FACULTY OF ENGINEERING UNIVERSITY OF PORTO

Prevention, Remediation and Valorization of Salt Impacted Soils

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Thesis submitted to the Faculty of Engineering, University of Porto

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Abstract

Soil salinization is a widespread soil degradation problem with severe long-term implications. In the present work a holistic approach was undertaken to address this issue. An evaluation of the impact of three different soil salinization processes was carried out followed by the assessment of preventive and remedial options.

The effects of the three salinization processes tested (episodic seawater inundation, irrigation with brackish water and capillary rise of saline groundwater) were found to be significantly dependent on soil characteristics. These results are particularly relevant for the agronomic management of irrigation water.

As a novel preventive measure, constructed wetlands were considered as a viable option to protect soil from salinization induced by saline wastewaters. The literature review done indicated some gaps regarding the experimental approaches to this technology and, as a result, several suggestions for standardization processes are proposed. Regardless, some conditions were identified where the effect of plants was likely to be more significant. These conditions were chosen for the experimental work done to maximize the probability of positive plant impact on the treatment.

Lab scale constructed wetlands were setup and a series of tests were conducted. As saline wastewater rarely contains only salts but also nutrients, the impact of salt on nutrient removal was important to assess. Tests done demonstrated the potential of *Spartina maritima* and *Juncus maritimus* for saline wastewater treatment. However, salt removal, although existent, is unlikely to be of practical use due to the long hydraulic retention time required.

Remediation of salt affected soils was also assessed with an emphasis on phytoremediation. An initial literature review provides a detailed comparison of this technology with the more traditionally one of chemical amendments application, as well as several other aspects, such as novel combinations to enhance the efficiency of phytoremediation, as well as the impact of climate change on its application.

Similarly to what was referred before, the literature review also provided clues to better focus the experimental assays done. In this case, emphasis was given to the unique potential of phytoremediation under non-leaching conditions, which was compared with chemical amendments for a non-calcareous saline sodic soil performed with leaching. While chemical amendments proved to be robust and yielded high salt removal, tests with plants, under these very specific conditions, were inconclusive. Although phytoremediation can still occur under leaching conditions, the unique advantage of phytoextraction being the removal of salts upwards, rather than to groundwater, was not decisively proven.

Resumo

A salinização do solo é um problema generalizado de degradação do solo com graves implicações a longo prazo. No presente trabalho foi usada uma abordagem holística para abordar esta questão. Inicialmente foi testado o impacto de três diferentes processos de salinização do solo, seguido da avaliação de opções preventivas e de remediação.

Verificou-se que os efeitos dos três processos de salinização testados (inundação episódica de água do mar, irrigação com água salina e ascensão de água subterrânea salina por capilaridade) dependem significativamente das características do solo. Estes resultados são particularmente relevantes para a gestão agronómica de água de irrigação.

Como uma nova forma de prevenção, leitos plantados, foram consideradas como uma opção viável para proteger o solo da salinização induzida por águas residuais salinas. A revisão bibliográfica efetuada indicou várias lacunas em relação às abordagens experimentais desta tecnologia e, como resultado, são propostas várias sugestões para processos de padronização. Independentemente desta limitação, foram identificadas algumas condições onde o efeito das plantas pode ser mais significativo. Estas condições foram escolhidas para o trabalho experimental realizado posteriormente, para maximizar a probabilidade de encontrar um impacto positivo da planta usada no tratamento.

Múltiplos leitos de plantas à escala laboratorial foram instalados e vários testes foram conduzidos. Dado que as águas residuais salinas raramente contêm apenas sais, o impacto do sal sobre a remoção de nutrientes foi também um aspeto importante a avaliar. Os testes realizados demonstraram o potencial de *Spartina maritima* e de *Juncus maritimus* para tratamento de águas residuais salinas ricas em nutrientes. No entanto, a remoção de sal, embora existente, é de utilidade prática improvável devido ao longo tempo de retenção hidráulico necessário.

A remediação de solos afetados pelo sal também foi avaliada, com ênfase na fitorremediação. Uma revisão inicial da literatura forneceu uma comparação detalhada desta tecnologia com a mais tradicional aplicação de químicos, e mostrou ainda vários outros aspetos, tais como novas combinações para aumentar a eficiência da fitorremediação, bem como o impacto das mudanças climáticas na sua aplicação.

De forma semelhante ao que foi referido anteriormente, a revisão da literatura também forneceu pistas para melhor focar os ensaios experimentais a realizar. Neste caso, foi dada ênfase ao potencial único da fitorremediação em operar sob condições de não-lixiviação, o qual foi comparado com adição de químicos, para remediação de um solo sódico salino não calcário. Embora a adição químicos provasse ser uma técnica robusta, com elevada remoção de sal, os testes com plantas nestas condições específicas, foram inconclusivos. Apesar de a fitorremediação ainda poder ocorrer sob condições de lixiviação, a vantagem exclusiva da fitoextração, com a remoção de sais do solo para os tecidos vegetais em vez de os lixiviar para águas subterrâneas, não foi ainda decididamente comprovada.

Aos meus pais e irmã que sempre me apoiaram

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Abbreviation and symbols

AMF – Arbuscular Mycorrhizal Fungi

ARH – Administração da Região Hidrográfica

ASTM - American Society for Testing and Materials

B - Baffled

BCF – BioConcentration Factor

BOD – Biochemical Oxygen Demand

CEC – Cation Exchange Capacity

CF – Continuous Flow

COD – Chemical Oxygen Demand

CW – Constructed Wetland

DB – Denitrifying Bacteria

DW – Dry Weight

EC – Electrical conductivity

EN – European Norm

EPA – Environmental Protection Agency

ESP – Exchangeable Sodium Percentage

FWS – Free Water Surface

GR – Gypsum Requirement

HRT – Hydraulic Retention Time

HSD - Honest Significant Difference

HSSF – Horizontal Subsurface Flow

IC – Inorganic Carbon

IPCC - Intergovernmental Panel on Climate Change

ISA – Ionic Strength Adjustor

LOI – Loss On Ignition

M – Moderate

MS – Mineral Soil

NP – Norma Portuguesa

NS – New Substrate

NT – Not Tested

O – Outdoor

OC – Organic Carbon

OM – Organic Matter

OS – Organic Soil

PAH – Polycyclic Aromatic Hydrocarbons
PGPB - Plant Growth Promoting Bacteria
RE – Removal Efficiency
RHSSF – Recirculating Horizontal Subsurface Flow
RSC – Residual Sodium Carbonate
RSD – Relative Standard Deviation
SAR – Sodium Adsorption Ratio
SP – Saturation Percentage
SS – Statistically Significant
ST – Subtropical
T – Tropical
TDS – Total Dissolved Salts
TGR – Theoretical Gypsum Requirement
TKN – Total Kjeldahl Nitrogen
TN – Total Nitrogen
TOC – Total Organic Carbon
TON – Total Organic Nitrogen
TP – Total Phosphorus
TPH – Total Petroleum Hydrocarbons
UC – Unplanted Control
VSSF – Vertical Subsurface Flow

1 Introduction

1.1 Motivation and Relevance

Salt affected soils are a worldwide problem (Figure 1.1) which severely limits agricultural productivity. Soils with this problem account for close to 910 million ha worldwide (Rengasamy, 2006; Wicke et al., 2011) with an estimated increase of 16% per year, which is equivalent to 60% of all arable area in the World (Metternicht and Zinck, 2008). Although most cases of soil salinization are naturally occurring, roughly 8.4% of this area is attributed to human induced salinization. This represents a more severe soil degradation problem than compaction, water logging, pollution or acidification (Metternicht and Zinck, 2008).

From Figure 1.1 it can be seen that there is some juxtaposition of saline or saline sodic areas with arid and semi-arid climates, primarily in northern Africa, the Middle East and Australia. However, that is not always the case, with vast extensions of salt affected soils in the US and former USSR, which can be attributed to poor irrigation practices rather than naturally occurring.

Soil salinization in Europe has been classified as the second biggest threat to soils and salinized soils occupy roughly 1 to 3 million hectares in Europe, value only exceeded by soil sealing and land take (Van-Camp et al., 2004; Montanarella et al., 2016). In the particular case of Portugal, over 104.000 ha are salt affected and over 1.3 million ha are at a high risk (by estimation) of salinization or are already salinized, almost exclusively un the Alentejo region alone, representing over 56.5% of the total regional area (ARH, 2012).

The Alqueva dam and associated irrigation scheme under development, located in the Alentejo, aims in part, to reduce the desertification that affects the region. However, the Guadiana river, which supplies the Alqueva dam, receives drainage water from 300.000 ha of Spanish irrigated land, and over 2 million population equivalents of wastewater, contributing to an increase of river salinity and of other contaminants (Melo and Janeiro, 2005). Furthermore, the irrigation water will drain back to the Alqueva reservoir, increasing the concentration of dissolved salts. As a result, only 17% of the intended 110.000 ha of irrigated soils are recommended for use without limitations, while 33% should not be irrigated (Melo and Janeiro, 2005).

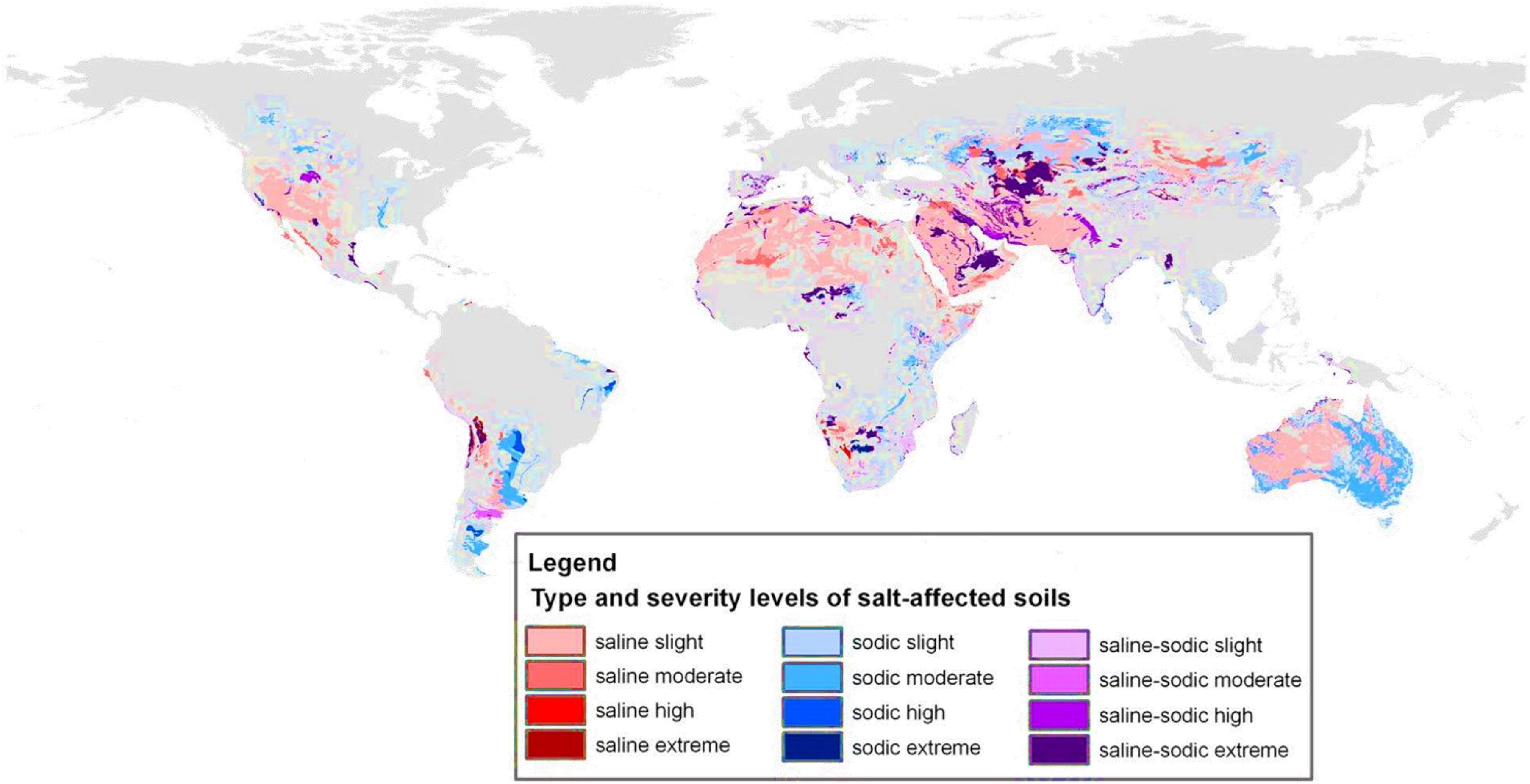

Figure 1.1- Worldwide saline, sodic and saline-sodic soils distribution (adapted from (Wicke et al., 2011))

It can be concluded that urgent steps to prevent or remediate soil salinization in the World in general, and in Portugal in particular, are necessary.

As a first step towards this objective, terms used need to be clarified and contextualized to explain the phenomena involved. First and foremost, soil salinization is mainly described by salinity and sodicity.

Salinity refers to the total quantity of dissolved salts and can be measured by Electrical Conductivity (EC), while sodicity refers to the relative quantities of sodium cations, expressed by sodium adsorption ratio (SAR) or exchangeable sodium percentage (ESP). Salt affected soils can therefore be divided into three different types based on these two main parameters (US Salinity Laboratory, 1954; Qadir et al., 2000):

- saline soil if EC is higher than 4 dS m^{-1}, with SAR below 13 or ESP below 15;
- sodic soil if EC is lower than 4 dS m^{-1}, but SAR is above 13 or ESP is above 15;
- saline sodic soil if both EC is higher than 4 dS m^{-1} and SAR is above 13 or if ESP is above 15.

Sodicity is a very crucial parameter as it has an impact on soil physical characteristics: excess SAR and/or ESP % leads to slaking, swelling and dispersion of clay aggregates, leading to hard setting, reduced hydraulic conductivity, impaired air movement, runoff, and more importantly, limited leaching capacity (Figure 1.2).

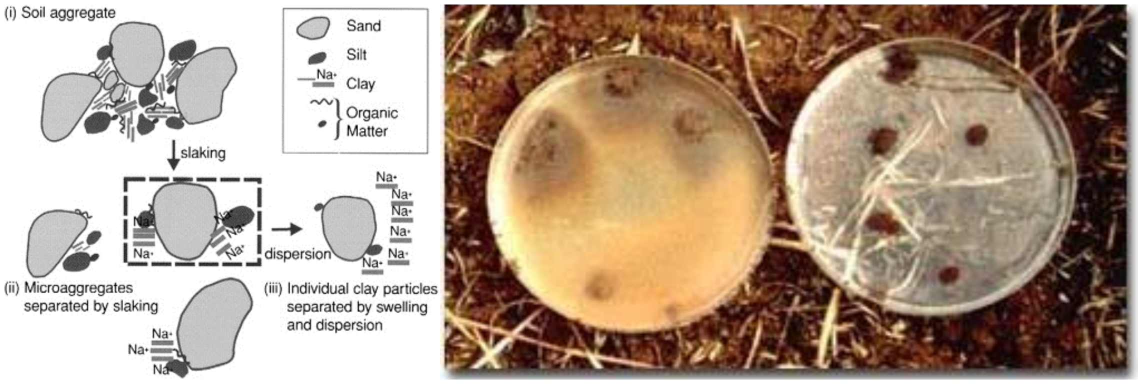

Figure 1.2 – Schematic representation of soil aggregate dispersion process (left) and example of dispersed, structureless soil (left) compared to a non-salt affected soil (right) (Sources: http://vro.depi.vic.gov.au/ ; http://origin-ars.els-cdn.com/)

Prior to other measures, a more detailed knowledge of soil salinization processes and the impact of climate change is required to provide context, particularly for preventive measures.

There are several mechanisms by which climate change can impact soil salinity, namely: 1) the increased occurrence of seawater inundation in coastal areas (either episodic or permanent); 2) the increased capillary rise of saline groundwater due to increased evaporation and 3) the expansion of irrigated lands and the increasing use of poor quality brackish water as a response to the scarcity of freshwater sources (Szabolcs, 1974).

However the exact impact of these mechanisms on different soils is poorly explored in the literature. Existing studies have not yet tested the effects of seawater temporary inundation for agricultural coastal soils, but rather either evaluated the impact on saline wetlands (Kirwan and Guntenspergen, 2012) or the effects of rare and extreme occurrences, such as tsunamis (McLeod et al., 2010; Yoshii et al., 2013), a phenomenon with clearly distinctive characteristics and impacts.

Finally, the ion distribution resulting from saline capillary rise, as well as the height of the capillary fringe and dependency on soil characteristics, is also seldom considered (Ibrahimi et al., 2013).

To mitigate the soil salinization problem, several approaches can be simultaneously applied. These can be divided into three main groups:

- Prevention – a set of measures to prevent the contact of salts with unaffected soils. This group includes measures such as capillary barriers, irrigation control, and salt removal during saline wastewater treatment, among others.
- Remediation – a group of techniques that aim to remove excess salinity / sodicity from affected soils. Currently at varying degrees of development and implementation in the field, these techniques range from the most common added chemical amendments, to phytoremediation, and even electrochemical techniques.
- Valorization – attempts to explore the more limited potential of salt affected soils through the adaptation of agricultural activities, also known as biosaline agriculture.

However, all these approaches remain severely hampered by a lack of information on their efficiency.

One approach to prevent soil salinization is to treat saline industrial wastewater. Several industrial processes produce this type of wastewater with variable salinity and nutrient loads. These industries include conventional and unconventional oil and gas exploitation (Lutz et al., 2013), aquaculture (Jesus et al., 2014), tannery (Calheiros et al., 2012), winery production (Ioannou et al., 2015), and agricultural drainage waters (Qadir et al., 2003), amongst others.

The treatment of these wastewaters would require removal of both salts and nutrients in order to prevent eutrophication and salinization. However, typical wastewater treatment facilities have low tolerance to salinity as well as no capacity to remove salts. Though, a constructed wetland with halophytic plants (Shelef et al., 2012) can have the potential to perform this simultaneous treatment.

Although it is a well-established fact that constructed wetlands can, in fact, tolerate high salinity and remove nutrients (Buhmann and Papenbrock, 2012; Jesus et al., 2014) it is not yet clear the feasibility or the mechanisms of salt removal.

This happens because, despite its potential, constructed wetlands have often been described as a ‘black box’ (Langergraber, 2008) that is, the mechanisms by which treatment of salt and/or nutrients is achieved, as well as their respective contribution, remain associated with a high degree of uncertainty.

Although plants are one of the most defining characteristic of a constructed wetland, their contribution to the treatment remains a subject of debate and controversy (Karathanasis et al., 2003; Lee and Scholz, 2007; Fia et al., 2014; Marchand et al., 2014). Clarifying the role of plants is important for both experimental and full scale designs and future research efforts.

To enable the use of constructed wetland technology as a viable option to prevent salinization, more practical information on its efficiency and the role of plant species is required.

Regarding the recovery of already salinized soils, there are several remediation options, which can be grouped into three specific types: leaching, addition of organic / chemical amendments with leaching and vegetative bioremediation with salt tolerant plants. However, when faced with high sodicity, leaching fails to provide adequate treatment.

In such cases, chemical amendments are the most commonly used method. These chemicals work by promoting the dissolution of native minerals with calcium which, in turn, will react with adsorbed sodium through cation exchange. Alternatively, these chemicals may also result in a direct addition of calcium cations for ion exchange with the adsorbed sodium, both followed by leaching.

One other option is phytoremediation or vegetative bioremediation of salt affected soils. This technique can simply be defined as the cultivation of salt accumulating or salt tolerant plants for the reduction of soil salinity and / or sodicity (Qadir and Oster, 2002).

A simplified comparison of these two techniques indicates that chemical amendments present several disadvantages, mainly the need of leaching, reducing their potential use to locations where water quality and quantity is not an issue (Qadir et al., 2001). Phytoremediation, on the other hand, seemingly is less costly, may not require excessive water for leaching and also prevents salt leaching to groundwater (Rabhi et al., 2009).

However, these techniques are currently at very different stages of development and field application: while chemical amendments have been applied for decades, phytoremediation for salt affected soils is more recent. This large discrepancy in information quantity and quality precludes an informed decision and adequate comparison of the applicability of both techniques.

As a result, a higher research effort on phytoremediation, particularly on the direct and indirect mechanisms by which plants contribute to the remedial process, is required. A focus should be given to the aspects that only phytoremediation has the potential to provide, as opposed to chemical amendments, namely the potential for simultaneous production of a cash crop, or the use of non-leaching conditions to protect important groundwater reservoirs.

Regardless of the wider application in the field, the use of chemical amendments still has some unknown aspects that require clarification. One of such aspects is the applicability to non-calcareous soils. This type of soil precludes the use of chemical or organic amendments, which promote the dissolution of native minerals, as one of the remedial mechanisms at play. As a result, some options, such as acid addition are no longer viable, while others may or may not have reduced efficiency.

1.2 Objectives and thesis outline

The present thesis aims to contribute to the prevention and remediation of salt affected soils. Considering the knowledge gaps found in the literature, the objectives of this thesis were established with the goal to contribute to our collective understanding of salinization processes and preventive and remedial techniques. As a result, three main objectives can be detailed as follows:

1) To explore various scenarios of soil salinization processes under climate change conditions in order to improve current knowledge on these phenomena and enhance preventive measures;
2) Evaluate constructed wetland technology as a method for simultaneous control and prevention of salinization and eutrophication originated from saline industrial wastewaters;
3) To test remediation options for non-calcareous soils with a particular focus on phytoremediation

Regarding the outline, this thesis is divided into seven chapters. After the present introductory chapter 1, chapters 2 through chapter 6 refer to scientific articles with the same title, which are either already published (chapters 2, 4 and 5), submitted (chapter 6) or in preparation to be submitted (chapter 3).

Chapter 2 relates to the evaluation of three different salinization processes, expected to increase in frequency with climate change, and their impact on two very different soils. The analyzed processes include seawater inundation, capillary rise of saline groundwater and irrigation water of poor quality.

Chapters 3 and 4 focus on Constructed Wetland technologies and their applicability to prevent both salinization and eutrophication. More specifically, Chapter 3 reviews the controversial contribution of plants in nutrient removal, aiming to simultaneously clarify the existing doubts in the scientific literature and identifying operational conditions under which improved removal efficiencies can be expected from a Constructed Wetland.

This acquired knowledge is subsequently applied in the design of tests performed in Chapter 4. In this chapter, three different halotolerant or halophytic plants are tested in a series of experiments (with expanded clay as substrate and under hydroponic conditions) to analyze the potential for simultaneous nutrient and salt removal of a synthetic industrial wastewater in lab-scale constructed wetlands.

Chapters 5 and 6 focus on remediation of salt affected soils, more specifically on phytoremediation. As a relatively novel approach, phytoremediation of salt affected soils remains poorly understood. Chapter 5 aims therefore, to review the limits of applicability of this technology, comparing its efficiency with more traditionally used techniques, and exploring the effect of climate change and how to adjust its use in accordance.

Chapter 6, on the other hand, focus on tests aiming to clarify important research gaps detailed in Chapter 5, namely the efficiency of both phytoremediation and chemical amendments when applied to a non-calcareous soil, as well as the potential of vegetative phytoremediation under non-leaching conditions.

Finally, chapter 7 aims to sum up the main conclusions and implications of this work from an engineering and practical perspective, as well as to provide suggestions for future work.

1.3 Scientific literature output

Papers published in international journals with peer review:
Jesus, J; Danko, A.S; Borges, M-T. “Phytoremediation of salt-affected soils: a review of processes, applicability, and the impact of climate change” (2015) Environmental Science and Pollution Research: doi: 10.1007/s11356-015-4205-4
http://link.springer.com/article/10.1007%2Fs11356-015-4205-4#enumeration.

Jesus, João; Castro, F.; Niemelä, A; Borges, M-T; Danko A.S. “Evaluation of the Impact of Different Soil Salinization Processes on Organic and Mineral Soils” (2015) Water, Air and Soil Pollution 226(4) doi: 10.1007/s11270-015-2373-y
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Jesus JM, Cassoni AC, Danko AS, Fiúza A, Borges M-T. Role of three different plants on simultaneous salt and nutrient reduction from saline synthetic wastewater in lab-scale constructed wetlands. Science of The Total Environment. doi: 10.1016/j.scitotenv.2016.11.074
http://www.sciencedirect.com/science/article/pii/S004896971632513X.

Jesus JM, Danko AS, Fiúza A, Borges M-T. Comparison of vegetative bioremediation and chemical amendments for non-calcareous highly saline-sodic soil remediation (submitted to Environmental Pollution).

Jesus JM, Danko AS, Fiúza A, Borges M-T. Effect of plants in constructed wetlands for organic carbon and nutrient removal: a review of contributing factors for higher impact and experimental standards recommendations (to be submitted to Critical Reviews in Environmental Science and Technology).

Book chapters with peer review:

Jesus, J., Danko, A. S., Fiuza, A. F., and M. T. Borges. 2015. "Phytoremediation in Saline Conditions. Chapter for the Book – Soil Remediation - Applications and New Technologies." Editors: Tomás Albergaria and Henri Nouws. Science Publishers doi: 10.1201/b19916-11 http://www.crcnetbase.com/doi/10.1201/b19916-11.

Posters in scientific meetings:

Jesus J., Gonçalves A., Mina I., Borges Maria-Teresa (2013) "Halotolerant Plants for Phytoremediation in Saline Environments", Abstract & Poster, 4ª Workshop Annual BioPlant, FCUP, 2013.

Oral Communications in scientific meetings:

Jesus J., Danko A., Fiúza A., Borges Maria-Teresa (2013) "Phytoremediation of salt affected soils" in 2nd Symposium on Subsoil Characterization and Remediation, Lisbon, 16 September 2013.

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2 Evaluation of the impact of different soil salinization processes on organic and mineral soils

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2.1 Keywords

Soil salinization; seawater inundation; brackish irrigation; capillary rise.

2.2 Abstract

Soil salinization is a worldwide problem of which secondary salinization is increasingly more frequent, threatening agricultural production. Salt accumulation affects not only plants but also the physio-chemical characteristics of the soil, limiting its potential use. Climate change will further increase the rate of salinization of soil and groundwater as it leads to increased evaporation, promotes capillary rise of saline groundwater as well as increased irrigation with brackish water. Episodic seawater inundation of coastal areas is likely to increase in frequency as well.

This work analyzed three types of salinization: seawater inundation (by irrigating soils with a 54 dS m^{-1} NaCl solution); saline groundwater capillary rise (soil contact with a 27 dS m^{-1} NaCl solution) and irrigation with two types of brackish water with different residual sodium carbonate (RSC). Two soils were used: a mineral soil (7.0% clay; 0.7% organic matter) and an organic soil (2.7% clay; 7.4% organic matter).

The tested soils had different resilience to salinization: The mineral soil had higher SAR due to low levels of calcium + magnesium but had higher leaching efficiency and more limited effects of RSC. The organic soil however, was more prone to capillary rise but seemingly more structurally stable.

Our results suggest that short term inundation with seawater can be mitigated by leaching although soil structure may be affected and that capillary rise of brackish groundwater should be carefully monitored. Also, the impact of irrigation with brackish water with high RSC can be inferior in soils with higher exchangeable acidity.

2.3 Introduction

Soil salinization is a worldwide problem threatening agricultural production, therefore, limiting its expansion is needed in order to support the projected increase in human population. As much as 10 billion hectares of soil are, in varying degrees of severity, already affected by salinization (Yensen and Biel 2006) with a potential increase of up to 16% per year (Aydemir and Sünger 2011). Secondary salinization due to poor irrigation practices is responsible for 24.6 to 61.5% of all irrigated salt affected soils (Pitman and Läuchli 2004; Mateo-Sagasta and Burke 2012), as well as for an estimated 25% of all saline groundwater (Weert et al. 2009).

Excess salt accumulation affects not only plants (Aslam et al. 2011) but also the chemical and physical characteristics of the soil. Excessive concentrations of sodium (expressed by sodium adsorption ratio (SAR) or exchangeable sodium percentage (ESP)) affects soil structural stability and particle aggregation due to slaking, swelling and dispersion effects. This leads to hydraulic and drainage problems which may culminate in water logging and lateral dispersion of salts (Qadir and Schubert 2002). High salinity also affects soil pH and nutrient concentrations as well as heavy metal mobility and bioavailability (Khodaverdiloo and Taghlidabad 2013).

Climate change will further increase the rate of salinization of both soil and groundwater. There are several mechanisms by which climate change can impact soil salinity, namely 1) the increased occurrence of seawater inundation in coastal areas (either episodic or permanent), 2) the increased capillary rise of saline groundwater due to increased evaporation and 3) the expansion of irrigated lands and the increasing use of poor quality brackish water as a response to the scarcity of freshwater sources (Szabolcs 1974; Jesus et al 2015). These mechanisms can be interconnected in numerous ways. For instance, seawater inundation can directly impact soil but also leach and/or intrude into a groundwater aquifer turning it brackish, and also, either through capillary rise (Chagué-Goff et al. 2014) or use in irrigation, affect soil salinity (Violette et al. 2009) (Rengasamy 2006). In addition, irrigation with brackish water can degrade groundwater resources which in turn could be used again as irrigation water and thusly potentially trigger a vicious cycle at a regional level (Schofield et al. 2001).

All of these mechanisms congregate mostly in coastal areas, particularly in dry or seasonally dry climates where groundwater levels are usually shallow. However, although salinization of groundwater is certainly an unfavorable process, it seems inevitable that irrigation with this poor quality water will have to be faced as a potential mitigation strategy for climate change effects due to the decrease in fresh water resources.

It is clear, therefore, that an assessment is needed of how these increasingly common salinization processes affect soil quality. However, the periodic and short term seawater inundation of agricultural and coastal soils is rarely analyzed in the literature, except in the most extreme events such as tsunamis (McLeod et al. 2010; Yoshii et al. 2013), or is focused on its negative impact on coastal wetlands (Kirwan and Guntenspergen 2012) rather than on the physio-chemical impacts on agricultural soils. Regarding saline water capillary rise, there are some works that evaluate and model (Jorenush and Sepaskhah 2003) this phenomenon, but few analyze the specific ion distribution over time and depth. Concerning brackish water irrigation, studies rarely consider the effect of residual sodium carbonate (RSC) (Prasad et al. 2001; Minhas et al. 2007) and there is a lack of consideration of different soil types and characteristics, which might affect the soil response to varying types of brackish water irrigation.

Finally, the use of leaching with poor quality water to control or remediate salinized soils has been thoroughly tested and applied in the field (Murtaza et al. 2009) but the impact of the application of this low quality water on soil quality has yet to be fully explored.

The objective of this work is to simulate three different types of salinization processes (seawater inundation, saline groundwater capillary rise and irrigation with two brackish waters of different composition) and to evaluate their respective effects on the quality of two types of soils: a mineral and an organic soil.

2.4 Materials and methods

2.4.1 Soil characteristics

This study involved the use of two different soils: a clear white soil, herein denoted as mineral soil (MS) and a dark brown soil, herein denoted as organic soil (OS). These two soils were specifically chosen due to their differences in organic matter and particle size compositions. The soils were air dried before being stored in the dark at room temperature. Representative samples for analysis were collected based on the Portuguese norms NP EN 932-1 and 932-2 2002 for sample collection and reduction techniques. The soils were sieved according to ASTM norms using the following sieve sizes:25; 20; 12,5; 10; 8; 6,3; 5; 4; 2; 1 mm and 500 and 250 µm. Wet sieving, due to the high organic content of the OS, was avoided and instead the fraction under 250 µm was analyzed by a laser diffraction particle size analyzer (Mastersizer 2000). A combination of agitation in water and ultrasound was used to disperse microaggregates, instead of using chemical dispersant and wet sieving. Texture was then assessed based on the feret diagram.

Soil density was calculated using a pycnometer (for aggregates between 0.063 to 31.5 mm) using the method described in NP EN 1097-6 2003, while bulk density was calculated using a graduated beaker of 100 mL. Soil saturation percentage was obtained by method 27a of U.S. Salinity Laboratory (1954), derived from a saturated soil paste prepared by method 2 of the same reference. The qualitative lime content test was prepared based on method 23a effervescence test of U.S. Salinity Laboratory (1954). Loss on ignition tests were performed in accordance to Heiri, Lotter et al. (2001). Cation exchange capacity (CEC) was tested using the methylene blue adsorption method described in Aprile and Lorandi (2012).

Soil water extractions were performed in accordance to U.S. Salinity Laboratory (1954) for saturated soil paste of 1:1, 1:2 or 1:5 soil to water ratios, followed by filtration with Whatman® 2.5 µm filter paper. To convert from soil to water ratios to saturated values, the following formula was used: denominator value of ratio (for example 5 in a 1:5 extraction) * 100/saturation percentage.

