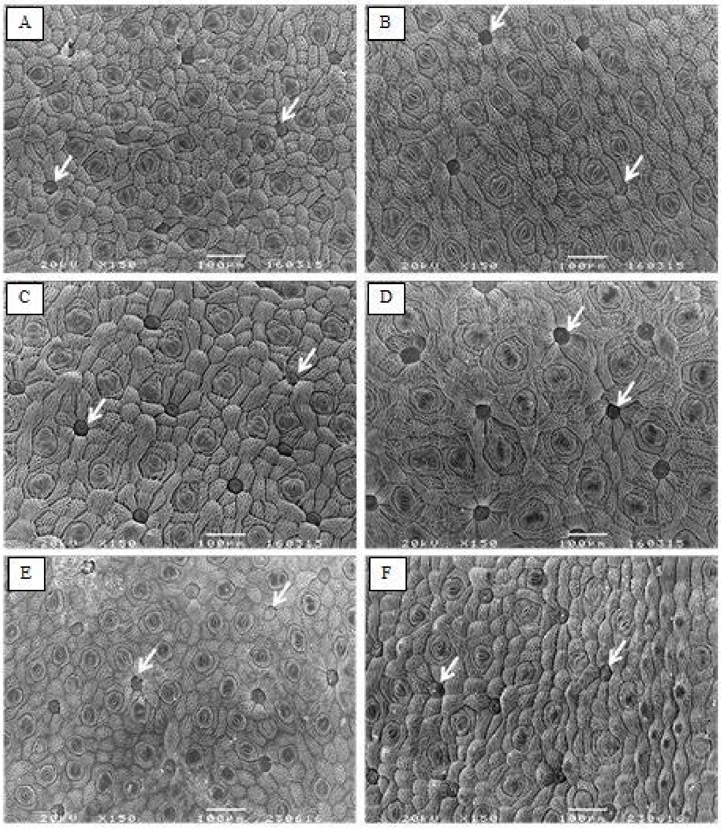


Figure III.4. *Limonium algarvense* SEM micrographs of leaves epidermal surface in plants irrigated with estuarine water. Different sized polyhedral epidermal cells, stomata, and salt glands (arrows) found in both adaxial (left images) and abaxial (right images) epidermal surfaces. A – B. control (C^{-}); C – D, Tec180 Technosol; E – F, Tec360 Technosol.

In C^{-} plants, leaves cross sections showed a compact symmetric mesophyll (Figure III.5A), with 2–3 layers of palisade parenchyma cells near both epidermis and about 5–7 rows of dense spongy parenchyma, almost without intercellular spaces. In all specimens, leaves sections showed many vascular bundles of similar dimensions and arranged linearly, and the cell content was dense and quite visible (Figure III.5B). No significant differences (p >0.05) were found between plants in the distribution of stomata and salt glands nor in leaf anatomy.

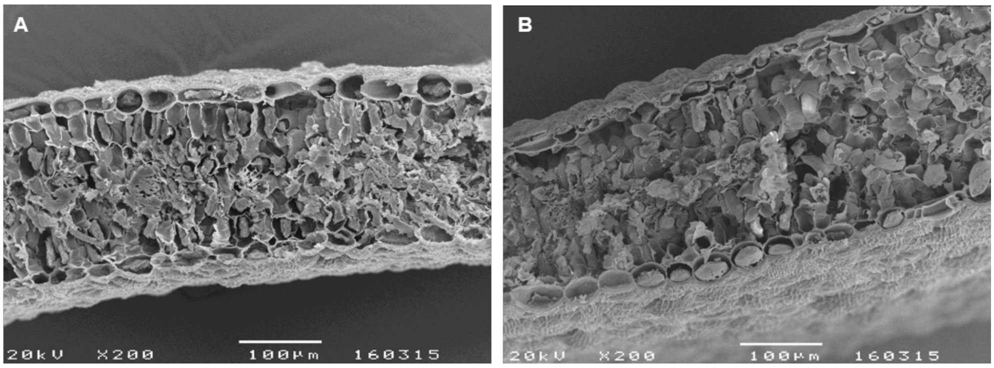


Figure III.5. *Limonium algarvense* leaves cross sections in plants irrigated with estuarine water. A and B SEM micrographs. A. Control (C^{-}) plant leaf cross section with a compact symmetric mesophyll; **B.** Leaf cross section of plant grown in Tec180 Technosol with a symmetric mesophyll.

Evaluation of physiological parameters

Most of the surviving plants showed high NDVI and PRI indexes but no significant differences ($p > 0.05$) among them were detected (Table III.5). The only Tec180 plant irrigated with estuarine water that survived to end of the experiment showed the lowest values of NDVI and PRI, an indication of an impaired physiological status.

For total ascorbate the plants previously irrigated with estuarine water showed lower ascorbate content than plants only irrigated with deionised water (Table III.5).

Table III.5. Physiological parameters measured in plants cultivated in commercial substrate (*C*⁻) and amended sediments (Tec180 and Tec360) from both Assays (A1 and A2) at the end of estuarine water irrigation (T1) and at the end of the experiment (T2). For the same parameter and reference period (T1 and T2), means with different letter are significantly different ($p < 0.05$, ANOVA followed by Tukey test) from each other. The standard deviation is in parenthesis. *Physiological parameter value of one individual. The other two individuals succumbed during the experiment.

Parameter	T1					T2				
	C⁻	A1		A2		*C*⁻	A1		A2	
		Tec180	Tec360	Tec180	Tec360		Tec180*	Tec360	Tec180	Tec360
AsT (µmol/g)	0.269^{a} (0.052)	0.508^{b} (0.254)	0.344^{ab} (0.126)	–	–	0.751^{a} (0.022)	0.231^{b} (0.090)	0.402^{abc} (0.162)	0.483^{ac} (0.108)	0.467^{bc} (0.009)
NDVI	0.684^{a} (0.022)	0.254^{b} (0.145)	0.531^{ab} (0.065)	0.659^{ab} (0.010)	0.644^{ab} (0.018)	0.680^{a} (0.022)	0.280^{a} (0.282)	0.653^{a} (0.028)	0.675^{a} (0.013)	0.681^{a} (0.024)
PRI	0.005^{a} (0.007)	0.024^{b} (0.002)	0.005^{a} (0.001)	0.003^{a} (0.017)	0.004^{a} (0.017)	0.026^{a} (0.004)	0.185^{a} (0.152)	0.024^{a} (0.006)	0.020^{a} (0.013)	0.024^{a} (0.010)

Principal Component Analysis

The PCA allowed to correlate all the chemical, morphometric and physiological parameters with each other and with the development of plants from different treatments: *C*⁻, Tec180 and Tec360 from A1 and A2 assays. No data were utilised from the *C*⁺ sediment because all plants succumbed during the estuarine water treatment. The first two components represented 79% of data variation (Figure III.6) but the third one only demonstrated 9% of variation and was not correlated ($r < 0,55$) with any variable; for this reason, was not represented in this analysis.

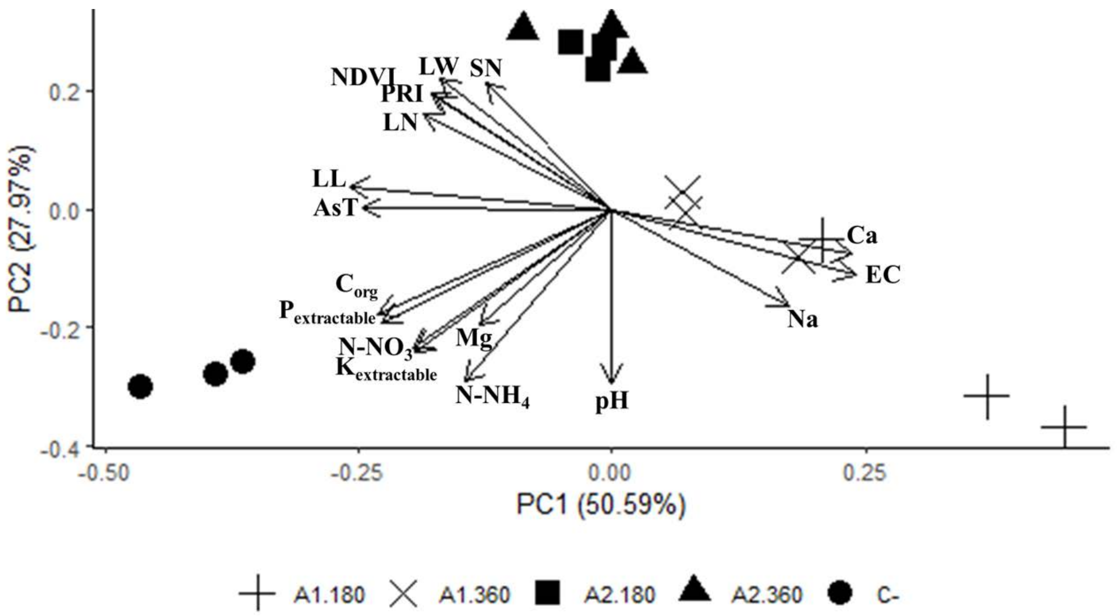


Figure III.6. The first two axes of Principal Component Analysis based on the fifteen parameters measured in the end of the study (T2). The pH, electrical conductivity (EC), organic carbon (C_{org}), $N\text{-}NH_4$, $N\text{-}NO_3$, extractable P and K, Na, Ca and Mg, ascorbate (AsT), photochemical reflectance index (PRI), normalized difference vegetation index (NDVI), leaf number (LN), scape Number (SN), leaf length (LL) and leaf width (LW) in different conditions: commercial substrate (*C^-)*, amended sediments (Tec180 and Tec360) from A1 (A1.180, A1.360) and A2 (A1.180, A1.360) assays. Percentages of total variance explained by the functions are given in parenthesis.

The PCA showed clearly three groups: *C^-* substrate, A1 assay and A2 assay. As it would be expectable, the first group has a high C_{org} concentration that was strongly positively correlated with high concentrations of extractable P (*r* = 0.99) and K (*r* = 0.95), $N\text{-}NO_3$ (*r* = 0.92), and AsT (*r = 0.77*) in this substrate (Figure III.6; Table III.6). By contrast Na, Ca and EC were strongly correlated among them (0.73 < *r* < 0.82). These parameters showed the lowest values in *C^-* substrate but they presented the highest values in assay A1 (Figure III.6; Table III.6). Plants from this assay formed two groups; the first group with one of the A1.Tec180 and all A1.Tec360 individuals that survived to changes in the irrigation water regime, and the second group with the remaining A1.180 individuals that succumbed after these changes. The A2 assay was better represented in PC2 than in PC1, having Tec180 and Tec360 plants grouped together (Figure III.6). The parameters highly correlated with PC2 were the pH (*r* = -0.81) and $N\text{-}NH_4$ (*r* = -0.80) (Table III.7). As can be seen in table III.3, these two parameters had the lowest values in A2 in comparison with A1 and *C^-* (Table III.3).

Table III.6. Correlation matrix between variables measured at the end of experiment (T2).

	C	$N\text{-}NH_4$	$N\text{-}NO_3$	K	P	Na	Ca	Mg	AsT	LN	SN	LL	LW	NDVI
$N\text{-}NH_4$	0,86													
$N\text{-}NO_3$	0,92	0,90												
K	0,95	0,93	0,93											
P	0,99	0,87	0,93	0,96										
Na	-0,33	0,04	-0,25	-0,17	-0,30									
Ca	-0,63	-0,32	-0,58	-0,47	-0,60	0,78								
Mg	0,61	0,65	0,61	0,67	0,65	0	-0,24							
AsT	0,77	0,45	0,66	0,60	0,75	-0,62	-0,82	0,39						
LN	0,37	-0,01	0,23	0,21	0,37	-0,77	-0,68	0,14	0,50					
SN	0,10	-0,28	-0,06	-0,04	0,11	-0,58	-0,44	-0,09	0,31	0,87				
LL	0,75	0,40	0,58	0,60	0,76	-0,54	-0,75	0,46	0,85	0,69	0,55			
LW	0,22	-0,14	0,01	0,05	0,20	-0,45	-0,57	0,09	0,58	0,50	0,49	0,74		
NDVI	0,28	-0,04	0,11	0,12	0,24	-0,46	-0,66	0,04	0,63	0,44	0,41	0,72	0,96	
PRI	0,27	-0,03	0,12	0,12	0,23	-0,43	-0,66	0,04	0,61	0,42	0,36	0,69	0,94	0,99

Table III.7. Correlation matrix between the first two principal components (PC1 and PC2) on the normalized data and the original variables measured at the end of experiment (T2)

	PC1	PC2
C	-0,85	-0,49
$N\text{-}NH_4$	-0,54	-0,8
$N\text{-}NO_3$	-0,72	-0,65
K	-0,71	-0,66
P	-0,84	-0,52
Na	0,64	-0,45
Ca	0,88	-0,21
Mg	-0,49	-0,54
AsT	-0,91	0,01
LN	-0,69	0,44
SN	-0,46	0,58
LL	-0,95	0,10
LW	-0,63	0,60
NDVI	-0,66	0,54
PRI	-0,64	0,52

Discussion

In Mediterranean region, aquaculture in the estuarine environment use abandoned or inactive coastal salt production areas like *Salinas* (Rosa et al., 2012) as found in the saltmarsh area in south-eastern Portugal. However, historic uses of saltmarshes have led to vegetation impoverishment and habitat degradation (Almeida et al., 2014). In this study tailored soils were constructed using bottom sediments accumulated in aquaculture ponds and disposed on their walls (*marachas* and slopes) and adjacent landscape to cultivate valued marine halophyte *L. algarvense* under different irrigation regimes.

The initial (bottom) pond sediments presented a pH value ~3 that contrasted with alluvial soils developed in the nearby area that presented higher pH (Guadiana saltmarshes, pH 6.22–8.8) (Camacho et al., 2014; Simões et al., 2011), or waterlogged sediments of other estuaries (*e. g.*, Tagus River estuary, pH 6.8–7.2) (Caçador et al., 2009; Santos et al., 2017a). Low pH values for bottom sediments in aquaculture ponds were also reported for intensive aquaculture in Asia (Senarath and Visvanathan, 2001; Mandario et al., 2019). Anoxia waters' lead to sulfides formation that under oxidation conditions generates sulfuric acid (Boman et al., 2010). The fishes farmed in these ponds have a high protein diet and most N is excreted by their gills as ammonia (NH_3), and only a small part is lost as solid wastes (Craig and Helfrich, 2002; Matos et al., 2006). In aqueous solution, NH_3 acts as a base by acquiring hydrogen ions from H_2O to yield N-NH_4 (ammonium) and hydroxide ions (Kotz et al., 2009), explaining the high N-NH_4 concentration as found in the initial sediments (Table III.3).

The characteristics of the wastes used in this study, such as the alkaline pH of *BA*, high C_{org} content of *AW*, *CoW* and *CW*, high extractable P concentration of *AgW* and *CoW*, and high extractable K content of *CoW* allowed improvement of sediment properties. Thus, the mixture of these organic amendments revealed to be adequate to improve soil fertility and structure as they are coarser than the sediments texture (clay loam to clay). Macía et al. (2014) also showed that organic amendments like sewage sludge and wood chips from pruning plants, with a high C_{org} concentration, ameliorate the fertility and texture of dredged marine sediments contributing to better plant growth and development. The *AgW, AW, CW* wastes have also been used in the rehabilitation of mine wastes with low pH value, low C_{org} and extractable P and K concentrations (Santos et al., 2014; Santos et al., 2016a). The estuarine water used in assay A1 presented characteristics of the estuarine water within the intertidal range of the Guadiana estuary (Delgado et al., 2009; Camacho et al., 2014). The SAR was very high, unsuitable to irrigate glycophytes (non-salt-tolerant plants) but tolerated by halophytes (Flowers and Colmer, 2008).

In the present study, plants cultivated in the initial sediments irrigated with estuarine water died probably due to high clay and silt contents of this substrate together with the high Na content in the irrigation water. This led to a lack of structure with a high sediment compaction resulting in adverse physical conditions for plant growth. The plants revealed a lower development in Technosols with a high EC and SAR. This index represents the hazard of soil damage due to excessive sodium in irrigation waters (Suarez, 1981; Robbins, 1984; Ayers and Westcot, 1985; Kazemi et al., 2018) like soil clays dispersion originating soil structure degradation (Barzegar et al., 1994). In A1 assay, two plants cultivated in Tec180 died after changing the irrigation water regime; nonetheless all the plants raised in Tec360 survive. The latter seemed well adapted for plants growth probably due to low SAR and/or high C_{org} content contributing to sulfides oxidation decrease. Altogether, these parameters contribute to a better soil structure in Tec360 in comparison with Tec180. The individuals cultivated in Tec360 were able to develop under both irrigation regimes, indicating that the 360 g/kg of amendments application allowed improvement of sediment properties. Although Tec360 plants irrigated with deionised water had a greater development than those irrigated with estuarine water, both assays enhanced vegetative and reproductive growth.

No significant differences in the distribution of stomata and salt glands nor in leaf anatomy between plants from different treatments were detected. Nevertheless, leaves SEM analysis revealed that saline water treatments induce a decrease in stomatal density and stomatal indexes as already demonstrated in other species (Ouyang et al., 2010). Salt glands distribution did not differ among leaves from distinct treatments, revealing that glands pattern is conserved as found in other *Limonium* species (Akhani et al., 2013). The observed differences in the salt gland index suggest that the upper face is more exposed to the salt deposits and its elimination is more pressing in this surface. As found in other halo-xerophytic plants (Bezic et al., 2003; Rančić et al., 2019), control plants presented palisade parenchyma cells next to both epidermis and a spongy parenchyma with few intercellular spaces. However, contrasting responses upon saline water irrigation were observed in other halophytes like a decrease in epidermal and mesophyll thickness as in *Bruguiera parviflora*, or an increase in epidermal thickness, palisade and spongy mesophyll as found in *Atriplex halimus* (Parida et al., 2004). The total ascorbate concentration analysis support that the plants previously subjected to saline water irrigation exhibited higher stress tolerance than plants only irrigated with deionised water. Total ascorbate usually increases upon stress conditions as in *Vitis* *vinifera* exposed to heat stress with previous acclimation to moderate drought, heat, and light stresses (Carvalho et al., 2016).

