ORIGINAL RESEARCH PAPER

Harnessing sediments of coastal aquaculture ponds through Technosols construction for halophyte cultivation using saline water irrigation

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Abstract

The Mediterranean aquaculture has been developed mostly in brackish 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 characterized 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

1. 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 brackish 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 (Claudet and Fraschetti, 2010; Simard et al., 2008). 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, 2014). 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. 2018; 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.

2. Materials and Methods

2.1. 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).

2.2. 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 salt marshes 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. 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, all other pots (with and without plants) were irrigated with brackish water (Table 1), which was collected from a channel located in the estuary of the river Guadiana that brought estuarine water into the aquaculture ponds.

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; Fig. 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.

2.3. Experimental monitoring and analytical methods

The brackish water were 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; de Varennes, 2003).

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 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 brackish 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 brackish 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%.

2.4. 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.

2.5. Scanning electron microscopy (SEM) analyses

Leaves from plants grown in Tec180 and Tec360 Technosols and irrigated with brackish water were analysed by SEM. They were fixed in a 2.5% glutaraldehyde solution in 0.1 M 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 adapted. All measurements and counts related with these micromorphological characteristics were done on random fields, always at comparable leaf situations and magnifications.

2.6. 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 Normalised 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).

2.7. 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.

3. Results

3.1. 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, Na (3.47 g/L), Mg and Ca and a low concentration of nitrates (< 0.5 mg/L) (Table 1). The SAR (123.31) was very high (Table 1).

The initial sediments from aquaculture ponds were very acid (pH 3.12) and had low organic C, extractable P and K (Table 3). By opposition they showed a high EC value and a high elemental sulfur (7.6 g/kg) and $N\text{-}NH_4$ concentrations. The $N\text{-}NO_3$ content in initial sediment was lower than *C⁻* but higher than amended sediment (Technosols Tec180 and Tec360, Table 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 $N\text{-}NH_4$ than $N\text{-}NO_3$ as in the initial sediments (Table 2). The wastes from distilleries (*AW* and *CW*) also had acid pH, low EC and high concentration of C_{org} (Table 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-NH_4 but lower N-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 2). Nonetheless, as expected it had the lowest C_{org} of all organic wastes (Table 2).

3.2. 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 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 3). The Technosols, after the plants were irrigated with brackish 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 brackish water (A1) or only irrigated with deionised water ($p = 1.26 \times 10^{-5}$) (A2) (Table 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 3). The EC values also decreased from T1 to T2 for both assays and Technosols (Table 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 3) due the highest level of wastes application used as amendments and consequently the C_{org} content present in wastes (Table 2). In T2, there was a significant (p = 0.04) difference in C_{org} content among different irrigation regimes in Tec180 (Table 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 3). In both assays there was a decrease in N-NH_4 content in both Technosols (Table 3).

What regards to K and P contents, the amendments (Table 2) improved significantly ($p < 1 \times 10^{-7}$) the initial sediment chemical composition (C^{+}), which was poor in K and P (Table 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 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 4). The Technosols previously irrigated with brackish 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 4).

3.3.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 (Fig. 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 (Fig. 3A).

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: $p = 6.26 \times 10^{-3}$) (Fig. 3A). In the latter, the number of scapes was also significantly higher in plants irrigated with deionised water than with brackish water (Tec180: $p = 1 \times 10^{-6}$; Tec360: $p = 8.56 \times 10^{-4}$) (Fig. 3B).

3.4. 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; Fig. 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 (Fig. 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.

In C^- plants, leaves cross sections showed a compact symmetric mesophyll (Fig. 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 (Fig. 5B). No significant differences ($p > 0.05$)

were found between plants in the distribution of stomata and salt glands nor in leaf anatomy.

3.5. 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 5). The only Tec180 plant irrigated with brackish 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 brackish water showed lower ascorbate content than plants only irrigated with deionised water (Table 5).

3.6. Principal Component Analysis

The PCA allowed to correlate all the physic, 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 utilized from the C^+ sediment because all plants succumbed during the brackish water treatment. The first two components represented 79% of data variation (Fig. 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. 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-NO_3 ($r = 0.92$), and AsT ($r = 0.77$) in this substrate (Fig. 6; Table S1). 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 (Fig. 6; Table S1). 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 this changes. The A2 assay was better represented in PC2 than in PC1, having Tec180 and Tec360 plants grouped together (Fig. 6). The parameters highly correlated with PC2 were the pH (r = -0.81) and N-NH_4 (r = -0.80) (Table S2). As can be seen in table 3, these two parameters had the lowest values in A2 in comparison with A1 and C^- (Table 3).

4. Discussion

In Mediterranean region, aquaculture in the brackish 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., 2017). Low pH values for bottom sediments in aquaculture ponds were also reported for intensive aquaculture in Asia (Mandario et al., 2019, Senarath and Visvanathan, 2001). 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 nitrogen 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 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 brackish 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 brackish 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 (Ayers and Westcot, 1985; Kazemi et al., 2018, Robbins, 1984; Suarez, 1981) 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 deionized water had a greater development than those irrigated with brackish 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 salt marshes only inundated during the highest tides (Caperta et al., 2017; Costa et al., 2014). In this study plant growth was greater under freshwater than under brackish water conditions, reflecting this species adaptation to both halophilic and fresh water environments (facultative halophyte).

5. 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 utilized within a context of soil salinization and scarcity of fresh water in a scenario expected with the sea level rise, as well as in the recovery of degraded areas.

Acknowledgements

The authors gratefully acknowledge Ana Paula Paes (LEAF/ISA) for help in plant maintenance, Erika Santos (LEAF/ISA) for help in some analytical determinations, and Telmo Nunes (FCUL) for excellent technical assistance in SEM images.

Thanks to the owner of the Atlantik Fish company (Lima Cabrita group) located in Castro Marim and Vila Real de Sto António Natural Reserve, Algarve, for providing materials (sediments and estuarine water).

This work was funded by Portuguese national funds through Fundação para a Ciência e a Tecnologia (www.fct.pt/) project PTDC/AGRPRO/4285/2014 and LEAF UI/AGR/04129/2013, grant SFRH/BD/130256/2017 attributed to AC.

Competing interests

The authors have no conflicts of interest to declare.

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Tables and Figures legends

Table 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; de Varennes, 2003)

Table 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.

Table 3. Characteristics of the commercial substrate (C^-), unamended (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.

Table 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) at the end of brackish 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.

Table 5. Physiological parameters measured in plants cultivated in commercial substrate (C^{-}) and amended sediments (Tec180 and Tec360) at the end of brackish 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.

Figure 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).

Figure 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 saline 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 brackish water; **E.** and **F.** Plants grown in Tec360 irrigated with brackish water, showing one scape (F).

Figure 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.

Figure 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.

Figure 5. ***Limonium algarvense* leaves cross sections in plants irrigated with brackish 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.

Figure 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-NH_4, N-NO_3, extractable P and K, Na, Ca and Mg, ascorbate (AsT), photochemical reflectance index (PRI), normalised 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.

Supplementary material

Table S1. Correlation matrix between variables measured at the end of experiment (T2).

Table S2. 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)