Electrical conductivity (EC) was determined with a WTW Tetracon® 325 conductivity electrode while pH was determined using a WTW pH-electrode SenTix 21. pH was determined using ratios of 1:1 soil to water extractions, 1:2 soil to 0.01 M $CaCl_2$ solution and 1:1 soil to 1 M KCl solution. Calcium + magnesium were determined simultaneously following EPA method (# 130.2 Hardness, Total (mg L^{-1} as $CaCO_3$) (Titrimetric, EDTA). Sodium was analyzed using a HANNA FC 300 B Na^+ electrode connected to a HANNA HI 4214 benchtop measuring unit after daily calibration. Important soil physical and chemical characteristics are shown in Table 2.1.

Table 2.1 - Characterization of the Organic Soil (OS) and Mineral Soil (MS) used in this work.

	OS	MS		OS	MS
Sand (%)	75.29	60.0[a]	EC (dS m^{-1})	0.90	1.28
Silt (%)	22.61	28.0[a]	Ca^{2+}+Mg^{2+} (meq L^{-1})	3.05	4.18
Clay (%)	2.11	7.0[a]	Bulk density (kg L^{-1})	1.10	1.16
Texture	Loamy sand	Sandy loam	Soil density (kg L^{-1})	2.14	2.68[a]
Saturation percentage (%)	33.0	46.3	pH water (1:1)	7.22	7.49
Lime content	not detected	not detected	pH 0.01 M $CaCl_2$ (1:2)	5.76	5.88
CEC (meq 100 g^{-1})	42.3	179.7	pH 1 M KCl (1:1)	4.80	4.09
OM (%)	7.4	0.7[a]	Exchangeable acidity (meq 100 g^{-1})	0.25	1.85

([a]Results obtained from Carvalho, M. 2014, unpublished data)

Screening tests for possible heavy metal contamination were done using of a Portable Analytical X-Ray Dispersive Energy Fluorescence Spectrometer (Innov-X System), and no abnormal level of metals was detected in either type of soil (data not shown).

2.4.2 Experimental setup

Soils were filled into acrylic columns with a height of 25.5 cm and an internal diameter of 5 cm. The bottom of each column was packed with glass wool to a height of 5 cm for drainage. The soils were subsequently added to the columns up to 10 cm height above the glass wool, allowing them to fall freely to avoid undue compaction and to retain bulk densities similar to the original values referred in Table 1. The columns were perforated at the bottom to allow for drainage and were set vertically in metal stands at 10 cm above the lab benchtop for the tests with seawater inundation. Soil loaded columns were placed in an oven at 40ºC (for the capillary rise tests) or at 60º C (for the irrigation tests). Although used for practical reasons, these temperatures simulate salt accumulation at a much faster rate than what happens in the field. Nevertheless, these temperatures remained within acceptable values according to Berglund et al. (2010).

2.4.3 Seawater inundation

The objective of this experiment was to simulate the effects of seawater inundation into the two soils (MS and OS, each one having different physical and chemical characteristics) to see how these soils responded. Two sets of columns were prepared in triplicate for each type of soil. Before testing, the soils were wetted with distilled water to saturation and allowed to drain. This was done in order to avoid hydrophobicity problems from using dry soils and corresponding drainage problems, as observed in preliminary tests. The experiment was divided in two phases: phase one salinized the soil using a saline solution, (herein referred to as samples of “soil after salt addition”) and the saline leached solutions (herein referred to as “salt solution leached”) were collected and tested; and phase two, in which the salinized soil was leached twice with distilled water.

For the salt addition phase, 2 pore volumes of a prepared solution using NaCl (Merck; ≥ 99.5%) at seawater salinity level (EC = 54 dS m-1, pH 6.02, sodium levels: 13950 mg L-1) were added. Due to differences in porosity the volumes added were different: 157.6 and 235.2 mL, for OS and MS, respectively. The leachate obtained was collected and analyzed. Contact time between the soil and saline solution was dependent on the infiltration rate of each replicate and therefore varied in each column. The EC and pH of the leachate and soil samples were assessed, along with calcium + magnesium and sodium levels.

During the second phase of the test (leaching phase), leaching with distilled water was performed to simulate heavy rain events. Distilled water was added at 2 pore volumes (157.6 mL for the OS and 235.2 for the MS). The volume of distilled water was allowed to leach through the soil once and then leaching was repeated with the same volume (as listed above) of new distilled water. Both the first and the second washing leachates were collected and analyzed at the end of the experiment. The EC and pH of the leachates were assessed, along with calcium + magnesium and sodium levels.

2.4.4 Capillary rise of saline groundwater

A test designed to simulate soil salinization due to capillary rise was conducted using the same experimental set up described above, but with a soil height of approximately 20 cm in each column. Two sets of columns were prepared in triplicate with each soil type and were placed in a container with a 27 dS m^{-1} saline solution (prepared with NaCl (Merck; ≥ 99.5%), at a height corresponding to that of the glass wool in the columns. The value of salinity used was similar to the one reported for saline groundwater in Fan et al. (2012). The level of water in the container was maintained by the addition of the 27 dS m^{-1} saline solution whenever needed. If there was no visible capillary rise to the top of the 20 cm soil height in the columns, the unsaturated soil was removed and not replaced.

Initially, column top soil samples were collected at different times (every 2 days) in order to assess the evolution of salinity over time and tested for moisture content, EC, pH and calcium + magnesium levels. After 5 days, all of the soil was removed from the columns but carefully separated into two soil depths. Samples of 0-10 cm and 10-20 cm depths were acquired to assess variability of saline groundwater effects with depth, with EC, pH, calcium + magnesium and sodium analyses performed.

2.4.5 Irrigation with brackish water

The objective of this test was to simulate a moderately severe case of irrigation of two types of soils (MS and OS) with brackish water (secondary salinization) and to assess the leaching potential. A column set up similar to the one previously described was used. This test was also composed of two phases: the salt addition phase, in which two different irrigation solutions were added to the soils at a rate simulating normal irrigation practices (the volume added was just enough to compensate for evaporation and limited to no leaching), and a leaching phase, in which the same irrigation solution was added in excessive volume to promote leaching.

The irrigation solutions used have similar EC values, resembling mildly brackish water but with varying RSC. One of the irrigation solutions used was composed of NaCl 1g L^{-1}, $CaCl_2.2H_2O$ 1.325 g L^{-1} and $NaHCO_3$ 1.85 g L^{-1}, resulting in a theoretical EC of 5.73 dS m^{-1} (actual EC of 5.20 dS m^{-1}, or 8.8% lower), SAR of 13.08 and RSC of 4.00. The other irrigation solution was composed of NaCl 2.3 g L^{-1} and $CaCl_2.2H_2O$ 1.325 g L^{-1}, resulting in a theoretical EC of 5.77 dS m^{-1} (actual EC of 6.10 dS m^{-1} or 5.7% higher), SAR of 13.21 and RSC < 0. All chemicals used were p.a. grade. Irrigation was performed by adding a few drops at a time to each triplicate column, more closely resembling surface drip irrigation than ponding as an irrigation method.

In the first phase (or the salt addition period), 67 mL of each solution were initially added to all soil columns (approximately 75% of the saturation percentage of the MS) and three subsequent irrigations were made during 4 days to compensate for evaporation. Salt build up was monitored by small sample collections (approximately 7 g of soil at 1:5 soil to water extraction) which were then used to determine EC and pH, as well as soil moisture (data not shown). Final soil samples were collected (65 g) to evaluate EC, pH, calcium + magnesium and sodium concentration (salt addition phase soil samples).

In the second phase of this test, leaching was performed at 1.2 pore volumes with the two different solutions used above for irrigation (which had similar EC and SAR, but with different RSC values). This resulted in the addition of 96 mL of irrigation solution in the OS and 145 mL in the MS. Soil samples were collected (designated “soil after leaching”), as well as the leaching solutions, and monitored for pH, EC, calcium + magnesium and sodium.

2.4.6 Statistical analysis

All statistical tests were performed using the Stastistica 8.0 software (StatSoft, Inc, Tulsa, USA). Normality and homogeneity of variances were tested by Shapiro-Wilk test and by Levene's test and Brown–Forsythe test, respectively. T-tests were performed for comparison between two independent sets of data at $\alpha = 0.05$. When normality was not verified, Mann Whitney U test was used (with respective formula for effect size, r). The tests performed with Mann Whitney U are indicated with * in the Tables and use $\alpha = 0.10$. Dependent paired t-tests were performed to compare soil samples before and after leaching.

2.5 Results

2.5.1 Seawater inundation

The results obtained for the seawater inundation test are shown in Table 2.2. It can be seen that the EC values of both soils samples after salt addition are similar to that of the salt solution used, as well as the respective sodium levels. Despite this, the SAR level in the MS is almost double that of the OS, likely due to differences in calcium + magnesium concentrations between the soils. Despite having higher initial values of soluble calcium + magnesium and pH, the MS was the most affected by the simulated salinization process. In this phase, the EC of the salt solution leached is within expected levels, considering the dilution with the distilled water that was previously added to the soils (which would result in predicted values of 23.4 and 24.5 dS m^{-1} for the MS and OS, respectively).

Similar trends in sodium and calcium + magnesium values can be observed in these leachates, but calcium + magnesium is likely to be higher in the OS leachate. Nevertheless, in this case the values were not statistically different due to the presence of a large outlier. In addition, there is a reduction in pH in both cases (due to the application of simulated seawater) but it is significantly higher in the solution obtained from the MS (p = 0.006), with a large size effect of over 0.5.

Table 2.2 - Seawater inundation tests. Salt addition phase refers to the addition of a 54 dS m^{-1} solution and monitoring soil samples and leached salt solutions. Leaching phase refers to washing with distilled water after salt addition and assessment of leached solutions (*Mann-Whitney U test, all remaining statistical analysis use independent t-tests; r indicates effect size, the results are expressed as units ± % relative standard deviation).

	Salt addition phase							
	Soil after salt addition				Salt solution leached			
			Statistics				Statistics	
	MS	OS	p-value	r	MS	OS	p value	r
EC (dS m^{-1})	56.6±12.4	55.5±24.3	0.823	0.01	29.3±22.7	24.1±15.4	0.297	0.26
pH	4.7±2.5	7.0±2.8	0.000	0.99	4.1±2.9	5.73±12.3	0.006	0.87
Ca^{2+}+Mg^{2+} (meq L^{-1})	2.2±17.9	9.9±55.3	0.071	0.60	19.4±5.7	21.9±39.0	0.631	0.06
Na^{+} (meq L^{-1})	548.8±13.3	490.9±27.3	0.540	0.10	271.5±19.5	215.4±22.2	0.225	0.34
SAR	537.7±22.5	245.3±44.4	0.036	0.71	87.2±17.4	70.9±47.8	0.491	0.13
	Leaching phase							
	1st Distilled water leached solution				2nd Distilled water leached solution			
EC (dS m^{-1})	21.9±18.7	19.8±44.0	0.719	0.04	2.9±35.5	3.49±33.3	0.587	0.08
pH	4.0±2.1	5.8±5.2	0.001	0.96	5.0±10.4	6.9±5.5	0.007	0.86
Ca^{2+}+Mg^{2+} (meq L^{-1})	5.6±5.7	11.5±28	0.035	0.71	0.3±27.7	1.7±103.6	0.081*	-0.71
Na^{+} (meq L^{-1})	205.3±34.1	138.6±43.1	0.278	0.28	16.3±38.9	18.6±29.8	0.658	0.05
SAR	121.7±31.0	57.0±35.0	0.058	0.63	43.3±49.3	24.2±22	0.206	0.36

The distilled water leaching phase, repeated twice to simulate heavy rain, was able to reduce sodium levels in both soils. However, it also leached out calcium + magnesium, leaving these soils more vulnerable to a rapid increase in SAR if a salt addition event reoccurred. As expected, leaching of calcium + magnesium was more visible in the OS due to its higher initial concentration in these ions.

A simplified mass balance demonstrates that the value for total salts removed by both leaching events in the MS was, on average, 57.5%, of which over 88% occurred in the first leaching event. In the OS this value is slightly lower, with 50.6% of salts removed, 85%

of which occurred in the first leaching event. In the case of sodium, there was a 53% removal in the MS (93% in the first leaching event) and 38.7% in the OS (88% in the first leachate).

2.5.2 Saline groundwater capillary rise

This test was designed to simulate the effects of capillary rise of saline water through the soil profile and a summary of the results obtained can be found in Table 2.3.

Table 2.3 – Saline groundwater capillary rise simulation results with two types of soils: Top soil samples – different times, refers to capillary rise evolution; Soil samples – different depths, refers to assessment of vertical distribution of salts (*Mann-Whitney U, all remaining statistical results are independent t-tests; r indicates effect size, the results are expressed as units ± % relative standard deviation).

	Top soil samples - different times							
	1st extract				2nd extract			
	MS	OS	Statistics		MS	OS	Statistics	
			p-value	r			p-value	r
EC (dS m^{-1})	0.9±35.8	4.7±39.6	0.025	0.75	7.2±39.5	20.9±64.6	0.161	0.43
pH	6.9±11.0	6.3±1.1	0.08*	0.71	5.1±16.7	6.5±2.3	0.055	0.64
Ca^{2+}Mg^{2+} (meq L^{-1})	5.3±31.1	62.2±40.0	0.017	0.80	60.6±43.3	256.0±60.6	0.098	0.54
	Soil samples – different depths							
	0-10 cm				10-20 cm			
EC (dS m^{-1})	12.6±18.9	22±18.6	0.026	0.75	21.5±17.2	36.6±23.2	0.048	0.67
pH	3.5±4.4	5.8±5.3	0.000	0.97	3.5±3.1	6.2±4.0	0.08*	-0.71
Ca^{2+}Mg^{2+} (meq L^{-1})	57.0±7.2	123.6±11.0	0.001	0.94	22.8±25.2	43.1±36	0.100	0.53
Na+ (meq L)	107.0±10.7	141.8±8.7	0.023	0.76	88.7±13.9	102.3±9.1	0.201	0.37
SAR	16.1±24.4	23.8±38.2	0.254	0.31	26.5±13.8	24.7±15.6	0.592	0.08

Due to its physical characteristics, the OS had a higher capillary fringe (20 cm) than the MS (17 cm) and reached the maximum level of soil depth tested (20 cm), although it is possible that it would have been higher if more soil height was available. These soil samples were small and tested at high dilution rates, which explains the high variability observed in this phase, in particular for calcium + magnesium results. Nevertheless, in both extracts, both the EC and calcium + magnesium values were superior in the OS than in the MS, and an increase over time is clearly visible, as expected.

When all of the soil was removed from the columns and analyzed at two different depths, several differences between the soil types in terms of EC, sodium and calcium + magnesium levels were observed in the top 10 cm of soil. The OS, due to its higher capacity for capillary rise, showed higher values for all variables, except SAR. Lack of a statistically significant difference between the two soils for SAR values (despite large differences in calcium + magnesium, as well as in sodium) may have been due to concurrent increases of sodium and calcium + magnesium in such a way that it prevented any difference in the ratio between these concentrations when expressed as SAR.

At a higher soil depth (10-20 cm) the differences for all parameters are less pronounced, certainly because the forced evaporation created in the experimental design was not enough to reach this depth. This would explain lower calcium + magnesium and sodium levels observed at this depth, when compared to the first 10 cm of soil, resulting in the existence of few statistically different cases between soil types.

The pH of the MS, as previously observed in the seawater inundation tests, is significantly lower than the value for the OS at both soil depths.

Further statistical analysis (paired t-tests, data not shown in Table 2.3) comparing different soil depths for the same soil type reveal that only calcium + magnesium content is significantly different between the two soil layers in the OS ($p = 0.0033$; $r = 0.99$), although differences in pH and sodium were close to be nearly significant ($p = 0.051$, $r = 0.90$; $p = 0.069$, $r = 0.87$, for pH and sodium, respectively), while SAR is very similar between soil depths for this soil ($p = 0.869$, $r = 0.02$). Concerning the MS, the same statistical test shows that there are more visible differences at different soil depths, mainly in calcium + magnesium ($p = 0.008$; $r = 0.98$) and SAR ($p = 0.033$; $r = 0.94$), with no other variables assayed having p values close to the 0.05 limit of significance.

2.5.3 Irrigation with brackish water

Irrigation tests with two different brackish water solutions (EC = 5 dS m^{-1}, but different RSC) were conducted in both MS and OS in order to simulate salt accumulation due to poor irrigation practices. Table 2.4 summarizes the main results obtained for the two experimental phases, salt addition and leaching.

Table 2.4 - Results of Irrigation with brackish water tests: Salt addition phase refers to the utilization of two different solutions ("RSC" and "no RSC" are solutions with or without residual sodium carbonate); Leaching phase refers to washing the soils with the same solutions as above (*Mann-Whitney U, all remaining statistical results are independent t-tests; r indicates effect size, results expressed as units ± % relative standard deviation).

	Salt addition phase							
	MS				OS			
	RSC	no RSC	Statistics		RSC	no RSC	Statistics	
			p-value	r			p-value	r
EC (dS m^{-1})	4.8±5.5	8.6±17.5	0.012	0.83	6.1±25.3	10.7±8.9	0.012	0.83
pH	7.2±3.9	5.3±2.4	0.000	0.97	7.4±2.5	7.1±4.0	0.194	0.38
Ca^{2+}+Mg^{2+} (meq L^{-1})	7.4±4.1	52.2±26.9	0.005	0.88	9.5±19.1	71.6±21.6	0.002	0.92
Na^{+} (meq L^{-1})	25.6±2.4	34.3±20.0	0.093	0.55	29.3±14.9	47.2±23.8	0.061	0.62
SAR	13.3±3.0	6.7±7.1	0.081*	0.71	13.4±6.0	8.00±27.6	0.016	0.80
	Leaching phase – Leachate solutions							
EC (dS m^{-1})	4.9±5.3	6.4±2.3	0.001	0.95	6.16±6.7	7.7±4.4	0.008	0.86
pH	5.0±3.8	4.8±1.9	0.134	0.47	6.1±1.7	6.0±2.2	0.157	0.43
Ca^{2+}+Mg^{2+} (meq L^{-1})	41.6±3.7	66.4±3.2	0.081*	-0.72	58.9±18.0	82±4.3	0.023	0.76
Na^{+} (meq L^{-1})	34.6±7.7	41.2±1.2	0.014	0.82	31.4±16.3	34.6±10.6	0.424	0.17
SAR	7.6±8.6	7.2±0.50	0.190*	0.53	5.9±23.1	5.4±12.9	0.633	0.06
	Leaching phase – soil after leaching							
EC (dS m^{-1})	3.4±3.2	5.1±6.5	0.001	0.95	3.8±4.1	6.5±12.9	0.005	0.88
pH	6.8±1.3	5.5±6.1	0.003	0.91	7.6±0.9	7.1±2.1	0.004	0.90
Ca^{2+}+Mg^{2+} (meq L^{-1})	15.3±39.6	41.5±7.0	0.003	0.92	8.7±9.3	42.4±11.4	0.000	0.97
Na^{+} (meq L^{-1})	22.0±12.7	28.0±4.1	0.026	0.75	27.3±13.5	31.1±15.3	0.343	0.22
SAR	8.5±35.7	6.2±1.5	0.663*	0.18	13.2±18.0	6.7±11.7	0.011	0.83

In the salt addition phase of the test, the irrigation of the MS and OS with either RSC or no RSC solutions yielded similar results. Sodium levels were 34 and 61% higher (MS and OS, respectively) when irrigation with no RSC was performed. However, as expected, calcium + magnesium concentrations were the parameter that changed more significantly when irrigation with no RSC was applied, with increases of 605 and 654% for MS and OS, respectively. This was accompanied by an EC increase of 79 and 76%, respectively, when compared with irrigation with high RSC. It is worthy to note that calcium + magnesium levels increase in the MS irrigated with RSC after leaching.

In the leaching phase, however, results obtained for the RSC and no RSC leachate solutions varied considerably. EC and calcium + magnesium levels were statistically different in both soils for samples with high RSC versus no RSC, although only the amount of sodium removed was significant in the MS. Since leaching occurred at such a different rate between soil types, with different removal of contaminants, the soil samples acquired after leaching reflected that difference: in the MS, SAR was not statistically different between high RSC and no RSC treatments, while in the OS, only sodium content was not statistically different between treatments. Despite this, the trends are similar to the results obtained before leaching: EC, with calcium + magnesium and sodium are higher after irrigation with no RSC solution, while pH and SAR are lower when compared with levels obtained after irrigation with high RSC water.

Furthermore, a statistical comparison between soil types (data not shown in Table 2.4) was performed. In the salt addition phase of the test, only the pH is significantly different when comparing the soil types with no RSC ($p = 0.0005$, $r = 0.96$), which may indicate that the increase of pH due to carbonate addition compensates for the potential pH drop normally seen in the MS. In the leachate solution samples, every single parameter was different in the MS treated with no RSC solution when compared with the OS in the same situation. For the high RSC solution, sodium and SAR levels in the leachate were not statistically different between the different soil types. Comparing the two soils, based on the soil samples obtained after leaching, it can be observed that the EC ($p = 0.02$, $r = 0.76$ for high RSC and $p = 0.049$, $r = 0.66$ for low RSC) and pH ($p = 0.0002$, $r = 0.98$ for high RSC and $p = 0.0020$ $r = 0.93$ for low RSC) are statistically different in both low and high RSC level treatments.

2.6 Discussion

This study examined the impact of three different but often interconnected salinization processes: short term seawater inundation, capillary rise of brackish groundwater and irrigation with brackish water of varying qualities. The impacts of these processes on two different soil types (MS and OS), as well as the implications of the obtained results, are analyzed herein.

Detailing the soil response to specific tests, the MS showed a higher SAR value than the OS after initial salt addition in the seawater inundation test. However, leaching with distilled water (simulating heavy rain) removed less calcium + magnesium and more sodium in MS, resulting in a larger SAR reduction (indicated by large values of SAR in the two leachates) with an estimated value of SAR similar to that of the OS at the end of the test (data not shown). These results may be due to the lower infiltration rate already mentioned for the MS, which increases the contact time of both the salt solution and the leaching solution. However, if this infiltration rate is significantly reduced, it may lead to higher salt content due to evaporation, and leaching would become increasingly less effective. An example of the impact of this situation can be found in (McLeod et al. 2010) for instance, where drainage problems prevented the natural restoration of adequate soluble salts levels in soils affected by the 2004 tsunami, despite the large amount of available rainfall for leaching.

In this test, the first leaching event removed the largest quantity of salts, as previously reported by other authors (Qadir et al. 2003): in the MS, the first leaching removed an estimated 14 kg of dissolved salts and 4.7 kg of sodium for every cubic meter of leaching water applied, while the second leaching event only removed 1.9 kg of total salts and 0.38 kg of sodium per applied cubic meter. In the OS, a similar reduction was found (12.7 kg of total salts and 3.2 kg of sodium in the first and 2.2 kg of total salts and 0.43 kg of sodium per applied cubic meter in the second leaching event). However, leaching may not always be desirable, since nutrients may also be leached away as indicated in our experiment by the values of dissolved calcium + magnesium that were effectively reduced by 36 and 82% in the MS and OS, respectively. Natural leaching due to heavy rain can lead to further salinization of groundwater resources after inundation events like tsunamis (Violette et al. 2009). To improve leaching and prevent water logging, the installation of a drainage system may be required (Ritzema et al. 2008). In extreme cases where inundation is frequent, biosaline agriculture of halophytes with high salinity and flooding tolerance may be an option (Glenn et al. 2013).

In the capillary rise test, the capillary fringe was higher in the OS, reaching the top 20 cm, while in the MS it only reached 16 or 17 cm. This is likely due to the different pore sizes of both soils (macro but especially micropores). Furthermore, organic content is likely to aid in capillary rise. This can be seen in other studies in which organic matter addition increased the extent of capillary rise (Eusufzai and Fujii 2012) as well as water retention (Pandey and Shukla 2006). In the soils tested, the accumulation of calcium + magnesium observed on the surface layer of the soil is caused by the addition of sodium, which displaced the calcium + magnesium from the exchange sites deep in the soil. Afterwards, the capillary rise transported these ions upwards (since they are now dissolved in the soil solution) onto the soil surface, where constant evaporation and compensatory rise of saline water in cycles further contributed to their accumulation. Since the OS has more total calcium + magnesium, the expected larger concentration of these ions on the soil surface was in fact observed. Ibrahimi et al. (2013) used a similar lysimeter setup and observed the same salt accumulation pattern due to capillary rise, but they additionally observed that groundwater table variations may lead to further accumulation, as opposed to a stable groundwater level (as simulated in our work). Potential mitigation solutions for capillary rise include the control of the water table by planting deep rooted and salt tolerant trees with high water consumption (Herron et al. 2003) and by applying physical capillary barriers made up of coarse materials, which can retard capillary rise (Ityel et al. 2014).

Irrigation with brackish water with high levels of RSC and salinity was also tested in this work with results that agree with those that can be found in the literature. Irrigation with brackish water with high RSC resulted in increased soil EC, pH and SAR, as well as in reduced infiltration rates as seen in Choudhary et al. (2010). In Minhas et al. (2007) similar trends were found: ESP increased likely due to the precipitation of calcite and gypsum. However, in an experiment in which the saturated soil paste extract was concentrated multiple times through evaporation, some sodium precipitation was detected, possibly contributing to ESP reduction. This fact may also help to explain why there was less sodium in soils irrigated with a higher RSC solution than in our work, as some portion of sodium might have precipitated.

Based on our results obtained after leaching, it is not clear whether the RSC affected salt leaching or if leaching increased the solubility of potentially precipitated calcium or magnesium carbonates, which resulted in a simultaneous reduction in EC levels. However, irrigation with RSC seems to have negatively impacted sodium mobility, which points more strongly to a problem of infiltration rate rather than precipitation.

Application of organic matter could be employed to decrease pH and dissolve calcium and/or magnesium carbonates (Qadir et al 2005), therefore limiting the negative effects of the applied irrigation water with high levels of RSC. However, this organic matter would also increase the cation exchange capacity of the soil and likely lead to further sodium accumulation. Furthermore, the positive impact of organic matter is only likely to be significant when the soil is calcareous and pH reduction mobilizes significant amounts of calcium to assist in the displacement of sodium on the cation exchange sites of the soil (Choudhary et al. 2011). In the case of the two soils tested in this work, the addition of organic matter would not make sense in the case of OS. For the MS, the exchangeable acidity would be much more significant in reducing pH than the addition of organic matter.

Comparing the initial irrigation solutions with the resulting leachate, the latter actually had a lower SAR value than the original. Consequently, a reuse of this leachate could prove beneficial in different aspects: 1) it would ensure that salt leaching, as well as nutrient and water loss, into groundwater is minimized (Glenn et al. 2009), 2) in the case of the MS leachate, the low pH could prove to be beneficial in mobilizing calcium, particularly in calcareous soils (Sadiq et al. 2007), 3) the required drainage system would also prevent water logging due to flooding and/or structural damages (as seen in the MS) caused by sodium (Ritzema et al. 2008). However, the main problems associated with this potential approach are the high cost of an adequate drainage system and that the leaching rate would likely have to be less frequent than the one simulated in this work. This would result in leachates with higher EC than observed. Several options could be chosen to ensure good soil quality for agricultural production depending on the level of the resulting EC. These include traditional physical desalinization treatments (Abulnour et al. 2003; Bunani et al. *in press*), a focus on salt tolerant plants production or fiber crop production (Barbosa et al. *in press*) or even, if appropriately managed and studied, mixing the saline leachate with high quality water to increase available water quantity at the expense of quality (Barnes 2012).

Ultimately, the different responses of the two soils to salinization were related to their initial characteristics throughout all of the three tests performed: the high exchangeable acidity and low exchangeable bases of the MS helped to explain the pH reduction and higher SAR developed with salt addition, while the OS, with its high organic content, had qualitatively higher structural stability, as well as higher capillary strength and lower SAR values.

2.7 Conclusions

In this work, the two different soils tested had different responses to the salinization processes, with important consequences to potential mitigation strategies. The MS was more prone to SAR accumulation due to low levels of native calcium + magnesium, but had higher leaching efficiency and more limited effects of RSC due to its high exchangeable acidity. The OS, on the other hand, was more prone to capillary rise, but seemingly was more structurally stable than the MS.

Short term inundation with seawater can be partially mitigated by leaching with high quality water and/or rainfall whenever it is available, although soil fertility and structure may be negatively affected. Capillary rise of brackish groundwater should also be carefully monitored in coastal agricultural areas, where perched water tables are frequent. In soils not yet affected by salinity, sodium and calcium + magnesium were mobilized deeper in the soil and transported upwards. This led to the accumulation of these cations at the surface of the soil and/or at the limit of the capillary fringe as demonstrated in this work.

In the tested conditions, irrigation can be maintained within acceptable limits of EC and SAR for salt tolerant crops using brackish water with high RSC, provided that frequent leaching is maintained as well. Soils with high exchangeable acidity are able to counteract the negative effects of carbonates in the irrigation water, although this capacity is likely limited over time.

Future research on soil salinization should focus on remediation options and how their efficiency might be affected by different soil types.

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3 Effect of plants in constructed wetlands for organic carbon and nutrient removal: a review of contributing factors for higher impact and experimental standards recommendations

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3.1 Keywords

Constructed wetlands; impact of the plants; performance comparison; plant uptake; experimental standards

3.2 Abstract

The present review details the effect of plants on removal efficiency of constructed wetlands by focusing on literature that includes unplanted controls for organic carbon and nutrient (N and P) removal. The contribution of plant direct uptake is also assessed. Although it was found that several studies showed no statistical differences between planted and unplanted controls, some factors were found that help maximize the effect of plants. This study intends to stimulate a discussion on the controversial role and effect of plants in a constructed wetland, as well as to provide suggestions and recommendations for a normalization of experimental procedures in this field.

3.3 Introduction

Constructed wetlands have been applied worldwide as a treatment option for a great variety of industrial and domestic wastewaters such as tannery (Calheiros et al., 2012), municipal (Jinadasa et al., 2008) and aquaculture (Jesus et al., 2014) to name a few. As a greener and cheaper alternative to more traditional and centralized treatment options, constructed wetlands play a major role in controlling inputs of contaminants into both fresh and marine water ecosystems.

However, this technology has yet to be fully understood, that is, while its removal efficiency is proven, the mechanisms by which treatment is achieved, as well as their respective contribution, remain associated with a high degree of uncertainty. One such aspects refers to the controversial role of vegetation in promoting treatment (Karathanasis et al., 2003; Lee and Scholz, 2007; Fia et al., 2014;Marchand et al., 2014).

Vegetation, either emergent, floating or submersed, is one of the most defining features of a constructed wetland. Regardless, doubts remain on which and how significant is the effect of plants to the treatment (Vymazal, 2011).

Clarifying these aspects is of extreme importance for the design of new constructed wetlands and management of existing ones. Comparisons of different studies in constructed wetlands, however, are always limited by the diversity of experimental conditions between different studies and should be interpreted with care.

The information that can be extracted from reviewing several studies is, therefore, limited by the lack of standards related to research and reporting in constructed wetlands, both at the laboratory and in field tests. Among other aspects, there are no standards on minimum number of replicates, preferred statistical analytic tests or the prerequisite of unplanted controls in most / all studies. On the other hand, strict standardization might limit the exploration of novel aspects. Therefore, a normalization procedure, both easily applied and yet capable of contributing to cement and consolidate CW technology, is required. As the goal of all scientific studies is to increment our collective knowledge on any given topic, some standardization is a pre requisite to enable a study contribution to the literature.

Nevertheless, despite limitations, several relevant reviews of the constructed wetland technology have provided important insights when appropriately focused on specific topics. For instance, Zhang et al. (2015) reviewed a variety of different constructed wetlands in tropical and subtropical climates, while Wu et al. (2014) detailed novel developments to increase CW performance and Brisson and Chazarenc (2009) analyzed in detail the impact of the choice of different plant species in overall performance.

Still, a poorly explored perspective relates to the effect of plants on treatment efficiency when compared to unplanted controls. In research articles that have such controls, comparisons are often qualitative, i.e., it is only highlighted whether or not plant systems outperformed unplanted controls, even if at times only by 1-5% difference in efficiency. In the rare cases when statistical analysis are performed for this comparison, several studies report no significant differences for organic carbon and nutrient removal for instance Calheiros et al., (2007) and Klomjek and Nitisoravut, (2005), heavy metals (Marchand et al., 2014), fecal and total coliforms (Karathanasis et al., 2003) and antibiotic removal (Cardinal et al., 2014; Verlicchi and Zambello, 2014). Even review papers such as that of Vymazal (2011) only report comparisons on relative terms such as positive, negative or no effect of plant presence.

Furthermore, other reviews often evaluate the potential of different plant species without taking into account the removal observed in the unplanted control. Although important (Brisson and Chazarenc, 2009), this perspective is limited to comparisons of the whole system and might under or overestimate the relative performance of different plant species.