In natural environments *L. algarvense* grows in the upper part of saltmarshes only inundated during the higher tides (Costa et al., 2014; Caperta et al., 2017). In this study plant growth was greater under freshwater than under estuarine water conditions, reflecting this species adaptation to both halophilic and freshwater environments (facultative halophyte).

Conclusions

In conclusion, our findings revealed that Technosols constructed with a mixture of organic wastes used as amendments can ameliorate the fertility and structure of sediments from coastal aquaculture ponds with adverse properties for vegetation development and the recovery of degraded ecosystems. Further, these results support that an endemic species with potential application in the agro-food and pharmaceutical industry can be grown using saline water irrigation. In this context, plants presented the highest growth and development in Tec360 irrigated with estuarine water. It can be concluded that *L. algarvense* as part of the Iberia halophilic vegetation with high conservationist value together with its economic potential can be utilised within a context of soil salinisation and scarcity of freshwater in a scenario expected with the sea level rise, as well as in the recovery of degraded areas.

Chapter IV. *Conservation of a critically endangered endemic halophyte of west Portugal: a microcosm assay to assess the potential of soil technology for species reintroduction*

Cortinhas, A., Ferreira, T. C., Abreu, M. M., Caperta, A. D., 2021. Conservation of a critically endangered endemic halophyte of west Portugal: a microcosm assay to assess the potential of soil technology for species reintroduction. Front. Ecol. Evol., 9, 267. https://doi.org/10.3389/fevo.2021.604509

Abstract

The soil system has been frequently overlooked during plant reintroduction planning and practice since working with soils and plant roots can be difficult, particularly in saline environments. Coastal saline environments are major contributors to regional and global biodiversity and an important source of endemic species. However, various species are in decline or considered threatened, particularly halophytes (salt tolerant) due to negative anthropic impacts. The Lusitanian endemic halophyte *Limonium daveaui* formerly had a large distribution range along the west coast of Portugal but currently it shows a restricted distribution in the Tagus estuary. Field surveys revealed that this critically endangered species forms few local populations with small size invaded by exotic species. In this study, we investigated the potential utilisation of Technosols, an innovative sustainable, ecological engineering method combined with estuarine water irrigation for potential *L. daveaui* reintroduction in native habitats. Seed germination percentages were evaluated in different environmental conditions. Through a microcosm assay, a Technosol was constructed using a Sodic Fluvisol with a mixture of low value inorganic and organic wastes, which were chemically characterised. Plants were cultivated in the Fluvisol and Technosol and irrigated with estuarine water collected in the nearby area. To assess plant growth, morphometric parameters and the plants' physiological status were assessed and the fresh and dry biomass determined. Results showed that seed germination was higher on moist filter paper with deionised water than in Fluvisol or Technosol. Plants grown in Technosol had a greater development, with higher values of photosynthetic indexes and biomass production than in Fluvisol. Our findings provide a basis for future in situ conservation studies and support the idea that eco-friendly soil technology approaches are beneficial to conserve rare halophyte species.

Keywords: Estuarine Water, *Limonium daveaui*, Fluvisol, Tailored soil, Plant restoration, Soil Technology, Wastes

Introduction

Reintroduction of native species is an important issue in conservation and has been increasingly acknowledged in international treaties and legislation, particularly endemic species (Maunder, 1992; Godefroid et al., 2011; Maschinski and Albrecht, 2017). Conservation of coastal endemics from rocky cliffs, sand dunes and saltmarshes is challenging due to the high degree of habitat specialisation of these species and their vulnerability to disturbance by several natural factors, invasive species and human activities (Baumberger et al., 2012; Martins et al., 2013; Caperta et al., 2014; Lechuga-Lago et al., 2017; Olsson et al., 2019).

The Portuguese coast is rich in endemic flora due to a singular biogeographic position (Braun-Blanquet et al., 1972; Asensi et al., 1993; van der Maarel and van der Maarel-Versluys, 1996; Costa et al., 2012) and about 35 % of all Portuguese Natura 2000 habitats consist of coastal habitats (Costa et al., 2007; Duarte et al., 2016). The critically endangered endemic species *Limonium daveaui* Erben had a large distribution in the marshes of the Tagus Estuary (SW Portugal, SW Europe) in the past (Erben, 1978; 1993; Franco, 1984; Espírito-Santo et al., 2012; Caperta and Carapeto, 2020) but currently it is narrowly distributed in this area (Caperta and Carapeto, 2020). Among the reasons invoked for the continuous decline of the species in this estuary are the deterioration of its habitat quality due to invasive species (*e. g.*, *Carpobrotus edulis*) and anthropic pressures (Caperta and Carapeto, 2020) like industry activities (petrochemical and steelwork) with huge requirements for space and suitable terrain for industrial complexes in the Lisbon Metropolitan Area (Costa, 2013; Fernandes et al., 2020). Moreover, the Tagus estuary is also affected by other anthropic activities (chemicals, ship construction and repair and cement manufacture), agriculture-fertilizers and pesticides (Duarte et al., 2014). Due to these activities, this estuary was considered as contaminated since 1985 (Figueres et al., 1985), with high levels of anthropic Hg in Fluvisols (Cesário et al., 2016).

A detailed understanding of the biology and microenvironmental requirements including soil is needed for successful reintroduction of rare species (Dunwiddie and Martin, 2016; Holl and Hayes, 2006). Conservation practitioners are increasingly using diverse soil-based technologies to propagate and restore rare plant species (Haskins and Pence, 2012). One of the most challenging aspects of rare species reintroduction is the low success obtained when seeds rather than seedlings are used to start new populations *in situ* (Davy, 2002; Godefroid et al., 2011; Albrecht and Maschinski, 2012). Physically adverse environments and soil disturbance have also been shown to limit the possible success of species reintroduction using directly sown seeds (Wang et al., 2014; Menges et al., 2016; Cao et al., 2018; van Regteren et al., 2020), as observed in saltmarshes where soil disturbance reduces successful seedling establishment (Balke et al., 2011). In the surface layer of sediments/soils, poor drainage may result in the build-up of potentially toxic dissolved species, high salinity, and anoxia that make seed germination difficult (Spencer et al., 2017). An alternative environmentally friendly management of Fluvisols is via elaboration of Technosols, man-made tailored soils, whose properties and pedogenesis are dominated by their technical origin (IUSS Working Group, 2015). These soils could be elaborated from wastes and employed in the subsequent recovery of degraded and/or polluted soils, improving their physicochemical and biological properties (Macía et al., 2014; Cortinhas et al., 2020), while contributing to the circular economy (Breure et al., 2018).

This study aims to assess the potential of tailored soils to improve (i) soil properties, (ii) seed germination, (iii) seedling establishment and (iv) plant growth of an endemic, critically endangered species, through a microcosm assay using estuarine water irrigation. The study also hopes to develop awareness of eco-friendly soil technologies for conservation of rare species.

Material and Methods

Study area and species

The Fluvial beach "Praia do Sobralinho", Sobralinho, in the Tagus Estuary (38°54'16.1"N, 9°01'09.0"W), in the Municipal Council of Vila Franca de Xira (https://www.cm-vfxira.pt/), is integrated in the Lisbon metropolitan area, on the west coast of Portugal (ICNF, 2017) (Figure IV.1). The saline tide reaches about 50 km upstream from the river mouth, near Vila Franca de Xira (Guerreiro et al., 2015).

The upper saltmarsh area encompasses plant communities with diverse halophytes found in NATURA 2000: Habitat 1420 Mediterranean and thermo-Atlantic halophilous scrubs (*Sarcocornetea fruticosi*) and in the priority Habitat 2130 Mediterraneo-Atlantic fixed grey dunes (APA, 2011), where the Lusitanian endemic *Limonium daveaui* (syn. *Limonium auriculae-ursifolium* (Pourr.) Druce subsp. *lusitanicum* (Pignatti) Pignatti; *Limonium ramosissimum* (Poir.) Maire subsp. *confusum* (Gren. et Godr.) Pignatti (Pignatti, 1971)) thrives (Espírito-Santo et al., 2012). This species comprises perennial plants with a basal rosette, and oblanceolate-spatulate glabrous, three parallel veined leaves. The inflorescences (scapes) are branched in the lower third, with a few sterile branches, and present bluish violaceous flowers blooming between June and August (Erben, 1978, 1993; Franco, 1984).

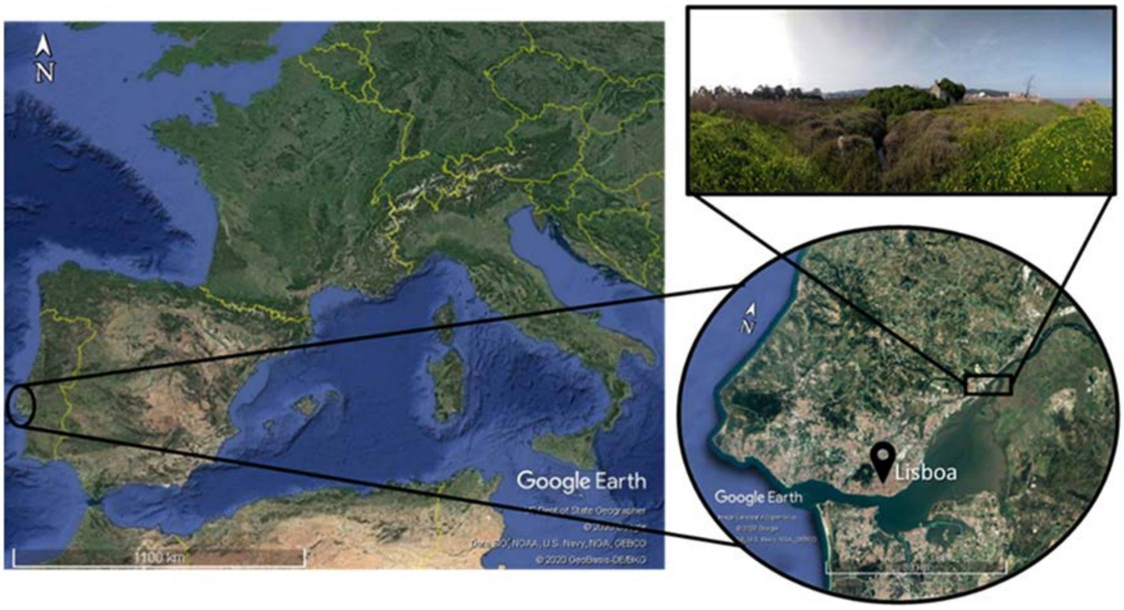


Figure IV.1. The study area located in Sobralinho saltmarsh area, on the right bank of the river Tagus, in the Municipal Council of Vila Franca de Xira, west Portugal.

Microcosm assay

A microcosm assay was set up with a Sodic Fluvisol (Eutric) (FLU; IUSS Working Group, 2015) collected in Sobralinho saltmarsh area, which was dried and sieved (< 2 mm) and a Technosol (TEC). The TEC was produced with 85% of FLU and 15% of organic/inorganic wastes mixture that were added and mixed manually. The wastes mixture used as amendments was constituted by sludge and waste kieselguhr from breweries, medium sand (0.25 < Ø < 0.5 mm), gravel limestone (2 mm < Ø < 5 mm) and residual biomass obtained from pruning, in the proportion 1.5:0.5:3:2:3, respectively. The FLU and TEC were potted and incubated at 70% of the maximum water-holding capacity, in the dark, for 28 days. After the incubation, and before seedling transplantation, composite samples from each pot (0–15 cm of depth) were collected and analysed. This process was repeated at the end of the assay. For the microcosm assay, the seedlings were obtained from seeds germinated on wet filter paper with deionised water and after 2.5 months (~4 cm) they were transplanted to the substrata, in five replicates, irrigated with estuarine water collected from a channel located in the Tagus estuary, keeping the substrata at 70% of the maximum water-holding capacity (400 ml).

Fluvisol, amendments and estuarine water characterisation

The FLU, amendments and TEC were characterised following methods in Póvoas and Barral (1992) for: pH and electric conductivity (EC) in a water suspension (1:2.5 *m*/*V*); extractable K ($K_{extract}$; Egner–Riehm method) and P ($P_{extract}$; Olsen method); total N (N_{total};

Kjeldahl method); organic C (C_{org}) by wet combustion; micronutrients (Lakanen and Erviö, 1971). For the FLU, the cation exchange capacity (CEC) by 1 mol/dm^3 ammonium acetate, particle size distribution analysis by sieving and sedimentation, and multielemental concentration by ICP-MS after acid digestion (Activation Laboratories, 2020) were determined.

The estuarine water was analysed for: pH (potentiometry), EC (conductivimetry), Cl^- (Mohr, 1945), $HCO3^-$ (titration method using HCl solution methyl orange as indicator), Na, Ca, Mg, K, P, Fe, Zn, Mn and Cu (atomic absorption spectrometry). The Na adsorption ratio (SAR) was calculated by ([Na]/(([Ca] + [Mg])1/2)) (Ayers and Westcot,1985, Lesch and Suarez, 2009).

Germination tests

The *L. daveaui* seeds were collected in Samouco salterns complex and germinated in transparent boxes containing filter paper, TEC and FLU, respectively. Each box contained 36 seeds equally distant from each other and 170 ml of deionised water. The boxes were exposed at the temperature 20 – 25 ºC with a 16 h light and 8 h dark photoperiod, in a controlled chamber (Rumed) as described in Róis et al. (2012).

Biomass, morphometric, and physiological parameters

To understand and compare the evolution of plant vegetative and reproductive growth in the substrata, morphometric and photosynthetic parameters were measured every 2.5 months, one measure in each season of the year. The measurements were taken at transplantation, in winter (T0); in spring (T1), in summer (T2) and at the end of the microcosm assay, in autumn (T3). The number of live and dead leaves, leaf length and width, scape number and length were determined. For leaf size measurements about three leaves/individual were labelled (15 leaves/treatment) at the beginning of the assay (T0). The Photochemical Reflectance Index (PRI) was measured with a PlantPen model PRI 200, which determined the photosynthetic light use efficiency in these leaves. The Normalized Difference Vegetation Index (NDVI) that is an indicator of chlorophyll content in plants and directly related to their photosynthetic capacity was also evaluated using the same leaves, with a PlantPen model NDVI 300. These measures were non-destructive, they were obtained through an optical window in the instruments. At the end of the experiment, the fresh and dried biomass of both aerial parts and roots were determined in a digital analytical balance (Sartorius ENTRIS124-1S, 120 × 0.0001 g, Sartorius Stedim Biotech North America Inc., New York, USA) The fresh biomass was oven-dried at 60 °C, until a constant weight was reached.

Statistical analysis

The germination and biomass data were analysed by one-way ANOVA (substrata) and the remaining data by two-way ANOVA (Time × Substrata). When the data were statistically significant ($p < 0.05$), all pairwise-comparisons were carried out by Tukey HSD post hoc test. A Correlation Matrix Principal Component Analysis (PCA using normalised data) was performed and the Pearson correlation coefficient ($r > 0, 7$). was used to correlate the nutrient concentrations of substrata in T0 and the morphometric and photosynthetic indexes in T3. The tests were made using the statistical software R studio version 1.1.423 for Windows.

Results and Discussion

Characterisation of soils, amendments, and irrigation water

The FLU had a silty clay loam texture, was slightly alkaline, with a high EC and low values of C_{org}, N_{total} and extractable P (Table IV.1, Figure IV.2A and Fig IV.3A). These data indicate that this soil is saline-sodic with a 25% of $Na_{exchangeable}$ and 26.28 of SAR. The multielemental composition of the Fluvisol showed that concentrations of the potentially hazardous elements were below the maximum allowed values for agricultural use (APA, 2019), except for As concentration (15.6 mg/kg), which was slightly higher than the allowed concentration limit (11 mg/kg; APA, 2019). This can be attributed to a widespread industrial waste such as wastes resulting from chemical and metallurgical plants located close to the river Tagus, deposited in the estuary in the past (De Bettencourt, 1988).

The FLU had a high $Na_{exchangeable}$ percentage and SAR contributing to soil aggregate degradation and colloid dispersion, promoting clogging of soil pores and making plants root penetration difficult. This leads to a lack of drainage, water puddle formation and oxygen deficiency (Brady and Weil, 2008). Further, the ratio [Ca]/[Mg] <1.5 indicates very unfavourable conditions for soil physical properties (INIA-LQARS, 2000). For the saline-sodic soil reclamation it is necessary to reduce the $Na_{exchangeable}$ percentage replacing the Na^+ ion to the Ca^{2+} ion, in the exchange complex (Brady and Weil, 2008). While some studies favor gypsum use (Ca_2SO_4) to form Na_2SO_4 that can be leached (Mozheiko, 1969; Carter et al*.,* 1977), other works support the application of organic compounds in addition to the gypsum to improve soil nutrition, aggregation, water-holding capacity, infiltration rate and decrease in EC (Hanay et al., 2004; Diacono and Montemurro, 2015). Tailored soils can be utilised as an alternative to improve salt-affected soil properties (Macía et al., 2014; Cortinhas et al., 2020). Moreover, the cultivation of species well adapted to the saline environments (halophytes) contribute to soil recovery (Manousaki and Kalogerakis, 2011; Devi et al., 2016; Liang et al., 2017). These plants can survive in saline environments due to protection mechanisms such as the Na^+ and Cl^- compartmentation, Ca^{2+} and K^+ uptake, succulence, synthesis of particular compounds and the presence of salt glands (Caperta et al., 2020; Flowers et al., 2010; Song and Wang, 2015).