An example can be considered to illustrate how the relative performance of plant species can be under or overestimated: comparing BOD_5 removal efficiency of the plant *Typha angustifolia* system from Jinadasa et al. (2008), with that of *Spartina patens* from Klomjek and Nitisoravut (2005), the conclusion would be that the latter system had higher removal efficiency since it removed 75.3% instead of 53.8% in the first one. However, the *Spartina patens* planted system only removed 6% more than its unplanted counterpart, while the *Typha angustifolia* planted system removed 17% more than the control. In terms of the potential of the plant species to increase removal efficiency, the question may arise on which species should be the one considered to have more potential.

A possible method to partially isolate the effect of the plants on treatment efficiency is to evaluate the contribution of plant uptake, since doubts persist on the relevance of this removal mechanism (Vymazal and Kröpfelová, 2008; Ye et al., 2016). Clarification of this aspect is important to assess the need to harvest plants in order to remove nutrients from the system.

The present review aims to detail and compare the effect of plants on removal efficiency of constructed wetlands by focusing on literature that includes unplanted controls, particularly for organic carbon and nutrient (N and P removal). More precisely, this work has the following specific objectives:

- Characterize and compare removal efficiency of several studies normalized for the removal of unplanted controls;
- Tentatively identify experimental conditions that maximize the effect of plants;
- Compare the contribution of plant uptake on removal efficiency;
- Contribute to a proposal of standardization procedure for future work to enhance the precision and validity of the observed trends;

3.4 Search methodology, study selection and analytic strategy

Considering the objectives of this study, search was focused on published articles having unplanted controls describing organic carbon and nutrient (N and P) removal in a constructed wetland. A preliminary search was performed in ScienceDirect using the keyword “constructed wetlands” and 2012-2016 as the range. This analysis was intended as a preliminary assessment of how many recent studies might use unplanted controls and statistical analysis. The first 200 results out of thousands retrieved were analyzed for content, with around 106 found to be outside the scope of this review (not focused on organic carbon or nutrient removal). Of the remaining 94 articles, it was found that 64% of them did not have unplanted controls and 51% lacked statistical comparative analysis and only 24% had both. This analysis indicated that restricting the research to studies with both unplanted controls and statistical analysis would be too restrictive and limiting.

Subsequent search, therefore, include the results of multiple web based libraries (http://link.springer.com/; http://www.tandfonline.com/ and sciencedirect.com) with the same keyword. Studies were selected based on the following premises:

1) Studies of constructed wetlands, of any size, with unplanted controls;
2) Studies that include multiple parameters of organic and nutrient removal;
3) Studies with results expressed in such a way as to allow rapid and simple comparison with others.

Several articles were excluded by a variety of reasons, but mostly due to lack of precision on data reporting such as no data for the control system, unrealistically large hydraulic retention time, different and therefore uncomparable removal efficiency calculation, etc., which precluded a precise comparison.

To enable an easier comparison, the large volume of data gathered was divided into two major Tables: Table 3.1, focusing on experimental conditions for each test, while Table 3.2 details removal efficiency of control systems and the difference of efficiency between planted systems and unplanted control.

An initial extensive collection of comparable experimental parameters was performed. Ultimately, however, not all data acquired was found in sufficient quantity or quality to be reported. It was therefore chosen to report the following parameters only: plant species, system type, substrate used, HRT, number of replicates, wastewater type, study duration and plant density (Table 3.1).

The data is organized by experimental setup with an associated test number meaning that the same article can be referenced several times if it tested different conditions simultaneously.

Data on removal efficiency for several parameters was calculated by subtracting the removal percent obtained for the unplanted control from the percent removal efficiency obtained for planted systems. The analyzed parameters (Table

3.2) focused on organic matter removal (BOD_5, COD) and nutrient removal (N and P). Nitrate was not included as only a small minority of studies report data on its removal efficiency.

It was also identified which of the differences obtained in percent removal efficiency represented a statistically different result between planted and unplanted systems. When available, the present study reports the results of the statistical tests from the original articles. However, as many studies lacked such statistical analysis, an assumption was used denoting likely statistically significant differences (represented as Ya, meaning statistically significant differences assumed, in Table 3.2).

The previous assumption was based on a simplified and hypothetical experimental design setup with three replicates for planted and unplanted situations each. The data for each parameter were considered to have a relative standard deviation lower than 5%, normal distribution and homogeneous variance. This scenario is more likely to overestimate the effect of the plants rather than underestimate it, as several reviewed studies have less than 3 replicates and a relative standard deviation higher than 5%.

As the data from this constructed scenario had normal distribution and homogeneous variance, a t-test could be applied and, under the set conditions, an average 13% percent removal efficiency difference of planted over unplanted system would be required to obtain a statistically significant ($p < 0.05$) and positive effect of the plant in the treatment.

Therefore, this simplified metric was applied and is referenced in Table 3.2, i.e., all data points from studies without statistical analysis and with removal efficiency difference over +13%, were marked as assumed statistically different (Ya).

After an analysis of the variations of experimental conditions of selected studies from Table 3.1, a more in-depth analysis of removal efficiency difference was performed.

The analytic strategy used can be described as follows: Removal efficiency differences were analyzed by either the main contaminant parameters (organic matter, phosphorus, total nitrogen and ammonia) or by varying experimental conditions. The analysis for the main parameters was initially performed by aggregating descriptive and generic statistics, with particular emphasis on the difference between statistically vs. non-statistically significant results. This general analysis is followed by the description of more specific trends that may be identified for each parameter.

In contrast, for the analysis of the impact of varying experimental conditions a focus was given to comparisons from within the same study or, alternatively, between very similar experimental tests from different studies, to attempt to tentatively identify under which conditions the effect of plants is more relevant to treatment efficiency.

3.5 Analysis of Constructed wetland studies

The experimental conditions of the studies that met our criteria can be found in detail on Table 3.1.

Table 3.1 - Experimental conditions of selected studies ordered by constructed wetland system type (FWS followed by HSSF and VSSF) and detailing plant species used, system type, substrate, hydraulic retention time (HRT), number of replicates, wastewater type, test duration and plant density (Notes: [a] - Nº of experimental units per treatment, [b] - Varying C/N ratios used, DB - denitrifying bacteria added; CF - continuous flow), [S1] - Salinity level of 2.5%, [S2] - salinity level of 0.5, [S3] - salinity level of 2; O – Outdoor, T – Tropical, ST - subtropical M – Moderate, No indication - indoors, temperate; - - no information).

Source	Test Nº ref	Plant Species	System Type / Location	Substrate	HRT (days)	Nº of units[a]	Wastewater type	Test duration (days)	Plant Density (plant m^{-2})
(Jinadasa et al., 2008)	1	*Scirpus grossus*	FWS / O / T	coarse soil + gravel	1	1	municipal	510	4
(Jinadasa et al., 2008)	2	*Typha angustifolia*	FWS/ O / T	coarse soil + gravel	1	1	municipal	510	4
(Klomjek and Nitisoravut, 2005)	3	*Typha angustifolia* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	4	*Digitaria bicornis* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	5	*Leptochloa fusca* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	6	*Cyperus corymbosus* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	7	*Spartina patents* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	8	*Vetiveria zizaniodes* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Klomjek and Nitisoravut, 2005)	9	*Brachiaria mutica* [S1]	FWS	Sand + Soil	5	3	municipal	60	11
(Akratos and Tsihrintzis, 2007)	10	*Phragmites australis*	HSSF / O / M	medium gravel	6 to 20	1	municipal (simulated)	730	-
(Akratos and Tsihrintzis, 2007)	11	*Typha latifolia*	HSSF / O / M	medium gravel	6 to 20	1	municipal (simulated)	730	-
(Calheiros et al., 2007)	12	*Canna indica*	HSSF / O / M	Filtralite MR 3-8	6.8	1	tannery	391	10
(Calheiros et al., 2007)	13	*Typha latifolia*	HSSF / O / M	Filtralite MR 3-8	6.8	1	tannery	391	10
(Calheiros et al., 2007)	14	*Phragmites australis*	HSSF/ O / M	Filtralite MR 3-8	6.8	1	tannery	391	10
(Calheiros et al., 2007)	15	*Stenotaphrum secundatum*	HSSF/ O / M	Filtralite MR 3-8	6.8	1	tannery	391	10
(Calheiros et al., 2007)	16	*Iris pseudacorus*	HSSF/ O / M	Filtralite MR 3-8	6.8	1	tannery	391	10
(Calheiros et al., 2007)	17	*Canna indica*	HSSF/ O / M	Filtralite MR 3-8	3.4	1	tannery	511	10
(Calheiros et al., 2007)	18	*Typha latifolia*	HSSF/ O / M	Filtralite MR 3-8	3.4	1	tannery	511	10
(Calheiros et al., 2007)	19	*Phragmites australis*	HSSF/ O / M	Filtralite MR 3-8	3.4	1	tannery	511	10
(Calheiros et al., 2007)	20	*Stenotaphrum secundatum*	HSSF/ O / M	Filtralite MR 3-8	3.4	1	tannery	511	10
(Calheiros et al., 2007)	21	*Iris pseudacorus*	HSSF/ O / M	Filtralite MR 3-8	3.4	1	tannery	511	10
(Camacho et al., 2007)	22	*Phragmites australis*	HSSF/ O / M	gravel	7.6	1	municipal (simulated)	300	-
(Camacho et al., 2007)	23	*Lythrum salicaria*	HSSF/ O / M	gravel	7.6	1	municipal (simulated)	300	-
(Camacho et al., 2007)	24	*Cladium mariscus*	HSSF/ O / M	gravel	7.6	1	municipal (simulated)	300	-

Source	Test N° ref	Plant Species	System Type / Location	Substrate	HRT (days)	N° of units[a]	Wastewater type	Test duration (days)	Plant Density (plant m^{-2})
(Camacho et al., 2007)	25	*Iris pseudacorus*	HSSF/ O / M	gravel	7.6	1	municipal (simulated)	300	-
(Ciria et al., 2005)	26	*Typha latifolia*	HSSF/ O / M	gravel	4 to 7	1	raw municipal	730	3
(Coleman et al., 2001)	27	*Typha latifolia*	HSSF / O	gravel	6 to 8	2	pre-treated municipal	365	10
(Coleman et al., 2001)	28	*Scirpus validus*	HSSF/ O	gravel	6 to 8	2	pre-treated municipal	365	10
(Coleman et al., 2001)	29	*Juncus effusus*	HSSF/ O	gravel	6 to 8	2	pre-treated municipal	365	10
(Coleman et al., 2001)	30	mixture of plants	HSSF/ O	gravel	6 to 8	2	pre-treated municipal	365	10
(Gao et al., 2015)	31	*Phragmites. australis* [S2]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Gao et al., 2015)	32	*Canna indica* [S2]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Gao et al., 2015)	33	*Scirpus validus* [S2]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Gao et al., 2015)	34	*Phragmites australis* [S3]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Gao et al., 2015)	35	*Canna indica* [S3]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Gao et al., 2015)	36	*Scirpus validus* [S3]	HSSF / O / ST	gravel + soil	3	3	saline municipal	113	15
(Idris et al., 2012)	37	*Arundo donax* [S2]	HSSF / O / T	gravel	10.6	3	dairy processing	568	4.68
(Idris et al., 2012)	38	*Phragmites australis* [S2]	HSSF / O / T	gravel	10.6	3	dairy processing	568	12.5
(Kaseva, 2004)	39	*Typha latifolia*	HSSF / O / T	gravel 8-25 mm	2.5	1	pre-treated municipal	42	-
(Kaseva, 2004)	40	*Phragmites mauritianus*	HSSF / O / T	gravel 8-25 mm	2.5	1	pre-treated municipal	42	-
(Madera-Parra et al., 2015)	41	*Gynerum sagittatum*	HSSF	gravel	3	2	landfill leachate	60	17
(Madera-Parra et al., 2015)	42	*Heliconia psittacorum*	HSSF	gravel	3	2	landfill leachate	60	17
(Madera-Parra et al., 2015)	43	*Colocasia esculenta*	HSSF	gravel	3	2	landfill leachate	60	17
(Mbuligwe, 2004)	44	*Typha latifolia*	HSSF / O / T	sand	1.2	1	pre-treated municipal	36	6
(Mbuligwe, 2004)	45	*Colocasia esculenta*	HSSF/ O / T	sand	1.2	1	pre-treated municipal	36	6
(Sousa et al., 2011)	46	*Spartina alterniflora*[S3]	HSSF / O / T	oyster shell + sand	0.25	1	Aquaculture shrimp	28	18 kg
(Toscano et al., 2015)	47	*Vetiveria zizaniodes*	HSSF / O / T	volcanic gravel	13	2	tertiary municipal	270	4
(Toscano et al., 2015)	48	*Myscanthus x giganteus*	HSSF / O / T	volcanic gravel	13	2	tertiary municipal	270	4
(Toscano et al., 2015)	49	*Arundo donax*	HSSF O / T	volcanic gravel	13	2	tertiary municipal	270	4
(Toscano et al., 2015)	50	*Phragmites australis*	HSSF O / T	volcanic gravel	13	2	tertiary municipal	270	4
(Wang et al., 2016)	51	*Typha orientalis*	HSSF / O	sand	1	4	artificial	56	40
(Wang et al., 2016)	52	*Scirpus validus*	HSSF/ O	sand	1	4	artificial	56	40

Source	Test Nº ref	Plant Species	System Type / Location	Substrate	HRT (days)	Nº of units[a]	Wastewater type	Test duration (days)	Plant Density (plant m^{-2})
(Wang et al., 2016)	53	*Canna indica*	HSSF/ O	sand	1	4	artificial	56	40
(Wang et al., 2016)	54	*Iris tectorum*	HSSF/ O	sand	1	4	artificial	56	40
(Wang et al., 2016)	55	*Typha orientalis*	HSSF/ O	sand	2	4	artificial	56	40
(Wang et al., 2016)	56	*Scirpus validus*	HSSF/ O	sand	2	4	artificial	56	40
(Wang et al., 2016)	57	*Canna indica*	HSSF/ O	sand	2	4	artificial	56	40
(Wang et al., 2016)	58	*Iris tectorum*	HSSF/ O	sand	2	4	artificial	56	40
(Wang et al., 2016)	59	*Typha orientalis*	HSSF/ O	sand	3	4	artificial	56	40
(Wang et al., 2016)	60	*Scirpus validus*	HSSF/ O	sand	3	4	artificial	56	40
(Wang et al., 2016)	61	*Canna indica*	HSSF/ O	sand	3	4	artificial	56	40
(Wang et al., 2016)	62	*Iris tectorum*	HSSF/ O	sand	3	4	artificial	56	40
(Wang et al., 2016)	63	*Typha orientalis*	HSSF/ O	sand	4	4	artificial	56	40
(Wang et al., 2016)	64	*Scirpus validus*	HSSF/ O	sand	4	4	artificial	56	40
(Wang et al., 2016)	65	*Canna indica*	HSSF/ O	sand	4	4	artificial	56	40
(Wang et al., 2016)	66	*Iris tectorum*	HSSF/ O	sand	4	4	artificial	56	40
(Zhang et al., 2012)	67	*Typha angustifolia*	HSSF / O / T	gravel	2	3	artificial	> 60	14-15
(Zhang et al., 2012)	68	*Typha angustifolia*	HSSF / O / T	gravel	4	3	artificial	> 60	14-15
(Zhang et al., 2012)	69	*Typha angustifolia*	HSSF (CF) / O / T	gravel	2	3	artificial	> 60	14-15
(Zhang et al., 2012)	70	*Typha angustifolia*	HSSF (CF) /O / T	gravel	4	3	artificial	> 60	14-15
(Abdelhakeem et al., 2016)	71	*P. australis*	VSSF	gravel 5-10 mm	0.5	-	raw municipal	240	67
(Abdelhakeem et al., 2016)	72	*P. australis*	VSSF (CF)	gravel 5-10 mm	0.5	-	raw municipal	240	67
(Abdelhakeem et al., 2016)	73	*P. australis*	VSSF	vermiculite 5 mm	0.5	-	raw municipal	240	67
(Abdelhakeem et al., 2016)	74	*P. australis*	VSSF (CF)	vermiculite 5 mm	0.5	-	raw municipal	240	67
(Mustapha et al., 2015)	75	*Cyperus alternifolius*	VSSF / O / T	Gravel + sand	2	2	Refinery wastewater	365	-
(Mustapha et al., 2015)	76	*Cynodon dactylon*	VSSF / O / T	gravel + sand	2	2	Refinery wastewater	365	-
(Sarmento et al., 2011)	77	*Cyperus sp*	VSSF	pea gravel	3	1	swine	> 90	10.6
(Shao et al., 2014)	78	*Phragmites australis* + DB	VSSF / O	soil + sand	7	3	river water (simulated)	16	42
(Shao et al., 2014)	79	*Phragmites australis*	VSSF/ O	soil + sand	7	3	river water (simulated)	16	42
(Shao et al., 2014)	80	*Phragmites australis* + DB	VSSF/ O	soil + sand	7	3	municipal (simulated)	16	42

Source	Test N° ref	Plant Species	System Type / Location	Substrate	HRT (days)	N° of units[a]	Wastewater type	Test duration (days)	Plant Density (plant m^{-2})
(Shao et al., 2014)	81	*Phragmites australis*	VSSF/ O	soil + sand	7	3	municipal (simulated)	16	42
(Tee et al., 2012)	82	*Typha latifolia*	VSSF / O	rice husks + gravel	2	2	municipal + nitrogen spike	90	44
(Tee et al., 2012)	83	*Typha latifolia*	VSSF / O	rice husks + gravel	3	2	municipal + nitrogen spike	90	44
(Tee et al., 2012)	84	*Typha latifolia*	VSSF / O	rice husks + gravel	5	2	municipal + nitrogen spike	90	44
(Tee et al., 2012)	85	*Typha latifolia*	Baffled / O	rice husks + gravel	2	2	municipal + nitrogen spike	90	44
(Tee et al., 2012)	86	*Typha latifolia*	Baffled / O	rice husks + gravel	3	2	municipal + nitrogen spike	90	44
(Tee et al., 2012)	87	*Typha latifolia*	Baffled / O	rice husks + gravel	5	2	municipal + nitrogen spike	90	44
(Yalcuk, 2012)	88	*Typha angustifolia*	VSSF	zeolite + gravel	4.2	1	70% cheese whey powder	100	-
(Yalcuk, 2012)	89	*Typha angustifolia*	VSSF	zeolite + gravel	4.2	1	40% cheese whey powder	100	-
(Zhao et al., 2010)*	90	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Zhao et al., 2010)*	91	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Zhao et al., 2010)*	92	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Zhao et al., 2010)*	93	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Zhao et al., 2010)*	94	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Zhao et al., 2010)*	95	*Lythrum salicaria*	VSSF	Gravel + slag	1.5	1	Simulated municipal[b]	270	16.7-20
(Arunbabu et al., 2015)	96	*Axonopus compressus*	RHSSF	6-10 mm gravel	10	2	synthetic greywater	50	-

* These experimental setups differ on C/N ratios used.

As also observed by Brisson and Chazarenc (2009), most studies have little or no replication (no more than one experimental unit per treatment test), as well as either no, or misapplied, statistical analysis, often based on repeated measures, which is significantly less robust than statistical analysis of different replicated units.

Although not referenced in Table 3.1, another important aspect refers to the scale of the treatment systems, as most studies can be considered lab scale microcosms or small scale outdoor systems. Interpretation of data from microcosms has to be made with care, as these studies should be followed by field tests and validated under more realistic conditions. Furthermore, such small scale tests should be accompanied by more replicates. However, it is not surprising that most studies selected have small scales as it is extremely rare that a large scale test includes an unplanted control system.

Several studies were conducted outdoors but with varying conditions, as some were protected from the effect of the rain by covering structures, while others were not. Report of climatic conditions is also often short or completely missing.

The values of hydraulic retention time referenced in Table 3.1 averaged 4.1±3.0 days with a minimum of 0.25 and a maximum of 13 days, with 75% of the cases below 5 days (with a clear outlier of 20 days found in Akratos et al (2007) excluded from this analysis). As for plant density, average values of 25±18 plants per m2, with a minimum of 3 and a maximum of 67, were found and finally, test duration averaged 210±186 days ranging from 16 to 730 days, with 75% of the data below 332 days.

From the experimental data analyzed it is also important to point out that not all of them are reported in every study, which makes comparisons between studies far more difficult. Furthermore, hydraulic retention time may reflect continuous or batch setups, often without a proper distinction between these two systems.

Also, test duration is important to evaluate the maturity of the system and appropriate determinations of plant growth. The so-called start-up phase or effect, is characterized by highly erratic data when it comes to performance of the system. However, the duration or even the existence of this period remains under debate. Estimates vary widely: 7 months (Taylor et al., 2011), 1 year (Stefanakis and Tsihrintzis, 2012); 3 years (Molle et al., 2008). This period might have an important impact on whether or not the plant presence is statistically meaningful as, due to erratic data sets, large standard deviations among replicates might preclude the detection of a statistically significant differences, particularly in cases in which there is low number of replicates.

Substrate types used in these systems are mostly gravel, sand, natural soil or filtralite, alone or in combination. As for wastewater sources, the analyzed studies tested wastewaters from different industries such as dairy, tannery, swine production, aquaculture (both marine and freshwater) and refinery. Other studies tested municipal wastewater, either raw or pre-treated while the remaining mostly tested artificial or simulated wastewaters. This attests the great versatility of constructed wetlands, but also limits the quality of comparisons between studies.

3.6 Test comparison

Data on removal percentage difference between planted systems and unplanted controls were compared (Table 3.2) and are analyzed parameter by parameter (organic matter, phosphorus, total nitrogen and ammonia removal). Assumed statistical significance (Ya) is established on the result of a theoretical t-test ($p < 0.05$) based on the simplified scenario referenced before, which adopts that a difference larger +13% is assumed to be statistically significant.

Table 3.2 - Relative difference in removal efficiency between planted and unplanted treatments for different types of pollutants. (Notes: SS? Statistical significant? (only for positive differences); Y - Yes; N - No; Ya - Assumed statistical significance; NT = not tested; %UC - percentage removal of unplanted control - Δ% removal efficiency difference - planted minus unplanted treatments; BOD_5 - Biochemical Oxygen Demand 5 days ; COD - Chemical Oxygen Demand; DB - Denitrifying Bacteria; TP - total phosphorus; TN - Total Nitrogen; TON - Total Organic Nitrogen; TKN - Total Kjeldahl Nitrogen). Test reference number refers to data in Table 3.1; and the symbol (-) refers to no available data.

Test n° ref	Plant species	Organic matter removal				Phosphorus removal				Total nitrogen removal				Ammonia removal		
			UC%	Δ%	SS?		UC%	Δ%	SS?		UC%	Δ%	SS?	UC%	Δ%	SS?
1	*S. grossus*	BOD_5	37	22	Ya	TP	5	6	N	-	-	-	-	42	17	Ya
2	*T. angustifolia*	BOD_5	37	17	Ya	TP	5	4	N	-	-	-	-	42	15	Ya
3	*T. angustifolia*	BOD_5	70	5	N	TP	31	14	Ya	-	-	-	-	71	4	NT
4	*D. bicornis*	BOD_5	70	9	N	TP	31	-3	NT	-	-	-	-	71	-1	NT
5	*L. fusca*	BOD_5	70	5	N	TP	31	6	NT	-	-	-	-	71	3	NT
6	*C. corymbosus*	BOD_5	70	8	N	TP	31	4	NT	-	-	-	-	71	-4	NT
7	*S. patents*	BOD_5	70	6	N	TP	31	4	NT	-	-	-	-	71	-3	NT
8	*V. zizaniodes*	BOD_5	70	3	N	TP	31	7	NT	-	-	-	-	71	5	NT
9	*B. mutica*	BOD_5	70	8	N	TP	31	3	NT	-	-	-	-	71	-3	NT
10	*P. australis*	COD	87	-2	NT	TP	43	-22	NT	TKN	34	21	Ya	0	36	Ya
11	*T. latifolia*	COD	87	2	NT	TP	43	16	Ya	TKN	34	33	Ya	0	54	Ya
12	*C. indica*	COD	54	2	N	TP	-33	-7	N	TKN	26	0	N	20	0	N
13	*T. latifolia*	COD	54	3	N	TP	-33	10	N	TKN	26	-1	N	20	0	N
14	*P. australis*	COD	54	4	N	TP	-33	-13	N	TKN	26	1	N	20	-3	N
15	*S. secundatum*	COD	54	1	N	TP	-33	-20	N	TKN	26	1	N	20	-3	N
16	*I. pseudacorus*	COD	54	1	N	TP	-33	-53	N	TKN	26	1	N	8	-1	N
17	*C. indica*	COD	61	1	N	TP	-8	4	N	TKN	25	1	N	8	2	N
18	*T. latifolia*	COD	61	4	N	TP	-8	-4	N	TKN	25	-1	N	8	-1	N
19	*P. australis*	COD	61	2	N	TP	-8	-8	N	TKN	25	-2	N	8	1	N
20	*S. secundatum*	COD	61	1	N	TP	-8	0	N	TKN	25	-1	N	8	2	N
21	*I. pseudacorus*	COD	61	2	N	TP	-8	-4	N	TKN	25	1	N	8	0	N
22	*P. australis*	COD	81	0	NT	TP	18	-1	NT	TN	36	-2	NT	6	4	NT
23	*L. salicaria*	COD	81	9	NT	TP	18	23	Ya	TN	36	12	NT	6	20	Ya

Test nº ref	Plant species	Organic matter removal				Phosphorus removal				Total nitrogen removal				Ammonia removal		
			UC%	Δ%	SS?		UC%	Δ%	SS?		UC%	Δ%	SS?	UC%	Δ%	SS?
24	*C. mariscus*	COD	81	6	NT	TP	18	10	NT	TN	36	0	NT	6	-2	NT
25	*I. pseudacorus*	COD	81	8	NT	TP	18	18	Ya	TN	36	19	Ya	6	29	Ya
26	*T. latifolia*	COD	75	4	N	TP	31	8	N	-	-	-	-	10	9	N
27	*J. effusus*	BOD_5	69	-4	NT	-	-	-	-	TKN	29	19	Ya	30	20	Y
28	*S. validus*	BOD_5	69	1	NT	-	-	-	-	TKN	29	-3	NT	30	-5	NT
29	*T. latifolia*	BOD_5	69	7	NT	-	-	-	-	TKN	29	33	Ya	30	31	Ya
30	mixed species	BOD_5	69	5	NT	-	-	-	-	TKN	29	46	Ya	30	43	Ya
31	*P. australis*	COD	58	12	NT	TP	40	5	NT	TN	57	15	Ya	60	10	NT
32	*C. indica*	COD	58	7	NT	TP	40	2	NT	TN	57	11	NT	60	6	NT
33	*S. validus*	COD	58	5	NT	TP	40	1	NT	TN	57	5	NT	60	4	NT
34	*P. australis*	COD	48	8	NT	TP	32	8	NT	TN	46	4	NT	42	10	NT
35	*C. indica*	COD	48	4	NT	TP	32	6	NT	TN	46	0	NT	42	8	NT
36	*S. validus*	COD	48	6	NT	TP	32	14	Ya	TN	46	2	NT	42	10	NT
37	*A. donax*	BOD_5	66	3	N	TP	-3	3	N	TN	26	0	N	25	6	N
38	*P. australis*	BOD_5	66	-4	N	TP	-3	0	N	TN	26	7	N	25	14	Ya
39	*P. mauritianus*	COD	34	23	Ya	-	-	-	-	-	-	-	-	11	15	Ya
40	*T. latifolia*	COD	34	27	Ya	-	-	-	-	-	-	-	-	11	12	NT
41	*G. sagittatum*	COD	58	9	NT	PO_4-P	84	7	NT	TKN	66	2	NT	68	5	NT
42	*H. psittacorum*	COD	58	5	NT	PO_4-P	84	-7	NT	TKN	66	2	NT	68	1	NT
43	*C. esculenta*	COD	58	11	NT	PO_4-P	84	-7	NT	TKN	66	0	NT	68	4	NT
44	*T. latifolia*	COD	65	15	Y	PO_4-P	51	18	Y	-	-	-	-	63	11	Y
45	*C. esculenta*	COD	65	10	Y	PO_4-P	51	24	Y	-	-	-	-	63	12	Y
46	*S. alterniflora*	-	-	-	-	PO_4	34	18	Y	TN	67	-7	N	79	-13	N
47	*V. zizaniodes*	COD	55	13	Ya	PO_4	23	0	NT	TN	43	17	Ya	39	19	Ya
48	*M. x giganteus*	COD	55	12	NT	PO_4	23	-8	NT	TN	43	18	Ya	39	19	Ya
49	*A. donax*	COD	55	11	NT	PO_4	23	3	NT	TN	43	18	Ya	39	22	Ya
50	*P. australis*	COD	55	14	Ya	PO_4	23	0	NT	TN	43	20	Ya	39	21	Ya

Test n° ref	Plant species	Organic matter removal				Phosphorus removal				Total nitrogen removal				Ammonia removal		
			UC%	Δ%	SS?		UC%	Δ%	SS?		UC%	Δ%	SS?	UC%	Δ%	SS?
51	*T. orientalis*	COD	20	12	Y	-	-	-	-	TN	21	0	N	11	2	N
52	*S. validus*	COD	20	11	Y	-	-	-	-	TN	21	0	N	11	2	N
53	*C. indica*	COD	20	20	Y	-	-	-	-	TN	21	3	N	11	4	Y
54	*I tectorum*	COD	20	17	Y	-	-	-	-	TN	21	1	N	11	2	N
55	*T. orientalis*	COD	35	10	Y	-	-	-	-	TN	27	2	N	17	2	N
56	*S. validus*	COD	35	6	N	-	-	-	-	TN	27	2	N	17	1	N
57	*C. indica*	COD	35	16	Y	-	-	-	-	TN	27	4	N	17	5	Y
58	*I tectorum*	COD	35	5	N	-	-	-	-	TN	27	1	N	17	0	N
59	*T. orientalis*	COD	46	5	N	-	-	-	-	TN	30	2	N	22	2	N
60	*S. validus*	COD	46	5	N	-	-	-	-	TN	30	3	N	22	2	N
61	*C. indica*	COD	46	11	Y	-	-	-	-	TN	30	4	Y	22	6	Y
62	*I tectorum*	COD	46	7	N	-	-	-	-	TN	30	1	N	22	1	N
63	*T. orientalis*	COD	59	4	N	-	-	-	-	TN	32	1	N	27	2	N
64	*S. validus*	COD	59	5	N	-	-	-	-	TN	32	2	N	27	1	N
65	*C. indica*	COD	59	7	N	-	-	-	-	TN	32	5	N	27	5	Y
66	*I tectorum*	COD	59	5	N	-	-	-	-	TN	32	2	N	27	1	N
67	*T. angustifolia*	COD	90	3	N	TP	37	21	Y	-	-	-	-	46	47	Y
68	*T. angustifolia*	COD	93	3	N	TP	39	31	Y	-	-	-	-	49	46	Y
69	*T. angustifolia*	COD	88	3	N	TP	28	2	Y	-	-	-	-	47	22	Y
70	*T. angustifolia*	COD	91	5	Y	TP	26	21	Y	-	-	-	-	47	33	Y
71	*P. australis*	COD	43	40	Y	TP	7	12	N	-	-	-	-	22	-3	N
72	*P. australis*	COD	33	52	Y	TP	15	1	N	-	-	-	-	23	13	N
73	*P. australis*	COD	37	46	Y	TP	20	11	N	-	-	-	-	22	4	N
74	*P. australis*	COD	33	54	Y	TP	27	-3	N	-	-	-	-	37	11	N
75	*C. alternifolius*	COD	40	25	N	PO_4-P	17	26	N	-	-	-	-	5	63.3	Y
76	*C. dactylon*	COD	40	23	N	PO_4-P	17	25	N	-	-	-	-	5	65.0	Y
77	*Cyperus sp*	COD	66	2	N	TP	44	12	Y	TKN	29	9	Y	-	-	-

Test n° ref	Plant species	Organic matter removal				Phosphorus removal				Total nitrogen removal				Ammonia removal		
			UC%	Δ%	SS?		UC%	Δ%	SS?		UC%	Δ%	SS?	UC%	Δ%	SS?
78	*P. australis + DB*	COD	41	35	Ya	TP	24	66	Ya	TN	31	65	Ya	39	58	Ya
79	*P. australis*	COD	41	27	Ya	TP	24	2	NT	TN	31	3	NT	39	8	NT
80	*P. australis + DB*	COD	59	27	Ya	TP	60	28	Ya	TN	39	40	Ya	45	44	Ya
81	*P. australis*	COD	59	5	NT	TP	60	5	NT	TN	39	6	NT	45	8	NT
82	*T. latifolia*	COD	42	8	Y	-	-	-	-	TON	98	0	NT	26	9	NT
83	*T. latifolia*	COD	50	10	Y	-	-	-	-	TON	99	0	NT	35	35	Ya
84	*T. latifolia*	COD	59	10	Y	-	-	-	-	TON	100	0	NT	42	54	Ya
85	*T. latifolia*	COD	49	10	Y	-	-	-	-	TON	99	1	NT	35	39	Ya
86	*T. latifolia*	COD	60	7	Y	-	-	-	-	TON	100	0	NT	43	41	Ya
87	*T. latifolia*	COD	66	13	Y	-	-	-	-	TON	100	0	NT	49	50	Ya
88	*T. angustifolia*	COD	25	13	Y	PO_4-P	36	10	Y	-	-	-	-	63	6	N
89	*T. angustifolia*	COD	28	23	Y	PO_4-P	23	32	Y	-	-	-	-	66	-2	N
90	*L. salicaria*	COD	51	13	N	TP	60	5	N	TN	38	19	Y	-	-	-
91	*L. salicaria*	COD	52	16	N	TP	64	7	N	TN	46	5	N	-	-	-
92	*L. salicaria*	COD	47	17	N	TP	58	8	N	TN	41	-6	N	-	-	-
93	*L. salicaria*	COD	46	14	N	TP	35	24	Y	TN	25	1	N	-	-	-
94	*L. salicaria*	COD	52	16	N	TP	64	7	N	TN	46	5	N	-	-	-
95	*L. salicaria*	COD	41	18	N	TP	45	18	Y	TN	48	14	N	-	-	-
96	*A. compressus*	COD	85	10	NT	PO_4-P	59	9	NT	-	-	-	-	-	-	-

3.6.1 Organic carbon removal

For organic carbon removal, expressed in mg L-1 for either BOD_5 or COD, roughly 31 out of 72 statistically tested conditions were found to be affected by the presence of plants. Of these 31, however, 9 were assumed to be significant based on our simplification as referred above, as data were not analyzed statistically in the original source study. Therefore, only 22 out of 72 statistically tested conditions were demonstrably found to be affected by the presence of plants for this parameter, while 32 out of all 104 referenced conditions were not tested for statistical significance.

Average removal difference (Δ%) for organic carbon removal for statistically significant data points (including those assumed) is 19.89±12.91%, ranging from 4.8 to 54%, while for non-statistically significant results average results are 6.51±6.18%, with a range of -4% to 25%. These results are marked by clear outliers, particularly for non-significant results: for example, Mustapha et al. (2015) reported that planted treatment was 23-25% more efficient in organic carbon removal than unplanted treatments, but these results were not statistically different. This result may be due to insufficient number of replicates (in this case, duplicates), which precludes the use of a strong statistical test, regardless of the obtained differences. Other studies with non-significant results have replicates with large standard deviations e.g. Zhao et al. (2010).

All this points to the fact that more replicates could provide statistically different results, and an increased number of replicates should be considered to clarify the role and impact of the presence of plants in microcosm tests.

In stark contrast, Wang et al. (2016) obtained statistically different results with a low average removal difference of 10%, which is below the threshold value of 13% assumed in our study. However, the authors used 4 replicates, which had low standard deviations among them. Under the assumptions referenced above, the threshold value for statistical difference assumed in this study would decrease from 13 to 10% if 4, not 3, replicates were used.

In a study not included in Table 3.2, Taylor et al. (2011) tested 19 different plant species on their capacity to reduce COD levels of 490±4.3 mg L^{-1} from a synthetic wastewater, in a batch subsurface flow lab scale constructed wetland. Most tested plant species performed similarly at varying temperatures, while unplanted controls have a significantly lower performance at 4ºC: at 4º and 8ºC planted microcosms removed 25-30% more COD than unplanted controls, and <20% more at 16ºC, all of which were statistically different results. However, at 24ºC, no statistical differences in performance between planted and unplanted controls were detected, denotating the importance of temperature on the evaluation of the impact of the presence of plants.

Furthermore, this study also mentions that widely used and researched species, such as *T. latifolia* and *P. australis*, provided less effective treatment, again highlighting that different and less used species might provide added benefits over more traditionally used ones.

3.6.2 Phosphorus removal

For phosphorus removal, expressed in mg L^{-1} for either TP or PO_4^{3-}, roughly 19 out of 44 statistically tested conditions were found to be affected by the presence of plants. Of these 19, however, 7 were assumed to be significant based on our simplification as referred before, as data were not tested in the original source study. Therefore, only 12 out of 44 statistically tested conditions were demonstrably found to be affected by the presence of plants for this parameter, while 31 out of all 75 referenced conditions were not tested for statistical significance.

Average removal difference for statistically significant data points (including assumed) is 21.54±12.71%, ranging from 1.8 to 66.2%, while for non-statistically significant results average results are -1.0±14.88% with a range of -53.3% to 26%. As previously mentioned for organic carbon removal, outlier results, particularly for non-significant data, can be traced back to lower number of replicates or higher resulting standard deviations.

Even in tests where the experimental design was similar, a wide variety of phosphorus removal results was found for different plant species. For example, Camacho et al. (2007) obtained phosphorus removal of 10% for *C. mariscus* but 18% for *I. pseudacorus* when compared to unplanted controls (which the authors attributed to different plant growth).

Certain studies in Table 3.2, by testing the same plant species in different settings, were able to highlight important aspects that might impact phosphorus removal, such as the hydraulic conditions, since continuous flow promoted a higher difference between planted and unplanted microcosms than batch conditions in Zhang et al. (2012).

For phosphorus removal difference, calculated between planted and unplanted microcosms as shown in Table 3.2, there is a greater number of testing conditions where said difference is negative (i.e. there is phosphorus accumulation), when compared to other parameters such as organic carbon or total nitrogen. Akratos and Tsihrintzis (2007) attributed this lower removal by planted microcosms to reducing conditions, which can lead to the release of dissolved phosphorus, as well as to litter decomposition.

3.6.3 Total nitrogen removal

For total nitrogen removal, expressed in mg L^{-1} for either TN, TON, TKN, roughly 16 out of 49 statistically tested conditions were found to be affected by the presence of plants. Of these 16, however, 13 were assumed to be significant based on our simplification as referred above, as data were not tested in the original source study. Therefore, only 3 out of 49 statistically tested conditions were demonstrably found to be affected by the presence of plants for this parameter, while 33 out of all 82 referenced conditions were not tested for statistical significance.

Average removal difference for statistically significant data (including assumed values) is 24.65±14.78%, ranging from 4.2 to 65%, while for non-statistically significant results average results are 1.54±3.46% with a range of -7% to 13.72%.

It should be noted, however, that some statistically significant removal data referenced in Table 3.2 were only made possible due to the addition of denitrifying bacteria, such as is the case of Shao et al. (2014). In this study inoculum addition increased total nitrogen removal significantly, but as no replicates were made with just inoculum added and no plant, it is unclear whether or not such removal was due to the combined effects of both plant and inoculum presence, or if similar removal could be simply achieved by adding inoculum alone.

Similarly to what was found for phosphorus removal, Coleman et al. (2001) report again varying differences of removal efficiency between different plant species under the same experimental conditions, while Wang et al. (2016) tested 16 different combinations of conditions (Table 3.2), but only found one plant where TN removal was significantly and positively affected by the presence of plants which was *Canna Indica*.

3.6.4 Ammonia removal

For ammonia removal, expressed in mg L^{-1} for NH_4^+-N, roughly 34 out of 65 statistically tested conditions were found to be affected by the presence of plants. Of these 34, however, 21 were assumed to be significant based on our simplification as referred above, as data were not tested in the original source study. Therefore, only 13 out of 65 statistically conditions were demonstrably found to be affected by the presence of plants for this parameter, while 44 out of all 109 referenced conditions were not tested for statistical significance.

Average removal difference for statistically significant data points (including those assumed) is 29.83±17.64%, ranging from 3.80 to 65%, while for non-statistically significant results average results are 1.47±4.51% with a range of -13% to 13%.

Generally speaking, observed ammonia removal difference (Δ%) between planted and unplanted systems follows similar trends as total nitrogen, as can been seen in Table 3.2.

Wang et al. (2016) used a particularly high concentration of ammonia, up to 400 mg L^{-1}, to test the physiological responses to its exposure of four different wetland plants, finding that two out four plants were highly suppressed at values of 100 mg L^{-1}. This is yet another important aspect of research in constructed wetlands: to test the limits of application of this technology to varying concentrations and situations.

3.6.5 The effect of different experimental conditions

Other experimental conditions that might impact the removal efficiency of all analyzed parameters include the hydraulic retention time, substrate type and salinity. However, the goal of the present review is not to analyze the impact of varying conditions on overall removal efficiency, but is rather on the effect of the plants in removal, as an attempt to identify the conditions under which the effect of the presence of the plants is more significant.

For this purpose, average results of removal efficiency differences between planted and unplanted conditions, considering the wide variety of experimental conditions that vary simultaneously, can only provide a general overview. Instead, as mentioned previously, comparisons from within the same study or, alternatively, between very similar experimental tests from across different studies, might provide more precise insights.

In the case of varying hydraulic retention time, for example, in the tests conducted in Calheiros et al. (2007), HRT does not seem to impact the effect of the plants in any significant way, when comparing the same plant species at the two different HRT tested. The only exception to this trend was found for total phosphorus, but it was likely due to the very low initial values of TP used, and so any variation in concentration would reflect disproportionally on removal efficiency.

In the tests conducted in Wang et al. (2016), on the other hand, even though higher retention time always increased overall performance, all tested plants seemingly decreased their effect for COD removal at increasing HRT. Indeed, COD removal from planted systems decreased to the point that it was no longer statistically higher than unplanted controls. For the remaining parameters, the effect of increasing HRT was less evident: no trend can be seen for ammonia, but increased total nitrogen removal was found for two out of four tested plants.

Finally, Tee et al. (2012) tested simultaneously two different systems (vertical (VSSF) and baffled systems) as well as varying HRT. Again, although the baffled system had increased overall performance in COD removal, planted microcosms, when corrected for unplanted performance, did not indicate any relevant difference. The most significant difference, however, was found in ammonia removal: not only the baffled system performed better, but also the presence of plants had a seemingly larger effect, particularly for the lowest HRT of 2 days. At higher retention times, however, the effect of baffled system and the presence of plants were diluted and less expressive.

Salinity is yet another factor to consider. While testing in different salinity scenarios, Gao et al. (2015) found that salinity increases from 0.5 to 2.0% clearly and in a statistical significant way, decreased overall removal efficiency for all tested parameters (COD, TP, TN and ammonia). However, in the case of COD for all three tested plants, and also for TN and *Scirpus validus*, the reduction in the plant removal effect is lower than the overall removal decrease. On the other hand, for TP and ammonia, the effect of plants tended to increase with increasing salinity, despite overall reduction of removal efficiency.

For substrate type and hydraulic flow, tests conducted in Abdelhakeem et al. (2016) suggest that COD and ammonia overall removal efficiency, as well as the effect of plants, increases in continuous flow when compared to batch mode, but the opposite can be seen for TP. This study also suggests that vermiculite can increase overall TP removal, but this is likely a reflection of sorption by the substrate, as the effect of plants on treatment removal is not statistically different with vermiculite when compared to gravel.

Zhang et al. (2012), on the other hand, found that TP and ammonia overall removal efficiency (and with a statistically significant effect), as well as the effect of plants, decreases with continuous flow when compared to batch tests. The authors suggest that differences in ammonia removal could be due to the fact that a batch drain and fill mode presented more oxidized environmental conditions.

3.7 Contribution of plant nutrient uptake to removal efficiency

The contribution of plant uptake to removal efficiency is calculated by evaluating the total mass of nitrogen and phosphorus removed into plant tissues and divided by the total mass (not concentration) removed by the system. A new simple search (not meant to be exhaustive, but focused on comparable results) provides some indications of this parameter for different systems (Table 3.3).

Based on the search performed, the contribution of plant uptake (Table 3.3) to nutrient removal presents a wide range of values. These can be as low as 0.98% and -4.8% or as high as 93 and 74.87% for nitrogen and phosphorus, respectively.

Tests conducted within the same conditions but with different plant species (e.g., Wu et al 2011) stress the fact that plant contribution through uptake is specific to plant species, where different plants can have contributions several times higher than others.

Even the same plant species, tested in different conditions, can have widely different contributions to nutrient removal: for *Typha latifolia,* for instance, it ranges from 1.73% to 8.81% for nitrogen removal, while it ranges from 0.06 up to 74.87% for phosphorus; for *Canna indica* nitrogen and phosphorus removal uptake varies from 0.98 to 17.95% and 0.43 to 4.17%, respectively; and finally, *Phragmites australis* contributes to 7.15 to17.04% for nitrogen and 0.56 to 36.7% for phosphorus removal (Table 3.3).

Table 3.3 - The effect of plant nutrient uptake to the removal of nitrogen and phosphorus (in %) in different constructed wetlands simulated scenarios (FWS – Free water surface, SSF – Subsurface flow, HSSF - Horizontal subsurface flow, VSSF – vertical subsurface flow, B – baffled, NS – new substrate).

Plant species	%N	%P	System type	Notes	Source
Phragmites australis	16.75	36.71	FWS	1st year harvested	(Zheng et al., 2015)
Phragmites australis	17.04	34.19	FWS	1st year unharvested	(Zheng et al., 2015)
Phragmites australis	16.2	32.02	FWS	2nd year harvested	(Zheng et al., 2015)
Phragmites australis	16.32	35.93	FWS	2nd year unharvested	(Zheng et al., 2015)
Typha latifolia	4.02	35.53	VSSF	bottom aeration	(Tang et al., 2008)
Typha latifolia	3.94	42.54	VSSF	bottom aeration + NS	(Tang et al., 2008)
Typha latifolia	8.8	74.87	VSSF	middle aeration	(Tang et al., 2008)
Typha latifolia	8.19	69.42	VSSF	middle aeration + NS	(Tang et al., 2008)
Canna indica	17.95	4.17	Tidal	-	(Ye et al., 2016)
Arachis duranensis	21.5	10.4	SSF		(Van et al., 2015)
Evovulus alsinoides	1.3	-4.8	SSF	-	(Van et al., 2015)
Cyperus alternifolius	93	29.8	SSF	-	(Van et al., 2015)
Philodendron hastatum	16.8	3.5	SSF	-	(Van et al., 2015)
Canna indica	2.21	0.7	B HSSF	-	(Cui et al., 2015)
Canna indica	2.27	1.4	B VSSF	-	(Cui et al., 2015)
Canna indica	1.45	0.49	B Hybrid	-	(Cui et al., 2015)
Canna indica	0.98	0.43	HSSF	-	(Cui et al., 2015)
Phalaris arundinacea	46.3	45.9	SSF	lower input	(Březinová and Vymazal, 2015)
Phalaris arundinacea	6.4	3.1	SSF	higher input	(Březinová and Vymazal, 2015)
Typha orientalis	21	14.31	VSSF	-	(Wu et al., 2011)
Phragmites australis	14.29	10.76	VSSF	-	(Wu et al., 2011)
Scirpus validus	45.52	32.27	VSSF	-	(Wu et al., 2011)
Iris pseudacorus	51.89	34.17	VSSF	-	(Wu et al., 2011)
Phragmites australis	7.15	0.56	HSSF	-	(Meng et al., 2014)
Arundo Donax	7.49	0.36	HSSF	-	(Meng et al., 2014)
Typha latifolia	1.73	0.06	HSSF	-	(Meng et al., 2014)
Iris sibirica	19.86	13.19	VSSF	high nutrient	(Gao et al., 2014)
Iris sibirica	23.9	14.23	VSSF	medium nutrient	(Gao et al., 2014)
Iris sibirica	50.19	22.32	VSSF	low nutrient	(Gao et al., 2014)

Other parameters that are reported to influence plant uptake contribution include root surface area and root oxidizing capacity (Jiang et al., 2011), as well as aeration and substrate type (Tang et al., 2008), and loading rate (Gao et al., 2014; Březinová and Vymazal, 2015).

It is natural to assume that higher productivity might be linked to higher plant uptake as a whole, but that is not always necessarily the case. For instance, in Wu et al. (2011), among other examples, *Typha orientalis* had the highest aboveground biomass increase, but its contribution was 25% and 16% lower than *Scirpus validus* for nitrogen and phosphorus removal, respectively.

Yet another important aspect for a correct assessment of the contribution of plant uptake to nutrient removal refers to nutrient translocation to the aboveground biomass. This aspect is made more difficult to analyze by the fact that not all studies differentiate between below and aboveground biomass when measuring, or reporting, the acquired data of nutrient accumulation in plant tissues. Aboveground accumulation is preferred, in order to enable nutrient removal from the constructed wetland through harvesting of aerial biomass.

Plant harvest should be planned in order to maximize nutrient removal and plant recovery and also taking into consideration maximum productivity due to seasonality issues (Weller et al., 2016). Yet maximum nutrient accumulation and maximum aerial plant biomass do not necessarily occur simultaneously (Kyambadde et al., 2005).

Unfortunately, the few studies found that evaluate the contribution of plant uptake often lack unplanted controls with which to compare this specific plant contribution to its overall effect, i.e., what is the exact role of plant uptake on increased treatment efficiency provided by planted systems over unplanted ones.

3.8 Recommendations for normalization procedures

Throughout this study some trends were identified where the effect of plants to the treatment is more noticeable. However, a significant number of tested conditions analyzed reported a small or non-existent effect of plants. To the different results obtained, experimental planning, design and analysis can have played a role.

In an ideally designed and executed experimental system, the lack of differences would simply reflect that the tested planted species, under the tested conditions, are unable to positively affect treatment efficiency. However, it is unclear, in many instances, whether the experimental design and data analysis negatively affected the outcome.

The importance of a minimum number of replicates and its impact on data interpretation has been already highlighted in the present work. Furthermore, the comparisons made in this study were also severely hampered by a significant number of articles which could not be used, as they failed to either perform or report data on unplanted controls.

In order to be able to increase in the future the number of identified trends regarding the effect of plants in the future, as well as strengthening the validity of the existing ones, several suggestions and recommendations are herein described (Table 3.4), particularly focused for lab scale or outdoor small scale studies (although some may also apply to the monitoring of full scale constructed wetlands).

In terms of experimental conditions, the use of unplanted controls and at least 3-5 replicates should be a minimum requirement. Several studies tend to use just one replicate for the unplanted control, but for statistical purposes, a similar number of replicates for all tested conditions is a pre-requisite. Autoclaved unplanted controls could provide information on abiotic removal processes, and should be particularly considered for parameters that are expected to be highly impacted by sorption processes.

Table 3.4 - Recommendations and suggestions for normalization, divided by experimental conditions, monitoring needs and data reporting and analysis, with a suggested level of recommendation associated (required, recommended or highly recommended).

Experimental conditions	Level
- Unplanted controls	Required
- Autoclaved unplanted controls	Recommended
- 3-5 replicates, equal number to all conditions tested, including controls	Required
- Synthetic, lab prepared wastewater	Highly recommended
- Greenhouse or chamber with adjustable temperature, humidity, photoperiod & light intensity	Recommended
- Tests without substrate (hydroponics)	Recommended
Monitoring needs	
- Climatic conditions such as temperature, humidity, illuminance and rainfall when applicable	Required
- Evaporation and transpiration measurements	Required
- Biofilm formation and/or microbial count	Recommended
- Redox potential measurements	Recommended
- Plant growth (parameters such as dry mass, plant height, etc.)	Required
- Plant tissue sample analysis	Highly recommended
Data reporting and analysis	
- Report all the following experimental conditions: Plant species, hydraulic retention time, system type, substrate, nº growth days, plant density, nº of replicates per treatment	Required
- Detailed description of the system such as substrate type and layers, sampling points, water flow, etc.	Required
- Report mass balance and percent removal efficiency simultaneously	Required
- Proper statistical analysis with effect size analysis and methods description	Required

Higher standardization and use of more replicates for unplanted controls might open up possibilities to extract more information when comparing studies. For instance, studies using different substrates, tested under similar conditions, provide indications of which substrate can provide better conditions for microbial biofilm growth and/or higher removal by sorption.

Furthermore, steps should be taken to minimize variability as much as possible. For that purpose, the use of synthetic laboratory prepared wastewater with precisely set and unchanging concentrations can provide more standardized information regarding the system capacity for treatment, and particularly the effect of the plants at least in an initial phase of the study. For similar reasons, climatic variability should also be controlled either by conducting the tests in sheltered greenhouses or alternatively in a chamber with controllable temperature, humidity, light period and intensity. Although these conditions do not reflect the real variability that may be encounter in the field, they should be preferred when the goal is to test the validity of constructed wetlands as a technology, particularly for less explored or completely novel applications.

Tests without substrate, i.e., in hydroponics conditions are recommended to better isolate the effect of plants, particularly plant uptake (Jiang et al., 2011; Keizer-Vlek et al., 2014), and should be used in combination with tests using substrates under similar conditions whenever possible.

Regarding monitoring needs, measurement of relevant climatic conditions, when applicable, should be seen as a requirement, with basic parameters such as air temperature, humidity, illuminance and rainfall. Evaporation and transpiration evaluation should also be a requirement to enable an improved mass balance analysis of contaminant removal.

Suggested parameters also include biofilm formation and/microbial count as well as the measurement of redox potential.

Finally, plant parameters are of extreme relevance and the evaluation of plant growth through a variety of means (plant height, dry mass variance, etc.) should also be a requirement. Furthermore, plant tissue sample analysis is highly recommended for contaminants that can be uptaken by the plants, such as N and P, salts and heavy metals.

Data reporting should be emphasized with as much information as possible on the experimental conditions and the system setup.

For the analysis of removal data, ideally a mass balance or load removal efficiency should be used for reporting the efficiency of these systems (Naylor et al., 2003; Bateganya et al., 2015; Toscano et al., 2015). However, mass balance is a lot less referenced than the more traditional calculation of removal efficiency RE(%) = [(Ci – Cf) / Ci] * 100 (where Ci and Cf refer to initial and final concentrations, respectively). This calculation, however, does not take into account the potentially larger reduction in wastewater volume in planted treatments, when compared to unplanted treatments, which might explain why differences in efficiency might not be statistically evident.

On the other hand, however, from a legislative point of view, most obligatory legal parameters refer to contaminant concentrations, not loads. In such cases, concentration alone is the most important parameter to monitor, regardless of the associated scientific adequacy.

As a result, both removal efficiency calculations should be made and reported, to more easily enable the comparisons of multiple studies and legal standards.

Furthermore, statistical analysis reported in the literature are, at times, non-existent, flawed or incomplete for the purposes of the analysis of the effect by plants. For instance, the use of dependent, rather than independent based statistical tests when comparing data sets through time hamper a correct interpretation of said data sets.

Additionally, statistical significance should be carefully interpreted: to aid in such future interpretation the effect size should also be initially calculated and reported as suggested in other fields (Briner and Kirwan, 2017), to enable a quick comparison of different studies with different number of replicates, as well as to differentiate between small, medium and large effects of the presence of plants.

Ultimately, however, the question might still persist: what is the required difference in removal efficiency between planted and unplanted systems required to make technical and/or economic sense to add plants to the treatment system?

Most, if not all of the suggestions and recommendations given are meant to be simple and relatively cheap ways to normalize and improve experimental setups and so contribute to answering the question posed above.

3.9 Final considerations

The effect of plants and associated rhizosphere on removal efficiency, based on the literature reviewed in this study, was found not to be always significant. In fact, in a large portion of the studies having statistical analysis, it was found no significant differences between planted and unplanted controls in 57% of the cases for organic carbon removal, 57% for total phosphorus, 67% for total nitrogen and in 48% of the cases analyzed for ammonia removal. Even taking into consideration the statistical assumption used in this study (which disproportionally favors positive statistical significance) to expand this analysis to more studies, these percentages actually increased only by 2 to 8% (except for total nitrogen).

These values are extremely high and should be a cause of reflection. Still, by analyzing the results of several studies, trends can potentially be identified and so, even seemingly negative results could provide information on the limits of constructed wetland technologies. Unfortunately, as mentioned throughout this study, lack of standardization severely weakens any trend identified.

As such, few trends can be identified and have limited validity. For instance, the results from some studies seem to suggest that lower hydraulic retention time and increasing salinity favor the effect of plants over unplanted controls for nutrient removal, while the impact of continuous flow, when compared to batch systems, showed conflicting data and was therefore inconclusive. Finally, the contribution of plant uptake for nitrogen and phosphorus removal in a Constructed Wetland seems to be positively affected by lower wastewater nutrient concentrations.

In the end, this study aims to spark a discussion on the need and nature of normalized procedures for constructed wetland research, providing suggestions and recommendations for that purpose, as well as highlighting the yet controversial role and effect of plants in a constructed wetland.

3.10 Acknowledgments

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4 Role of three different plants on simultaneous salt and nutrient reduction from saline synthetic wastewater in lab-scale constructed wetlands

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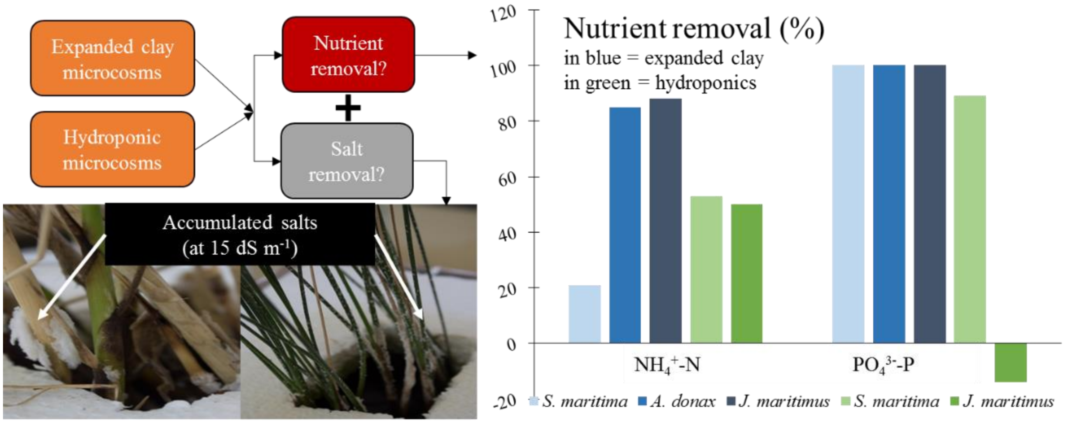
4.1 Highlights

- Simultaneous removal of salt and nutrients was tested under hypersaline conditions.
- Planted microcosms have higher ammonia and phosphate removal than unplanted ones.
- The tested plants have limited salt removal capacity for reasonable HRT values.
- Saline wastewater treatment in constructed wetlands is feasible with tested plants.

4.2 Keywords

Saline wastewater; Halophytes; Salt uptake; Nutrient removal; Constructed wetland; Hydroponics;

4.3 Graphical abstract



4.4 Abstract

Constructed Wetlands (CWs) can be a valuable technology to treat high salinity wastewaters but it is not known their potential for removal of both nutrients and salt, and the type of plants to use. This study evaluated the effect of three plants on salt reduction and simultaneous nutrient removal in CWs microcosms with expanded clay and in hydroponic conditions. Initial values of the synthetic wastewater tested were EC = 15 dS m^{-1}, SAR = 151; NH^{4+}-N = 24 mg L^{-1}; PO$_4^{3-}$-P = 30 mg L^{-1} and NO^{3-}-N = 34 mg L^{-1}.

With expanded clay CW removal efficiency for NH4^{+}-N was 21, 88 and 85%, while for NO^{3-}-N, it was 4, 56 and 68% for *Spartina maritima*, *Juncus maritimus* and *Arundo donax*, respectively. PO$_4^{3-}$-P was adsorbed completely in the expanded clay. However, in hydroponic system, removal efficiencies for NH^{4+}-N were 53 and 50%, while PO$_4^{3-}$-P removal was 89 and -14% for *Spartina maritima* and *Juncus maritimus*, respectively. Nutrient removal in planted microcosms was statistically higher than unplanted controls for NH4+-N and PO$_4^{3-}$-P.

However, salt removal was apparent in the hydroponic system only after 23 days of HRT, despite clear salt excretion visible in both *Spartina maritima* and *Juncus maritimus*.

This study demonstrates the potential of two halophytic plants for saline wastewater treatment. However, salt removal in such a scenario could not be well documented and might prove to be impractical in future work.

4.5 Introduction

Several industrial processes produce high strength wastewater with variable salinity and nutrient loads. These industries include conventional and unconventional oil and gas exploitation (Lutz et al., 2013), aquaculture (Jesus et al., 2013), tannery (Calheiros et al., 2012), winery production (Ioannou et al., 2015), and agricultural drainage waters (Qadir et al., 2003), amongst others.

The treatment of these wastewaters is necessary to reduce pollution and eutrophication risk in the discharge area due to organic matter and excess nutrients. However, the presence of dissolved salts can severely hinder the ability to reduce contaminants by conventional wastewater treatment plants due to microbial inhibition (Khengaoui et al., 2015). Furthermore, these treatment plants have limited to no capacity to remove the dissolved salts, which precludes discharge into sensitive surface waters without significant negative impacts on these ecosystems.

Additionally, considering existing and future freshwater scarcity, significant efforts have to be made for alternative water resources, particularly for agricultural irrigation in semi-arid regions. Reuse of treated wastewater is a potential solution but its use may be limited by salinity. Dissolved salts present in irrigation water can directly affect plant growth, accumulate in the soil and lead to soil structure degradation and water logging (US Salinity Laboratory, 1954. Ahmad et al 2013, Wang et al 2014). Several guidelines have been proposed for a threshold value of salinity that could be applied to soil that fall within a broad range between 0.5 dS m^{-1} to up to 2 dS m^{-1} (US Salinity Laboratory, 1954) depending on the plant culture in question, as well as the accompanying sodicity hazard (measured by the sodium adsorption ratio (SAR) or residual sodium carbonate (RSC). Nevertheless, moderately saline wastewaters can have variable EC values ranging from 1.27–8.12 dS m^{-1} from hydroponic systems (Chondraki et al., 2012) to 11-14 dS m^{-1} from cheese manufacturing (Prazeres et al., 2016). A treatment system that could reduce excess nutrients and salt simultaneously would be ideal, enabling either discharge to surface waters or reuse for irrigation or other purposes.

One type of wastewater treatment system that could potentially be able to perform such simultaneous treatment is constructed wetlands (CWs) (Shelef et al., 2012). CWs using halotolerant or halophytic plants are capable of reducing nutrient and organic loads in these high salinity wastewaters (Buhmann and Papenbrock, 2012; Jesus et al., 2013). Although, it is still unclear whether or not salt absorption by the plants is an effective method for salt removal within a CW (Shelef et al., 2012), since most studies using saline wastewater and CW treatment focus mostly or exclusively on plant salt tolerance rather than on salt removal.

However, the possibility of phytoremediation for salt removal in soils is already well established (Qadir and Oster, 2002; Qadir et al., 2006), although the removal mechanisms are still under debate. Therefore, this technology could be adapted to constructed wetlands if salt uptake is an important mechanism (Rabhi et al., 2010).

Plants have different salt tolerance mechanisms which might impact treatment efficiency (Yensen and Biel, 2006). This includes exclusion (plants that prevent salt from entering their tissues), accumulation (plants adapted to accumulation of salts within their tissues through various means) and excretion (plants with salt glands or trichomes capable of excreting excess salts). Although, plants with demonstrably high salt tolerance and biomass productivity might also be used for biosaline agriculture for forage or as a biofuel feedstock (Abideen et al., 2011). This is true even for those species exhibiting low salt removal.

Still another aspect not properly explored in the literature is the impact of salinity, or specific salts, on nutrient adsorption by CWs substrate. In particular, adsorption of phosphate to the substrate is considered the main removal mechanism of this nutrient in a CW, and several articles attempt to find the best performing substrate (Vohla et al., 2011). Nevertheless, increases in salinity might lead to desorption of nitrogen ions and improved phosphate removal by precipitation (Jun et al., 2013), or alternatively, decrease phosphate adsorption by competition for adsorption sites (Zhang and Huang, 2011).

Studies testing constructed wetland performance under high salinity conditions are not abundant in the literature and more so regarding the potential for salt removal. Furthermore, in the works investigating the high salinity CW issue, like the ones of Shelef et al (2012) and Gao et al (2015) for example, the objectives are focused either on salt or on nutrient reduction, but not on both aspects. Also, the adequate plant type and role under such conditions is still under debate. Therefore, it is not yet certain the feasibility of salt removal in CW. Additionally, studies are needed to analyze whether or not nutrient and salt can be removed simultaneously, and what is the role played by substrate and/or plants in such a scenario.

The objective of this study is to evaluate the impact of three different plants on the simultaneous removal or reduction of salt (EC, Na^+, Cl^-, SAR) and nutrients (NO^{3-}-N, NH^{4+}-N and PO_4^{3-}-P) using CW microcosms containing expanded clay, and also under hydroponic conditions. The plants were chosen based on known or expected different responses to salinity. The first one is *Arundo donax,* which is a halotolerant, high biomass productive plant that is very easy to propagate (Ceotto and Di Candilo, 2010). The second plant is *Spartina maritima,* which is an obligatory halophyte and tolerant to flooding and submersion (Duarte et al., 2013). It also has low biomass productivity and is capable of salt excretion via salt glands. Finally, the last plant is *Juncus maritimus*, which is tolerant to flooding (Álvarez Rogel et al., 2001) and also a halophyte with intermediate biomass productivity.