Table IV.1. Chemical characteristics of the Fluvisol and amendments (mean ± sd, n=3) collected in Sobralinho, in the Municipal Council of Vila Franca de Xira, west Portugal. Electrical conductivity (EC) is given in mS/cm; C_{org}, N_{total}, $K_{extractable}$ and $P_{extractable}$, in g/kg; $Na_{exchangeable}$, $K_{exchangeable}$, $Mg_{exchangeable}$, $Ca_{exchangeable}$ and Cation Exchangeable Capacity (CEC) in $cmol_c$/kg; ESP – Exchangeable sodium percentage [(($Na_{exchangeable}$/CEC)*100); Ayers and Westcot, 1985].

Parameters	Fluvisol	Wastewater sludge	Waste *kieselguhr*
pH	8.03 (0.23)	8.47 (0.15)	7.17 (1.12)
EC	5.60 (1.28)	3.18 (0.41)	0.77 (0.45)
C_{org}	19.96 (2.07)	223.13 (19.55)	31.33 (16.37)
N_{total}	1.69 (0.17)	42.19 (4.06)	10.95 (4.33)
$Na_{exchangeable}$	6.54 (0.31)	–	–
$K_{exchangeable}$	2.11 (0.07)	–	–
$K_{extractable}$	0.86 (0.03)	1.92 (2.05)	0.13 (0.02)
$Mg_{exchangeable}$	10.27 (0.31)	–	–
$Ca_{exchangeable}$	10.26 (0.26)	–	–
$P_{extractable}$	0.01 (7.40 x10^{-4})	1.38 (0.31)	0.24 (0.08)
CEC	26.07 (0.50)	–	–
ESP	25.10 (1.33)	–	–

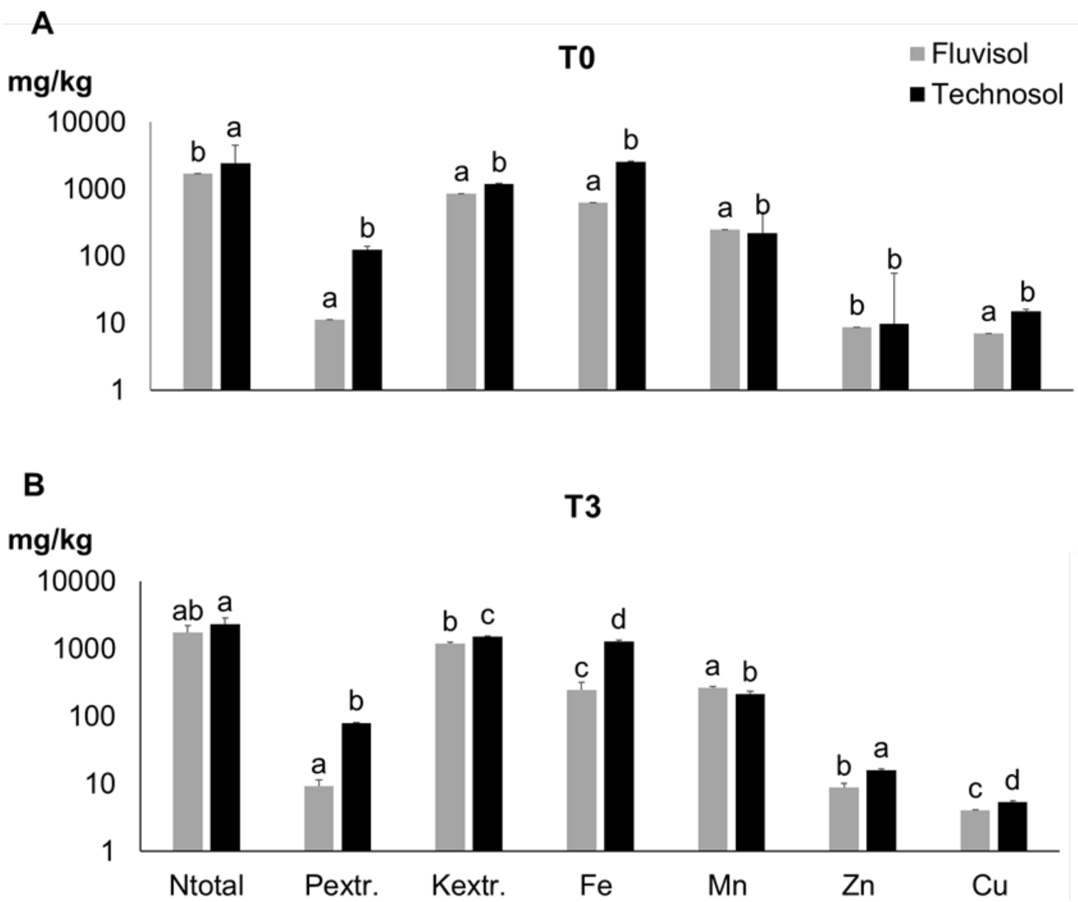


Figure IV.2. Nutrients concentration in Fluvisol and in Technosol (means ± sd; n =5) in the beginning (T0) (A) and at the end (T3) of assay (B). Different letters for the same element indicate significant differences between time and substrata (*p* < 0.001, two-way ANOVA (time x substrata) followed by Tukey HSD test).

In this study, the TEC soil fertility was increased due to high content of N_{total}, C_{org}, extractable P and K present in sludge and waste kieselguhr (Table IV.1). The residual biomass applied as a source of C and other nutrients in the long run by slow mineralisation also contributed to improve soil texture/aggregation. The addition of sand and limestone gravel enhanced the texture and permeability of the Fluvisol used in the TEC construction (Figure IV.3B and IV.3D) as also shown previously (Macías et al., 2014; Cortinhas et al., 2020). No significant differences among TEC and FLU were found in terms of pH and CE. Nonetheless, the TEC presented a significant increase in the concentration of all determined nutrients, mainly in P ($p = 2.16 \times 10^{-5}$), Fe ($p = 2 \times 10^{-6}$) and Cu ($p = 2 \times 10^{-6}$), except for Mn and Zn ($p = 1,07 \times 10^{-7}$) (Figure IV.2A). The soil improvement in these nutrients is principally related to the wastewater sludge that was rich in P, Fe and Cu (Table IV.1). The mixture of amendments was suitable to improve soil fertility, texture, and structure (Figure IV.3B) as well as soil permeability and drainage avoiding waterlogging. These latter two soil properties are extremely important since the remaining populations of *L. daveaui* in the Tagus estuary thrive in Fluvisols and saline sands, mainly inundated with salty water by the equinoctial tides (Caperta and Carapeto, 2020).

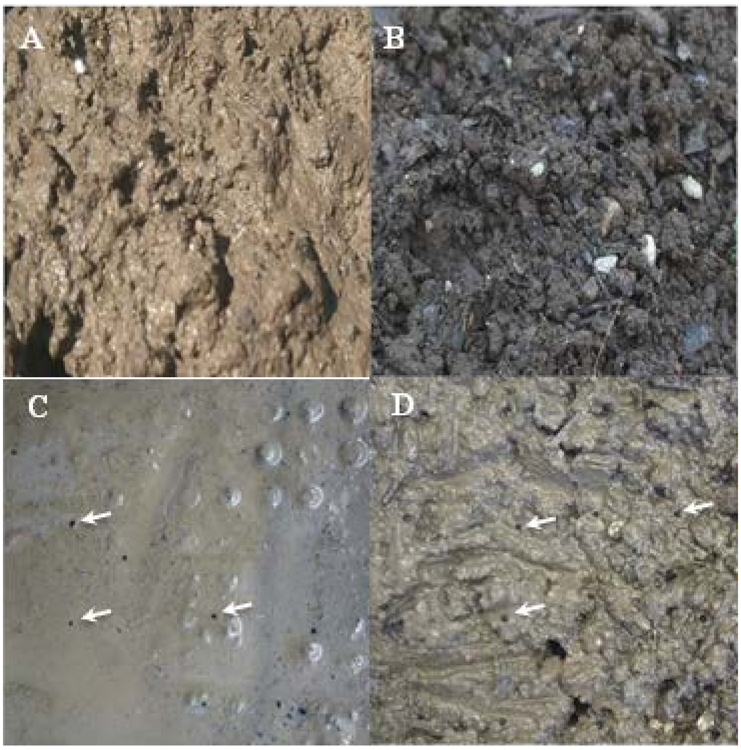


Figure IV.3. Physical appearance of Fluvisol (A) and Technosol (B). The Fluvisol was collected in Sobralinho saltmarsh area in the Municipal Council of Vila Franca de Xira, west Portugal. The Technosol was produced with Fluvisol and organic/inorganic wastes as amendments. Seed germination (white arrows) on a water film retained in the Fluvisol surface **(C)** and in the Technosol surface, where the water film was not present **(D).**

The estuarine water was strongly saline, had a neutral pH and high concentrations of Cl^- (207 mmol/L), HCO_3^- (4.37 mmol/L), Na^+ (187 mmol/L), K^+ (4.02 mmol/L) and Mg^{2+} (22.14 mmol/L) (Table IV.2) as was to be expected in estuarine water (Santos et al., 2017a; Cortinhas et al., 2020). The calculated SAR of water was high (35.4) but tolerated by halophyte species (Haro et al., 1993; Flowers and Colmer, 2008) (Table IV.2). The maritime fog mostly observed from November to February each year in the Lisbon area (Belo-Pereira et al., 2016), is deposited as occult precipitation by condensing or sublimating directly onto plant surfaces (Karavoltsos et al., 2017), probably contributing to the water balance of plants in these saline habitats.

After plant growth (T3), the soils irrigated with estuarine water show no change in pH values, however the EC values of FLU (5.6 dS/m) and TEC (5.9 dS/m) had a significant ($p < 1 \times 10^{-7}$) increase (34.03; 32.48 dS/m, respectively) due to the high salinity of estuarine water. In T3 (Figure IV.2B), the organic C, N_{total} and extractable P concentrations did not show significant variations compared to T0 (Figure IV.2A). At the end of the assay (Figure IV.2B), the Zn content of TEC increased significantly as well as the extractable K in both soils ($p < 5 \times 10^{-6}$). In contrast, the Fe and Cu values decreased significantly ($p < 9 \times 10^{-4}$) from T0 (Figure IV.2A) to T3 (Figure IV.2B).

Table IV.2. Chemical characteristics of irrigation water (mean ± sd, n=3) collected in Sobralinho, in the Municipal Council of Vila Franca de Xira, west Portugal. The electrical conductivity (EC) is given in mS/cm and anions and cations concentrations in mmol/L; SAR - sodium adsorption ratio [([Na] /(([Ca] + [Mg])$^{1/2}$)); Ayers and Westcot, 1985; Lesch and Suarez, 2009].

Chemical characteristics of estuarine water	
pH	7.78 (0.41)
EC	22.03 (7.09)
Cl^-	207 (75.05)
HCO_3^-	4.37 (0.89)
Na^+	187 (58.16)
Ca^{2+}	5.24 (1.49)
Mg^{2+}	22.14 (7.59)
K^+	4.02 (1.34)
P	2.59×10^{-2} (1.55×10^{-2})
Fe	4.86×10^{-4} (6.38×10^{-4})
Zn	8.58×10^{-5} (4.53×10^{-5})
Mn	1.77×10^{-3} (1.98×10^{-3})
Cu	1.31×10^{-4} (1.20×10^{-4})
SAR	35.43 (5.81)

Seed germination and plant growth

The findings of a significantly different ($p = 5.95 \times 10^{-8}$) higher seed germination percentage in deionised water and in FLU compared to TEC (Figure IV.4) can be due to the effect of high salinity (*i. e*., lowest osmotic potential) on *Limonium* seed germination (Boorman, 1967; Luque et al., 2013; Al Hassan et al., 2017) as well as of other halophyte species (Debez et al., 2018; Lombardi et al., 2019).

Due to FLU low permeability, a water film is retained on the surface of the substrate allowing the seeds to float (Figure IV.3C), whereas at the surface of the relatively porous TEC this water film does not occur (Figure IV.3D). Although Fluvisols and rocky deposits seem to be suitable for halophyte seed germination, for endemic *Limonium* species seed germination by directly sowing them in the field did not prove to be successful (Ana D. Caperta and Vasco Silva, unpublished research).

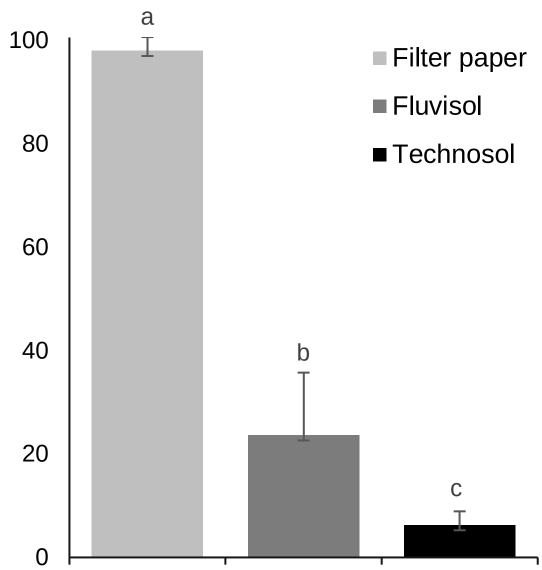


Figure IV.4. The percentage of *Limonium daveaui* seeds germination (mean ± sd; n = 144). The substrata (moist filter paper, Fluvisol and Technosol) with different letters are significantly different ($p < 0.05$, one-way ANOVA (substrata) followed by Tukey HSD test).

Transplanting and even translocating plants to start new populations of rare and endemic species have shown better results than using seeds in situ (Davy, 2002; Godefroid et al., 2011; Albrecht and Maschinski, 2012). A comparison of plants grown in FLU and in TEC irrigated with estuarine water (Figure IV.5) revealed that there were no significant differences for the growth parameters measured in plants growing in both soils. However, there was a significant increase in the leaf number in T2 ($p \leq 6.51 \times 10^{-4}$) followed by a small decrease in T3 (Figure IV.5). Plants grown in TEC presented smaller leaves than plants cultivated in FLU. Nonetheless, the former had a higher number of leaves, which can contribute to a higher photosynthetic rate (Figure IV.5). Between T2 and T3, the plants started to bloom, which may have influenced a reduction in leaf number. The plants cultivated in TEC had a higher vegetative and reproductive growth and produced flowering stems that emerged earlier than in FLU. Although only one individual per treatment presented a flowering stem, the plant grown in TEC produced more and larger scapes than that in FLU (Figure IV.5). The TEC substrate, rich in essential nutrients and with better texture and structure for plants allowed better plant development. These findings suggest that plant cultivation in Technosols contributes to the reproductive success of *L. daveaui* as also found previously in *Limonium algarvense* (Cortinhas et al., 2020).

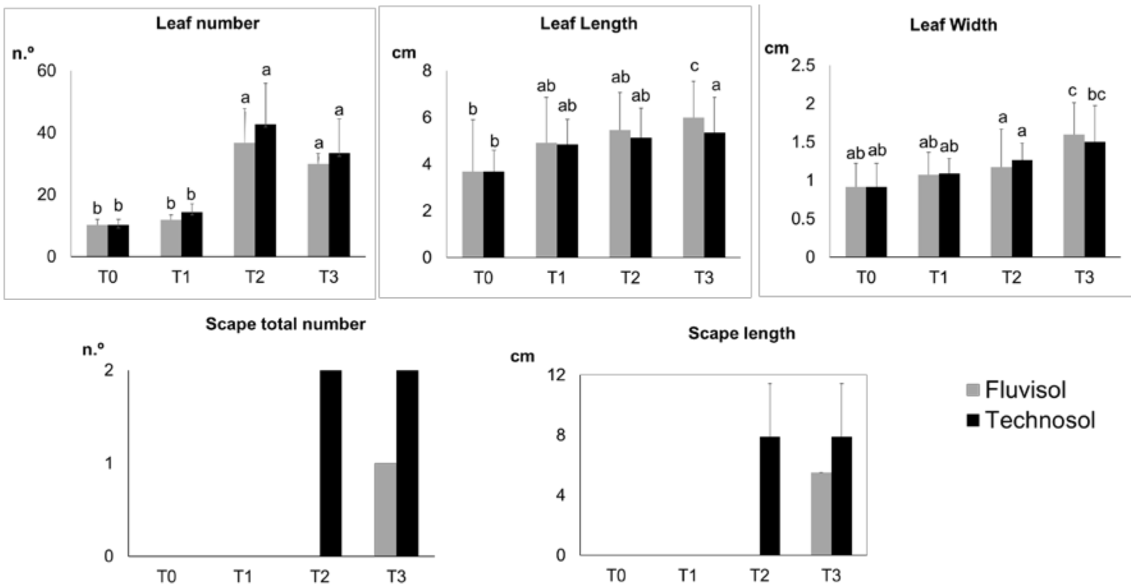


Figure IV.5. Plant growth parameters assessed during the microcosm assay (mean ± sd (except scape total number); n= 15). T0 - transplantation, in winter; **T1** - Spring; **T2** - Summer; and **T3** - Autumn. Different letters meaning significant differences (p < 0.0 5, two-way ANOVA (time x substrata) followed by Tukey HSD test).

In terms of the physiological status, there were no significant differences in NDVI and PRI between plants raised in different substrata (Figure IV.6). However, the plants cultivated in FLU presented lower values in all the reference periods than individuals grown in TEC, supporting that this soil was a more stressful environment for plants than TEC (Figure IV.6). The NDVI and PRI values of plants raised in both substrata dropped significantly ($p < 2.4 \times 10^{-6}$) after the spring (T1). While the PRI value only reduced significantly from T1 to T2 ($p = 5 \times 10^{-7}$), the NDVI value decreased significantly between T1-T2 ($p < 1 \times 10^{-7}$) and T2-T3 ($p < 1 \times 10^{-7}$). Stress factors such as leaves senescence, salinity, light excess, or high temperature or in combination can lead to PRI and NDVI reduction (Bieto and Talón, 2008; Yudina et al., 2020). In this study, reduction of these indexes at least could be associated to leaves senescence that occurred when plants start flowering.