These plants have rarely (*Arundo donax)* or never (*Spartina maritima* and *Juncus maritimus*) been tested under constructed wetlands scenarios, despite their potential applications in other phytotechnologies such as soil remediation (Conesa et al., 2011; Mesa et al., 2015).

For a more complete picture of nutrient removal in constructed wetlands treating highly saline wastewater, tests with expanded clay as a substrate and tests in hydroponics were performed under planted and unplanted conditions. This was done to pinpoint the direct plant contribution on the removal of nutrients and salts.

4.6 Materials and methods

4.6.1 Synthetic saline wastewater preparation

A high salinity synthetic wastewater was prepared with NaCl (9.6 g L^{-1}) at 15 dS m^{-1} (resulting in Na^+ concentration of 3806 mg L^{-1} and Cl^- of 5867 mg L^{-1}). Final nutrient concentrations were 30 mg L^{-1} of PO_4^{3-}-P (prepared from analytical grade, $NaH_2PO_4{\cdot}H_2O$); 24 mg L^{-1} NH_4^+-N (prepared from analytical grade NH_4Cl) and/or (depending on the experiment), 34 mg L^{-1} of NO_3^--N (prepared from analytical grade $Ca(NO_3)_2$ and resulted in 2.43 meq L^{-1} of Ca^{2+} in the final solution). This solution had a resulting in SAR of 151 and pH close to 7. Micronutrients were also added at 50% of Hoagland solution (Hoagland and Arnon, 1950) strength, resulting in final concentrations of 0.25 mg L^{-1} Bo, 0.25 mg L^{-1} Mn; 0.025 mg L^{-1} Zn; 0.01 mg L^{-1} Cu; 0.005 mg L^{-1} Mo and 0.25 mg L^{-1} Fe. As no organic carbon source was added, BOD_5 level was assumed to be close to zero as previously tested in other studies with hydroponic wastewaters (Gagnon et al., 2010).

The generic saline wastewater formulation used was chosen so that the results obtained could be of value to a multitude of applications in hypersaline wastewater treatment. Selected nutrient concentrations match the range of several different wastewater types, but more closely resemble values found in hydroponic wastewaters (Park et al., 2008; Gagnon et al., 2010; Park et al., 2015), while also having low or zero BOD_5.

4.6.2 Greenhouse Microclimate Characterization

The location chosen for the tests was the window gallery of the building of the Department of Biology, University of Porto, with South/South-West solar exposure. Temperature, humidity (hygrometer TFA-DostmannTM) and light intensity (Lutron LX-150 lux meter) were monitored. The average conditions in the microcosm studies (February and June 2016) were as follows (n = 55): Temperature 14.5 ± 2.9◦C; Humidity 63.51 ± 7.29% and illuminance 4377.21 ± 3164.84 lx. Daylight hours were 10.1 hours in the Winter (minimum) and peaked at 15.1 hours in the Spring.

4.6.3 Plant Origin and Maintenance

Arundo donax specimens were collected in Vila Nova de Gaia, Portugal at approximately 500 m from the sea and were kept in the indoor location described above for three years before being used in the present experiments. They were collected with an intact rhizome, and the stem was cut on site to approximately 10 cm height. The specimens were subsequently planted in an expanded clay of a diameter between 3–8 mm (ARGEX), and watered with tap water or full strength Hoagland solution. Two weeks before the beginning of the assays, these plants were transplanted to an expanded clay with a diameter between 8–12.5 mm (ARGEX), and left to acclimate.

Spartina maritima and *Juncus maritimus* were collected in October 2015 in Viana do Castelo, Portugal in the estuary of Lima river from a natural wetland. Plants were removed with intact rhizomes and stems. They were subsequently planted in commercial turf for development for at least 2 weeks and were watered with a 10 dS m^{-1} saline solution. These plants were transplanted to an expanded clay with a diameter between 8–12.5 mm (ARGEX) and maintained for several months with saline, full strength, Hoagland solution. Plants showing poor adaptation, namely low shoot and leaf production, were discarded.

4.6.4 Microcosm studies with substrate (expanded clay)

Microcosms were set up in opaque grey polyvinyl chloride plastic containers (food grade; with 0.345 m × 0.255 m × 0.165 m length, width and height, respectively, representing 0.088 m^2 of surface area and a total useful volume of 10 L). The substrate used in the microcosms was a washed expanded clay with a diameter between 8–12.5 mm (ARGEX). The total volume of expanded clay was approximately 8 L per microcosm. The saline solution was added at 1.25 L per microcosm, and was completely replaced at the end of each HRT through a perforated tube placed in the container and a hand pump. Samples of treated wastewater were collected with a large syringe. This setup was meant to simulate a vertical subsurface constructed wetland (VSSF) operated in batch conditions.

Four different conditions were tested in triplicate for a total of 12 microcosms (randomly distributed): substrate only (3 microcosms); substrate and *Spartina maritima* (3 microcosms, 8-10 cm diameter clumps, 17-22 stems per microcosm); substrate and *Juncus maritimus* (3 microcosms, 8-10 cm diameter clumps, 44-62 stems per microcosm) and substrate and *Arundo donax* (3 microcosms, 8-10 cm diameter clumps, 11-12 stems per microcosm). Plants were previously adapted to the microcosms (namely to the type of substrate) and moderate salinity, as referred above.

The hydraulic retention time (HRT) tested (in batch) was 4 days, with irrigation using the synthetic wastewater (with ammonia and nitrate as the N sources). Three consecutive runs of four-day HRT tests were performed in the first set of tests. Another set of tests with three runs of four-day HRT each was also performed with the same saline water as described above, but this time without any ammonia added (to test for nitrate uptake only). It is important to point out that the substrate was flushed with tap water several times to ensure that no salts remained, since they accumulated from previous test runs. The time interval between the first and second set of tests with four-day HRT was 51 days. During this time the plants were maintained in the same synthetic wastewater with a HRT of 7 days. Nevertheless, due to strong evapotranspiration, these tests were not conclusive for nutrient and salt removal, and therefore, they are not presented.

Samples were collected at the end of each run and analyzed for nutrients (N from ammonia or nitrate, salts, pH and electrical conductivity. Microclimate conditions (humidity, temperature, and light intensity) were monitored daily. In all of the experiments performed, plant survival, number of green and dry shoots, stems and leaves, as well as the height of tagged stems, was registered weekly. Visual inspections for signs of toxicity (necrosis, malformations, etc.) were conducted daily.

4.6.5 Microcosm studies without substrate (hydroponics)

Microcosms were set up in clear polyethylene plastic containers (with 0.28 m × 0.20 m × 0.14 m length, width and height, respectively, and a 0.056 m^2 surface area). The boxes were covered in black plastic sheet to prevent the influence of sunlight. Substrate was not used in this experiment to simulate hydroponic conditions. As such, plants were immersed in three liters of saline solution with the same synthetic wastewater composition referred above (with N from both ammonia and nitrate). Styrofoam covers were used to support the plants. This volume was required to cover all the root system of the tested plants. Additionally, aeration was provided by an aquarium air pump and small aquarium air stones.

Three different conditions were tested in triplicate for a total of 9 microcosms (randomly distributed): synthetic saline wastewater solution only (control, 3 microcosms); *Spartina maritima* (3 microcosms, 6-8 cm diameter clumps, 14-17 stems per microcosm); *Juncus maritimus* (3 microcosms, 8-10 cm diameter clumps, 36-38 stems per microcosm).

The hydraulic retention time (HRT) tested (in batch) was 4 days. Three runs of four-day HRT tests were performed sequentially to assess temporal variations in microcosm behavior. Samples were collected and analyzed for nutrients, salts, pH and electrical conductivity. Microclimate conditions (humidity, temperature, and light intensity) were monitored daily.

After completion of these tests, an additional extended test was performed only for these hydroponic conditions. The HRT was increased (with evaporation compensation) to 23 days in order to assess salt removal over a longer period of time. Afterwards, the plants were washed with deionized water and accumulated salts were determined.

Plant growth and survival were monitored in all of the experiments done by counting the total number of stems and leaves and respective conditions (green or dry). For the purpose of monitoring stem height, five stems were marked per microcosm, and height was measured on a weekly basis. It should be noted that this monitoring started only after the first run of the four-day HRT test.

4.6.6 Chemical Analysis

Electrical conductivity (EC) was determined with a WTW Tetracon® 325 conductivity electrode, while pH was determined using a WTW pH electrode SenTix 21. Calcium + magnesium were determined simultaneously following EPA method (# 130.2 Hardness, Total (mg L^{-1} as $CaCO_3$) (Titrimetric, EDTA). Sodium was analyzed using two HANNA FC 300 B Na^+ electrodes while chloride was analyzed using a HANNA HI 4107 Chloride combination electrode. Ammonia-nitrogen was analyzed using a Thermo Scientific 9512HPBNWP high performance ammonia electrode. All ion selective electrodes used were calibrated immediately before use with freshly prepared calibration solutions.

Nitrate was evaluated by ion chromatography (Thermo Scientific™ Dionex ICS-3000) using Dionex Ionpac AS9-1HC Thermo column conductivity detector; elution was performed with 12 mM of $NaCO_3$ + 5 mM $NaHCO_3$ with a flow of 1.0 mL/min at 25°C. Phosphate (PO_4^{3-}), was measured spectrophotometrically at 885 nm using a kit (Merck 1.14848.0002).

4.6.7 Statistical Analysis

Prior to statistical analysis, outliers falling outside of the [Q1 − k (Q3 − Q1), Q3 + k (Q3 − Q1)] range (with $k = 0.7$) were excluded (Ben-Gal, 2005), for $n > 3$ (for $n = 3$, no outliers were removed for the statistical analysis).

Statistical analyses were performed using Stastistica 8.0 software (StatSoft, Inc, Tulsa, USA). Shapiro-Wilk test was used to test for normal distribution. Subsequently, Levene's test was performed to test for homogeneity of variances. For normally distributed and with homogenous variance data sets, the t-test or ANOVA test were performed and depended on whether two or more variables were tested. For normally distributed, but with heterogeneous variance data, Welch's t-test or Welch's ANOVA test were performed instead. For significant ANOVA results, post hoc Tukey was used for equal number of data points and unequal N HSD was used for unequal number of data points.

For non-normally distributed data sets, Mann-Whitney U or Kruskall Wallis tests were performed, depending on whether two or more variables were tested. Significance level was $p < 0.05$ for normal distributed data points, and $p < 0.10$ for non-normally distributed data sets. When applicable, values are presented as the mean ± standard deviation of the population (stdev.p).

For dependent samples, paired dependent t-test or Wilcoxon matched pairs tests were used for normal or non-normally distributed data sets, respectively.

4.7 Results

4.7.1 CW Microcosm behavior with substrate (expanded clay)

The simulated VSSF constructed wetland systems were tested with a synthetic saline wastewater to evaluate nutrient and salt removal. A first set of tests was done using a four-day HRT, in batch, for three consecutive runs and the synthetic saline wastewater changes in composition are detailed in Table 4.1.

Table 4.1 - Synthetic saline wastewater final values for salts and ammonia-nitrogen after treatment in lab-scale constructed wetlands with expanded clay only (control) and planted with three different plants, after three consecutive runs of 4 days retention time each. Different letters in the same column indicate statistically different results between treatments for each parameter per retention time, i.e, results that share at least one letter are not statistically different.

Treatment	EC (dS m^{-1})	pH	Cl^{-} (mg L^{-1})	$Ca^{2+}+Mg^{2+}$ (meq L^{-1})	Na^{+} (mg L^{-1})	SAR	NH_4^{+}-N (mg L^{-1})
4 day HRT - 1st							
S. maritima	12.0±1.6^{b}	7.88±0.06^{b}	3045±489^{b}	13.7±3.0^{ab}	2185±301^{b}	37±8^{ab}	5.0±2.2^{b}
J. maritimus	14.8±1.0^{ab}	7.88±0.06^{b}	3359±90^{ab}	25.3±7.6^{b}	2457±74^{ab}	31±4^{b}	3.7±0.7^{b}
A. donax	11.1±1.3^{b}	8.09±0.06^{a}	2309±381^{b}	11.1±0.2^{a}	2095±322^{b}	39±6^{ab}	5.7±1.9^{b}
Unplanted	19.8±4.3^{a}	8.23±0.04^{a}	4507±559^{a}	7.8±3.4^{a}	3203±388^{a}	77±22^{a}	19.8±4.0^{a}
4 day HRT - 2nd							
S. maritima	15.1±0.7^{b}	7.55±0.04^{b}	5668±293^{b}	10.6±0.8^{ab}	4618±164^{b}	87±3^{b}	17.8±1.5^{ab}
J. maritimus	16.8±0.9^{ab}	7.50±0.05^{b}	5786±331^{ab}	17.5±3.8^{b}	5308±110^{ab}	80±12^{b}	14.5±1.7^{ab}
A. donax	16.5±0.1^{ab}	7.50±0.04^{b}	5783±98^{ab}	8.7±0.1^{ab}	5491±107^{ab}	115±3^{ab}	8.4±5.5^{b}
Unplanted	17.9±0.8^{a}	7.94±0.12^{a}	6614±300^{a}	7.1±1.5^{a}	5931±320^{a}	140±19^{a}	38.8±3.2^{a}
4 day HRT - 3nd							
S. maritima	16.7±0.5^{a}	7.47±0.04^{ab}	4982±220^{a}	9.4±0.7^{ab}	3293±98^{a}	66±1^{a}	18.9±4.1^{a}
J. maritimus	17.7±1.6^{a}	7.40±0.11^{b}	5057±498^{a}	13.1±2.7^{b}	3496±420^{a}	60±7^{a}	2.8±0.9^{a}
A. donax	18.1±0.2^{a}	7.61±0.07^{ab}	5239±119^{a}	8.7±0.7^{ab}	3524±65^{a}	73±2^{a}	3.7±2.3^{a}
Unplanted	17.4±0.3^{a}	7.77±0.15^{a}	5062±205^{a}	7.3±0.9^{a}	3472±100^{a}	79±6^{a}	23.8±12.4^{a}

It can be seen that EC, sodium and chloride follow the same trend in all tested runs, which was expected. Also, these parameters are always higher in the unplanted treatment when compared to planted treatments for the first two HRTs runs. However, only *Arundo donax* and *Spartina maritima* showed statistically lower values compared to the control for these parameters in the first HRT run, and only *Spartina maritima* in the second run. No significant changes were observed between different treatments for these three parameters during the last period. This may indicate a saturation of salts in plant tissues or lower plant growth. Also, considering initial values of Na^{+} = 3806 mg L^{-1} and Cl^{-} = 5867 mg L^{-1}, accumulation of these salts can be observed in the second HRT period, potentially due to higher evaporation rates. However, the accumulation is always higher in the unplanted system, as previously mentioned.

Regarding pH, unplanted controls have higher values than planted treatments. In fact, expanded clay is known for increasing water pH, and plants may excrete organic compounds to counteract or buffer this increase (Chen et al., 2016).

As to calcium and magnesium levels, Table 4.1 shows that they are initially high and steadily decrease along the consecutive runs. Considering that calcium is added through the irrigation solution at a concentration of 2.43 meq L^{-1}, the high levels found possibly derive from released calcium from either the plants or the expanded clay. This is most likely due to cation exchange reactions with sodium. However, the unplanted control presents a lower release of calcium and/or magnesium during all three HRT periods.

These differences in calcium and/or magnesium content reflect on the values of SAR. In all three runs, the unplanted control had a higher SAR value than the planted microcosms, although no statistical differences could be found in the last run.

Necrosis or drying was not apparent in the plants through visual inspection at this stage, and all tested specimens remained alive (0% mortality rate). Excreted salts were more clearly visible in *Spartina maritima* and some in *Juncus maritimus,* but no evidence of this behavior was observed in *Arundo donax*.

A second set of tests with the same HRT was performed much later (approximately 51 days apart), but was carried out with synthetic saline wastewater without ammonia (Table 4.2).

Table 4.2 - Final values for dissolved salts and nitrate-nitrogen of synthetic saline wastewater (without ammonia nitrogen added) after treatment in microcosms with expanded clay only (unplanted controls), or planted with three different plants. The tested retention time was 4 days, and three consecutive runs were performed. Different letters in the same column indicate statistically different results between treatments for each parameter per retention time, i.e, results that share at least one letter are not statistically different.

Treatment	EC (dS m^{-1})	pH	Cl^{-} (mg L^{-1})	Ca^{2+}Mg^{2+} (meq L^{-1})	Na^{+} (mg L^{-1})	SAR	NO$_3^-$-N (mg L^{-1})
4 day HRT - 1st							
S. maritima	14.6±0.6^{a}	7.77±0.05^{a}	5073±212^{b}	4.1±0.2^{a}	3189±164^{b}	97±3^{a}	25.1±5.8^{a}
J. maritimus	18.9±1.1^{b}	7.56±0.14^{a}	6492±524^{a}	5.8±0.7^{a}	3969±239^{a}	102±2^{a}	25.8±7.7^{a}
A. donax	19.5±0.8^{b}	7.65±0.04^{a}	6888±217^{a}	4.3±0.2^{a}	4128±165^{a}	123±6^{b}	35.4±9.0^{a}
Unplanted	17.1±0.9^{ab}	7.69±0.06^{a}	5179±214^{b}	3.2±0.0^{b}	3563±221^{ab}	123±7^{b}	23.2±8.2^{a}
4 day HRT - 2nd							
S. maritima	16.6±1.3^{b}	7.79±0.09^{a}	7740±213^{a}	4.1±0.4^{ab}	4184±37^{a}	128±6^{ab}	29.0±7.2^{a}
J. maritimus	20.5±1.5^{a}	7.62±0.23^{a}	8551±415^{a}	5.4±0.7^{b}	4493±492^{a}	119±8^{a}	31.0±5.1^{a}
A. donax	19.1±1.0^{ab}	7.69±0.05^{a}	8039±230^{a}	4.1±0.2^{ab}	4049±187^{a}	123±2^{a}	24.8±20.7^{a}
Unplanted	18.1±0.5^{ab}	7.71±0.14^{a}	7904±339^{a}	3.3±0.3^{a}	4228±263^{a}	144±4^{b}	36.7±12.1^{a}
4 day - 3rd							
S. maritima	15.9±1.7^{a}	7.81±0.08^{a}	6459±863^{a}	4.0±0.2^{a}	3647±333^{a}	112±8^{a}	29.9±10.1^{a}
J. maritimus	20.7±2.4^{a}	7.63±0.18^{a}	8148±1107^{a}	4.9±0.6^{a}	4582±372^{a}	127±2^{a}	25.6±10.6^{a}
A. donax	18.7±1.7^{a}	7.74±0.02^{a}	8126±788^{a}	4.3±0.2^{a}	4343±315^{a}	129±11^{a}	16.9±19.7^{a}
Unplanted	19.4±0.6^{a}	7.81±0.01^{a}	8660±578^{a}	3.4±0.5^{a}	4313±289^{a}	145±20^{a}	33.4±12.4^{a}

In this second set of tests with a four-day HRT, EC, sodium and chloride all follow similar trends again. However, unlike the tests with ammonia and nitrate as N sources, trends between these three parameters are less consistent throughout time. Initially, *Arundo donax* microcosms had higher levels of these three parameters than the remaining treatments, but this trend changed in the second HRT run. By the third HRT run, all treatments reveal statistically similar values for these three parameters. These results are similar to those from the previous experiments (Table 4.1).

As for pH, there were no statistical differences observed for different treatments along this set of HRT tests. After such a prolonged time in contact with the synthetic wastewater, a potential impact of expanded clay on the value of pH is no longer noticeable.

Similarly to pH, calcium and magnesium levels also seemed to have stabilized and showed a much lower concentration than reported in Table 4.1. However, it is still possible to see a difference in calcium and magnesium levels during the first two HRT runs, in which unplanted treatments remain with lower levels of calcium and magnesium released when compared to the remaining treatments.

These differences in calcium and/or magnesium content once again reflect on SAR values. The unplanted control showed a higher SAR value than the planted microcosms in all three HRT runs performed. Although, it should be noted this was not always statistical different.

At this stage, some dry leaves and necrosis were apparent in plants, particularly for *Arundo donax,* but all tested specimens remained alive (0% mortality rate). As previously noted, excreted salts were more clearly visible in *Spartina maritima* and some in *Juncus maritimus,* but evidence of similar behavior was not found in *Arundo donax*.

As for nitrogen removal in simulated saline wastewater effluent, ammonia-nitrogen removal (Table 4.1) was initially high with values on the order of 17.5±16.6% in the control and varied between 76.1-84.7% in the planted treatments. Initial removal in the control is likely due to adsorption onto clay aggregates, which may have also occurred to a certain extent in the planted treatments. Still, this effect either disappears or is not as strong by the second HRT run, since the control registers a negative removal (accumulation) and the planted treatments have lower efficiencies. Finally, ammonia removal seems to have stabilized by the third HRT run, with *Juncus maritimus* and *Arundo donax* achieving a very good performance (88.2±3.6% and 84.7±9.52%, respectively), while *Spartina maritima* showed a low and unstable ammonia removal percentage (21.1±17.7%).

Regarding nitrate removal (Table 4.2) the simulated VSSF CW behaved more inconsistently and with higher standard deviation and clear outliers than those found for ammonia removal in the second set of tests performed compared to the previous test. While *Spartina maritima* and the unplanted control had an inconsistent removal throughout time, both *Juncus maritimus* and *Arundo donax* had consistent improvements, if data is corrected for outliers, with an initial removal efficiency of 9.0±5.3% and 9.3±3.0%, respectively (n=2, one outlier removed) which increased up to 55.8±21.6% and 68.1±5.9% during the last HRT test (n=2, one outlier removed).

The assessment of phosphate removal by the plants in these microcosm tests was hampered by the strong adsorption initially observed, where 100% removal observed during the first four-day HRT run. Since one of the main goals of the test was to compare different plant species with unplanted microcosms, such rapid and complete adsorption of phosphate precluded the detection of any kind of plant effect. Therefore, phosphate was only monitored later in hydroponic studies.

Regarding plant growth, a clear differentiation between different plants was observed (Figure 4.1). In the first set of tests during the four-day HRT tests, it can be seen that *Spartina maritima* initially outperformed the remaining plants with 6.5% height increase during the initial 8 days, compared to 4.3% and 1.5% for *Juncus maritimus* and *Arundo donax,* respectively. However, during the seven-day HRT tests, *Juncus maritimus* surpassed *Spartina maritima,* and at the end of this stage, the cumulative percentage growth was 34.6% for *Juncus maritimus*, 23.7% for *Spartina maritima* and 10% for *Arundo donax*.

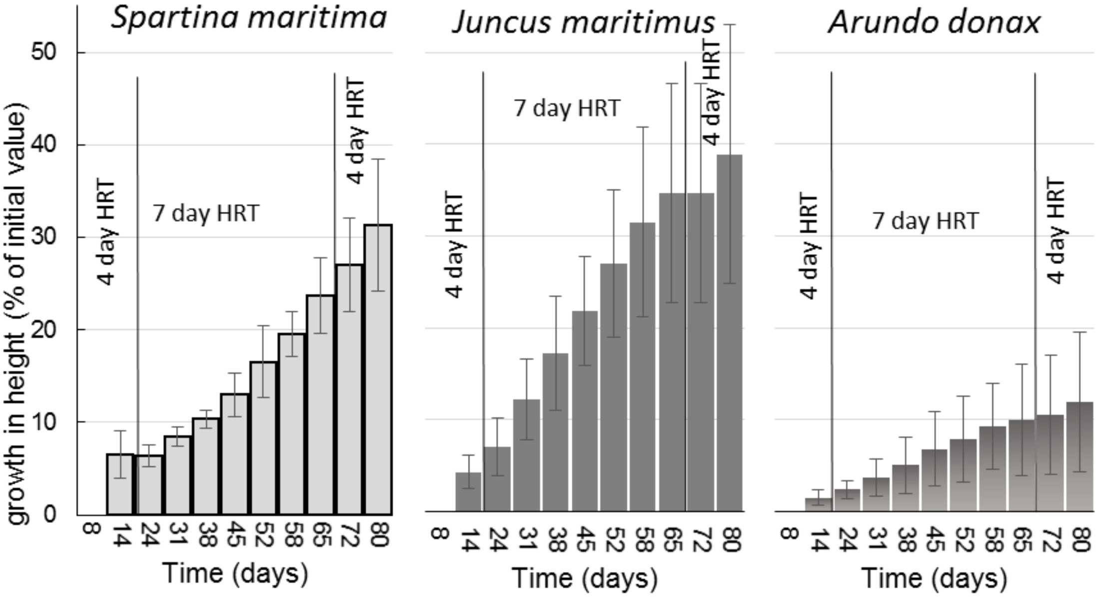


Figure 4.1 - Growth in height as percent of initial value (%) for the three plants studied in expanded clay microcosms (*Spartina maritima*, *Juncus maritimus*, *Arundo donax)* using synthetic saline wastewater and different HRT (first set of tests at 4 day HRT and second stage at 7 day HRT with complete saline solution, and second set of tests at 4 day HRT without ammonia). (Note: monitoring started at the second run of HRT=4 days, resulting in no data for the first 8 days).

Finally, during the last set of the four-day HRT tests, *Spartina maritima* continued to grow in height but *Juncus maritimus* initially stabilized, and *Arundo donax* showed no clear growth trend. These results seemingly suggest that *Spartina maritima* performs better with a four-day HRT and *Juncus maritimus* performs better with a seven-day HRT. Although anecdotal, this trend provides at least a clue to the fact that HRT might impact plant health and growth during saline wastewater treatment, and should be further investigated in future research efforts. However, this result may also be due to the introduced changes in N source, since only nitrate and not ammonia was present at this last set of four-day HRT tests.

Throughout the entire study (including the data not shown for the seven-day HRT tests), *Spartina maritima* had statistically higher stem height growth than *Arundo donax*, and *Juncus maritimus* had even taller stems, since growth was statistically higher than the other two plants. *Juncus maritimus* stems grew 3 times more on average than *Spartina maritima* and 7.4 times more than *Arundo donax*. A similar trend is visible for number of stems (green, dry or total; data not shown), although not statistically different in this case, and was most likely due to the high variability that was observed.

In addition, the variation in number of leaves and stems in each plant species between initial and final values was not statistically different for any plant species. However, plant height was statistically larger for *Spartina martima* and *Juncus maritimus*. This indicates a significant growth of these plants in the conditions tested in the expanded clay CW.

With these data it becomes apparent that *Juncus maritimus* was the plant that performed better concerning growth in the tested conditions and *Arundo donax* was the one that performed the worst, since no growth and visible signs of saline stress were observed.

4.7.2 Microcosm studies without substrate (hydroponics)

A hydroponic system was operated with synthetic saline wastewater to evaluate nutrient and salt removal without the potential interference of the substrate. A hydroponic system operated in batch was simulated with a four-day HRT and was repeated sequentially for three times with this value (Table 4.3). The tested conditions include microcosms planted with *Spartina maritima,* with *Juncus maritimus* and unplanted controls (all in triplicate).

Table 4.3 - Synthetic saline wastewater final values for salts and nutrients after treatment in hydroponic system with saline wastewater only (control) and planted with two different plants, after consecutive 4 days retention time assays. Different letters in the same column indicate statistically different results between treatments for each parameter per retention time, i.e, results that share at least one letter are not statistically different (N.A. - not available).

Treatment	EC (dS m^{-1})	pH	Cl$^-$ (mg L^{-1})	Ca^{2+}Mg^{2+} (meq L^{-1})	Na$^+$ (mg L^{-1})	SAR	NH$_4^+$-N (mg L^{-1})	PO$_4^{3-}$-P (mg L^{-1})
4 day HRT - 1st								
S. maritima	18.1±0.5^a	6.45±0.42^a	5895±714^a	5.5±0.5^a	4436±92^a	116±5^a	15.6±3.0^a	30.0±3.0^a
J. maritimus	18.0±0.1^a	5.85±0.15^a	5178±152^a	6.1±0.2^a	4413±44^a	110±1^a	10.6±0.5^b	30.4±1.4^a
Unplanted	18.1±0.3^a	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4 day HRT - 2nd								
S. maritima	18.5±0.5^a	6.20±0.22^a	7235±145^a	5.2±0.3^a	4391±112^a	118±2^a	11.5±0.5^a	20.9±14.0^a
J. maritimus	19.0±0.5^a	6.09±0.25^a	7473±111^a	5.8±0.6^a	4355±100^a	112±4^a	12.1±0.6^a	25.2±14.3^a
Unplanted	18.2±0.4^a	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4 day HRT - 3rd								
S. maritima	18.9±0.3^a	6.09±0.13^a	7489±344^a	5.3±0.2^a	4233±123^a	113±5^a	11.3±0.3^a	3.3±2.2^a
J. maritimus	19.9±0.3^a	5.28±0.09^b	7661±187^a	5.5±0.2^a	4200±120^a	110±2^a	12.0±0.6^a	34.1±2.6^b
Unplanted	18.2±0.2^a	7.20±0.06^c	N.A.	N.A.	N.A.	N.A.	22.3±0.6^b	35.8±0.5^b

Globally, it can be seen that electrical conductivity (EC), chloride and sodium concentrations did not differ significantly among different treatments with plants, with usually higher EC than initially added. This was possibly due to evapotranspiration in the microcosms.

pH values are lower than those obtained with the CW setup since the lowest values measured in hydroponics for *Spartina maritima* and *Juncus maritimus* were of 6.09 and 5.28 respectively, compared to 7.47 and 7.40 in the CW setup), which reflects the lack of the expanded clay substrate. These values steadily decreased over time, possibly reflecting the effect of nitrification.

In addition, Ca^{2+}+Mg^{2+} and SAR values did not vary significantly among planted treatments. However, ammonia-nitrogen levels were initially lower with *Juncus maritimus* (10.6±0.5 mg L^{-1} compared to 15.6±3.0 mg L^{-1} with *Spartina maritima*), but were statistically similar among different plants during the second and third HRT runs. Results from both plants show statistically lower ammonia levels than the unplanted control (with 22.3±0.6 mg L^{-1} NH4$^+$-N).

As for phosphate, a decreasing trend in concentration can be observed for *Spartina maritima* (30.0±3.0; 20.9±14.0 and 3.3±2.2 mg L^{-1} PO$_4^{3-}$-P for the first, second and third HRT runs, respectively), with a final concentration statistically lower than results from both *Juncus maritimus* (34.1±2.6 mg L^{-1}) and unplanted controls (35.8±0.5 mg L^{-1}).

In terms of nutrient removal, results obtained for hydroponics are far more stable than the ones of the tests performed with expanded clay as the substrate. For example, ammonia removal was fairly consistent with removal efficiencies of 34.8±12.6%, 52.2±2.2% and 53.1±1.4% during each of the three HRT runs with *Spartina maritima,* and 55.8±2.1%, 49.8±2.6% and 49.61±2.5% removal efficiencies for *Juncus maritimus* during the same time period.

For phosphate, only *Spartina maritima* obtained a positive and consistent removal during the last HRT run, with 89.0±7.2% removal efficiency.

For a clearer evaluation of salt removal in the hydroponic system, a mass balance was performed using a simple conversion of electrical conductivity to dissolved salts (TDS (mg L^{-1}) = EC * 0.640) (Ali et al., 2012). The results suggest a removal efficiency of total salts and sodium removal in the first HRT run between 7.6-7.7% and 1.6-2.1%, respectively with both plants tested, which quickly decreases in the subsequent HRT runs. However, for the simulated wastewater that was kept in the microcosms for a total HRT of 23 days, the final salt removal mass balance was 10.4±4.0% and -1.0±2.5% for total salts, and 13.3±4.2% and 0.5±1.7% for sodium removal for *Spartina maritima* and *Juncus maritimus*, respectively.

Salts removed by both plants were clearly visible. To better test for accumulated salt quantities, the plants were washed with a known volume of deionized water and dissolved salts were determined. The results again seem to indicate a better performance for *Spartina maritima,* with total salt mass recovery of 629±33 mg compared to only 440±67 mg found for *Juncus maritimus*. Sodium values obtained were 174±10 mg and 131±30 mg, respectively, while chloride was determined to be 365±13 mg and 235±35 mg, respectively for *Spartina maritima* and *Juncus maritimus*.

4.8 Discussion

The objectives of this study were to test whether or not halophytic and halotolerant plants could actively contribute to the treatment of saline wastewater for either nutrient removal, salt removal, or both simultaneously. Taking into consideration that nutrient and salt removal by plants are usually analyzed individually by different studies, these topics shall be discussed separately in this section.

4.8.1 Nutrient removal under high salinity

Nutrient removal results obtained in this study are within the results found in other works at similar salinity and HRT. A compilation of relevant data on this subject to help comparisons with results from the present work can be seen in Table 4.4. The selection of studies cited was not meant to be comprehensive but intended to include works that showed some similarity with the present study as to salinity, nutrient type assayed and HRT used. The choice was difficult because there is not much literature where nutrient removal was studied under high salinity (0.6 to 3.1 % salt content).