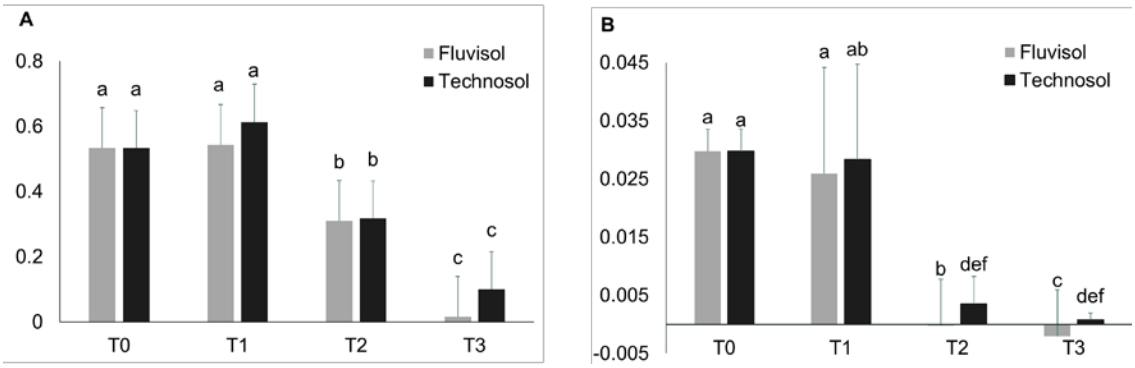


Figure IV.6. The NDVI (A) and PRI (B) indexes evaluated during the microcosm assay in different reference periods (mean ± sd; n= 15). T0 - transplantation, in winter; **T1** - Spring; **T2** - Summer; and **T3** - Autumn. Different letters meaning significant differences ($p < 0.05$, two-way ANOVA (time x substrata) followed by Tukey HSD test).

The dry mass of roots, aerial part and flowers represented ~40% of their weight. The aerial (leaves and scapes) and roots biomass did not show significant differences among substrata (Figure IV.7B). However, plants from TEC showed high flowers production than FLU (Figure IV.7B). The roots of plants cultivated in FLU were concentrated in the bottom of pots (Figure IV.7A), while in plants grown in TEC roots spread uniformly all over the substrate (Figure IV.7A). The deficient FLU structure, due to colloid dispersion, is an obstacle to roots penetration and oxygen circulation. The poor structure and the compaction due to high $Na_{exchangeable}$ percentage and SAR can lead to plant death as found previously in Cortinhas et al. (2020).

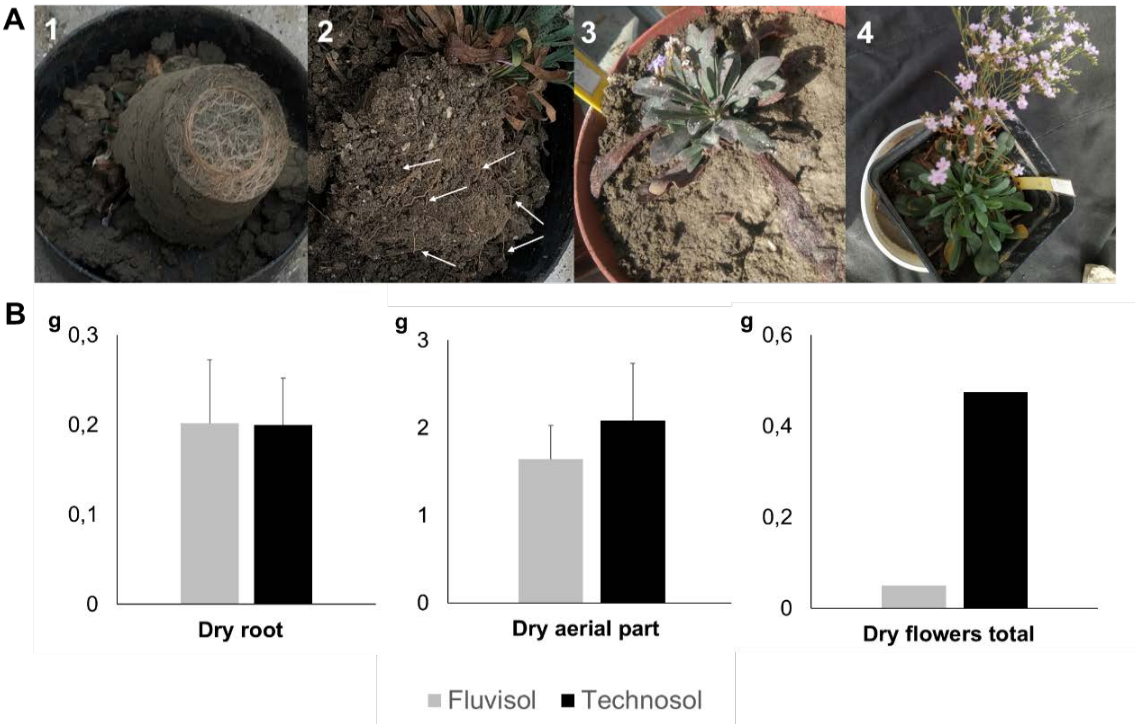


Figure IV.7. Biomass production at the end of the microcosm assay using estuarine water irrigation. A. Fresh biomass produced. **A1.** Plant roots cultivated in Fluvisol concentrated at bottom of the pot. **A2.** Plant roots (white arrows) grown in Technosol spreading uniformly over the soil **A3.** Plant grown in Fluvisol exhibiting one scape. A4. Plant cultivated in Technosol showing two scapes. **B**. Dry biomass of leaves, roots, and flowers (mean ± sd (except dry flowers total); n= 5). The aerial part represents the leaves and scapes; * - only one individual produced scapes. No significant differences were found between substrata ($p > 0.05$, one-way ANOVA followed by Tukey HSD test).

Principal Component Analysis (PCA)

The PCA correlated nutrient concentrations determined in substrata in the beginning of the assay (T0) with the morphometric parameters and the photosynthetic indexes measured in plants at the end of the assay (T3). The first two components explain 71.14% of data variation. The third principal component only corresponded to 12.78% of variation and did not show correlation with any of the evaluated variables ($r < 0.60$); as such, it was not represented in this analysis.

The first principal component represented 53.12% of data variation with the pH and nutrients being the variables having the highest correlation ($r > 0.70$) with that component

(Figure IV.8; Table IV.3). This contributes to clearly separate the individuals cultivated in TEC from plants cultivated in FLU (Figure IV.8).

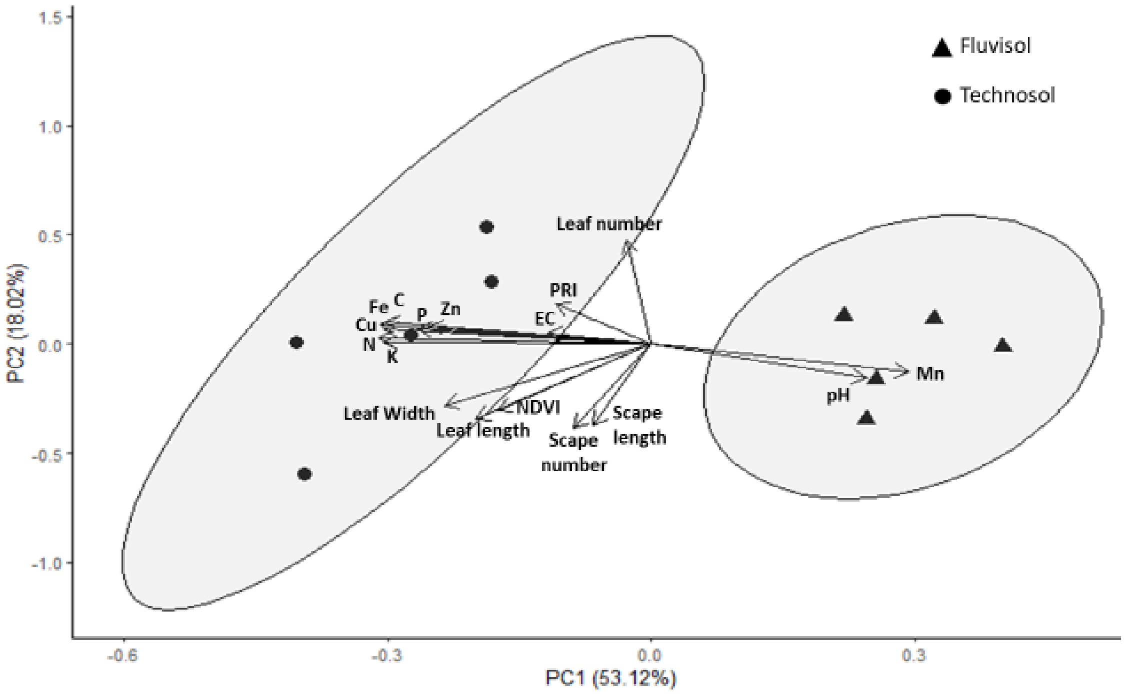


Figure IV.8. The first two axes of Principal Component Analysis. This analysis was based on pH, CE, organic carbon (C), nutrients concentration (N total (N), extractable P (P), extractable K (K), Fe, Mn, Zn, Cu) determined in the soils in the beginning of the assay (T0) and the morphometric parameters (leaf number, leaf length, leaf width, scape number, scape length) and photosynthetic indexes ((normalized difference vegetation index (NDVI) and photochemical reflectance index (PRI)) measured in plants at the end of the assay (T3)**.** Percentages of total variance explained by the functions are given in parenthesis.

Table IV.3. Correlation matrix between the first two principal components (PC1 and PC2) on the normalized data and the original variables. This matrix includes the pH, CE, organic carbon (C) and nutrient concentration (N total (N), extractable P (P), extractable K (K), Fe, Mn, Zn, Cu) values determined in the soils in the beginning of the assay (T0) and morphometric parameters (leaf number, leaf length, leaf width, scape number, scape length) and photosynthetic indexes (Normalized Difference Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI)) obtained in plants at the end of the assay (T3).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
K	-0,96	0,02	0,06	0,12	0,02	-0,02	0,25	-0,03	-0,01	-0,30
P	-0,84	0,13	0,35	0,23	-0,31	-0,10	-0,01	-0,06	0,06	-0,44
N	-0,97	0,05	0,09	0,17	-0,08	-0,06	-0,04	-0,02	0,01	-0,36
C	-0,95	0,18	0,02	0,14	0,16	0,09	0,09	0	-0,03	-0,29
EC	-0,37	0,09	-0,31	-0,83	-0,25	-0,02	0,08	-0,07	0	-0,55
pH	0,77	-0,29	0,35	0,31	-0,23	-0,15	0,13	-0,03	-0,06	0,36
Fe	-0,97	0,17	0,04	0,16	0,06	0	0,01	0,02	0	-0,36
Mn	0,92	-0,23	0,09	0,10	0,13	-0,01	-0,23	-0,09	0,02	0,57
Zn	-0,80	0,15	0,34	-0,20	-0,37	0,04	-0,22	0,07	-0,05	-0,59
Cu	-0,96	0,13	0,10	0,16	-0,05	0,04	-0,17	-0,05	0,01	-0,34
LN	-0,09	0,87	-0,13	0,05	0,27	0,38	-0,03	-0,04	-0,02	-0,21
LL	-0,55	-0,56	-0,61	0,10	0,06	0,02	0,02	0,03	0,03	0,17
LW	-0,74	-0,51	-0,33	-0,02	0,23	-0,14	-0,05	-0,01	-0,02	0,06
SN	-0,28	-0,70	0,59	-0,15	0,10	0,21	0,04	-0,05	-0,04	0,39
SL	-0,21	-0,68	0,61	-0,19	0,13	0,26	0,02	0,04	0,05	0,41
NDVI	-0,63	-0,62	-0,44	0,04	0,05	-0,03	-0,13	-0,03	-0,04	0,15
PRI	-0,34	0,34	0,54	-0,29	0,49	-0,40	-0,04	0,01	0	-0,32

The PCA revealed that TEC had higher nutrient concentrations (except for Mn) but lower pH value than FLU. All the nutrients showed correlation (*r* >0.70) among them (Table IV.4) and the pH was negatively correlated with C, Fe, and Cu, (-0.77< *r* < -0.70) but positively with Mn (*r* = 0.78). In the second principal component, which represented 18.02% of data variability (Figure IV.8), leaf and scape number were the parameters that mostly contributed to the observed variability (*r* ≥ 0.70; Table IV.3). All the growth parameters and the values of the photosynthetic indexes were also higher in the plants cultivated in the TEC than plants grown in the FLU. The NDVI was strongly correlated (0.95 < *r* < 0.96) with leaf size (Table IV.4).

Table IV.4. Correlation matrix between variables. This matrix includes the pH, CE, organic carbon (C) and nutrient concentration (N total (N), extractable P (P), extractable K (K), Fe, Mn, Zn, Cu) values determined in the soils in the beginning of the assay (T0) and morphometric parameters (leaf number, leaf length, leaf width, scape number, scape length) and photosynthetic indexes (Normalized Difference Vegetation Index (NDVI) and Photochemical Reflectance Index (PRI)) obtained in plants at the end of the assay (T3).

	K	P	N	C	EC	pH	Fe	Mn	Zn	Cu	LN	LL	LW	SN	SL	NDVI
P	0.84															
N	0.95	0.92														
C	0.96	0.80	0.94													
EC	0.26	0.11	0.22	0.21												
pH	-0.65	-0.40	-0.66	-0.77	-0.61											
Fe	0.95	0.86	0.98	0.99	0.21	-0.75										
Mn	-0.92	-0.78	-0.88	-0.90	-0.52	0.78	-0.91									
Zn	0.69	0.86	0.81	0.69	0.44	-0.55	0.76	-0.76								
Cu	0.90	0.90	0.98	0.94	0.21	-0.70	0.97	-0.85	0.84							
LN	0.09	0.03	0.08	0.32	0.04	-0.47	0.25	-0.25	0.07	0.19						
LL	0.5	0.18	0.47	0.43	0.24	-0.46	0.44	-0.42	0.10	0.40	-0.34					
LW	0.68	0.37	0.65	0.62	0.29	-0.59	0.63	-0.55	0.32	0.60	-0.33	0.90				
SN	0.28	0.26	0.24	0.17	-0.04	0.10	0.15	-0.05	0.30	0.21	-0.56	0.18	0.37			
SL	0.20	0.19	0.16	0.10	-0.06	0.12	0.09	0.01	0.26	0.14	-0.53	0.12	0.30	0.99		
NDVI	0.54	0.29	0.55	0.47	0.26	-0.47	0.49	-0.43	0.26	0.50	-0.42	0.96	0.95	0.35	0.28	
PRI	0.34	0.34	0.34	0.40	0.11	-0.32	0.40	-0.3	0.38	0.35	0.22	-0.33	0.08	0.18	0.18	-0.20

Conclusions

This work contributes to the knowledge of *L. daveaui* microenvironmental requirements particularly soil properties. The findings demonstrated that eco-friendly soil technologies have an enormous potential to improve the reintroduction success of this species. As such, plants will be pre-grown in the Technosol and then will be planted on the sites in modified soil. The amendments that have shown suitable to improve the physical and chemical proprieties of the saline-sodic Fluvisol will be added and mixed with this soil. The plants will be subjected to irrigation with estuarine water collected directly from a channel located in the Tagus estuary. From a circular economy perspective, this approach that uses cost-effective subproduct/wastes contributes to enhance soil fertility and structure, valuing underused resources such as saline soils, not used in conventional reintroduction schemes, but appropriate for halophyte species.

Chapter V. *Sustainable cultivation of succulent halophytes using resources from a degraded estuarine area through soil technologies approaches and saline irrigation water*

Cortinhas, A., Ferreira, T. C., Abreu, M. M., Caperta, A. D. Sustainable cultivation of succulent halophytes using resources from a degraded estuarine area through soil technologies approaches and saline irrigation water. Land Degradation & Development (*Revision being processed* – LDD-22-0140.R1).

Abstract

The degradation of estuarine areas has been a growing concern in recent years, as this ecosystem offers several services. Several species of halophytes such as *Arthrocnemum* and *Suaeda* species produce compounds with food, pharmaceutical and/or medicinal applications that have been overexploited, threatening species in their native habitats. The main goal of this study is to use green technologies for sustainable cultivation of halophytes *Arthrocnemum macrostachyum* and *Suaeda vera* approaches. Saline Fluvisol from a marginal estuarine area and organic and inorganic residues were used as amendments to construct a tailored soil for plant cultivation. Seeds collected in natural populations were pretreated with two dormancy breaking compounds and germinated in different substrata. A microcosm assay using different substrata and saline irrigation concentrations was established. Results showed that the seed germination percentage was highly influenced by the substrate in absence of salinity but slightly by the pre-treatments. Plant's growth and development assessed through morphometric parameters, biomass, and mortality percentage were more affected by the cultivation substrate than by the irrigation concentrations. The principal component analysis clearly distinguished plants cultivated in the Fluvisol from plants grown in the tailored soil that were strongly and positively correlated with nutrients, biomass, and morphometric parameters. This study contributes to a sustainable management of marginal lands using valuable halophytes and to reduction of overexploitation of plants resources.

Keywords: Amended Soils, *Arthrocnemum Macrostachyum*, Brackish Irrigation, Saline Marginal Land, *Suaeda Vera*.

Introduction

Land degradation is one of the main threats to agriculture since soil degradation can be caused by several processes such as erosion, compaction, pollution, organic matter decline and salinisation/sodification (Lal et al., 1989; Olsson et al., 2019). The latter affects 480,000 ha of fertile soils and can result from natural processes as intertidal areas or marine sediment deposition (Estrela et al., 1997; Gonçalves et al., 2015; Shao et al., 2018) or can be human induced as overexploitation of freshwater aquifers or irrigation with low-quality water. The estuarine marshes are considered one of the most productive ecosystems due to numerous ecosystem services (*e. g.*, coastal protection against to erosion, carbon sequestration, habitats, among others) that they offer (Barbier et al., 2011; Li et al., 2014; Peres et al. 2016). However, these ecosystems are strongly threatened by human exploitation leading to habitat degradation, pollution, and commonly used as wastes deposit of several human activities (*e. g.*, industry) (Serafim et al., 2013; Santos et., 2017a, Rajasree and Deo, 2018) and sea-level rise (Duarte et. al, 2008; Hussain et al., 2020).

Soil salinity, namely high soluble salts concentration (mostly NaCl), is a major factor limiting plant development in coastal areas (Li et al., 2014). The Na accumulation in soil contributes to colloids dispersion leading to soil permeability and aeration decrease (Lakhdar et al., 2009) and can also contribute to pH increase to alkalinity that limits the nutrients availability to plants (Machado and Serralheiro, 2017). However, tailored soils construction is a sustainable technology that allows to improve the physical (*e. g.*, aggregation), chemical (*e. g.*, nutrients concentration) and biological (e. g., soil biota) soil properties (Séré et al., 2010; Santos et al., 2017b). This soil management technique has been used to reclaim degraded soils allowing the plants' growth (Abreu and Magalhães, 2009; Macía et al., 2014; Fourvel et al., 2019; Cortinhas et al., 2020, 2021; Otremba et al., 2021).

Saline soils are inappropriate to most conventional crops that are salt sensitive (commonly designated as glycophytes) but can be an opportunity to cultivate valuable halophytes that cope with saline soils and saline irrigation water (Aronson et al., 1985; Glenn et al., 1991; Ventura et al., 2013; Panta et al., 2014). Halophytes (salt tolerant plants) can develop and complete their life cycle at levels up to 200 mmol NaCl (Flowers and Colmer, 2008). To avoid the salt stress, halophytes present different defense mechanisms like accumulation of ions Na^+ and Cl^- in the root parenchyma organs, salt bladders and salt glands, accumulation of non-toxic compounds in the cytoplasm and/or antioxidant molecules production (Breckle, 1995; Kefu et al., 2002; Ventura et al., 2014; Ghezlaoui, 2018). For example, the succulent *Arthrocnemum macrostachyum* (Moric.)

Moris and *Suaeda vera* Forssk. ex J. F. Gmel. accumulate Na^+ and Cl^- ions in the stems' vacuoles and in the leaves' vacuoles, respectively (Guilló et al., 2013; Elbar and Mohamed, 2013) allowing a decrease in water potential and permitting water uptake by plants even in salinity conditions (Song and Wang, 2015).

Halophytes' seeds germination and seedling establishment in saline soils are critical phases due to poor drainage that may result in the build-up of potentially toxic dissolved species, osmotic pressure, and anoxia (Keiffer and Ungar, 1995; Muñoz-Rodríguez et al., 2017). Salinity conditions can influence the biosynthesis of abscisic acid, a phytohormone promoter of seeds' dormancy (Khan and Gul, 2006; Gul et al., 2013; Li et al., 2016). The reactive oxygen species such as hydrogen peroxide (H_2O_2) contribute to the dormancy breaking through abscisic acid catabolism and biosynthesis of gibberellins that promotes germination (Liu et al., 2010; Wojtyla et al., 2016). Several studies have demonstrated the H_2O_2 improve the salinity tolerance of crops (Conner, 2008; Barba-Espín et al., 2010; Kilic and Kahraman, 2016; Bouallègue et al., 2017; Li et al., 2017) however few studies have addressed its role in halophyte plants: *Suaeda fruticosae* and *Limonium stocksii* (Hameed et al., 2009; Hameed et al., 2016) and *Chenopodium quinoa* (Hajihashemi et al., 2020). The sulfuric acid (H_2SO_4) is commonly applied in the chemical scarification of seeds with a thick coat such as *Suaeda maritima* (Mariko et al., 1992), *Prosopis juliflora* (Zare et al., 2011), *Sphaerophysa kotschyana* (Yildiztugay and Kucukoduk, 2012) and *Cressa cretica* (Etemadi et al., 2020).

Succulent halophytes from genera *Arthrocnemum* and *Suaeda* species have edible tips and produce edible seed oil (Weber et al., 2007; Wang et al., 2012). Both the vegetative and reproductive organs of these species are rich in antioxidants, proteins, fiber, and polyunsaturated fatty acids that are essential for the human diet (Custódio et al., 2012; Chamkouri et al., 2015; Song and Wang, 2015). In the last decades, the interest in compounds produced by halophytes increased (Aronson, 1985; Glenn et al., 1991; Weber et al., 2007; Barreira et al., 2017) and most studies are based on plant material exploited in natural habitats. By contrast, a few studies focus on sustainable cultivation strategies and methods to produce these plant species (Ventura et al., 2013).

This study aims to (i) test the influence of two dormancy breaking treatments in the germination percentage of *A. macrostachyum* and *S. vera* germinated in different substrata; (ii) assess cultivation of these halophyte species in a tailored soil by harnessing a saline marginal soil and inorganic and organic residues, irrigated with distinct saline water concentrations. The results will allow disclosing the effects of a recovered degraded soil (tailored soil) and the salinity effects on the growth and development of both species.

Materials and Methods

Study species

The *A. macrostachyum*, a member of Amaranthaceae, is a subshrub with succulent stems, articulated cylindric branches, pairwise fused leaves, fused opposite bracts three-flowered cymes and black seeds with a thick testa (Castroviejo, 1990; Piirainen et al., 2017). This species is adapted to extreme habitats and occurs in the high marshes across Mediterranean Europe, in the north and east of Africa and southwest Asia (Álvarez-Rogel et al., 2001; Piirainen et al., 2017; eHALOPH, 2021a). In Portugal, it is present in estuarine marshes with large populations in the southern estuaries (Carapeto et al., 2021a). The *A. macrostachyum* can be considered as a functional food due to having a nutritional profile suitable for human consumption rich in nutrients, vitamins, proteins, flavonoids, phenolics and tocopherols compounds and polyunsaturated fatty acids (Kalra, 2003; Hameed et al., 2011; Custódio et al., 2012; Barreira et al., 2017).

The *S. vera* belongs to Amaranthaceae and is a subshrub with glabrous stems prostrate to erect, alternate succulent linear-oblong leaves, hermaphrodite flowers in the leaf axils in three-flowered cymes and black seeds with a crustaceous coat (Pedrol and Castroviejo, 1990; Fernández-Illescas et al., 2010). This species dominates anthropically affected marginal zones is present in all Mediterranean region and in Moroccan and Portuguese Atlantic coasts, in high marshes (Álvarez-Rogel et al., 2001; eHALOPH, 2021b; Carapeto et al., 2021b). Some studies indicate *S. vera* as a source of proteins and vitamin compounds (Vizetto-Duarte et al., 2019; Duarte et al., 2022).

The Fluvisol, amendments, tailored soil, and estuarine water

The Fluvisol (FLU) and estuarine water (VF) were collected in an abandoned and degraded estuarine area, in the Fluvial beach “Praia do Sobralinho” (Sobralinho, Tagus Estuary - 38°54'16.1"N, 9°01'09.0"W), in the Municipal Council of Vila Franca de Xira, in the west coast of Portugal. In this area, pavilions, warehouses and corporate offices, unused land, roads, and railways were observed.

A tailored soil (TAIL) was produced using sieved (< 2 mm) and dried FLU, organic and inorganic amendments following the methodology in (Cortinhas et al., 2021). In brief, sludge, and waste kieselguhr from breweries, quartz sand, gravel limestone and residual biomass from pruning were added to FLU and mixed manually.

The FLU, amendments, TAIL and VF were previously characterised in Cortinhas et al. (2021). The FLU is strongly saline (EC 5.6 mS/cm) and slightly alkaline (pH 8.0) with low values of $C_{organic}$ (20.0 g/kg) and $P_{extractable}$ (0.01 g/kg). The sludge and waste kieselguhr have a high $C_{organic}$ (223; 31.3 g/kg), N_{total} (42.1 g/kg; 11.0 g/kg), P (1.4 g/kg; 0.2 g/kg) and K (1.9 g/kg; 0.1 g/kg) increasing the $C_{organic}$ and nutrients content of FLU. The TAIL presents high values of $C_{organic}$ (27.8 g/kg), N_{total} (2.5 g/kg), $P_{extractable}$ (0.1 g/kg) and $K_{extractable}$ (1.2 g/kg) (Cortinhas et al., 2021). The VF is neutral (pH 7.8) with high EC (22.0 mS/cm), high concentration of Cl^- (207 mmol/L), HCO_3^- (4.4 mmol/L), Na^+ (187 mmol/L), K^+ (4.0 mmol/L) and Mg^{2+} (22.1 mmo/L). The Na adsorption ratio is high too (35.43) (Cortinhas et al., 2021).

Germination tests

The seeds of *A. macrostachyum* and *S. vera* were collected in natural populations during the summer of 2018. Then, the seeds were observed in a Stereo Microscope (ZEISS Stemi DV4) to assess the seed heteromorphism. The seeds were germinated in transparent boxes (10.5 × 10.5 × 6.5 cm) containing filter paper, the FLU or the TAIL, respectively. Each box had 36 seeds equally distant from each other and 170 ml of deionised water. The boxes were exposed to a temperature 20 – 25 ºC with 16 h light and 8 h dark photoperiod, in a controlled chamber (Rumed). For *A. macrostachyum* and *S. vera* the germination tests were made in three replicates, *i. e.*, three boxes by substrata: (i) seeds without chemical pre-treatment; (ii) seeds previously treated with a 0.03% H_2O_2 solution (5 min immersed and then deionised water washed to remove the solution) (H_2O_2); and (iii) seeds pre-treated with a 98% H_2SO_4 solution (5 min immersed and then deionised water washed to remove the solution) (H_2SO_4). The seeds germination (SG) expressed in percentage [SG = Number of germinated seeds / Number total of seeds) x 100] (Luo et al., 2017) was determined.

Microcosm assays

Pots containing 1.150 kg of FLU or TAIL, respectively, were incubated at 70% of the maximum water-holding capacity (400 ml) for 28 days in the dark. Then, the *A. macrostachyum* and *S. vera* seedlings previously germinated in filter paper were transplanted to the pots. In each substrate, the plants were irrigated with estuarine water (VF) or saline solutions with different NaCl concentrations: deionised water (0), 200 mmol/L NaCl solution (200) and 400 mmol/L NaCl solution (400). Due to low number of *A. macrostachyum* seedlings obtained in this study, it was not possible to assess the plant growth irrigated with deionised water. At the end of the microcosm assay, the mortality percentage (MR) per treatment expressed in percentage [MR = (Number of succumbed individuals / Number of individuals transplanted)] was calculated and morphometric characters such as main stem length, number of primary branches and primary branches length were measured, and the fresh biomass were determined.

Statistical analysis

The seed germination and mortality percentage were analysed by a generalised linear model with a binomial distribution logit and link function with two factors (seed germination (substrate × treatment); mortality percentage (substrate × irrigation)) followed by a least-squares means test with Tukey adjustment ($p < 0.05$)

The morphometric characters data, except of the number of primary branches, and biomass data, obtained in the several treatments were tested by a two-way ANOVA (Substrate × Irrigation) followed by Tukey HSD test ($p < 0.05$) used to contrast the means. To compare the number of primary branches between treatments, a generalised linear model with a Poisson distribution and logit link function with two factors (Substrate × Irrigation) was fitted followed by a least-squares means test with Tukey adjustment ($p < 0.05$). A Correlation Matrix Principal Component Analysis (PCA using normalized data) was performed, and the Pearson correlation coefficient ($r > 0.7$) was used to correlate the chemical characteristics of substrata in the beginning of the assay and morphometric and biomass parameters obtained in plants at the end of the assay. The tests were performed using the R studio version 1.2.1335 software.

Results

The *A. macrostachyum* and *S. vera* germination

The *A. macrostachyum and* S. vera presented black seeds with a thick coat without showing seed heteromorphism. The seed germination of *A. macrostachyum* did not present significant differences ($p = 0.3523$) among the pre-treatments but had significant differences between the substrata ($p = 2.352e^{-13}$). The *A. macrostachyum* showed a very low germination percentage obtaining the highest value for seeds pre-treated with H_2SO_4 and germinated in filter paper (20%), followed by germination in the same matrix without the pre-treatment (16%) (Figure V. 1A). Regarding *S. vera*, germination was significantly influenced by the pre-treatment and substrate; however, the type of substrate ($p= 1.761e^{-15}$) presented higher significant differences than the type of pre-treatment performed ($p = 0.01241$). The *S. vera* seeds germination showed better performance in filter paper when the seeds were pre-treated with H_2O_2 (49%), followed by seeds germinated in the same matrix without pre-treatment (35%), and seeds germinated in the TAIL pre-treated with H_2SO_4 (35%) (Fig V. 1B). The *S. vera* had the highest germination percentage in all substrata regardless of the pre-treatment in comparison with *A. macrostachyum* (Figure V.1). Both species presented the highest germination percentage in environments of total absence of salinity.

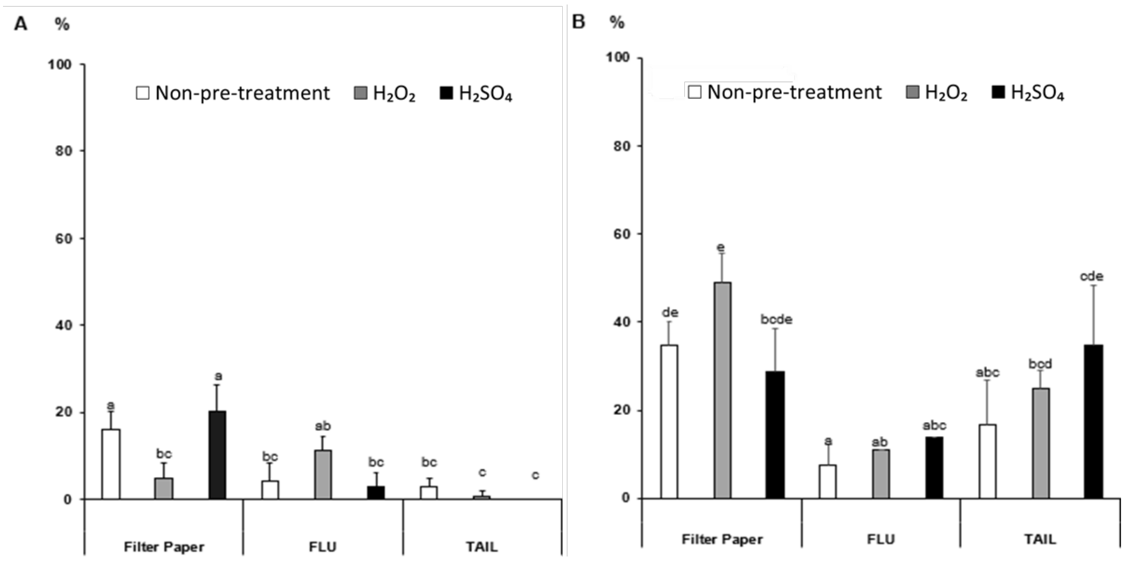


Figure V.1A. The percentage of *Arthrocnmeum macrostachyum* (A) and *Suaeda vera* (B) seeds germination (mean ± sd; n = 144). The substrata (moist filter paper, Fluvisol (FLU) and Tailored soil (TAIL) with different letters are significantly different [generalised linear model with a binomial distribution logit and link function with two factors (Substrate × Treatment) followed by a least-squares means test with Tukey adjustment ($p < 0.05$)].

Plant growth

Mortality and morphometric parameters

Globally, the mortality percentage of *A. macrostachyum* did not show significant differences between the substrata ($p > 0.05$) or irrigation with the different saline aqueous solutions ($p > 0.05$). Except for plants irrigated with solution 200, which presented the minimum and maximum value of mortality percentage (TAIL, 6%; FLU, 38%), respectively, significant differences between the substrata ($p = 0.03$) (Figure V.2) were found. By opposition, *S. vera* mortality percentage was affected by irrigation ($p = 0.03$) and strongly influenced by the type of substrata ($p = 1.632e^{-10}$) (Figure V.2). The plants grown in the FLU presented the highest values of mortality rate (75 % - 100 %) and all individuals cultivated in this substrate irrigated with the solution 400 died. In both substrata, the irrigation with solution 0 and VF water showed to be the most favourable to the survival of plants and the solution 400, the most harmful (Fig V.2). The mortality percentage of *S. vera* was higher than *A. macrostachyum* in all common treatments, except for plants cultivated in the TAIL irrigated with VF water (Figure V.2).

At the end of the assay, both species presented individuals grown in the TAIL with a stem significantly ($p < 2e^{-16}$) larger than those cultivated in the FLU. The plants grown in the TAIL and watered with solution 0 or VF water presented the largest stem. The length of *S. vera*'s stems was more than twice the length of *A. macrostachyum*'s stems (Figure V.2). Nonetheless, the number of primary branches was similar between species. For both halophytes this number was clearly higher ($p = 2.2e^{-16}$) in individuals transplanted to the TAIL than individuals transplanted to the FLU substrata. In the *A. macrostachyum* assay, in the TAIL substrate, plants irrigated with saline solution 200 had more primary branches. By contrast, in *S. vera* assay, the plants with highest number of primary branches were those cultivated in the TAIL irrigated with solution 0. For both halophytes, among plants cultivated in the TAIL, those irrigated with VF water showed fewer primary branches (Figure V.2). Nevertheless, these individuals and those planted in the TAIL irrigated with solution 0 presented the largest primary branches. The individuals transplanted to the TAIL ($p = 2.85e^{-07}$) showed significantly larger primary branches than individuals transplanted to the FLU. As for stem length, *S. vera*'s primary branches were more than twice the length of *A. macrostachyum*'s primary branches too. *Suaeda vera* also shows a higher heterogeneity primary branches length than *A. macrostachyum*, as it is demonstrated through a high standard deviation (Figure V.2).