However, comparisons are still difficult as other aspects of the experimental setup remain different between studies, as is the substrate and plant used. In fact, while most studies cited tested gravel, soil, sand or a combination of them as substrate, only the present study (out of the cited studies in Table 4.4) tested expanded clay and hydroponic conditions. Furthermore, only one other study was performed under VSSF (Wang et al 2010), while six tests were performed under HSSF, five in free water surface mode (FWS) and two in hybrid CWs. In terms of test duration, around six studies have similar or inferior duration than the present study, while six others have longer durations. The plants used were also different. Finally, only the present study tested for simultaneous removal of salt and nutrient.

When tested with a substrate, both *Juncus maritimus* and *Arundo donax* globally showed a performance in line with other works for N removal. However, a low ammonia removal and negative nitrate removal were obtained for *Spartina maritima*. Nonetheless, when tested under hydroponic conditions, *Spartina maritima* performed better than *Juncus maritimus*, particularly regarding phosphate removal (Table 4.4).

Table 4.4 - Comparison of nutrient removal efficiency (%RE – percent removal efficiency for NH_4^+-N, PO_4^{3-}-P/TP, NO_3^--N) between this and other literature works using saline wastewater treatment in constructed wetland systems. (HRT – Hydraulic retention time; * - in hydroponic system; N.A. – not available) Sources: [1] – This study; [2] - (Sousa et al., 2011); [3] - (Su et al., 2011); [4] - (Wang et al., 2010); [5] - (Lin et al., 2005); [6] - (Shi et al., 2011); [7] - (Klomjek and Nitisoravut, 2005); [8] - (Gao et al., 2015); [9] - (Idris et al., 2012).

Source	Plant species	HRT (days)	Salinity %	NH_4^+-N		PO_4^{3-}-P / TP		NO_3^--N	
				Initial	RE %	Initial	RE %	Initial	RE%
[1]	*S. maritima*	4	0.9	24	21	30	100[a]	34	4
[1]	*J. maritimus*	4	0.9	24	88	30	100[a]	34	56
[1]	*A. donax*	4	0.9	24	85	30	100[a]	34	68
[2]	*S. alterniflora*	0.25	2.0	2.4	66	1.6	51	1	41
[3]	*A. marina*	2	3.1	1.5	43	1.4	26	0.1	27
[3]	*R. stylosa*	2	3.1	1.5	50	1.4	27	0.1	25
[3]	*L. racemosa*	2	3.1	1.5	35	1.4	30	0.1	38
[4]	*K. candel*	1.5	1.0	1.2	82	0.1-0.2	14	0.2-0.4	85
[5]	Two species	1.5-2	0.3	0.3	66	1.1	-8	6	-5
[6]	Several species	-	0.8	1.3	71	0.1	24	3	59
[1]	*S. maritima**	4	0.9	24	53	30	89	N.A.	N.A.
[1]	*J. maritimus**	4	0.9	24	50	30	-14	N.A.	N.A.
[7]	*T. angustifolia*	5	0.6	24	65	9	24	N.A.	N.A.
[7]	*D. bicornis*	5	0.6	24	62	8	41	N.A.	N.A.
[8]	*P. australis*	3	1.0	21	68	26	45	N.A.	N.A.
[8]	*S. validus*	3	1.0	21	62	26	47	N.A.	N.A.
[8]	*C. indica*	3	1.0	21	60	26	43	N.A.	N.A.
[9]	*A. donax*	10.6	0.6	160	31	65	0	N.A.	N.A.
[9]	*P. australis*	10.6	0.6	160	39	65	-3	N.A.	N.A.

[a] Data for the first HRT run with expanded clay as substrate.

In terms of ammonia-nitrogen removal, the conditions tested in this study more closely resemble those in Klomjek and Nitisoravut (2005) and Gao et al. (2015), which specifically used similar HRT and initial ammonia concentration. In comparison to these articles, the tested plants in this work were between 7-18% less efficient in removing ammonia in hydroponic conditions and 17-35% more efficient (without accounting for the low performing *Spartina maritima*) in the CW system.

As for phosphorus, Gao et al. (2015) tested a similar concentration at the same salinity and obtained a performance similar to this work. Although, total phosphorus, rather than phosphate, was analyzed and different plants were used.

Finally, comparisons of nitrate-nitrogen removal are complex since other studies tested lower concentrations and most of them also observed ammonia present in the saline wastewater used, as can be seen in Table 4.4.

It must be also noted that, when compared with other works cited, the present study tested two plants (*Spartina maritima* and *Juncus maritimus*) which, to the best of the authors' knowledge, were not yet tested under simulated CW conditions. Furthermore, most studies do not perform hydroponic assays as was done in this study, which enables a better evaluation of plant performance and contribution to nutrient removal.

Overall, nutrient removal in planted microcosms outperformed unplanted controls in terms of ammonia-nitrogen and phosphate in both expanded clay and hydroponic tests. However, nitrate removal was similar; therefore, no positive effect of plant presence was detected in the conditions assayed.

4.8.2 Salt removal in CW microcosms

Salt removal assessment proved to be extremely complex in the simulated CW with clay. Several physico-chemical parameters appeared to have acted simultaneously, thereby affecting data interpretation. This includes evapotranspiration (extremely relevant for HRT of 7 days, data not shown), potential salt adsorption to the substrate (expanded clay) and capillary rise above water level, all seemed to have played a role.

Evaporation naturally leads to higher concentration of salts; however, salts may precipitate and be physically trapped above the system final water height in a porous bed media. These salts may redissolve when new water is added, thereby affecting the salt removal results.

Salt precipitation may also occur at the clay surface (efflorescence) or within the matrix (subflorescence) and can also affect evaporation due to increasing osmotic potential (Hird and Bolton, 2016; Nachshon et al., 2011). Therefore, salt precipitation due to evaporation is a very complex physical process that is difficult to monitor and predict.

Capillary rise might also have affected salt distribution in the expanded clay. Salt crusts at the surface of the expanded clay were observed, even though the water level was several centimeters below the top surface. It has been shown that capillary rise in expanded clay had a significant impact on nutrient distribution when expanded clay is used for horticultural purposes (Meinken, 1997). In the case of salts, capillary rise followed by evaporation can lead to salt redistribution when considering the relatively high salinity tested. The impact of this redistribution in a CW is unclear, even for nutrient removal efficiency over time, and should be examined further in the future.

Evapotranspiration is reported as an important factor when evaluating removal rates in CWs based only on influent and effluent concentrations (Białowiec et al., 2014). As a result, several authors (Freedman et al., 2014; Shelef et al., 2012; Zheng et al., 2015) proposed the use of mass balance to more accurately evaluate CW performance. Even though calculations of mass loads takes into account the effect of evapotranspiration, these still do not consider phenomena such as capillary rise and precipitation.

Freedman et al. (2014) also found that evapotranspiration in a CW resulted in higher salinity at the outlet, despite plant uptake. This result was obtained in spite of the lower HRT used in their study compared with our system (two- versus four-day HRT), and their CW was operated under a continuous flow rather than as a batch test, which limits the effects of evaporation.

Hydroponic systems represent a more simplified means to evaluate such mass loads, as capillary rise and precipitation do not occur in such conditions. This technology was previously used for the evaluation of salt removal in CWs (Shelef et al., 2012).

Comparison of the data acquired in our hydroponic test with other published research is again difficult, since most studies with saline CWs do not focus on the potential for salt removal. To the best of our knowledge, Shelef et al. (2012) is the only published study which analyses salt removal in constructed wetlands. Shelef, et al (2012) reported that 2.67 g of sodium were removed with an initial EC value of 16 dS m^{-1} and an HRT of 10 days. This removal compares favorably to the average removal of 3.3 g of sodium obtained after 23 days in this study. However, these authors only detected 0.28 g of sodium accumulated in the analyzed plants, which indicates a sodium recovery of only 81%, denoting the difficulties of using mass balance approaches for this evaluation. It is unclear if similar recovery problems were at play in our study.

Regardless of the above mentioned difficulties in evaluation, ultimately salt removal was low for the HRT of 4 days tested and became only somewhat significant after 23 days of operation of the hydroponic system. This limited salt uptake might have been due to redeposition of excreted salts, low plant density tested in this study compared to the water added in the hydroponic system, or simply due to the low capacity for salt absorption by the tested plants.

However, several factors discussed in this article can help guide future research in constructed wetland. For instance, capillary rise redistribution of nutrients and salts as well as the importance of evapotranspiration, are aspects that are rarely discussed or taken into account.

Despite all these limitations, plant survival in the tested conditions with expanded clay was 100%. Although, *Arundo donax* exhibited several signs of toxicity such as increase in dry leaves, low growth and necrosis at the surface of the leaves. *Spartina maritima* and *Juncus maritimus* seemed to be well adapted to high salinity levels, as well as to the combination of the remaining conditions tested in expanded clay. Nevertheless, under hydroponic conditions, *Spartina maritimus* started exhibiting stress signs by the end of the experiments, which might indicate that these conditions may not be ideal for future, prolonged tests.

The plants tested in this study and others (Shelef et al 2012) have limited salt removal capacity for reasonable values of HRT. This suggests that plant application for salt removal is limited in CW scenarios.

However, the tested plants may still prove potentially useful in salt removal in soil, since the contact time between plants and salts is much higher (months rather than days). In addition, plants in a soil phytoremediation setting have several different mechanisms for salt removal, rather than solely accumulation in its aerial tissues (Qadir et al., 2006).

4.9 Conclusions

The objectives of this study were to evaluate the effect of planted vegetation on simultaneous salt and nutrient removal from a simulated saline wastewater. The impact of plant presence when compared with unplanted controls did not show any coherent trend for most salts and nitrate. Ammonia-nitrogen and phosphate reduction results were promising, with statistically lower NH_4^+-N and PO_4^{3-} in planted microcosms.

Noticeably, the three tested plants had a widely different performance throughout this study:

- *Arundo donax* was able to consistently remove nitrogen as ammonia and nitrate from a simulated saline effluent when planted in expanded clay. However, the highly inhibited growth (possibly due to salinity) precluded further tests with this plant under hydroponic conditions.

- *Juncus maritimus* was also able to remove considerable N as ammonia and nitrate when planted in expanded clay and irrigated with saline wastewater. In addition, it also removed ammonia in hydroponic conditions. It grew well in all tested conditions, but phosphate and salt removal were low, despite showing salt removal capacity.

- *Spartina maritima* grew moderately well in expanded clay with highly saline wastewater, but performed poorly and inconsistently in terms of nutrient removal. Nevertheless, ammonia-nitrogen, phosphate and some salts were removed under hydroponic conditions. In addition, this plant showed the highest salt excretion.

In sum, this study was able to demonstrate the potential of two of the three tested plants for implementation of saline wastewater nutrient removal in a CW. However, salt removal in such a scenario could not be well demonstrated and might prove to be impractical in future work.

Furthermore, this study also highlights important technical difficulties in monitoring nutrient and salt removal in CWs with substrates having high capillarity, as well as the effects of evapotranspiration. These aspects and their impact should be taken into consideration when designing future experiments and/or full scale applications.

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5 Phytoremediation of salt affected soils: a review of processes, applicability and the impact of climate change

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5.1 Keywords

Phytoremediation, saline soils, salt-affected soils, phytoextraction, climate change.

5.2 Abstract

Soil salinization affects 1-10 billion hectares worldwide, threatening the agricultural production needed to feed the ever increasing world population. Phytoremediation may be a cost-effective option for the remediation of these soils. This review analyzes the viability of using phytoremediation for salt-affected soils and explores the remedial mechanisms involved. In addition, it specifically addresses the debate over plant indirect (via soil cation exchange enhancement) or direct (via uptake) role in salt remediation. Analysis of experimental data for EC+SAR reduction and plant salt uptake showed a similar removal efficiency between salt phytoremediation and other treatment options, with the added potential for phytoextraction under non-leaching conditions.

A focus is also given on recent studies that indicate potential pathways for increased salt phytoextraction, co-treatment with other contaminants and phytoremediation applicability for salt flow control.

Finally, this work also details the predicted effects of climate change on soil salinization and on treatment options. The synergetic effects of extreme climate events and salinization is a challenging obstacle for future phytoremediation applications, which will require additional and multi-disciplinary research efforts.

5.3 Introduction

Salt affected soils can be defined as soils with high levels of dissolved salts and/or high concentrations of adsorbed sodium ions in the soil matrix (Qadir et al., 2000). They can be divided into three classes based on salinity and sodicity values, represented by electrical conductivity (ECe) and sodium adsorption ratio (SAR) or exchangeable sodium percentage (ESP): saline, saline-sodic and sodic soils. Saline soils are characterized by an ECe value of over 4 dS m^{-1} and SAR value below 13 or ESP values below 15. Sodic soils, on the other hand, are characterized by ECe values under 4 dS m^{-1} and SAR values above 13 or ESP values above 15. Saline-sodic soils show both ECe over 4 dS m^{-1} and SAR values above 13 or ESP values above 15 (Qadir et al., 2000).

The effects of high salt concentrations in soils are marked in plants, which exhibit physiological changes including stomata closure, hyper osmotic shock, inhibition of cell division and photosynthesis; however, the most common effects are nutrient imbalance, low osmotic potential and toxicity of specific ions such as Na^+ and Cl^-, resulting in plant growth inhibition or death (Aslam et al., 2011). Salinity, and especially sodicity, also contribute to soil degradation by destabilizing soil aggregation due to slaking, swelling and dispersion (in particular of the clay aggregates) which ultimately leads to hard setting, reduced hydraulic conductivity, impaired air and water movement, runoff and exposure to erosion. These effects on soil stability are shown in the lower water availability for plants and reduced root penetration, oxygen content and seedling emergence (Qadir and Schubert, 2002).

It is estimated that 1-10 billion hectares of salt affected soils exist worldwide (Yensen and Biel, 2006) in over 100 countries (Qadir and Oster, 2002), with a potential of 10 to 16% increase per year (Aydemir and Sünger, 2011). Soil salinization is particularly relevant in irrigated lands where 20 to 50% are considered salt affected, and has been shown to result in a decrease of crop yields (Pitman and Läuchli, 2004).

The rate of expansion of soil salinization worldwide is expected to increase due to climate change. This will lead to the use of lower quality water, to increased irrigation induced salinization and to the expansion of dryland salinization (by the increase of arid and semi-arid areas and desertification) and to sea level rise, directly contaminating nearby soils or indirectly affecting soils though saline intrusion in aquifers.

Leaching and chemical or organic amendments are the most frequently used methods for salt affected soil remediation. Leaching involves the application of excess water to promote the movement of soluble salts from the surface soil to deeper soil strata. However, this technique is restricted to saline soils as its effect on SAR is limited and it is even counterproductive since it reduces soil stability. Leaching depends on water availability and quality, as well as soil drainage and water table depth (Qadir et al., 2000). Another disadvantage of leaching is that such treatment reduces total nitrogen (TN), total organic carbon (TOC) and microbial activity and overall soil fertility (Laudicina et al., 2009). Chemical amendments are required for most sodic soils remediation. This process works by promoting ion exchange through the dissolution of existing $CaCO_3$ in the soil or by the addition of calcium cations, followed by leaching. Chemical amendments, such as gypsum ($CaSO_4$) as well as several other compounds ($CaCO_3$, $CaCl_2.2H_2O$, H_2SO_4, S, HCl, FeS_2, CaS_5, $FeSO_4.7H_2O$, $Al_2(SO_4)_3.18H_2O$), are able to reduce soil salinity and sodicity (Qadir, Schubert et al., 2001). However, some of these chemical amendments are limited to calcareous soils and all of them still depend on leaching, and all of the limitations that it ensues (Qadir et al., 2003). Lastly, organic amendments can also be employed, increasing native calcite dissolution as well as soil structure and aggregation and, by extension, drainage and hydraulic conductivity for improved leaching (Wong et al., 2009).

However, increasing prices of chemical amendments have forced farmers to look for alternatives (Qadir et al., 2001), namely in the form of phytoremediation.

Phytoremediation or vegetative bioremediation of salt affected soils can simply be defined as the cultivation of salt accumulating or salt tolerant plants for the reduction of soil salinity and / or sodicity (Qadir and Oster, 2002). Phytoremediation has several unique advantages over other salt remediation techniques. For instance, it can provide a more uniform removal of salts and at higher depths than gypsum (Qadir, et al., 2001), while also presenting the opportunity to treat salt and other pollutants simultaneously (Greenberg et al., 2007; Manousaki and Kalogerakis, 2011a; Shelef et al., 2012). Plants may be used to lower the water table and enhance drainage (Stirzaker et al., 1999). Salt uptake into the shoots also prevents their leaching to groundwater (Rabhi et al., 2009).

However, there are still many aspects about this process that need clarification, including the mechanisms (indirect or direct) by which plants are able to contribute to salt remediation, the testing conditions used (e.g., leaching) and their implications, the performance of specific plant species, and how environmental conditions may affect their performance. For instance, it is essential to identify which parameters are more relevant for practical applications, such as phytoextraction potential per dry weight versus quantity and quality of plant biomass produced (whether a plant with high bioaccumulation and low biomass is preferred over a plant with low bioaccumulation and high biomass production) as well as the uptake of specific ions over others (namely sodium) and, most importantly, whether it is technically feasible to enhance the desired traits to expand the applicability of phytoremediation and its efficiency.

It is also imperative to ascertain the impact of climate change, not only on all the parameters mentioned above, but also on the *in situ* conditions of soil salinization.

This review paper aims to evaluate phytoremediation as a viable treatment option for salt affected soils by exploring the mechanisms involved in the process and comparing its performance to the most widely used remediation techniques, especially under climate change scenarios, while also suggesting future research approaches.

5.4 Mechanisms involved in salt removal by plants

The mechanisms by which plants remove salt from the soil and the consequences of this process to soil properties are diverse (Figure 5.1). Although this complete and holistic approach on plant (and associated rhizosphere microorganisms) impacts in the soil system is not fully explored in the literature, the main mechanisms behind the actions shown are well established and recognized. Aside from increasing leaching conditions (Qadir et al., 2000; Qadir et al 2005), there are two main mechanisms for the role of plants in salt affected soils remediation. The first one is pH reduction, which increases the dissolution of $CaCO_3$ and, therefore, the available Ca^{2+} for cation exchange with sodium (Qadir et al., 2000; Qadir et al 2005; Rasouli et al., 2013; Walker et al., 2013). The second one is plant uptake of dissolved salts in general and / or sodium in particular (Rabhi et al., 2009; Shelef et al., 2012; Walker et al., 2013, Manousaki and Kalogerakis, 2011a). The relative importance of each of these two mechanisms is still a question of debate in the literature (Qadir et al., 2006; Rabhi et al., 2009). However, with the plant biomass obtained in the process, added value opportunities might be available such as their use as bioenergy crops or for celullose production (Abideen et al., 2011; Suer and Andersson-Sköld, 2011; Wang et al., 2011; Wicke et al., 2011; Glenn et al., 2013).

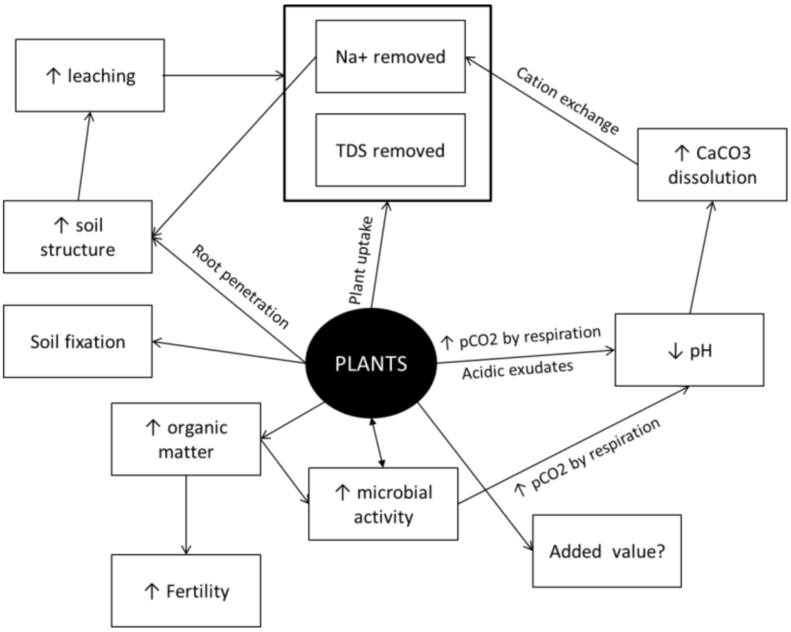

Figure 5.1 - Role of plants in salt affected soils remediation and possible variations in soil properties as a result of this process (based on Qadir, et al., 2000; Qadir, et al., 2006; Rabhi, et al., 2009)

Many research articles (Minhas et al., 2007; Shekhawat et al., 2006; Gharaibeh et al., 2011) have extensively suggested that plant salt uptake is small in comparison with salt input or salt content in a salinized soil and therefore CaCO3 dissolution would be the main mechanism of remediation. In particular, Qadir et al. (2000) concluded that even in the best possible scenario (high yield and high quality irrigation water) the plant in question (*Leptochloa fusca*) could only remove 90% of the salt added through the water used in the remediation process, and thus soil salinity would in fact increase, rather than decrease. However, other authors (Rabhi et al., 2010; Ammari et al., 2011; Shelef et al., 2012), demonstrate the potential for salt and, more specifically, sodium uptake. Rabhi et al. (2009) argue that Qadir and co-authors have neglected salt accumulation in plant shoots. In fact, in greenhouse experiments and under non-leaching conditions, Rabhi et al. (2009) found a significant decrease in sodium levels (up to 70%) and in overall salinity of contaminated soils.

Although both salt removal mechanisms are valid, the different perspectives discussed above may result from varying experimental conditions, namely leaching (e.g Qadir et al., 2000) or non-leaching conditions (e.g Rabhi et al., 2009), which influence plant uptake and accumulation in the shoots, or even result from utilization of different plant species. For practical purposes, however, it is crucial to clarify if plant uptake is or is not a significant mechanism of salt removal, since this may limit the phytoremediation approach to calcareous soils, as well as to situations in which water for leaching is available.

Plants referred in soil salt phytoremediation studies are either salt tolerant or halophytes. Within halophytes, salt uptake is highly dependent on plant species (Tipirdamaz et al., 2006). Yensen and Biel (2006) suggested a new classification system for halophytes, which partly accounts for their different behavior in salt remediation processes, and divides them into three groups: excluder, accumulator and conductor plants. Excluder and accumulator type of plants are well-known and applied classifications (Ammari, 2008; Gamalero et al., 2009; Shelef et al., 2012; Guittonny-Philippe et al., 2014); however, conductor plants is a relatively novel classification. Excluders prevent salts from entering their tissues as a salinity tolerance mechanism; accumulators uptake and accumulate salts in their tissues and the third type, called conductor plants, absorb salts and excrete them by salt glands, conducting the salts from the soil into the air. This classification and the mechanisms behind phytoremediation actions are the main factors for plant species selection and associated remediation efficiency.

Therefore, if $CaCO_3$ dissolution is the main mechanism for saline soil remediation, the most adequate plant characteristics for treatment would be plants with a higher capacity to increase pCO_2 and stronger and larger root systems. The type of salinity tolerance mechanism is, in this case, irrelevant, as long as plants are able to withstand high salinity. If plant uptake is a key element for successful remediation, excluder type plants are obviously not recommended and accumulator plants would be more appropriate, assuming they possess high total salts uptake (and more specifically sodium) and have high aerial biomass productivity. Perennial plants would also allow for a more extended active period of remediation throughout the year. For conductor type plants, screening could be done by identifying plants with salt glands or bladders or *in situ* visualization of excreted salt. Sufficient dispersion of salts by wind to avoid soil recontamination is unlikely, although more research is needed to verify this hypothesis (Yensen and Biel, 2006).

An improved clarification of plant contribution to the remediation process is needed, in particular the role of salt uptake in aerial biomass. If this is significant, it can extend the use of this technology to non-calcareous soils and / or under non-leaching conditions (Rabhi et al., 2009). Non-calcareous soils, which are also salt affected, still represent a significant problem. For instance, non-calcareous salt affected soils represent 30% (75000 ha) and 23.1% (294000 ha) of all salt affected soils in France and Hungary, respectively (Van-Camp et al., 2004). Therefore, the development of remediation techniques that are effective in these types of soils is of paramount importance.

5.5 Performance comparison and affecting parameters

A comparison of the available studies in the literature regarding the efficiency of phytoremediation for salt affected soils is challenging due to different testing conditions. Moreover, remediation techniques have a strong case specific component that cannot be fully accounted for in a review. Therefore, two different basic comparisons of salt phytoremediation information were made.

The first focused on comparing articles referring EC and SAR reductions from phytoremediation and chemical amendments tests (set # 1) and the second focused on plant salt uptake capacity (set # 2). It would be useful to classify the studied plants into one of the previously discussed categories (accumulator, excluder or conductor) but due to the relative novelty of this categorization, sufficient data has yet to be compiled. Furthermore, it will be seen in both sets that very few plant species were tested. As in other phytoremediation applications, authors prefer the use of plants that have been tested elsewhere to enable comparisons of other relevant or novel parameters tested.

The first set of data compares articles with appropriate levels of information for EC and SAR reduction capacity in the first 15 or 30 cm of soil (Table 5.1). EC and SAR reductions are based on treatment reduction minus control values, when such differences were not already taken into account. The first two studies analyzed can be considered a direct comparison between chemical remediation and phytoremediation, while the comparison between examples 3 and 4 is based on similar initial EC and SAR, but from different studies, introducing further variability.

Table 5.1 - Soil EC and SAR reduction through phytoremediation and chemical amendments using different plants (i = initial, f = final) (Sources: 1 - (Qadir et al., 1997); 2 - (Qadir et al., 2002); 3 - (Ravindran et al., 2007); 4 - (Abd Elrahman et al., 2012); 5 - (Rabhi et al., 2009); 6 - (Rabhi et al., 2010), 7 - (Aydemir and Sünger, 2011).

Amendment or plant species	EC_i (dS m^{-1})	EC_f (dS m^{-1})	EC Reduction (%)	SAR_i	SAR_f	SAR reduction (%)	Source
Sesbania aculeata	7.5	5.5	27	55.6	43.5	22	1 (1st year)
Leptochloa fusca	7.4	5.3	28	57.9	44.7	23	
Sorghum bicolor	7.8	6.4	18	62.3	55.1	12	
Gypsum	9.0	7.2	20	73.0	53.3	27	
Sesbania aculeata	5.5	4.4	20	43.5	30.1	31	1 (2nd year)
Leptochloa fusca	5.3	4.9	8	44.7	32.5	27	
Sorghum bicolor	6.4	6.0	6	55.1	40.0	27	
Gypsum	7.2	6.8	6	53.3	24.7	54	
Sesbania bispinosa	11.1	4.6	58	35.0	7.9	77	2
Leptochloa fusca	11.1	4.2	62	35.0	9.0	74	
Gypsum	11.1	4.0	64	35.0	15.0	57	
Sesbania bispinosa	10.3	6.8	34	65.9	35.0	47	
Leptochloa fusca	10.3	7.8	24	65.9	37.0	44	
Gypsum	10.3	7.8	24	65.9	30.0	54	
Sesbania bispinosa	8.4	6.7	20	68.9	40.0	42	
Leptochloa fusca	8.4	5.8	31	68.9	45.0	35	
Gypsum	8.4	7.0	17	68.9	50.0	27	
Suaeda maritima	4.9	1.3	72	15.6	2.81	82	3
Sesuvium portulacastrum	4.9	2.5	50	15.7	3.94	75	
Clerodendron inerme	4.8	2.6	45	15.5	4.50	71	
Ipomoea pes-caprae	4.7	3.1	35	15.6	5.13	67	
Heliotropium curassavicum	4.8	3.6	26	15.3	7.65	50	
Gypsum	6.3	4.78	24	14.9	4.9	67	4
Citric acid		5.08	19		9.0	40	
Farm manure		4.88	23		7.4	50	
Compost		5.02	20		8.1	46	
Sesuvium portulacastrum	19	9.1	52	2.1 (mg g^{-1} Na^+)	0.63	70	5
Arthrocnemum indicum		10.1	47		0.76	64	
Suaeda fruticosa		12.0	37		0.94	56	
Sesuvium portulacastrum	14.4	9.1	37	59	39	34	6
Lotus corniculatus	5.27	2.4	54	20.5	15.8	20	7
	8.37	2.8	67	24.2	19.1	17	

By analyzing data from Table 5.1, some trends are visible. For instance, for plants referred in more than one study, the higher the initial EC value, the higher the difference between initial and final EC values. This can be seen for *Leptochloa fusca, Sesbania aculeata* and *Sesuvium portulacastrum* studies from different sources. Furthermore, the final three tests were conducted in non-leaching conditions, further indicating the possibility of plant uptake as the most significant driving force for remediation. In the case of the study presented by Ravindran et al. (2007), EC and SAR values decreased from above recommended values for soil (EC > 4 dS m^{-1} and SAR > 13) to values that may be considered non-saline or sodic. Yet, the initial values were significantly lower compared to those of other studies, and in the other cases analyzed, phytoremediation must be maintained for a prolonged period of time for total remediation.

A direct comparison of phytoremediation with chemical amendments (Table 5.1), namely gypsum, shows that EC reduction does not appear to be markedly different between different treatments, regardless of remediation time. Both treatment types experienced a significant reduction of treatment rates for this parameter by the second year (Qadir et al., 1997) and with lower initial EC (Qadir et al., 2002), indicating that treatment efficiency is dependent, once again, on initial contaminant values. This is potentially due to salt dilution in the leaching water and, as a result, every leaching event, in terms of mass balance, removes less and less salts from the soil, thereby decelerating remediation rates.

The reverse effect is visible on SAR reduction with a slight decrease with increasing SAR values, as well as an improvement over time, possibly reflecting improved hydraulic characteristics of the soil for leaching. For this parameter there are observable, yet contradictory, differences between the two treatment types: while in Qadir et al. (1997) gypsum (compared to phytoremediation) had a significantly superior SAR removal (in particular in the second year), in Qadir et al. (2002) phytoremediation almost always showed superior SAR reduction over gypsum (including at higher depths). In the indirect comparison between sources 3 and 4 in Table 5.1, phytoremediation with different plants (with one exception) revealed higher EC and SAR removal than four different amendment types.

Regarding plant behavior, in Qadir et al. (2002), *Sesbania aculeata* had the highest yield, while *Leptochloa fusca* had the lowest yield for the same soil type, despite the fact that both plants showed similar effects on SAR reduction. This may reflect different responses to salt stress for these two plant species, which implies that, in cases where phytoremediation is mainly due to enhanced soil structure for leaching, above ground biomass is not an appropriate indicator of the potential of a plant for salt remediation.

The application of non-leaching conditions provides further information on salt uptake capacity of plants in soils. Rabhi et al. (2009) reported that in the field, *Suaeda fruticosa* contributed to desalination of the surrounding rhizosphere mostly by improved leaching due to enhancement of soil structure, while the contribution of *Arthrocnemum indicum* was by salt uptake. When both plants were tested in non-leaching conditions, the maximum salt uptake of *S. fructicosa* was in fact higher. It is possible, therefore, that *S. fructicosa* improves the structure of the soil in a more efficient way than *A. indicum*, possibly due to different root systems, and in such a way that leaching occurs too quickly to enable significant amounts of salt uptake.

On the other hand, Aydemir and Sünger (2011) showed a reduction of calcite levels in the saline-sodic soils tested but not in the non-saline soil while planted. The authors attributed this difference to lower levels of calcite in the non-saline soil, but it is also possible that this difference was due to the initial high pH of the saline-sodic soils and subsequent reduction of pH after phytoremediation. The mobilization of calcium ions by the dissolution of calcite may have enhanced sodium desorption but, without leaching, the exchanged sodium would recapture its place during cation exchange and recontaminate the soil (Qadir et al., 2001). Therefore, salt uptake by the plants was likely to be the most important removal mechanism, aided by the dissolution of calcite which made sodium more available for plant uptake.

The second set of data acquired on phytoremediation performance regards phytoextraction and includes eight different articles in which salt uptake by different plant species was analyzed (Table 5.2). Values for salt removal in kg ha^{-1} year^{-1} were extrapolated when needed by dividing salt uptake data by the duration of the experiments and multiplying by the number of days of the growing season for each plant species (365 days in the case of perennial plants). Therefore, for the purpose of this analysis, it was assumed that plant productivity and salt uptake did not change significantly over the course of the growing season. Although a clear simplification, this extrapolation was required to account for the varied duration of the different studies and therefore allowed a direct comparison of plant salt uptake data.

Table 5.2 - Salt uptake in mg g^{-1} dry weight (DW) and kg ha^{-1} $year^{-1}$ of different plant species in saline soil remediation applications (Sources: 1 - (Zhao et al., 2005); 2 - (Neves et al., 2007); 3 - (Rabhi et al., 2009) 4 - (Rabhi et al., 2010); 5 - (Ravindran et al., 2007); 6- (Aydemir and Sünger, 2011); 7 - (Gharaibeh et al., 2011); 8 - (Boonsaner and Hawker, 2012).

Plant species	ECi (dS m^{-1})	SARi	Salt uptake mg g^{-1} DW	Salt uptake in kg ha^{-1} $year^{-1}$	Source
Suaeda salsa	42	-	155 (Na^+)	2300 (Na^+ + Cl^-)	1
Kalidium folium	42	-	168 (Na^+)	2800 (Na^+ + Cl^-)	
Tetragonia tetragonioides	21	-	-	4760 (Na + Cl)	2
Sesuvium portulacastrum	9.1	-	163 (Na^+)	5376 (Na^+)	3
Arthrocnemum indicum	10.1	-	113 (Na^+)	1527 (Na^+)	
Suaeda fruticosa	12.0	-	176 (Na^+)	1726 (Na^+)	
S. portulacastrum	14.4	59	273 (Na^+)	1931 (Na^+)	4
Suaeda maritima	4.9	15.6	184 (TDS)	1512 (TDS) [a]	5
Sesuvium portulacastrum	4.9	15.7	147 (TDS)	1422 (TDS)[a]	
Clerodendron inerme	4.8	15.5	94 (TDS)	1189 (TDS) [a]	
Ipomoea pes-caprae	4.7	15.6	81 (TDS)	1079 (TDS) [a]	
Heliotropium curassavicum	4.8	15.3	71 (TDS)	976 (TDS) [a]	
Lotus corniculatus	5.27	20.5	-	91 (TDS)	6
	8.37	24.2	-	200 (TDS)	
Atriplex halimus	65.3	26.4	288 (Na^+)	2419 (Na^+)	7
Atriplex halimus[b]	65.3	26.4	304 (Na^+)	3192 (Na^+)	
Typha angustifolia	18.8	-	370 (TDS)	1200 (TDS)[c]	8
Acanthus ebracteatus		-	620 (TDS)	2400 (TDS)[c]	

[a] Values estimated by mass balance

[b] With gypsum added to the soil

[c] Assuming approximate productivity of 10 ton ha^{-1} $year^{-1}$

According to Table 5.2, values of salt uptake can vary from 91 kg ha^{-1} $year^{-1}$ for *Lotus corniculatus* to up to 5376 kg ha^{-1} $year^{-1}$ for *Sesuvium portulacastrum,* which are both halophytic plants. However, non-halophytes (or salt tolerant) can have significant salt uptake capacity as, for example, *Typha angustifolia* removed 1200 kg ha^{-1} $year^{-1}$, while others have the potential for salt uptake, but were never actually tested in the conditions necessary to be referenced in the present comparison. Initial EC plays a significant role in total salt uptake, as seen in Aydemir and Sünger (2011), where the higher the EC value, the larger the salt uptake capacity. On the other hand, simultaneous gypsum addition may also increase the salt uptake capacity by 132% (Gharaibeh et al., 2011).

A compromise between halophytic (or salt tolerant) crop yield and remediation goals may be required in harsh phytoremediation conditions, e.g., highly salt affected soils.

Depending on whether or not the aerial biomass obtained is to be used, remediation options may differ. For instance, Boonsaner et al. (2012), proposed an initial crop of *Glycine max* for salt remediation, alleging that the high salt uptake per dry weight and the small price of plant seeds would make the process viable.

The salt accumulation data shown in Table 5.2 needs to be analyzed in the proper context. Assuming a hypothetical case study of a medium textured soil with a water saturation percentage of 35% and bulk density of 1300 ton m^{-3}, EC of 20 dS m^{-1} and a remediation goal of lowering EC to 4 dS m^{-1}, then the mass of total dissolved salts that needs to be removed is 71.5 ton ha^{-1} at a soil depth of 1 meter. To achieve this by plant remediation, even with the best performing plant listed in Table 5.2 (*S. portulacastrum*), it would take approximately 13 years, not considering further salt inputs that might occur during that period. Considerations like these may have led many researchers to deem that phytoextraction alone is not a viable remediation option. However, most studies consider remediation of only the first 15 to 30 cm of soil as this is the most important depth for most agricultural crops. At a soil depth of 15 cm, the value of salts to be extracted in this scenario would be significantly lower, circa 10.725 ton ha^{-1}, reducing the remediation time needed to only 2 years.

With the goal of making a clear assessment of the potential of plant species to remediate salt affected soils, a bioconcentration factor (BCF), similar to that applied to heavy metals phytoremediation, could be used. However, the obtained BCF could be, as seen before for the plant performance in Table 5.1, dependent on initial salt concentration, as well as on productivity (Liang et al., 2009). Furthermore, a differentiation between BCF for total dissolved solids (TDS) and for sodium would be required to define salt hyperaccumulating plants. In addition, the perfect plant for remediation should have a high sodium uptake, but a low uptake of calcium and magnesium since they are stabilizing agents for the soil, and therefore contribute more quickly to SAR reduction.

The distribution of sodium within plant tissues is also a relevant aspect (Table 5.3) to assess salts translocation capacity to above ground biomass. This approach is needed not only to assess overall salt removal but more specifically to calculate the ratio between sodium to calcium and magnesium. Potassium is also a relevant ion, since a high K^+ / Na^+ ratio may indicate that the plant needs potassium to tolerate sodium toxicity. This could increase potential nutritional needs or indicate that the plant is highly selective to this cation over sodium. As such, sodium uptake would be smaller in the presence of high levels of potassium.

For example, comparing sodium uptake content alone, *Petrosimonia brachiata* and *Atriplex tatarica L.* could be classified as similar in their potential for SAR reduction in a contaminated soil (based only on data in Table 5.3). However, *P. brachiata* also showed a significantly larger concentration of calcium, magnesium and potassium when compared with *A. tatarica L.* Therefore, *Atriplex tatarica L.* seems to be a more appropriate choice for saline soil phytoremediation.

Table 5.3 - Ion distribution (in mmol L^{-1}) in different plants (Tipirdamaz et al., 2006) and plant tissues (Rabhi et al., 2010).

		Na^+	K^+	Ca^{2+}	Mg^{2+}	K^+ / Na^+	Cl^-
Halocnemum strobilaceum		3.30	0.25	0.20	0.13	0.08	2.04
Chenopodium album		1.54	0.78	0.03	0.34	0.51	1.70
Atriplex tatarica L.		1.42	0.29	0.01	0.37	0.20	2.24
Petrosimonia brachiata		1.42	0.46	0.05	1.49	0.32	3.14
Plantago maritima		1.33	0.26	0.46	0.24	0.20	0.78
Reaumuria alternifolia		1.44	0.30	0.28	0.57	0.21	1.79
Salicornia europaea		4.49	0.40	0.21	0.39	0.09	5.57
Sesuvium portulacastrum	Leaves	6.52	0.40	0.58	0.19	0.06	-
	Stems	3.81	0.78	1.12	0.21	0.21	-
	Roots	1.63	0.45	0.54	0.20	0.28	-

As previously mentioned, plant productivity is extremely relevant for overall salt phytoremediation. Even when phytoextraction values are extremely high, the impact of plants on soil remediation can be low due to low plant growth. This can be better understood using, for instance, the work of Goulet et al. (2005) on aluminum phytoremediation, were it was reported that in mesocosm tests, *Lemna minor* had an aluminum concentration capacity close to 5 times that of *Typha latifolia*, although the latter was responsible for 99% of the aluminum removed. In the situation under analysis, while above ground productivity is considered in the results expressed in Table 5.2, this value is dependent on a variety of conditions and further studies should be developed to assess productivity in saline environments closer to actual plant exposure in a salt affected soil.

Furthermore, productivity is dependent on plant density, which is yet another parameter that is far from being optimized in this context. So far, data shows that increasing density can result in decreased productivity, but it may also increase salt accumulation in plant tissues (Hansi et al., 2014), possibly determining an overall increase in salt uptake.

5.6 Opportunities for enhancing salt phytoremediation

In order to enhance salt phytoremediation there is a need to improve the two main mechanisms by which plants can remediate a salt affected soil: phytoextraction or leaching enhanced by plant roots. How these goals can be approached may differ: either by increasing salt uptake per unit of mass (through various methods, mostly biological) or by increasing tolerance to salinity stress and therefore increasing yield, which can create more leaching through larger and stronger roots and/or increased overall salt uptake. Also, different management techniques can contribute to an increase of the efficiency of the process. An analysis of the potential applicability of different enhancement techniques for phytoremediation of salt affected soils, which are summarized in Figure 5.2, may provide information of future research trends and co-treatment possibilities to enable the management of complex soil contaminations.

Many of the techniques that will be described have yet to be implemented and, in some cases, were not even tested in soil salinization processes or with halophytic plants. In some instances, therefore, the techniques presented were applied in other experimental contexts but can provide relevant information for the enhancement of salt affected soils phytoremediation.

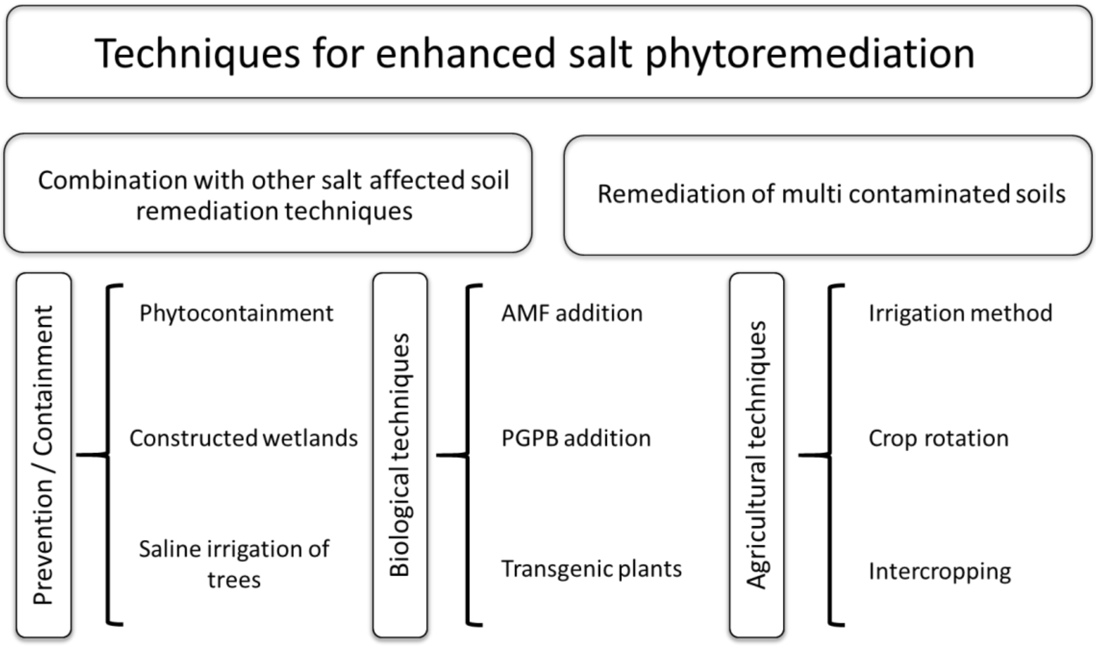

Figure 5.2 - Techniques for enhanced salt phytoremediation grouped by type

Combination of remediation techniques & multi contaminated soils

To increase plant salt remediation efficiency, there are opportunities for synergetic combinations between different treatment types for salt affected soils. For instance, Gharaibeh et al. (2011) described that phytoremediation and gypsum addition could increase plant phytoextraction capabilities and productivity as well as increasing overall salt removal. Theoretically, the plants without gypsum may have had a calcium deficiency (Zia et al., 2007; Ahmad et al., 2011) that was supplemented by gypsum addition, which resulted in increased productivity. However, it is more likely that gypsum increased the bioavailability of sodium ions by supplying calcium, which removed adsorbed sodium from soil particles. Additionally, plants may have decreased pH (Ghafoor et al., 2012) and therefore increased gypsum dissolution rates. These hypotheses, however, require further studies to be confirmed.

Nevertheless, other studies showed that the combination of plant remediation and amendments were not always beneficial. In fact, gypsum and H_2SO_4 decreased the productivity of Kallar grass (*Leptochloa fusca L.*) and Berseem (*Trifolium alexandrinum L.*) when compared to the control conditions (Zia et al., 2007). Reduced plant productivity was also obtained for the combination of S. *bispinosa* with H_2SO_4, especially in a high SAR environment, due to chemical burns of plant roots (Ahmad et al., 2011).

Therefore, doubts remain on the existence and importance of synergetic combinations of salt remediation treatments.

Phytoremediation can be applied for combined treatment of salt affected soils also contaminated with other pollutants. Several contaminant combinations have already been considered, for instance, the combined treatment of saline soil with organic degradation of polycyclic aromatic hydrocarbons (PAHs) or total petroleum hydrocarbons (TPHs) (Hue et al., 2002). The uptake of multiple heavy metals by a single plant species is possible (Satpathy and Reddy, 2013) and therefore simultaneous uptake of heavy metals and sodium is likely to be possible as well since some researchers have found a link between the root zone ionic strength and composition (i.e. saline levels and ion distribution) with the type of excreted salts from salt glands (Manousaki et al. 2008). Although, salinity does not seem to affect heavy metal phytoextraction in the same way for all metals (Manousaki and Kalogerakis, 2009).

Salinization Prevention or Containment

Controlling or limiting the salt flow is of the utmost importance to prevent further soil degradation through salinization processes. Hydraulic control of catchment areas is an important part of an integrated management policy to control salt flows. Phytohydraulic containment using salt tolerant trees has already been applied in the control of saline seepage, salt mobilization and capillary rise of salts from contaminated groundwater. By consuming large amounts of water in their growth, trees enable the reduction of the water table, decrease runoff and upflow of the groundwater, and can even phytoextract significant quantities of salt without deleterious effects (Crosbie et al., 2008; Rodríguez-Suárez et al., 2011).

To prevent further expansion of salt affected soils, intensifying and/or improving saline wastewater discharge control can have a significant impact. Several industries produce saline wastewater, ranging from fish farming activities to oil and gas extraction. Although phytoremediation has been successfully used to treat saline wastewaters (1 to 3.5% salinity levels) from a variety of industries such as aquaculture (Laudicina et al., 2009), tannery (Calheiros et al., 2012) or olive mill (Herouvim et al., 2011), the existing effluent excess salts were not considered a problem to be treated. Some studies with constructed wetlands have, however, demonstrated the ability of salt co-treatment with other pollutants (Lymbery et al., 2006; Jesus, et al., 2014), while in other studies, constructed wetlands were designed for the sole purpose of salt phytoextraction, with promising results (Shelef et al., 2012). Constructed wetlands may be a good option for intercepting non-point contaminants (from agriculture and greenhouse leachates, for instance) in catchments areas, avoiding salt discharge in the soils.

The reuse of several saline wastewater sources in phytoirrigation of trees is also under investigation which can prevent salt accumulation and/or leaching through the soil due to uptake by trees (Jordahl et al., 2004; Zalesny and Bauer, 2007; Smesrud et al., 2011).

Biological techniques

The application of arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB) has been extensively proposed in order to increase plant salt tolerance and promote the growth of plants in saline soils (Gamalero et al., 2009). These applications include not only agricultural crops but also salt marsh halophytes, potentially to increase their phytoextraction efficiency (de-Bashan et al., 2012). The subjects of plant salt tolerance and the impact of these applications have been extensively and adequately analyzed elsewhere (Evelin et al., 2009; Gamalero et al., 2009; Dodd and Pérez-Alfocea, 2012; Porcel et al., 2012). Therefore, in this review, only the potential of AMF and PGPB addition in the enhancement of salt affected soils phytoremediation (particularly in phytoextraction) will be explored since this was not yet addressed.

It is known that the addition of AMF affects plant accumulation of Na^+ and K^+, which may be relevant to phytoremediation of salt affected soils (Evelin et al., 2009; Gamalero et al., 2009; Cartmill et al., 2012; Ruiz-Lozano et al., 2012). There are reports of increased uptake of sodium and chloride with AMF, which can be accompanied by increased nutrient uptake and productivity (Evelin et al., 2009). In some other studies, however, there is reduced uptake of sodium (Abdel et al., 2011), which may be regarded as undesirable for phytoremediation goals. However, these results refer to glycophytic plants, as studies with AMF inoculation of halophytes with emphasis on salt phytoextraction are very rare. A recent example can be found in the work of Zhang et al., (2014) where AMF added to *Ricinus communis* lead to reduced EC and sodium values in the soil possibly (as reported by the authors) due to increased salt phytoextraction through the roots. The existence of limited studies on AMF addition to halophytes can be due to multiple reasons: there is a higher interest in applying AMF in crop plants to increase their yield, as halophytes have limited commercial uses; salinity limits the richness of naturally occurring AMF species (Krishnamoorthy et al., 2014), and many halophytic plants are considered to be non-mycorrhizal (Caravaca et al., 2005). Nevertheless, there are some clues to the potential of AMF addition for enhanced phytoremediation. In Zhang et al., (2011), for instance, despite the fact that AMF reduced sodium uptake in the aboveground biomass of the halophyte *Leymus chinensis* by 29%, the concurrent 222% increase in biomass lead to an overall increase of accumulated sodium by 130%. Furthermore, AMF addition can lead to an improvement of soil structure and therefore leaching of salts and soil remediation (Caravaca et al., 2005; Qin et al., 2015).

Regarding PGPB, there are several studies in which its utilization enhanced salt tolerance and therefore plant productivity under saline conditions. An extensive list of examples can be found in de-Bashan et al. (2012), and to illustrate this possibility two examples are referred in the present work: in Goswami et al. (2014), *Arachis hypogaea* treated with *Bacillus lincheniformis A2 s*howed an increase of 31% in plant length and 43% in fresh biomass at 50 mM NaCl while in Siddikee et al. (2011), several types of PGPB were tested and all led to improved root length, accumulation of dry matter in roots and reduction of ethylene stress levels, leading to increased salt tolerance in *Capsicum annum*

L. Yet, in this study, sodium uptake by the plants decreased. As previously noted for AMF, studies with PGPB application in halophytes are less common. However, Rueda-Puente et al.(2007) reported higher plant height, length of the root system and fresh biomass of the halophyte *Salicornia bigelovii* with the addition of PGPB in saline conditions, concluding that PGPB could be used reliably to promote the growth of halophytic plants. Although PGPB have been extensively studied for the enhancement of several different types of phytoremediation of contaminated soils (de-Bashan et al., 2012), in the bibliographic search undertaken in this work no studies were found that showed increased salt phytoextraction on a per mass basis in any plant as a result of PGPB addition. Nevertheless, similarly to what is observed with AMF, PGPB may increase overall salt phytoextraction by enhancing plant yield (Chang et al 2013).

The development and utilization of transgenic plants can also be a potential way to further increase plant salt tolerance. In Bhavanath et al. (2013) transgenic plants of *Jatropha curca*, in which the SbNHX1 gene was cloned from *Salicornia brachiata*, had a higher yield compared to wild types as well as a 43% increase in sodium uptake. Similar results are also reported in others studies (Rajagopal et al., 2007; Jha et al., 2011). Most studies with transgenic plants are focused on increasing salt tolerance, not necessarily on phytoextraction. However, by focusing on genes that regulate the plasma membrane-bound or vacuolar Na^+/H^+ antiporters, improved salt compartmentation in the vacuoles was observed (Saqib et al., 2005; Apse and Blumwald, 2007; Jha et al., 2011; Hasegawa, 2013), and therefore, increased phytoextraction is a by-product of these enhancements.. Curiously, Ruan et al. (2010) report the existence of 100 claims of plant transformation by genetic engineering aimed at increasing salt tolerance but, yet again, few of them focus on halophytes, as these plants are much more frequently used as the source of the gene rather than the tested plant.

Agricultural techniques

The way irrigation water is applied to a salt affected soil can also be optimized to enhance salt removal from both the soil and irrigation water. For instance, it has been reported that water logging tends to increase salt phytoextraction; therefore ponding could be used to enhance salt uptake by some halophytes (Barrett-Lennard and Shabala, 2013), as the foliar concentration of sodium may double (Carter et al., 2006). On the other hand, directly exposing plant leaf surface to the saline solution can increase salt foliar absorption (Qadir et al., 2000; Sultana et al., 2001; Chondraki et al., 2012).

A scheme for annual crops could be developed by crop rotation. This method has already been used in salt affected soil remediation and prevention of secondary salinization (Kaur et al., 2007; Zia et al., 2007; Mandare et al., 2008; Ahmad et al., 2011; Al Khamisi et al., 2013). Crop rotation in this context can take many forms, but mostly involves the use of either a halophytic plant or rice crop, followed by an economic crop (Ahmad et al., 2011): the first crop is salt tolerant and is used to leach the salts, particularly in the case of rice, and is intended to create more adequate conditions for the growth of the second, more economically valuable crop. This possibility is well explored elsewhere (Qadir et al., 2008).

Intercropping salt removing plants with existing crops could also be an option to prevent or remediate salt affected soils (Qureshi et al., 2003; Kan et al., 2008; Al Khamisi et al., 2013). An intercropping agroforestry scheme could provide simultaneous salt removal in a preventive approach (Kiliç et al., 2008). However, there seems to be conflicting results in the literature: Inal and Gunes (2008) concluded that interspecific root interactions might be helpful for mineral nutrition and salt tolerance in mixed crops, while Kurdali et al. (2003) found no substantial differences with the use of intercropping in a saline soil, and Patra et al. (2002) reported no changes in productivity with intercropping, but a significant sodium accumulation in one of the plants used (*Matricaria chamomila*), which increased with gypsum addition. Further studies are required to clarify the potential of intercropping on salt affected soils remediation.

Economically, crop rotations and intercropping may provide further income and cost less in fertilization and water use, since the leaching requirement is decreased in summer months.

5.7 Climate change: effects on soil salinization and adaptation measures

Climate change has repercussions in soil salinization expansion and prevalence, as well as in the remediation techniques that can be applied. Prevalent climatic conditions affect the choice of remediation technique in any given location and contamination scenario. However, regional predictions of future climatic changes should also be taken into account in the choice of a soil remediation technique, given the fact that remediation efforts may span a considerable amount of time and climate change may cause significant and ever evolving differences in contaminant concentration and biochemical parameters (Bradford et al., 2010; Van den Berge et al., 2011).

The impact of climate change on soil salinization expansion is difficult to assess (Schofield and Kirkby, 2003). In the case of Europe, current levels of soil salinization are estimated at 50 million ha. However, Szabolcs (1974) estimated an increase, due to direct and indirect impacts of climate change, of at least 26.7 million ha by the year 2050, a 53.4% increase in Europe alone. The expected increase would be due to expansion of arid and semi-arid environments, sea level rise and irrigation (Van-Camp et al., 2004; Tóth et al., 2008). In Australia, one of the most affected countries by both salinity and climate change, beyond the confirmed 1.047 million ha of salt affected soils, there are an additional 1.7 million ha estimated cases of salinization or in risk of salinization (Jardine et al., 2007).

Therefore, a flexible remediation technique that is both adaptable to climatic changes and environmentally sustainable, is required (Hou and Al-Tabbaa, 2014; Hou et al. 2014). Climate change impacts on remediation efficiency are already being evaluated for other types of contaminants and remedial techniques, such as bioaugmentation, organic amendments and acid mine drainage remediation (Al-Tabbaa et al., 2008; Anawar, 2013). Also, new management options are being considered for field remediation scenarios such as accelerating the initiation of the restoration process to prevent further deterioration from climate change or the application of compensatory restoration, when necessary (Harris et al., 2006; Rohr et al., 2013).

Climate change can have unpredictable and even contradictory impacts on phytoextraction techniques. Rajkumar et al. (2013) review several possible implications of climate change on metal phytoextraction capabilities of various plants, with examples in which climate change lead to increasing or decreasing metal uptake, depending on plant type, and tolerance to metal ions. Also, ion competition for cation exchange sites between salts and heavy metals was shown by Hamzenejad Taghlidabad et al. (2014). For salt phytoextraction, however, there is little information on the impact of climate change. Therefore, further studies are necessary to increase the robustness of all remediation techniques to climate change and of soil salinization remediation techniques in particular. In 1996, IPCC (Intergovernmental Panel on Climate Change), addressed this issue within the broader future scenarios of “warm and dry” and “warm and wet” climates. The impact of the two scenarios set up by the IPCC on irrigated soils, in existing salt affected soils, and in their expansion, is explored in Table 5.4.

Table 5.4 - Climate change effects on multiple parameters, including salt affected soils, within two future scenarios: warm and dry & warm and wet conditions Sources 1 - (IPCC 1996); 2 - (Szabolcs 1990); 3 - (Van-Camp et al., 2004); 4 - (Ritzema et al., 2008), 5 - (Horneck et al., 2007), 6 - (Daily 2005).

Effects on	Scenario warm and dry	Scenario warm and wet
Rainfall	Decreased [1]	Increased [1]
Water table	Decreased [1]	Increased [1]
Irrigation	Increased [2]	Normal or decreased
Drainage[a]	Increased [3]	Decreased [4]
Irrigated non-salt affected soils	Salt buildup by evaporation, use of brackish water or saline groundwater [2,3]	More leaching, less irrigation needed, more drainage necessary in some cases[1,4]
Existing Salt affected soils	Further salt buildup, less leaching and vegetation cover, more wind erosion and transport [2,3]	More leaching, reducing dissolved salts but decreasing soil structure [1,5]
Expansion of salt affected soils	Expansion of affected area to adjacent soils following the trend of aridity [1,2,3]	Leaching salts to groundwater or, if low drainage, lateral spread of salts and water logging [6]

[a] In case of if shallow aquifer occurrence

By comparing the two analyzed scenarios, it becomes clear that the “warm and dry scenario” is the most negative and influential one on soil salinization, as expected. However, the warm and wet scenario also presents significant, although rather unexpected, negative impacts. In particular, in situations where the water table is already high, the excess rainfall may create water logging and further agricultural damages. On the other hand, intensive rainfall will leach out the dissolved salts, further de-stabilizing the soil, and resulting in clay loss, macropore clogging and reduced permeability (Diamantis and Voudrias, 2008; Sahin et al., 2011). This will effectively transform a manageable saline-sodic soil into a hard set sodic soil (Horneck et al., 2007). This excess water can also create lateral transport of salts, propagating soil salinity to neighboring, potentially not yet affected soils, or forming saline seepage and waterlogging (Daily 2005; Dragovich and Dominis, 2008).

It is certain, however, that soil salinization is severely affected by climate change. Yet, the reverse effect, the impact of soil salinization on climate change, must also be considered, since the expansion of soil salinization translates into less CO_2 uptake due to its impact on plant yield (Setia et al., 2013). However, it might also negatively affect decomposition rates, thereby lowering CO_2 emissions. Overall, it has been found that soil salinization has a total negative effect on climate change, with an estimated past contribution of 2 Pg of CO_2 (Setia et al., 2013).

The “warm and dry scenario” is likely to present more challenges for phytoremediation, not only due to increased salt concentrations, but also due to the action of other simultaneous stress inducers, such as heat and drought. Therefore, there is more work developed to study the negative impacts of this scenario. Drought and heat stress in plants entails a very similar response to salt stress, since salt stress may also cause both osmotic imbalances and water absorption deficiencies. Heat and salinity stresses also have similar effects in the sense that both cause oxidative stress. As a result, foliar application of compatible solutes, such as proline and glycinebetaine, has been suggested to increase plant tolerance to heat and salt (Wahid et al., 2007). Accumulation of proline and glycinebetaine has been reported in salt accumulating plants, since they are used to maintain osmotic balance in the cell, which is disrupted by the presence of ions stored in the vacuoles (Abdel et al., 2011; Manousaki and Kalogerakis, 2011b). Therefore, these organic solutes do not necessarily preclude salt uptake and may be used to enhance it by increasing salt tolerance, although further studies are necessary to confirm or deny these hypotheses. Nevertheless, even if having neither a positive nor a negative effect on salt uptake, these solutes would increase tolerance to heat and salinity stress, enabling improved plant acclimation to heat waves. Hansi et al. (2014) indicated that the combination of heat and salt stress had less negative effects compared to each stress acting individually in tomato plants, possibly due to the increased glycinebetaine accumulation stimulated by both stress inducers.

Heat acclimation and foliar application of calcium may also be used to improve heat tolerance and again, in the case of calcium application, may simultaneously alleviate salt stress (Wahid et al., 2007).

Climate change will also increase the occurrence of extreme events, namely floods, specifically in the “warm and wet scenario”. Plants have varying resistance to flooding, which are mainly limited by oxygen deprivation. Physiologically, plants adapt to this situation through the growth of adventitious roots containing aerenchyma (Carter et al., 2006).

In temperate climates, in which dryland salinity is not a severe problem, halophytes can be found mostly in wetlands and are likely to be acclimated to both salinity and flood conditions. In arid or semi-arid regions, however, continuous flood conditions are unlikely, and acclimation to this type of stress is rarer, since it is exacerbated by uneven distribution of precipitation, such as in monsoon areas (Akhter et al., 2004).

5.8 Final considerations and perspectives

Salt affected soils threaten global agricultural productivity. Although several remediation techniques have been successfully developed and implemented, there are still various situations for which there is a lack of appropriate technical solutions. Under certain circumstances, phytoremediation can be the best option from both technical and economical perspectives.

In this work, we intended to clarify the efficiency of the phytoremediation approach for the salt remediation of soils. A thorough review of the available literature, and in particular of the research directly comparing phytoremediation with other approaches, reveals removal efficiency similar to other techniques. However, debate on the main mechanism behind salt phytoremediation has yet to be settled and requires a more focused research effort to assess the contribution of phytoextraction to the remedial process. Furthermore, recent research in the field of phytoremediation, and particularly phytoextraction, hints at several new possibilities to increase the efficiency and quality of the treatment of salt affected soils (combination of treatment types, mixed plant cultures, biostimulation, etc.) or expand to new applications such as co-treatment and salt flow control measures. Nevertheless, these novel applications are still in their infancy and further development is essential.

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6 Comparison of vegetative bioremediation and chemical amendments for non-calcareous highly saline-sodic soil remediation

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6.1 Highlights

Amendments and phytoremediation were tested for a non-calcareous saline-sodic soil.

Plant salt uptake was comparable to others and had little effect on soil parameters.

Plants may still be useful in a water scarcity scenario using extended remediation time.

Chemical amendments proved effective but choice may depend on economic constrains.

Applied together, these techniques might decrease remedial time even without leaching.

6.2 Keywords

Chemical amendments; vegetative bioremediation; gypsum; salt uptake; highly saline-sodic soil

6.3 Abstract

Salt affected soils cover a wide area, limiting agricultural production worldwide. Several remediation options are available and include chemical amendments and vegetative bioremediation, but several aspects of each remedial process are not yet fully understood. Therefore, the goal of this work is to study the application of both techniques in a highly saline scenario and provide insights into the limits of the application of this technology.

Two chemical amendments ($CaSO_4$ and $CaCl_2$) and two plant species (*Juncus maritimus* and *Spartina maritima*) were tested to improve the quality of a non-calcareous saline-sodic soil with an initial electrical conductivity (EC) value of 20 dS m^{-1} and sodium adsorption ratio (SAR) of 45.

Vegetative bioremediation experiments were performed under non-leaching conditions. The salts showed a redistribution with an increase at the soil surface and a decrease in depth due to capillary rise. In such conditions, there was no evident positive effect of plant presence on soil parameters. However, tested plants survived and grew, and accumulated and excreted dissolved salts and sodium comparably to other research in the literature. Regardless, the obtained results suggest that salt uptake by plants alone is not sufficient for adequate saline soil remediation, and therefore, other mechanisms may also play a significant role.

In terms of chemical amendments, both chemicals used proved effective and reduced non-calcareous saline soil parameters below the threshold values of 4 dS m^{-1} for EC and 7 for SAR. However, $CaCl_2$ was more effective and faster to remediate excess salt than $CaSO_4$, which was likely due to higher solubility. Therefore, it may be a viable, yet less tested, option for faster remediation processes.

The two tested remediation techniques could be used in combination for added benefits, but more research is needed.

6.4 Introduction

Soil salinization can be described as the excess of salts and/or of sodium ions, either in the soil solution or in its cation exchange sites (Qadir et al., 2000). This accumulation may derive from human activities, such as poor irrigation practices or excessive aquifer exploitation close to the sea, etc. Alternatively, salt accumulation may occur naturally, either due to saline groundwater contamination or geological deposition of former seas, amongst other phenomena. These type of soils can be divided into saline, sodic or saline-sodic, depending on their characteristics. Saline soil refers to soils with electrical conductivity (EC) values higher 4 dS m^{-1} and a sodium adsorption ratio (SAR) below 13, while sodic soil refers to soils with EC lower than 4 dS m^{-1} and a SAR higher than 13; and finally, saline-sodic refers to a combination of both types, in which both EC and SAR exceed the above mentioned limits simultaneously (US Salinity Laboratory, 1954; Qadir et al., 2000).