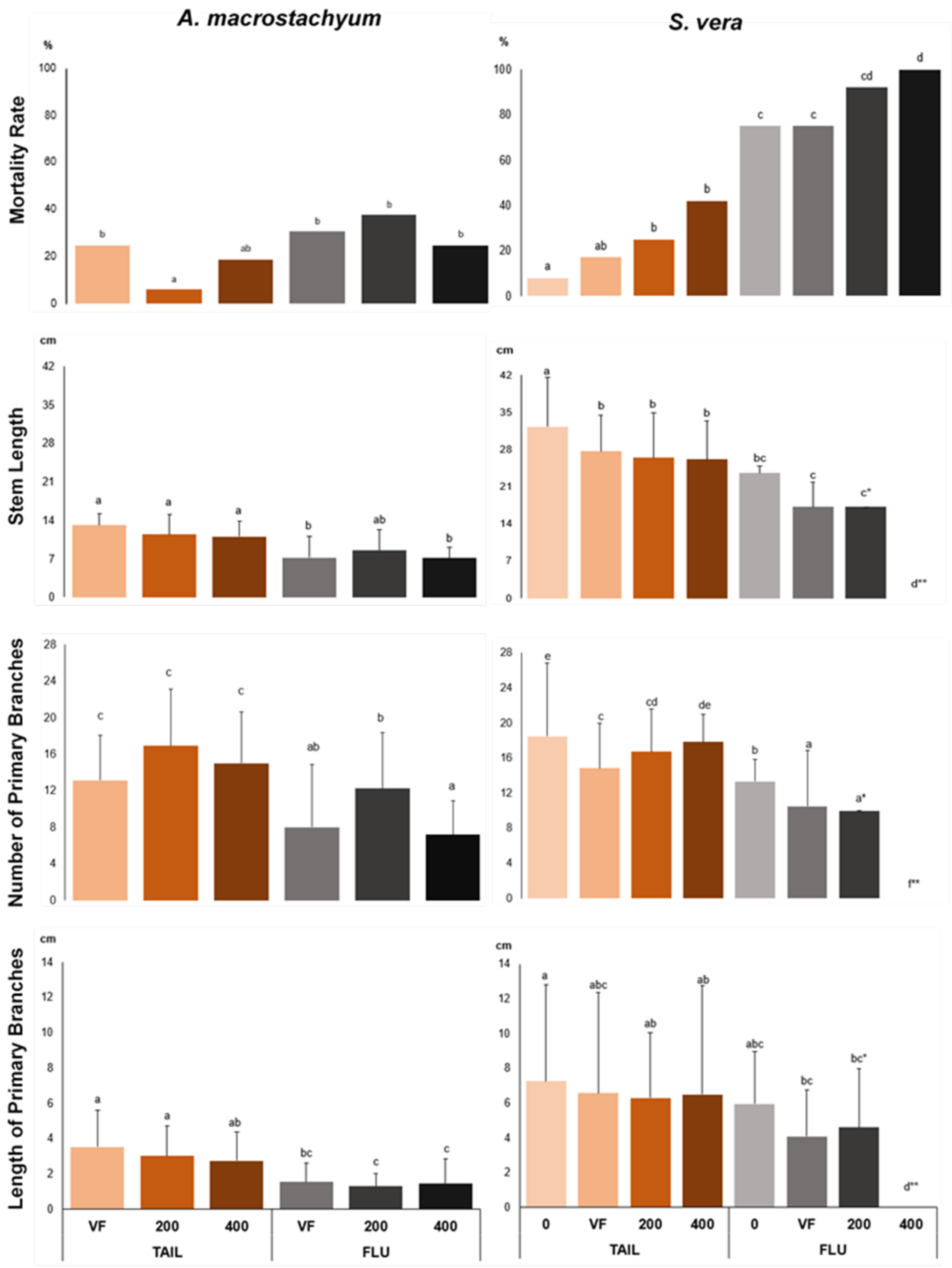


Figure V.2. Mortality rate and morphometric parameters (mean ± sd) of Arthrocnemum macrostachyum (n = 16) and Suaeda vera (n = 12) in different treatments determined at the end of assay. For each parameter, treatments with different letters are significantly different [generalised linear model with a binomial (mortality rate) and Poisson (number of primary branches) distribution and logit link function with two factors (Substrate × Irrigation) followed by a least-squares means test with Tukey adjustment (*$p < 0.05$*); two-way ANOVA (substrata $_x$ irrigation) followed by Tukey HSD test ($p < 0.05$) to analyse stem length and length of primary branches parameters]. Mortality rate (MR) was calculated according to the equation [MR= (Number of died individuals / Number of individuals transplanted)]. FLU – Fluvisol, TAIL – Tailored soil, 0 – deionised water, VF – estuarine water, 200 - 200 mmol/L NaCl solution and 400 - 400 mmol/L NaCl solutions. *Only one individual survived. **All individuals succumbed

Biomass

For both species, the fresh biomass of the aerial part and the fresh biomass of root was significantly higher ($p < 7.63\ e^{-11}$) in the TAIL than the FLU, but the influence of saline irrigation in fresh biomass was lower ($p < 3.31e^{-4}$) than in the substrata (Figure V.3). In the *A. macrostachyum* assay, the plants cultivated in the TAIL and irrigated with VF water (Figure V.3.A1.1) or solution 200 (Figure V.3.A1.2) showed the significantly highest yield of aerial part fresh biomass ($p < 0.009$) (Figure V.3.C1). The plants grown in the TAIL and irrigated with solution 400 (Figure V.3.A1.3) produced significantly ($p < 0.02$) less fresh biomass of aerial part than other individuals cultivated in the TAIL (Figure V.3.A1 and Figure V.3.A2), but significantly ($p < 0.001$) more biomass than individuals cultivated in the FLU (Figure V.3.A2 and Figure V.3.C1). However, the plants grown in the TAIL and irrigated with solution 400 (Figure V.3.A1.3) produced significantly ($p < 0.01$) more root biomass than all other plants (Figure V.3.A and Figure V.3.C1). In *S. vera* assay, plants cultivated in the TAIL irrigated with solution 0 (Figure V.3.B1.1) followed plants grown in same substrate irrigated with VF water (Figure V.3.B1.2) or solution 200 (Figure V.3.B1.3) produced significantly more biomass of aerial part than in all remaining treatments ($p < 0.02$)*.* The biomass of aerial part obtained in plants cultivated in the TAIL and irrigated with solution 400 (Figure V.3.B1.4) was significantly smaller ($p < 0.02$) than other plants developed in the TAIL (Figure V.3. B1) and did not show significant differences ($p > 0.05$) from plants cultivated in the FLU (Figure V.3.B2 and Figure V.3.C2). In this assay, the plants with significantly ($p < 0.01$) highest value of fresh root biomass were those transplanted to the TAIL and irrigated with solution 0 (Figure V.3.B1.1), followed by individuals grown in the same substrate but irrigated with solution 200 (Figure V.3.B1.2; Figure V.3.C2). In both assays, the roots of plants cultivated in the TAIL (Figure V.3.A1 and Figure V.3.B1) were thicker than the roots of plants cultivated in the FLU. The first ones spread over the substrate with several secondary roots (*e. g.*, Figure V.3.B1.1) by opposition to the latter ones which presented few secondary roots and were concentrated in the bottom of pots (*e. g.*, Figure V.3.B2.). Further, for both substrata, as the salt concentration increased, the plants seemed to have fewer and thinner roots.

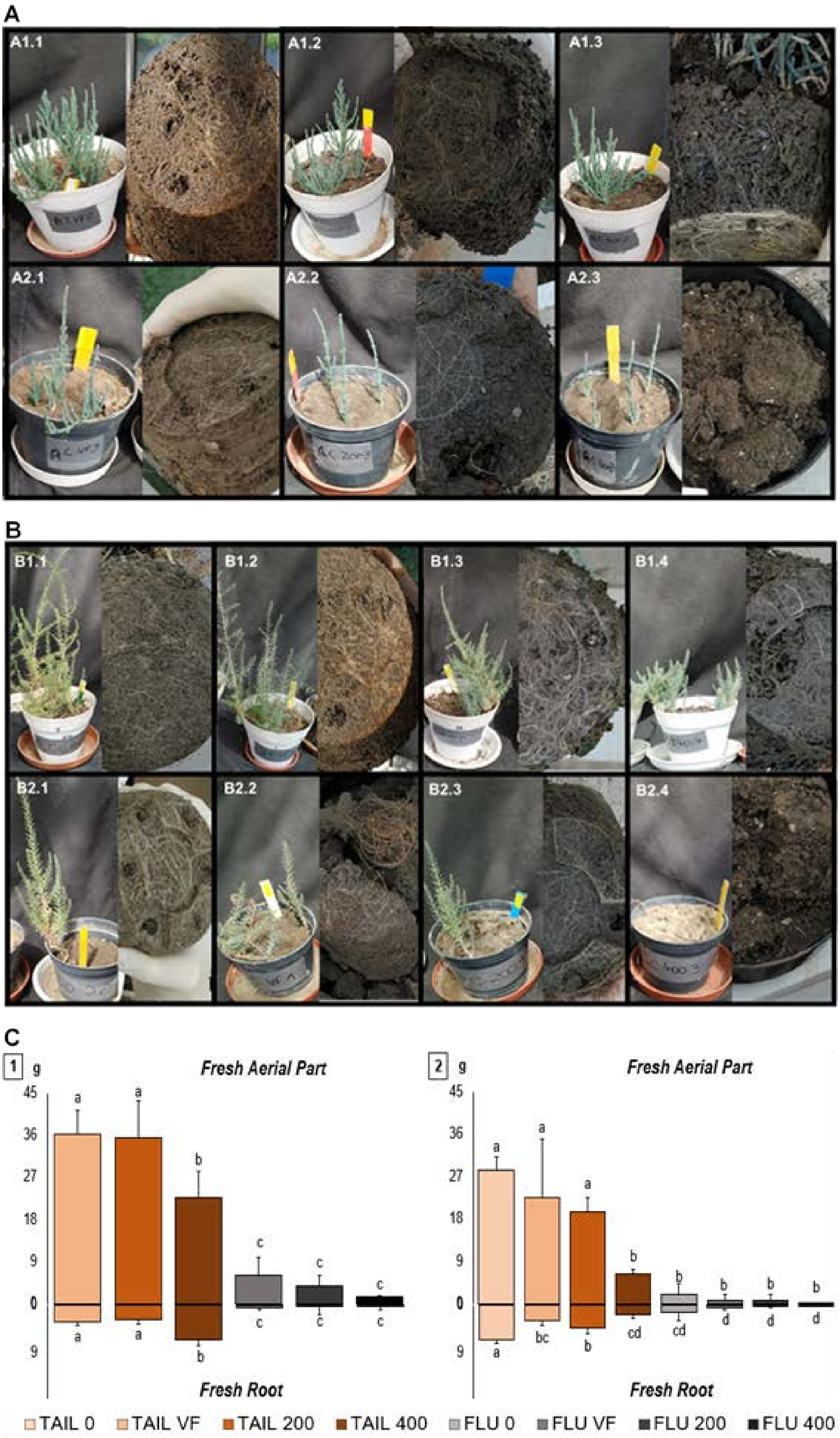

A
A1.1
A1.2
A1.3
A2.1
A2.2
A2.3
B
B1.1
B1.2
B1.3
B1.4
B2.1
B2.2
B2.3
B2.4
C
1 g
Fresh Aerial Part
Fresh Root
2 g
Fresh Aerial Part
Fresh Root
TAIL 0
TAIL VF
TAIL 200
TAIL 400
FLU 0
FLU VF
FLU 200
FLU 400

Figure V.3. Fresh biomass production of Arthrocnemum macrostachyum (A) and Suaeda vera (B) plants obtained at the end of the microcosm assay. The A. macrostachyum individuals plants grown in Tailored soil (A1) and Fluvisol (A2) and irrigated with estuarine water (A1.1; A2.1) and 200 (A1.2; A2.2) and 400 (A1.3; A2.3) mmol/L NaCl solutions; the S. vera individuals cultivated in Tailored soil (B1) and Fluvisol (B2) and irrigated with deionised water (B1.1; B2.1), estuarine water (B1.2; B2.2) and 200 (B1.3;B2.3) and 400 (B1.4; B2.4) mmol/L NaCl solutions. C. Fresh biomass weight of areal part and roots (means ± sd) of *A. macrostachyum* (n = 16) (1) and *S. vera* (n = 12) (2). For each parameter, treatments with different letters are significantly different (two-way ANOVA (substrata $_x$ irrigation) followed by Tukey HSD test; $p < 0.05$). FLU – Fluvisol, TAIL – Tailored soil, 0 – deionised water, VF – estuarine water, 200 - 200 mmol/L NaCl solution and 400 - 400 mmol/L NaCl solutions.

Principal Component Analysis (PCA)

The two Principal Component Analysis (PCA) based on the data collected in *A. macrostachyum* (Figure V.4.A) and *S. vera* (Figure V.4.B), respectively, correlated the pH, CE and nutrients concentration of substrata at the beginning of the assay, with the morphometric parameters and biomass determined at the end of the assay. Both PCA clearly showed two groups, the plants cultivated in the FLU and the plants cultivated in the TAIL. However, the concentrations of the irrigation solutions were not grouped so visibly, except for the *S. vera* plants grown in the TAIL and irrigated with solution 0, which were separated from the other individuals grown in the TAIL (Figure 4.B).

The first two components of each PCA explained 81.0 - 84.1 % (Fig V.4.A, *A. macrostachyum*, and Fig V.4.B, *S. vera*) of data variability, while the third component (not shown) only represents 6.5 - 6.8 % of data variation and did not show correlation with any variable ($r < 0.41$), except for the fresh root parameter ($r = 0.74$) in the PCA based on *A. macrostachyum* data. The first principal component of both PCA, were strongly correlated with all parameters ($0.62 < r < 0.96$), except EC that was strongly correlated ($0.78 < r < 0.86$) with the second principal component, and fresh root biomass that was correlated with the third component in PCA based on *A. macrostachyum* data (Table V.1 and Table V.2). For both PCA, the nutrients, biomass, and morphometric parameters, except fresh root in PCA of *A. macrostachyum,* were positively correlated ($0.66 < r < 0.95$) among them and negatively correlated with pH ($- 0.77 < r < - 0.66$) (Table V.3 and Table V.4). The PCA also showed that the TAIL had higher concentrations of all nutrients and presented higher values of morphometric parameters and higher values of biomass than plants grown in the FLU.

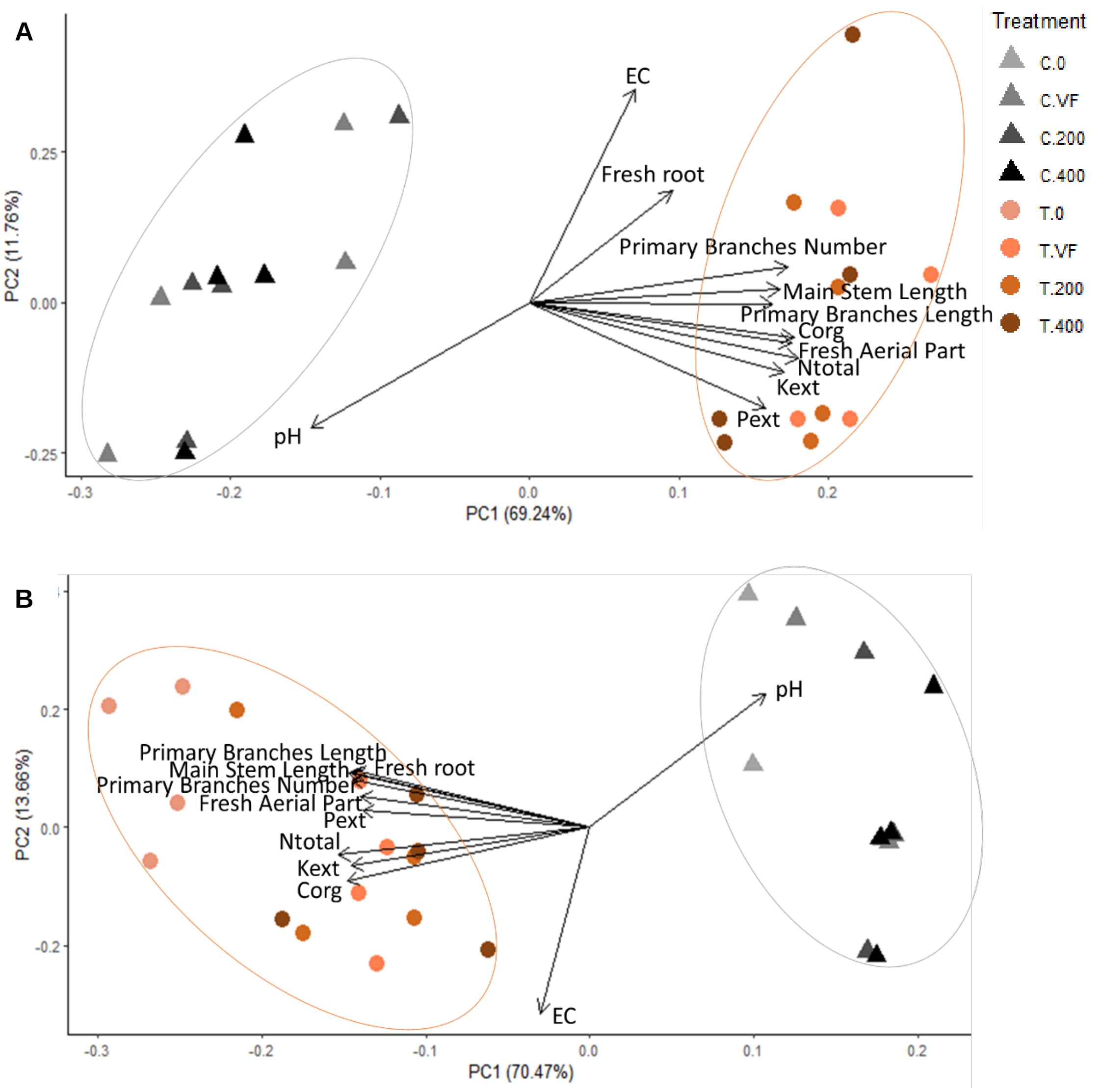


Figure V.4. The first two axes of Principal Component Analysis based on *Arthrocnmeum macrostachyum* (A) and *Suaeda vera* (B) data. These analyses correlate the pH, CE, organic carbon (Corg) and nutrients concentration (N total (Ntotal), extractable P (Pext) and extractable K (Kext) values determined in substrata and in the beginning of the assay with the morphometric (Length of Main Stem, Number of Primary Branches and Length of Primary Branches) and biomass parameters (Fresh Aerial Part and Fresh Root) obtained in plants at the end of the assay. Percentages of total variance explained by the functions are given in parenthesis. FLU – Fluvisol, TAIL – Tailored soil, 0 – deionised water, VF – estuarine water, 200 - 200 mmol/L NaCl solution and 400 - 400 mmol/L NaCl solutions.

Table V.1. Correlation matrix between the first two principal components (PC1 and PC2) on the normalized data and the original variables of Principal Component Analysis based on *Arthrocnemum macrostachyum* data. This matrix includes the pH, CE, organic carbon (Corg) and nutrient concentration (N total (Ntotal), extractable P (Pext) and extractable K (Kext) values determined in the substrata in the beginning of the assay and morphometric parameters (Length of Main Stem, Number of Primary Branches and Length of Primary Branches) and biomass parameters (Fresh Aerial Part and Fresh Root) obtained in plants at the end of the assay.

	PC1	**PC2**
Fresh Root	0.51	0.41
Fresh Aerial Part	0.94	-0.15
Number Primary Branches	0.92	0.13
Length Main Stem	0.89	0.05
Length Primary Branches	0.86	-0.01
Kext	0.91	-0.26
Pext	0.84	-0.39
Ntotal	0.96	-0.21
Corg	0.94	-0.13
EC	0.38	0.78
pH	-0.78	-0.45

Table V.2. Correlation matrix between the first two principal components (PC1 and PC2) on the normalized data and the original variables of Principal Component Analysis based on *Suaeda vera* data. This matrix includes the pH, CE, organic carbon (Corg) and nutrient concentration (N total (Ntotal), extractable P (Pext) and extractable K (Kext) values determined in the substrata in the beginning of the assay and morphometric parameters (Length of Main Stem, Number of Primary Branches and Length of Primary Branches) and biomass parameters (Fresh Aerial Part and Fresh Root) obtained in plants at the end of the assay.

	PC1	**PC2**
Fresh Root	-0.89	0.26
Fresh Aerial Part	-0.86	0.14
Number of Primary Branches	-0.90	0.22
Length of Main Stem	-0.91	0.25
Length of Primary Branches	-0.90	0.25
Kext	-0.90	-0.18
Pext	-0.86	0.08
Ntotal	-0.95	-0.13
Corg	-0.92	-0.24
EC	-0.19	-0.86
pH	0.67	0.62

Table V.3. Correlation matrix between variables of Principal Component Analysis based on *Arthrocnemum macrostachyum* data. This matrix includes the pH, CE, organic carbon (Corg) and nutrient concentration (N total (Ntotal), extractable P (Pext) and extractable K (Kext) values determined in the substrata in the beginning of the assay and morphometric parameters (Length of Main Stem, Number of Primary Branches and Length of Primary Branches) and biomass parameters (Fresh Aerial Part and Fresh Root) obtained in plants at the end of the assay.

	Fresh Root	**Fresh Aerial Part**	**Number Primary Branches**	**Length Main Stem**	**Length Primary Branches**	**Kext**	**Pext**	**Ntotal**	**Corg**	**EC**
Fresh Aerial Part	0.36									
Number Primary Branches	0.50	0.82								
Length Main Stem	0.44	0.80	0.89							
Length Primary Branches	0.41	0.80	0.89	0.78						
Kext	0.29	0.89	0.72	0.72	0.69					
Pext	0.32	0.80	0.74	0.77	0.71	0.83				
Ntotal	0.43	0.90	0.82	0.82	0.77	0.95	0.91			
Corg	0.39	0.92	0.78	0.75	0.74	0.98	0.78	0.95		
EC	0.23	0.24	0.43	0.37	0.29	0.20	0.09	0.21	0.26	
pH	-0.53	-0.7	-0.71	-0.65	-0.60	-0.65	-0.38	-0.67	-0.77	-0.57

Table V.4. Correlation matrix between variables of Principal Component Analysis based on Suaeda vera data. This matrix includes the pH, CE, organic carbon (Corg) and nutrient concentration (N total (Ntotal), extractable P (Pext) and extractable K (Kext) values determined in the substrata in the beginning of the assay and morphometric parameters (Length of Main Stem, Number of Primary Branches and Length of Primary Branches) and biomass parameters (Fresh Aerial Part and Fresh Root) obtained in plants at the end of the assay.

	Fresh Root	**Fresh Aerial Part**	**Number Primary Branches**	**Length Main Stem**	**Length Primary Branches**	**Kext**	**Pext**	**Ntotal**	**Corg**	**EC**
Fresh Aerial Part	0.89									
Number Primary Branches	0.88	0.72								
Length Main Stem	0.93	0.81	0.92							
Length Primary Branches	0.89	0.72	0.91	0.94						
Kext	0.65	0.70	0.71	0.69	0.72					
Pext	0.72	0.79	0.69	0.71	0.68	0.83				
Ntotal	0.74	0.79	0.77	0.76	0.77	0.95	0.91			
Corg	0.66	0.70	0.74	0.72	0.74	0.98	0.78	0.95		
EC	0.06	0.10	0.01	0.03	-0.01	0.20	0.09	0.21	0.26	
pH	-0.45	-0.45	-0.51	-0.49	-0.48	-0.65	-0.38	-0.67	-0.77	-0.57

Discussion

Regarding soil and irrigation, halophytes are less demanding than traditional crops, and the first ones have several potential applications such as phytostabilisation and soil rehabilitation (Cassaniti et al., 2012; Santos et al., 2015), biofuel (Sharma et al., 2016; Song and Wang, 2015), fodder crops (El Shaer, 2010; Glenn et al., 2013), and food complement due to compounds they synthesize (Barreira et al., 2017; Souid et al., 2019). Studies have reported heteromorphism in seeds of some halophytes, that differ in colour, size and shape (Gul et al., 2013) and relate this phenomenon with different germination percentages, namely in *Arthrocnemum indicum* and *A. macrostachyum* (Nisar et al., 2019), *Suaeda salsa* (Song and Wang, 2015) and *Suaeda araloscapica* (Wang et al., 2017). However, in the present study, no heteromorphic seeds were found in the collected fruits of both *S. vera* and *A. macrostachyum*. Regarding seeds germination, as found in previous studies both *A. macrostachyum* (Rubio-Casal et al., 2003) and *Suaeda vera* presented reduced germination in high salinity conditions (Muñoz-Rodríguez et al., 2017). Nevertheless, in this later study *A. macrostachyum* had a higher germination percentage than *S. vera*, contrasting with our results. In agreement with other works on halophytes, the abundant contact with freshwater favoured seed germination (Navarro and Guitian, 2003; Guan et al., 2010; Krauss and Ball, 2013). The osmotic stress induced by high salinity reduces the imbibition of water into the embryo leading a germination delay (Muñoz-Rodríguez et al., 2017). However, according to Navarro-Torre et al. (2016), *A. macrostachyum* germination can be increased using plant growth-promoting bacteria tolerant to salinity due to enzymatic activity, which contributes to cellular wall breaking and seeds' hydration.

Regarding seed dormancy breaking pre-treatments, H_2O_2 treatment did not influence seed germination of *A. macrostachyum* nor *S. vera*. According to the few available studies on the role of exogenous H_2O_2 in halophytes germination, H_2O_2 appears to improve germination of *Suaeda fruticosa*, *Chenopodium quinoa* and *Zygophyllum simplex* (Hammed et al., 2009; Hajihashemi et al., 2020; Hussain et al.,2022) but has no influence on *A. stocksii* germination (Hammed et al., 2016). As for pre-treatment with H_2SO_4, it only improved the germination percentage of *S. vera*'s seeds in the tailored soil. This contrasts with reports that H_2SO_4 ameliorates the seed germination of halophytes like *S. maritima* (Mariko et al. 1992), *Prosopis juliflora* (Zare et al., 2011), *Cressa cretica* (Etemadi et al., 2020) and *Lycium humile* (Palchetti et al., 2020). However, those studies were carried out in soilless systems and used different H_2O_2 or H_2SO_4 concentrations solutions as pre-treatments.

Previous studies reported that tailored soils production through wastes applications contribute to improve the structure, aeration, and nutrient concentration of substrates such as mining wastes (Asensio et al., 2013; Santos et al., 2017; Arán et al., 2021), dredged river/marine sediments (Macía et al., 2014; Mattei et al., 2016; Fourvel et al., 2019). The sand and limestone gravel enhanced the texture and permeability of the Fluvisol used in the construction of the tailored soil, while organic residues improved soil structure and fertility as was also observed by Macía et al. (2014) and Cortinhas et al. (2020; 2021). The addition of organic residues like biomass wastes obtained from pruning is a source of C and other nutrients, in the long run by slow mineralisation of these wastes, contributed to improve soil texture and aggregation. Whereas the soil fertility increased due to high content of Ntotal, Corg, extractable P and K present in the sludge and waste kieselguhr utilised as amendments (Cortinhas et al., 2021). In the case of halophytes, tailored soils also improved the production of *C. quinoa* (Castiglione et al., 2021), *L. algarvense* and *L. daveaui* (Cortinhas et al., 2020; 2021). The present study adds to this list the succulent halophytes *A. macrostachyum* and *S. vera* species since both species present the highest growth when cultivated in the constructed soil (TAIL). In other non-succulent halophytes' as in *Limonium* sp. both vegetative morphometric characteristics (leaf number, leaf length and width) and reproductive parameters (scape number and length) as well as dry biomass, increased in amended than in non-amended soil (Cortinhas et al., 2020, 2021). By contrast, in *Chenopodium quinoa* although stem length increased, the dry biomass did not enhance in an amended substrate (Castiglione et al., 2021).

Concerning the irrigation with aqueous solutions with different NaCl concentrations, previous studies in *A. macrostachyum*, carried out in sand/perlite culture irrigated with different NaCl solutions combined with a nutrient solution (Khan and Gul, 2002; Redondo-Gómez et al., 2010a) showed that this species has an optimal growth in the wide range 171–510 mmol/L NaCl. Contrastingly, in the conditions used in the current study *A. macrostachyum* plants cultivated in the tailored soil irrigated with 400 mmol/L NaCl solution had significantly lower aerial biomass than the remaining plants cultivated in the same substrate. Regarding *S. vera*, the 400 mmol/NaCl solution was also harmful to the plants' development. All the individuals grown in the Fluvisol and irrigated with this solution succumbed. The plants cultivated in the tailored soil watered with 400 mmol/NaCl solution presented lower growth by comparison with the plants irrigated with the other aqueous solutions. Other species of the same genus as *S. salsa* and *S. fruticosae* have an optimal growth at 200 mmol/L NaCl and a decrease at 400 mmol/L NaCl (Khan et al., 2000; Lu et al., 2003). In *S. vera*, although the plants irrigated with

200 mmol/L NaCl solution showed a good performance, they did not differ significantly from plants irrigated with deionised or estuarine water. The stress conditions, such as the structureless of the Fluvisol or the irrigation with 400 mmol/L NaCl solution induced a reduction in the number and thickness roots and induced a higher roots' concentration at the bottom of the pots, as also shown previously in other halophytes (*e. g.*, *Limonium daveaui*; Cortinhas et al., 2021).

Conclusion and perspectives

The seed germination of both species was favoured in salinity absence conditions. *Arthrocnemum macrostachyum* and *Suaeda vera* presented the highest growth and biomass production when cultivated in the tailored soil and irrigated with deionised water or estuarine water.

Overexploitation is one of the main threats to biodiversity contributing to habitat degradation. The sustainable cultivation technique described in the present study can play an important role in the conservation of *A. macrostachyum* and *S. vera* species and their habitat. Further, low-cost eco-friendly methods can be a potential rehabilitation technique of degraded estuarine areas. These valuable halophytes can be cultivated using resources from a degraded estuarine area while contributing to reduce overexploitation of plants resources. Further *in situ* studies are essential for large scale cultivation of such species and to validate these results.

Chapter VI. *General discussion, conclusions, and future perspectives*

General Discussion

Soil salinisation is one of the main threats to land degradation (Szabolcs, 1974; Olsson et al., 2019), and several studies on salinisation causes and/or consequences, namely on soil properties degradation, and on salt-affected soils reclamation have been published (Rengasamy, 2006; Lakhdar et al., 2009; Diacono and Montemurro, 2015; Amini et al., 2016; Daliakopoulos et al., 2016; Machado and Serralheiro, 2017; Saifullah et al., 2018; Leogrande and Vitti, 2019). Nonetheless, although saltmarshes are one of the most threatened habitats by increased soil salinisation due the sea level rise (FAO, 2021), there are few studies targeting saltmarshes' improvement of soil properties (Mao et al., 2016; Chaudhary et al., 2019; Ding et al., 2021; Gunarathne et al., 2020; Li et al., 2020), and most works are performed in inland areas (Akhter et al., 2004; Hanay et al., 2004; Mahmoodabad et al., 2013; Yazdanpanah et al., 2013; Nisha et al., 2018; Yang et al., 2018; Day et al., 2019). However, coastal salt-affected soils present different characteristics from dryland areas, namely the sea-water influence (Mao et al., 2016). Further, considering that soil degradation processes are much faster than the soil regeneration processes (European Commission, 2002) and the vital importance of the numerous services provided by saltmarshes (*e. g.*, high rate of carbon storage) (Barbier et al., 2011; Sousa et al., 2017), it would be expectable more studies on this subject. However, research on this topic is limited and studies focusing on saltmarshes improvement of saline-sodic soil properties are even scarcer (Mao et al., 2016; Li et al., 2020; Ding et al., 2021). Most works found on subject were performed in China (Mao et al., 2016; Li et al., 2020) since the Asiatic coastline is one of the most affected by soil salinisation in the world (FAO, 2021; Yang et al., 2018). Remarkably, the characteristics of soils approached in those studies are very similar to the Fluvisol studied in this thesis (FLU) (Chapter IV). Li et al. (2020) presented a silt-loam and saline-sodic Fluvisol with the same pH and CE values as FLU and slightly higher ESP than FLU. To decrease the ESP value, provide organic matter, and improve soil structure and permeability, the above referred authors used a mixture of commercial amendments, potassium-silicon-calcium microporous mineral fertilizer and furfural residue, as opposed to this PhD project in which only low-cost amendments were used. Ding et al. (2020) grew wheat on saline-sodic clayey soil with the same pH and ESP values as FLU and a slightly higher EC than FLU. Differing with the methodology used in the present doctoral dissertation, Ding et al. (2021) showed that the best option to reduce the ESP value was a combination of deep tillage and vermicompost, in a field assay monitored for two years. Mao et al. (2016) also demonstrated that gypsum application in silt-loam soil, in the limit between saline-sodic and sodic soil, decreases pH and ESP values while allowing bamboo cultivation, in a field study monitored for 18 months. In general, the main goal of these studies was to cultivate non-halophyte species, contrasting with this thesis' focus, which is to improve the physical, chemical, and biological properties of saline soil to cultivate halophylic species.

Regarding to studies on aquaculture waste valorisation, most of them only centre on wastewater (Fierro-Sañudo et al., 2015; Pinheiro et al., 2017; Gichana et al., 2018; Rodrigues et al., 2020). Although coastal earthen ponds are the most common aquaculture system (FAO, 2022a), there are few works on this system and on sediments' valorisation. In general, these sediments must be removed between fish crops and frequently, without any treatments, they are disposed on fertile lands affecting soil properties and groundwater (Muendo et al., 2014; Dróżdż et al., 2020). Van Tung et al. (2021) used aquaculture sediments as fertilizer showing that the application of 10 to 20 Mg/ha of compost from aquaculture sediments treated with microorganisms which degrade organic materials can improve cornfield yield up to 15%. Burducea et al. (2022) tested wheat cultivation in a mixture of aquaculture sediments with commercial peat, at different rates. Based on wheat's physiological and compounds characterisation, these authors concluded that wheat can be cultivated using 75% of sediment and 25% of commercial peat. In a study on halophytes growing in slopes of coastal earthen ponds, Colette et al. (2022) demonstrated that high saltmarshes' halophytes such as *Suaeda* sp., present a better development in aquaculture sediments than low saltmarshes' halophytes. The first species presented a high capacity to acquire nutrients due to a deeper root system, while contributing to aquaculture sediments' bioremediation too.

In this PhD project, halophytes cultivated using 74% of sediment and 26% amendments were also able to develop, but the individuals raised in non-amended aquaculture sediments did not survive (Chapter III). Nevertheless, sediments properties depend on several drivers, namely aquaculture system, fish species and fish feed (Datta et al., 2012; Granada et al., 2015). Burducea et al. (2022) and Colette et al. (2022) approach the carp and shrimp rearing, respectively, while this thesis considers seabass and seabream farming.

Most Technosols designed to improve sediment/soil properties are developed in a context of potentially hazardous elements and/or mining (Arbestain et al., 2008; Abreu and Magalhães, 2009; Macías-García et al., 2009; Hafeez et al., 2012; Asensio et al., 2013; Moreno-Barriga et al., 2017; Villenave et al., 2018; Santos et al., 2019; Arán et al., 2021; Otremba et al., 2021). The research using Technosol technology applied to salt-affect soils/sediments is limited and, in general, focuses on dredged marine sediments in harbour docks, which sometimes also present potentially hazardous elements and/or organic xenobiotics (Macía et al., 2014; Mattei et al., 2016). Further, most existing studies on salt-affect soils/sediments' valorisation just use one organic (Mattei et al., 2016; Chaudhary et al., 2019; Burducea et al., 2022) or inorganic amendment (Mao et al., 2016; Yang et al., 2018) and not an integrated solution, as the one offered by this thesis. In this PhD project, different organic and/or inorganic amendments were utilised, providing a more lasting integration of nutrients and C in the natural biogeochemical cycles (Macías et al., 2007), and halophytes cultivation, species well adapted to saline environments. In addition, the use of waste contributes to transforming waste into by-products that will incorporate other production, integrating a circular economy (Breure et al., 2018), the common goal of the Strategic Portuguese Plan for Non-Urban Waste 2030 (APA, 2022) and EU Soil Strategy for 2030 (European Commission, 2021).

This thesis, moreover, contributes to raising knowledge on cultivation of four understudied halophyte species. A search on Web of Science (ALL FIEDS: "name of each species") revealed that the succulent *A. macrostachyum* (123 results) and *S. vera* (34 results) present more studies, being *Limonium* species the least studied. There are only ten papers on *L. algarvense,* being one of them the Chapter III and only one paper on *L. daveaui,* the paper presented in the Chapter IV of this PhD project, respectively. Most studies on *A. macrostachyum* addressed plant-bacteria associations (Camacho et al., 2016; Mora-Ruiz et al., 2016; 2018; Navarro-Torre et al., 2017; 2018; 2020), bioactive compounds (Custódio et al., 2012; Rodrigues et al., 2014a; Abideen et al., 2015; Chekroun-Bechlaghem et al., 2019; El-Amier et al., 2021; Ghanem et al., 2021), phytoremediation (Redondo-Gomez et al., 2010b; Martínez-Sánchez et al., 2012; Conesa et al., 2011; de la Fuente, 2020; Mujeeb et al., 2021), and most of them use plant material collected in its natural habitat. Among all the works, only eleven studies present a plant growth assay and the majority address plant cultivation in an inert substrate (sand, perlite). Furthermore, these studies focus on the effect of saline irrigation with different aqueous solutions of NaCl combined with a nutrient solution on species growth (Khan et al., 2005; Redondo-Gómez et al., 2009), enzymes and compounds production (Ghanem et al., 2021), plant physiology (Trotta et al., 2012), or halophylic plant growth promoting-bacteria effects on plant development (Navarro-Torre et al., 2016). Concerning studies using soil as substrate, Paraskevopoulou et al. (2015) presented a similar methodology to the one used in this PhD project (Chapter IV). *Arthrocnemum macrostachyum* was cultivated in a sandy loam soil (15%) mixed with an inorganic amendment (pumice, 70%) and with the application (15%) of two other amendments: an organic amendment (grape marc compost) and/or an inorganic amendment (white peat). By contrast, the ratio of soil/amendment was different to the ratio applied in this thesis (Chapter IV). Further, the authors had a distinct goal from this PhD project, aiming at assessing a methodology to create an extensive green roof system in coastal areas. As mentioned previously, Paraskevopoulou et al. (2015) also placed greater focus on irrigation than on substrate, concluding that plants' height, diameter, and biomass irrigated with 30% of evaporation were higher than plants irrigated with 15% evaporation. Regarding substrate, these authors only indicate that plants growing in the substrate amended with perlite were the least tolerant to water stress, having a high number of plants succumbed during the summer.

Barcia-Piedras et al. (2019) used saline clay loam soil (25%) amended with inorganic amendments (silica 50% and perlite 25%) and salty water (171 mmol NaCl) to cultivate *A. macrostachyum* deionised. This study contrasts with this thesis' methodology in the ratio of soil/amendments applied, in the absence of organic amendments (Chapter IV) and irrigation with different aqueous solutions of NaCl concentrations (Chapter V). Besides, the authors presented a different aim of this dissertation reporting that *A. macrostachyum* can be used as an intercrop for soil desalinisation for glycophytes cultivation since this halophyte decreased soil salinity by 31% after 30 days.

Most research papers approaching *S. vera* are on species' ecology (Álvarez-Rogel, 2000; 2001; González-Alcaraz, 2014; Palacio et al., 2017), compounds it produces (Dudai et al., 2008; Vizetto-Duarte et al., 2019; Duarte et al., 2022), phytoremediation (Gómez-Garrido et al., 2018; Terreno et al., 2020; Naz et al., 2022), but not on cultivation techniques. However, in an experimental study on restoring a semiarid degraded area due to overgrazing, Pueyo et al. (2009) compared three restoration techniques: ploughing, damming and nurse-plant. The ploughing and damming break and roughen the soil surface, creating dams and micro catchments that increased macroporosity of the tilled layers, water infiltration and availability, and reduced water run-off (Pueyo et al., 2009). However, these techniques lead to organic matter and N content decreased by the removal of the topsoil layer. *Suaeda vera*, as a nurse-plant, protected the seedlings against grazing, and seedlings' growth enhanced of organic matter and nutrients contents in the soil (Pueyo et al., 2009). These authors concluded that the combination of those two types of techniques, with soil physical properties modifications and a nurse-plant could be the best option to improve the soil properties and to have success in the restoration of a degraded area. Despite the difference in scales between studies, this conclusion is in line with the results presented in this PhD project. After the application of amendments, there was an improvement in the soil properties (texture, structure, permeability, and fertility) and the species growth like *S. vera* that presented a higher vegetative and reproductive development in such conditions (Chapter III – Chapter V).

Regarding *L. algarvense*, most studies are on bioactive compounds produced by this species (Rodrigues et al., 2015; 2016; 2019) and only two (Rodrigues et al., 2020; 2021) present a plant growth assay, in addition to Chapter III of this thesis. Rodrigues et al. (2020; 2021) focused on the influence of water irrigation with different NaCl concentrations in compounds' production using only one substrate, a mixture of peat and perlite (3:1). In this PhD project, the influence of irrigation water and substrate on plant growth in *L. algarvense* was tested (Chapter III). Rodrigues et al. (2020) found the highest seed germination percentage of *L. algarvense* under deionised water conditions, agreeing with the results of *L. daveaui* germination (Chapter IV). Moreover, the above referred authors demonstrate that *L. algarvense* growth parameters such as leaves' size and scapes' number are also favoured by irrigation with deionised water, agreeing with the findings obtained in Chapter III of this thesis.

Studies on *A. macrostachyum, L. algarvense, L. daveaui* or *S. vera* cultivation are scarce or non-existent, but these species have a wide range of applications interesting for different industries, becoming very attractive species. Nonetheless, Halophytes present characteristics such as small seed size and low biomass index (Glenn et al., 2013), making their germination, and seedling to plant transition very sensitive stages. Further, seed germination and cultivation must also consider soil type and properties, soil salinity, and water irrigation salinity (Brown et al., 2014; Santos et al., 2016b). For studying one of the applications of these species (*e.g.*, antioxidant compounds production or phytoremediation of potentially hazardous elements contaminated soils) it is much easier to harvest the plant or part of them from their natural habitat than to germinate and cultivate plants. Nevertheless, species overexploitation, *i. e.*, a plant harvest higher than their reproductive output, can often depauperate species and degrade their native habitat. For example, *L. algarvense* is very attractive due to antioxidant and anti-inflammatory properties of compounds it produces (Rodrigues et al., 2016). However, this species as well as *A. macrostachyum*, *S. vera*, and *L. daveaui* are bioindicators of Mediterranean saltmarshes (Alfa, 2004) and form plant communities with high conservationist importance (Habitat Directive, 1992; Costa et al., 2014; Ramírez et al., 2021). Besides that, *L. algarvense* is restricted to the Iberian Peninsula and Morocco and has a 'Near Threatened' conservation status (Carapeto et al., 2020). The perennial and stable plant communities of saltmarshes, extremely specialised environments, constitute permaseries or permasigmetum, a climactic stage where no substitution perennial stages exist (Rivaz-Martínez et al., 2007). Thus, an unsustainable overcollection of plants can lead not only to biodiversity loss but also to habitat degradation, and in extreme cases, to their extinction.

Conclusions

In this PhD project, the number of halophytes in Portugal has been estimated for the first time. There are 163 species distributed among various orders and families of angiosperms being Caryophyllales and Amaranthaceae the most represented order and family, respectively, as found across the whole world. The compilation on halophytes' uses in Portugal revealed their potential as pharmaceuticals, nutraceuticals, food, and in phytostabilisation and rehabilitation of degraded and/or contaminated soils/sediments of estuarine areas. This thesis also contributed to improving knowledge of least-studied areas like improvement of soil properties from saltmarshes degraded by salinisation, mainly saline-sodic saltmarshes soils; valorisation of aquaculture sediments from earthen coastal ponds that are disposed on fertile lands affecting soil properties and groundwater; Technosol technology applied to salt-affected soils/sediments; and halophytes cultivation, namely *A. macrostachyum, L. algarvense, L. daveaui,* and *S. vera*.

The Technosol produced with a mixture of local wastes from fruit spirits distillation, substrate used in strawberry crops, coffee wastes, and biomass ashes, utilised as amendments, was suitable to increase the values of pH, C organic, nutrients and the structure of aquaculture sediments from earthen coastal ponds in the Guadiana estuary. Further, *L. algarvense* was not able to grow in sediments but was able to raise in the Technosol with mixture application at 360 g/kg and irrigated with estuarine water.

The tailored soil produced with a mixture of local amendments constituted by sludge and waste kieselguhr from breweries, medium sand, gravel limestone, and residual biomass obtained from pruning, was suitable to improve soil fertility, texture, and structure of the Sodic Fluvisol (saline-sodic) from saltmarsh area of Tagus estuary. The species *A. macrostachyum, L. algarvense, L. daveaui,* and *S. vera* presented the highest growth and biomass production when cultivated in the tailored soil and irrigated with deionised water or estuarine water from Tagus River.

Therefore, this PhD project presents a promising solution that approaches the freshwater scarcity, soil salinisation and the high percentage of non-reused waste, while answering challenges presented by society: how to valorise aquaculture sediments from coastal ponds in the Guadiana estuary, and how to valorise an abandoned and degraded saltmarsh area, in the Tagus estuary. The solution finding by this thesis consists in the waste valorisation and improvement of salt-affected sediments/soil properties through the Technosols construction to the add-value plant cultivation irrigated with estuarine water. These plants are important due to their role in ecosystems, conservation status, and their potential application in different industries such as feed, food, pharmaceutical, and nutraceutical. The cultivation of these halophytes not only constitute an alternative income source which does not depend on freshwater nor non-salt-affected soils and additionally, this solution can be the first step in saltmarsh rehabilitation.

Thus, the recent findings and future directions promote some of the sustainable development goals defined by the United Nations: waste reduction through a circular economy, farming practices to protect freshwater, sustainable agriculture, land degradation rehabilitation, and biodiversity conservation.

Future Perspectives

To validate the promising results obtained in this PhD project, *in situ* studies are needed by incorporating the selected low-cost amendments to improve sediments or saltmarsh soils, in the respective estuary and cultivation of the local species studied in this thesis. The recent findings and future directions will allow the evaluation of the potential growth of *L. algarvense* irrigated with estuarine water from Guadiana around aquaculture ponds, where the sediments have been deposited and the native species no longer grow. Moreover, it will also give an understanding if *A. macrostachyum*, *L. daveaui* and *S. vera* could be cultivated *in situ* irrigated with estuarine water from the Tagus estuary, in the currently abandoned and degraded saltmarsh area. These studies could contribute to saltmarsh rehabilitation, landscape recovery, and reintroduction of the critically endangered endemic *L. daveaui*, by avoiding species overcollection and preventing habitat loss.

Research Outcomes

Peer-reviewed publications

1. **Cortinhas, A.,** Ferreira, T. C., Abreu, M. M., Caperta, A. D. Sustainable cultivation of succulent halophytes using resources from a degraded estuarine area through soil technologies approaches and saline irrigation water. Land Degrad. Dev. (Revision being processed – LDD-22-0140.R1**).**

2. **Cortinhas, A.**, Ferreira, T. C., Abreu, M. M., Caperta, A. D., 2021. Conservation of a critically endangered endemic halophyte of west Portugal: a microcosm assay to assess the potential of soil technology for species reintroduction. Front. Ecol. Evol. 9:604509. doi: 10.3389/fevo.2021.604509 (Impact factor:4.171; Q1)

3. **Cortinhas, A.**, Caperta, A. D., Teixeira G., Carvalho, L., Abreu, M. M., 2020. Harnessing sediments of coastal aquaculture ponds through Technosols construction for halophyte cultivation using saline water irrigation. J. Environ. Manag. 261:109907 https://doi.org/10.1016/j.jenvman.2019.109907 (Impact Factor: 6.789; Q1) https://www.sciencedirect.com/science/article/pii/S0301479719316251

Book chapter

Cortinhas, A., Caperta, A. D., Custódio, L., Abreu, M. M., 2019. Halophytes Uses in Portugal: A Review, in: R. Tucker (Ed.), Halophytes: Identification, characterization and uses, Nova Science Publisher, New York, pp. 161-192. https://novapublishers.com/shop/halophytes-identification-characterization-and-uses/

Papers in Edited Proceedings with peer-review

1. **Cortinhas, A.,** Ferreira, T. C., Abreu, M. M., Caperta, A. D., 2022. Producing a Tailored soil, with an underused saline Fluvisol, for the conservation of a critically endangered species. *Symposium on Salt-affected Soils*. 20–22nd October 2021. Rome. p.63. https://www.fao.org/3/cb9565en/cb9565en.pdf

2. **Cortinhas, A.,** Ferreira, T. C., Abreu, M. M., Caperta, A. D., 2021. Cultivation of an economic potential halophyte using saline soil and saline water. *2019. Annual Meeting of the Soil Science Portuguese Society*: *Challenges of soil management in a climate change context*. 28th October, Portalegre, Portugal, p. 45. https://www.spcs.pt/wp-content/uploads/2022/04/Livro-de-Resumos-do-Encontro-Anual-das-Ciencias-do-Solo-EACS-2021_FINAL_21_Dez.pdf

3. **Cortinhas, A.**, Ferreira, T. C., Monteiro, F., Caperta, A. D, Abreu, M. M., 2019. Technosols approach to restore declining populations of *Limonium daveaui* Erben due to anthropogenic environmental problems. *XX Iberian Congress of Geochemistry*. 22 – 26th September, Évora, Portugal. Poster section, p. 109. http://www.cig2019.uevora.pt/pt/images/programadetalhado.pdf

4. **Cortinhas, A.,** Ferreira, T.C., Caperta, A. D., Abreu, M. M., 2019. The cultivation of Portuguese endemism *Limonium daveaui*, a new approach to its conservation. *2019 Annual Meeting of the Soil Science Portuguese Society*: *Soil – a priority target to combat desertification*. 17-18th June, Beja, Portugal, p. 29. http://www.eacs2019.uevora.pt/wp-content/uploads/2019/06/EACS_2019_LivroResumos.pdf

5. **Cortinhas, A.**, Caperta, A. D., Abreu, M. M., 2019. Technosols produced with sediments from Mediterranean aquaculture for the cultivation of halophyte *Limonium algarvense*. *5th Annual Congress on Plant and Soil Science*. 28th February – 01st March, London, UK. J Plant Physiol Pathol 7:8. Doi: 10.4172/2329-955X-C1-029

6. **Cortinhas, A.,** Caperta, A. D., Custódio, L., Abreu, M. M., 2018. Halophytes in Portuguese diet and impacts on human health. *International Seminar Health, Food and Environment: Sustainability and Challenges*. 11-12th October, Lisbon, Portugal. Poster section, p. 12. http://www.centrodehistoria-flul.com/uploads/7/1/7/0/7170743/livro_de_resumos.pdf

Communications in poster

1. **Cortinhas, A.**, Ferreira, T. C., Caperta, A. D., Abreu, M. M., 2021. New sustainable method of edible halophyte cultivation using underused resources. National Science Summit'21 – Science and Technology Summit in Portugal. June 28 – 30, Lisbon, Portugal. https://www.encontrociencia.pt/2021/?accao=posters

2. **Cortinhas, A**., Ferreira, T. C., Caperta, A. D., Abreu, M. M., 2021. Valorisation of cost-effective wastes and underused soils through Technosols construction for the *Suaeda vera* Forssk. ex J. F. Gmel. cultivation: a valued marine halophyte, EGU General Assembly 2021, online, 19–30 Apr 2021, EGU21-6811, https://doi.org/10.5194/egusphere-egu21-6811

3. **Cortinhas A.**, Ferreira T. C., Monteiro F., Caperta, A. D., Abreu M. M., 2019. The *Limonium daveaui* species conservation through Technosols use. National Science Summit'19 – Science and Technology Summit in Portugal. July 8 – 10, Lisbon, Portugal. http://www.encontrociencia.pt/home/

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