Depending on the source of salt contamination, EC and SAR values in the field can vary widely, with some locations exceeding EC of 65 dS m^{-1} (higher than seawater). Nevertheless, remediation objectives tend to target EC below 4 dS m^{-1} and SAR values below 7-13 (Ashworth et al., 1999).

There are several remediation options for salt affected soils, which can be grouped into three specific types: leaching, addition of either organic or chemical amendments and vegetative bioremediation with salt tolerant plants. Leaching is the most basic and applied technique and can be described as the addition of excessive water in order to move soluble salts through the soil to higher depths. There are various methods of water application with different possible outcomes. One such example is the addition of excessive water at each irrigation time to ensure salt drainage, and therefore, prevent soil salinization.

However, as a remediation option, leaching has no direct effect on SAR values, limiting its application to saline soils only. In addition, it is limited to areas with sufficient water of moderate to high quality (low salinity) and it is also dependent on soil hydraulic conductivity. Furthermore, it also depends on the existence of a deep groundwater table to limit potential capillary rise or water logging (see Qadir et al. (2000) for a thorough review of leaching techniques).

Chemical amendments are the most common method in order to overcome the above mentioned issues when remediating sodic soils. These chemicals work by promoting the dissolution of native minerals with calcium (usually $CaCO_3$) which, in turn, will react with adsorbed sodium through cation exchange. Alternatively, these chemicals may also result in a direct addition of calcium cations for ion exchange with the adsorbed sodium, both followed by leaching. Gypsum ($CaSO_4$) is the most common chemical for amendment, but there are several other chemical compounds available such as $CaCO_3$, $CaCl_2$, H_2SO_4, S, HCl,

FeS_2, CaS_5, $FeSO_4$, $Al_2(SO_4)_3$, each one with different effects and limitations (Qadir et al., 2001).

Chemical amendments also show several disadvantages, since they still require leaching of the soil to wash away the sodium that was removed. If there is no water for adequate leaching or if drainage remains limited despite treatment, the exchanged sodium can replace calcium ions supplied by the chemical amendments and recontaminating the soil (Qadir et al., 2001).

The addition of some acid chemical amendments may excessively decrease of soil pH and therefore, its applicability may be limited to calcareous soils. Furthermore, chemical amendments are of limited reactivity. This may be problematic since they are only able to react until equilibrium is reached between calcium and sodium on the cation exchange sites and is dependent on the quantity of calcium added. Therefore, chemical amendments do not have a sustained reaction over time (Qadir et al., 2003) and only those that add soluble calcium can be applied to non-calcareous salt affected soils.

Remediation tests of non-calcareous soils are scarce in the literature. Therefore, the effects of chemical amendments under such conditions are not yet fully understood. For instance, it is unclear whether the efficiency of gypsum is affected by this scenario, since gypsum is also applied to decrease pH and dissolve native $CaCO_3$ (Kim et al., 2016) in calcareous soils, but will have no such effect on non-calcareous soils.

Another alternative for salt affected soils remediation is vegetative bioremediation. In this technique, plants may serve a multitude of purposes ranging from physical to chemical influences (Jesus et al., 2015b). Physically, penetration of plant roots, particularly in a sodic soil, leads to improved soil structure and drainage. Plant root exudates increase organic content and improve microbial activity, leading to higher soil fertility. This also increases carbon dioxide partial pressure and leads to a reduction in pH. Consequently, this pH reduction increases the dissolution of native minerals containing calcium ions which, in turn, exchange with sodium. Finally, plants may also directly accumulate or excrete dissolved salts or sodium. However, the relative contribution and importance of each of these mechanisms is still unclear, particularly whether plant salt uptake and accumulation can provide sufficient treatment when isolated (i.e, in non-leaching conditions and/or non-calcareous soils (Jesus, et al., 2015b).

Not all plants are able to perform in such saline / sodic conditions. Therefore, plant selection is paramount, with preference for plants that are both halophytic and with high aerial biomass productivity. However, halophytic plants have different responses to salinity, which might affect their impact on salt remediation efforts.

As proposed by Yensen and Biel (2006), halophytic plants can be salt excluders, accumulators or conductors. This classification is extremely relevant for vegetative bioremediation as excluder type plants may be of little use or even counterproductive for the treatment effort, regardless of their salt tolerance. Alternatively, accumulator and conductors plants may accumulate sufficient salts in their tissues to positively impact soil salinization remediation. However, an important distinction between the two is whether or not wind salt dispersal is a valid decontamination procedure. This mechanism would remove the need for constant plant harvest, but remains poorly understood despite recent studies referencing its potential (McSorley et al., 2016a; McSorley et al., 2016b).

The goal of this study is to evaluate the effect of chemical amendments and vegetative bioremediation on a salt affected soil. However, only two chemical amendments and two plant species are being tested under highly saline conditions in order to limit the scope of the study. Furthermore, a non-calcareous was chosen to minimize the potential for native $CaCO_3$ dissolution as a removal mechanism.

Finally, unlike chemical amendment tests, vegetative bioremediation tests were conducted under non-leaching conditions with two different plant species not yet reported in the literature in such a scenario. This was intended to see the very limits of the applicability of this technology by simulating a scenario where leaching is not an option, whether due to water scarcity or high groundwater table, and where only vegetative bioremediation had the potential for remediation through phytoextraction (since chemical amendments without leaching are not effective).

In this way, it was also expected to eliminate the impact of leaching, pH reduction and native calcium minerals dissolution on remediation. Therefore, it would isolate the direct impact of plant salt uptake on salt removal.

6.5 Material and methods

6.5.1 Soil characteristics and contamination procedure

The saline soil tested was obtained by contaminating a non-saline sodic soil rather than using a naturally occurring saline-sodic soil. This was done in order to ensure more homogeneity in contamination values across the different experimental setups.

Soil characteristics can be found in Table 6.1. This soil was of the same origin as the 'organic soil' tested in Jesus et al. (2015a). Screening tests for possible heavy metal contamination were done using a Portable Analytical X-Ray Dispersive Energy Fluorescence Spectrometer (Innov-X System), and no excessive levels of metals were detected in the soil (data not shown).

Table 6.1 - Characterization of the soil used in this study prior to salt contamination.

Sand (%)	75.29	EC (dS m^{-1})	0.90
Silt (%)	22.61	Ca^{2+}+Mg^{2+} (meq L^{-1})	3.05
Clay (%)	2.11	Bulk density (kg L^{-1})	1.10
Texture	Loamy sand	Soil density (kg L^{-1})	2.14
Saturation percentage (%)	33.0	pH water (1:1)	7.22
Lime content	not detected	pH 0.01 M $CaCl_2$ (1:2)	5.76
CEC (meq 100 g^{-1})	42.3	pH 1 M KCl (1:1)	4.80
OM (%)	7.4	Exchangeable acidity (meq 100 g^{-1})	0.25

For the salt contamination process itself, several initial tests were performed to ensure reproducible results. The initial objective was to raise soil EC to roughly 20 dS m^{-1}. This value was chosen to simulate a relatively high EC (4.7-19 dS m^{-1}, Jesus et al., 2015b), which is within the range of other studies found for salt affected soil remediation and is comparable to other research under non-leaching conditions (14.4-19 dS m^{-1} (Rabhi et al., 2009; Rabhi et al., 2010)). For that purpose it was estimated that a concentration of 12.8 g L^{-1} of NaCl at 33% soil volume (i.e. equivalent to saturation percentage) would yield such value. However, to prevent or limit water erosion damages to soil structure, only half of the volume corresponding to the saturation percentage was used with double the concentration (25.6 g L^{-1} of NaCl for 16.5% soil volume). The resulting saline solution prepared using NaCl (Merck; ≥ 99.5%) was carefully and thoroughly distributed through the soil volume to ensure a homogenous distribution. Immediately afterwards, the soil was dried at 60ºC in multiple recipients for at least 15 hours with sporadic mixing to ensure maximum drying area and limited soil depth to avoid salt redistribution during drying.

After drying, soil samples were collected and tested for the main contamination parameters, namely, EC, Na^+ and Ca^{2+}+Mg^{2+} , and SAR, and the initial values were determined to be EC ≈ 20 dS m^{-1} and SAR ≈ 45. However, initial values were checked before each experiment to ensure more precise values.

6.5.2 Plant Origin and Maintenance

Two plants species were used, *Spartina maritima* and *Juncus maritimus*. These species were chosen based on their capacity for salt tolerance, removal and excretion (Jesus, et al. in press) and were collected in Viana do Castelo, Portugal, from a natural wetland in the estuary of the Lima river. Plants were removed with intact rhizomes and stems. They were subsequently planted in commercial turf for development for at least 2 weeks and were watered with tap water to leach as many salts as possible as well as to avoid more undue salt accumulation prior to tests. Plants showing poor adaptation, namely low shoot and leaf production, were discarded.

6.5.3 Vegetative bioremediation in microcosms

Vegetative bioremediation tests was performed indoors, under natural light, with two different plants, and under non-leaching conditions. These conditions were chosen as an attempt to isolate the direct contribution of the tested plants to soil salt removal as well as to simulate a scenario where leaching of any kind is not possible, precluding the use of amendments as a remedial option.

Microcosms were set up in small plastic vases (22 cm height and 24 cm diameter) with an approximate volume of 10 L. Approximately 9 liters of contaminated soil was carefully added to each vase.

Two different conditions were tested in triplicate for a total of 6 vases randomly distributed: soil planted with *Spartina maritima* (3 microcosms, 8-10 cm diameter clumps, 15-20 stems per microcosm) and soil planted with *Juncus maritimus* (3 microcosms, 8-10 cm diameter clumps, 25-40 stems per microcosm). To ensure similar exposure to sunlight the vases were randomly rotated every two weeks. To avoid either leaching or water logging conditions, irrigation with tap water was carefully performed in order to simply maintain the soil moisture as needed by adding approximately 0.5 liters of water per microcosm per week (procedure similar to Jlassi et al. (2013).

Soil samples were collected after a period of 110 days of treatment from the top 10 cm and bottom 10 cm for a total of 12 soil samples, and tested for EC, pH, sodium, calcium and magnesium, and SAR.

At the same time, the aboveground portion of both plants was washed with deionized water to test for excreted salts through the measurement of EC and sodium in the washing solutions.

Finally, plant tissue samples were collected before the start of the experiment to assess background levels of salt of transplanted plants. In addition, they were also collected at the end of the experiment to test for dry weight changes as well as to extract salts and sodium for analysis.

Salts were extracted with boiling water (Ravindran et al., 2007) and accumulated salts by the plants were evaluated by determining EC and sodium content.

6.5.4 Chemical amendments in column tests

Two chemical amendments were chosen for soil column tests: the more traditionally used gypsum (CaSO4) and the more soluble calcium chloride ($CaCl_2$).

To establish the amount of chemicals to be added, the theoretical gypsum requirement was obtained based on the following formula (Ashworth et al., 1999):

$$\mathrm{TGR} = 0.335 \times a^2 \left[\left(\frac{1}{b^2}\right) - \left(\frac{1}{c^2}\right)\right] \times \left(\frac{\%_{sat}}{100}\right)$$

Where TGR refers to theoretical gypsum requirement, a refers to Na^+ mmol L^{-1}, b refers to target SAR (which is 7 for ideal soil conditions (Ashworth et al., 1999), c to the original SAR value (in this case it was conservatively considered to be 40, even though it was later determined to be closer to 45) and %sat refers to the saturation percentage. The resulting TGR was 86.56 ton per ha, which is equivalent to 14.5 g $CaCl_2.2H_2O$ or 17.0 g $CaSO_4.2H_2O$ per soil column our in tests. These chemicals were thoroughly mixed with the contaminated soil prior to its addition to soil columns.

The soil columns used were acrylic and has a height of 25.5 cm and an internal diameter of 5 cm. The bottom of each column was packed with glass wool to a height of 5 cm for drainage. The soil (250 g) was subsequently added to the columns up to 10 cm height above the glass wool, allowing it to fall freely to avoid undue compaction and to retain bulk densities similar to the original values. The columns were perforated at the bottom to allow for drainage and were set vertically in metal stands at 10 cm above the lab benchtop.

Two leaching events were performed with 100 mL of deionized water and samples were collected for both soil (initial and final) and leaching water.

6.5.5 Chemical Analysis

Soil water extractions were performed in accordance to U.S. Salinity Laboratory (1954) for a saturated soil paste of 1:1, 1:2 or 1:5 soil to water ratios, followed by filtration with Whatman® 2.5 µm filter paper. To convert from soil to water ratios to saturated values, the following formula was used: denominator value of ratio (for example 5 in a 1:5 extraction) * 100/saturation percentage, when needed.

Electrical conductivity (EC) was determined with a WTW Tetracon® 325 conductivity electrode, while pH was determined using a WTW pH electrode SenTix 21. Calcium + magnesium were determined simultaneously following EPA method (# 130.2 Hardness, Total (mg L^{-1} as $CaCO_3$) (Titrimetric, EDTA). Sodium was analyzed using HANNA FC 300 B Na^+ electrodes. All ion selective electrodes used were calibrated immediately before use with freshly prepared calibration solutions.

6.5.6 Statistical Analysis

Statistical analyses were performed using Stastistica 8.0 software (StatSoft, Inc, Tulsa, USA). For testing the assumptions required for parametric tests, Shapiro-Wilk test was used for normality, and Levene's test for homogeneity of variances. As all tested data sets were both normally distributed and had homogenous variances, the t-test was performed for independent variables, while the paired dependent t-test was used for dependent variables, such as comparisons between final and initial values. The significance level for both tests was p value of < 0.05.

When applicable, values are presented as the mean ± standard deviation of the population (stdev.p).

6.6 Results and Discussion

6.6.1 Vegetative bioremediation tests

The main vegetative bioremediation results after 110 days of treatment are summarized in Table 6.2. Comparing EC among different experimental setups shows that EC in the bottom 10 cm of soil were reduced with both plants, while, simultaneously, the EC of the top 10 cm of soil increased substantially. More specifically, EC increase at the surface from initial values of 20.25±0.18 dS m^{-1} to 31.03±3.22 and 26.06±1.27 dS m^{-1}, while decreasing at higher depths to 16.17±1.57 and 14.09±0.61 dS m^{-1} for *Spartina maritima* and *Juncus maritimus*, respectively. Statistically higher results for this parameter were found for both plants when comparing EC at the surface with EC at higher depth. When compared to initial values through a paired-wise dependent t-test, only the EC value of the bottom 10 cm of *Juncus maritimus* microcosms was statistically different (lower) than initial values.

A similar trend can be seen for sodium, which also had a statistically different result for the same depth comparison, with sodium increasing at the soil surface and decreasing at higher depths. Comparing with initial values, sodium levels were only statistically different (higher) for the initial 10 cm in *Juncus maritimus* microcosms.

Average calcium and magnesium content in the soil (Table 6.2) tends to follow the same trend: cation levels increase in the soil at top 10 cm in both plants and decreases at depth. However, calcium and magnesium values were only statistically different for both depths in *Juncus maritimus* microcosms.

SAR values also follow the general trend, although to a lesser extent due to the influence of calcium and magnesium values. Although generically it can be said that SAR values decrease with depth and tend to increase at the surface, the upper soil surface values for *Spartina maritima* are statistically different from those at higher depth. This is likely a reflection of the high sodium content and is similar to the statistical results obtained for EC. As observed for sodium, SAR values at the bottom of *Juncus maritimus* microcosms were statistically lower than initial values.

However, it should be noted that no analyzed parameter revealed any statistically significant difference between different plants, for either the top or the bottom 10 cm of soil.

Table 6.2 - Initial and final EC, pH, Na^+, Ca^{2+}+Mg^{2+} and SAR values for vegetative bioremediation of highly salt affected non-calcareous soil at two different depths. Different letters in the same column for each plant species indicate statistically different results between different soil depths.

	EC ($dS\ m^{-1}$)	pH	Na^+ ($mg\ L^{-1}$)	Ca^{2+} Mg^{2+} ($meq\ L^{-1}$)	SAR
Initial	20.25±0.18	6.80±0.08	4639±266	36.4±1.4	47.3±3.5
Spartina maritima microcosms					
top 10 cm	31.03±3.22^a	5.29±0.06^a	6249±1601^a	55.7±15.7^a	51.1±5.9^a
bottom 10 cm	16.17±1.5^b	5.69±0.38^a	2858±580^b	27.3±3.5^a	33.4±4.8^b
Juncus maritimus microcosms					
top 10 cm	26.06±1.27^a	5.22±0.08^a	4300±291^a	44.8±0.7^a	39.5±2.4^a
bottom 10 cm	14.09±0.61^b	5.60±0.31^a	2534±156^b	22.5±2.5^b	33.0±2.7^a

Finally, all pH values decreased with depth for both plants tested, which is possibly a result of plant metabolism and may have increased pCO_2 (Qadir and Oster, 2002). Based on a paired dependent t-test, all pH values obtained in both depths with both plants are statistically lower than the initial value. This large decrease of pH values can have implications for soil remediation in saline and/or sodic calcareous soils, but is unlikely to have a strong effect on the tested soil due to the low calcium carbonate content.

The general trend referred before in which described parameters tend to increase at the surface and decrease with depth is very likely the result of capillary rise due in part to the non-leaching conditions applied in the test. The salts were possibly mobilized from the lower 10 cm of soil and transported upwards through capillary rise. Afterwards, evapotranspiration lead to its concentration on the soil surface, where they remained due to the lack of leaching conditions.

Tests were also performed to assess the extent of plant contribution to the results obtained. This was done by determining that amount of externally excreted salts at the surface of the leaves and stems, as well as salts internally accumulated within plant tissues.

After extraction with deionized water, it was found that *Spartina maritima* excreted an average of 90±42 mg of total salts and 25±16 mg of sodium, while *Juncus maritimus* excreted 134±43 mg of total salts and 29±2.4 of sodium. Although not statistically different, these results seem to suggest a better performance of *Juncus maritimus*. However, it is not clear whether some excreted salts fell back to the soil surface or were otherwise transported out of the microcosm (even though the microcosms were located within a closed building with no wind effect).

The results obtained for extracted salts in this study are significantly lower than those obtained in hydroponic conditions in prior studies (Jesus, et al. *in press*) with the same plant species. This difference may be due to variances in salt bioavailability between the hydroponic saline solution used and the salt contaminated soil tested in this study, as well as to the difference in experimental periods. Since soil tests were of longer duration, an unknown quantity of salts might have fall back to the soil after excretion.

However, based on the data obtained for excreted salts, there seems to be no evidence of any potential for soil remediation through haloconduction as proposed by Yensen and Biel (2006). This technique consists of salt removal through excretion by salt glands or bladders, followed by wind dispersion.

Regarding internally accumulated salts and sodium (Table 6.3), *Spartina maritima* accumulated statistically higher concentrations of both total salts and sodium when compared to *Juncus maritimus*. However, although both plant species had a survival rate of 100% in the tested conditions, *Juncus maritimus* grew significantly more (40 %) in terms of dry weight.

Table 6.3 - Total tissue salts (calculated based on EC), tissue sodium content and dry weight increase for *Spartina maritima* and *Juncus maritimus* remediating hypersaline non-calcareous soil. Different letters in the same line indicate statistically different results between treatments. (Please note that for salts and sodium removed in ton ha, a correction was made for the initial background concentrations of the transplanted plants).

Parameter	*S. maritima*	*J. maritimus*
Total salts (mg g of DW plant)	2206±17^{a}	1195±207^{b}
Sodium (mg g of DW plant)	554±56^{a}	299±39^{b}
Dry weight increase (g)	4.25±0.20^{a}	6.97±0.64^{b}
Salts removed (ton ha)	2.16±0.03^{a}	3.13±1.11^{a}
Sodium removed (ton ha)	0.35±0.21^{a}	0.68±0.23^{a}

To adequately evaluate accumulated salts, it is necessary though to incorporate into the calculation not only the dry weight increase, but also the initial background levels of sodium and salts in plant tissues before the start of the experiment.

This is due to the fact that the plants were transplants from the field, where they were exposed to high salinity. To the best of the authors' knowledge, this correction is rarely used or not at all in the literature, but if only dry weight increase and concentration in mg per g of DW was to be considered, we estimate that, in the present study, the final value in ton ha^{-1} would be overestimated by 4-8 times for total salts, and by 2.3 to 2.7 times for sodium calculations.

It would also incorrectly give higher values for *Spartina maritima* compared to *Juncus maritimus,* since the overall concentration in *Spartina maritima* is higher, but the initial (field) values in the tissues are also higher.

After the above mentioned correction, the final values indicate a higher salt and sodium absorption potential for *Juncus maritimus* when compared to *Spartina maritima.* The obtained values in ton per ha are moderately high, and clearly demonstrate removal potential for both plants, which is comparable or higher than other plants tested in similar conditions (i.e. non leaching condition). In fact, Ravindran et al. (2007) found total salt accumulation in six different plant species that ranged between 0.302 up to 0.504 ton ha, while Rabhi et al. (2009) found that *Arthrocnemum indicum, Suaeda fruticosa* and *Sesuvium portulacastrum* removed 0.711, 0.802 and 2.504 ton ha^{-1} of sodium, respectively. Finally, Jlassi et al. (2013) obtained values of 0.3 ton ha^{-1} of sodium for the plant *Sulla carnosa.*

It also important to point out that the same plant can show a wide different response, even when tested by the same research group. For example, while in Rabhi et al. (2009) the plant *Sesuvium portulacastrum* removed up to 2.504 ton ha^{-1} of sodium, in Rabhi et al. (2010) the potential of the same plant species was reduced to 1 ton ha^{-1} of sodium, despite similar experimental periods (170 days vs. 189 days), similar initial EC (19.6 vs. 14.4 dS m^{-1}) and even a higher dry weight per plant (5.58±0.25 vs. 8.28±0.45 g plant^{-1}).

Analyzing both soil and plant data (Table 6.2 and 6.3) obtained in the present work, it would seem that the results are at odds with each other. Although, plant tissue samples demonstrated high salt and sodium accumulation, the soil results seem to suggest that plants have limited or no potential for salt affected soil remediation (at least in the non-leaching conditions tested).

There are several explanations for this situation. First, it is possible that the quantities of salts removed by the plants were not significant enough to create a demonstrable effect in soil parameters. Since this trend may change with time, an extension of the experimental period would be advisable. Second, the effect of capillary rise may mask any obvious effect of plant salt uptake on soil parameters.

As described before, there are two main mechanisms (aside from leaching) for vegetative bioremediation of salt affected soils. The first one is the reduction of pH, which increases the dissolution of native $CaCO_3$, and consequently increases available $Ca2^+$ for cation exchange with sodium. The second reason is the direct plant uptake of salts or sodium. However, the relative importance of each of these two mechanisms remains under debate. Some researchers consider plant uptake is unlikely to have a strong impact on soil parameters (Qadir et al., 2006), while others suggest that plant uptake may be sufficient for remediation (Rabhi et al., 2009).

This work was partially designed to help address this issue by testing a highly saline, non-calcareous soil under non-leaching conditions. This limits or even eliminates both the impact of leaching and the pH reduction in the treatment. Although comparable to other studies, our results suggest that plant uptake, had seemingly little effect on soil parameters under the conditions tested. However, this result is only valid for the tested plants species and it does not necessarily mean that different species or even a longer period of growth would change the outcome.

Assuming an annual growth similar to that obtained for the 110 days of our test, the average total salt removal would be 7.2 and 10.4 ton ha^{-1}, while sodium removal would account for 1.2 and 2.3 ton ha^{-1} for *Spartina maritima* and *Juncus maritimus*, respectively. Similar results were observed in a study from Rabhi et al. (2009) where removal was determined to be between 0.711 and 2.5 ton Na^+ ha^{-1} under non-leaching conditions with a comparable growth period of 170 days. Even though this is the case, the impact on soil parameters (such as EC and SAR) may only show up after longer periods of time than the one used in the present study.

In fact, remediation goals are often measured in years, not months, and as a result, the potential of vegetative bioremediation in a situation of water scarcity, where leaching is not an option, is not entirely ruled out based the results obtained. Therefore, further studies are required to clarify its potential, as other remediation techniques, such as chemical amendments, are not applicable in such a scenario.

6.6.2 Chemical amendment tests

Two chemical amendments, namely calcium sulfate and calcium chloride, were added to highly salt contaminated non-calcareous soil in order to test for reduction in EC, sodium and SAR values (Table 6.4).

Two leaching events with 100 mL of deionized water each were performed shortly after amendment addition to wash away accumulated salts. Analyses of the resulting leachates from the different amendments showed significant differences (Table 6.4). For example, EC and calcium are always higher for $CaCl_2$ when compared to $CaSO_4$, which is to be expected considering the higher solubility of $CaCl_2$. In addition, pH values tend to be lower for $CaCl_2$ leachates. However, sodium and SAR do not follow any obvious trend. In the first leachate sodium and SAR are higher for $CaSO_4$, while the reverse is observed in the second leachate.

Table 6.4 - Initial and final EC, pH, Na^+, Ca^{2+}+Mg^{2+} and SAR values of the addition of chemical amendment for highly salt affected, non-calcareous soil remediation, as well as leachate characteristics. Different letters in the same column indicate statistically different results between treatments for each individual condition (Leaching 1, 2 and Final soil).

	EC ($dS\ m^{-1}$)	pH	Na^+ ($mg\ L^{-1}$)	Ca^{2+} Mg^{2+} ($meq\ L^{-1}$)	SAR
Initial soil	23±2	6.71±0.15	4557±152	36±3	46.6±0.7
			Leaching 1		
$CaSO_4$	35±3^a	5.40±0.05^a	3374±380^a	84±1^a	22.6±0.04^a
$CaCl_2$	132±2^b	4.69±0.002^b	3014±38^b	1890±80^b	4.3±0.04^b
			Leaching 2		
$CaSO_4$	4±1^a	6.42±0.13^a	375±91^a	41±4^a	3.6±0.7^a
$CaCl_2$	39±17^b	5.21±0.13^b	1282±354^b	437±144^b	3.8±0.5^a
			Final soil		
$CaSO_4$	3±1^a	7.14±0.12^a	323±31^a	27±1^a	2.8±0.5^a
$CaCl_2$	1±1^b	7.41±0.03^b	132±30^b	6±1^b	3.4±0.5^a

Regarding soil samples at the end of the test, the trends are reverse to what was seen in both leachates. For example, EC, calcium + magnesium and sodium values are statistically lower for $CaCl_2$ when compared to $CaSO_4$, while pH is higher. However, SAR values are not statistically different between both situations. Despite these differences, both amendments reduced EC and SAR values of contaminated soil to non-saline non-sodic soils (EC < 4 dS m^{-1} and SAR < 13).

Also, both amendments showed statistically lower levels than initial contaminated soils for EC, sodium, calcium + magnesium and SAR, and only the pH value for $CaSO_4$ amended soil is not different from initial values.

Although the amendments done introduced significant quantities of dissolved salts to the soil in general, and of calcium in particular, it is still possible to do a simplified mass balance for sodium. Initial value of soluble sodium in the contaminated soil was approximately 17.97 mg, on average. For $CaSO_4$ amended leachates, the first leaching event removed 14.7 mg, while the second removed 1.6 mg for a total of 16.3 mg out of 17.97 For $CaCl_2$, the first leaching event removed 13.1 mg, and the second 5.6 mg, for a total of 18.7 out of 17.97 mg.

However, it is important to point out that sodium was likely displaced from soil cation exchange sites into the leachates, which might explain the high removal in both cases. This is particularly true for the $CaCl_2$ amendment, where more sodium was removed than it was initially detected in the soil solution, indicating sodium mobilization from the exchange sites.

Comparisons of the data obtained in this study with others referred in the literature are presented in Table 6.5.

Table 6.5 - Comparison of reduction percentage in electrical conductivity (EC) and sodium adsorption ratio (SAR) between this study and others in the literature using chemical or organic amendments; (1) This study; (2) (Abd Elrahman et al., 2012) (3) (Diamantis and Voudrias, 2008) (4) (Qadir et al., 2002).

Source	Amendment	EC initial (dS m^{-1})	EC final (dS m^{-1})	EC % reduction	SAR initial	SAR final	SAR % Reduction
(1)	Gypsum	23±2	3±1	87	46.6±0.7	2.8±0.5	94
(1)	$CaCl_2$	23±2	1±1	96	46.6±0.7	3.4±0.5	93
(2)	Gypsum	6.3	4.78	24	14.9	4.9	67
(2)	Citric acid	6.3	5.08	19	14.9	9	40
(2)	Farm manure	6.3	4.88	23	14.9	7.4	50
(2)	Compost	6.3	5.02	20	14.9	8.1	46
(3)	Gypsum	3.0	0.91	70	8	1.6	80
(4)	Gypsum	11.1	4.6	64	35	15	57
(4)	Gypsum	10.3	7.8	24	66	30	54
(4)	Gypsum	8.4	7.0	17	69	50	27

Based on Table 6.5, the current study outperformed comparable studies in the literature. However, it is important to point out that there are significant differences in methodologies that help explain this discrepancy. For instance, gypsum requirement (GR) calculations differ among authors, and many studies do not test 100% GR as tested in this study.

Furthermore, field and lab tests naturally differ in efficiency and even the type of contamination is different among tests: for example, in the current study, citric acid addition would be unlikely to provide any improvement in soil condition, while farm manure or compost would likely be redundant considering the already high organic matter content of the tested soil.

Still, the tested amendments proved to be effective under the assayed conditions, but overall, $CaCl_2$ is technically more efficient. However, the different effects of both amendments have to be taken into consideration when choosing between them, depending on the type of contamination and subsequent remediation goals. For example, $CaCl_2$ is a more soluble salt than $CaSO_4$. As a result, the EC of leachates increases rapidly as seen in this work. This may either be a problem or an opportunity. For instance, considering the values obtained for calcium and magnesium in the leachate, this rapid dissolution of $CaCl_2$ may lead to a considerable amount of unreacted calcium to be rapidly leached. Although, if applied to sodic soil, this large increase in EC may help in soil stabilization (Horneck et al., 2007).

Considering gypsum as an alternative, the lower solubility allows for a more pronounced effect over time, and is less likely to waste calcium ions for cation exchange reactions with adsorbed sodium.

Other considerations include the difference in price and local availability of these compounds. For example, waste materials such as phosphogypsum, a by-product from the phosphate industry (Gharaibeh et al., 2010), is also often used. However, its lower quality translates into a trade off with treatment efficiency. Furthermore, these wastes may present further risks, since they might be radioactive (Olszewski et al., 2015).

Some authors have already suggested the combination of gypsum with $CaCl_2$, with reported similar amendment efficiency at a lower cost (Qadir et al., 2001). This process took advantage of the rapid dissolution of $CaCl_2$ with the more sustained release of calcium provided by gypsum even though it was done using calcareous soils.

6.7 Conclusions

The goal of this study was to clarify several aspects of two main remediation techniques for non-calcareous salt affected soil remediation, namely chemical amendments and vegetative bioremediation. This included determining the relative performance of CaCl2 and $CaSO_4$ in non-calcareous soil. In addition, the potential for salt uptake, as the sole mechanism for effective remediation in such conditions was tested for vegetative bioremediation.

Under the extreme scenario tested of high EC (20 dS m^{-1}) and SAR (45) with non-calcareous soil and under non leaching conditions, the plants tested (*Spartina maritima* and *Juncus maritimus*) had no demonstrable effects on soil salinity or sodicity after 110 days of testing.

In spite of this, the tested plants survived and grew, as well as accumulated and excreted significant quantities of dissolved salts and sodium. These results were similar to other plants in other tests referred to in the literature.

Even though our work is comparable in duration to other studies, the results suggest that prolonged tests in time might reveal demonstrable impacts of plant presence on soil parameters. In addition, the adequate survival and plant growth, along with the large pH decrease, indicates that the tested plants (even if eventually they were not effective in longer duration tests under non leaching conditions), still show a high potential for vegetative bioremediation under leaching conditions, particularly for calcareous soils.

Alternatively, the chemical amendments tested, proved to be extremely effective for non-calcareous soils remediation. Despite the simplified laboratory conditions, it can be concluded that $CaCl_2$ is more effective and faster, but is also likely to be more costly than $CaSO_4$. The initial high EC of the leachates may also preclude the use of $CaCl_2$, due to its high solubility, in certain local conditions.

Future work should consider the potential of the simultaneous application of both chemical amendments and vegetative bioremediation. The added calcium could increase soil structural stability, while simultaneously making exchangeable sodium more biologically available. This would allow for better root penetration and plant development, as well as potentially higher sodium uptake. As a result, this may preclude the need for leaching in a water scarcity scenario.

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7 Concluding Remarks

Taking into consideration the three main objectives previously detailed in the introduction, an evaluation of the practical implications that can be either concluded or tentatively deduced from the present study is presented. Although conclusions derived from each individual chapter have already been referenced in this study, a more holistic and general approach is still important to be done.

Regarding soil salinization risks, it was found that soil characteristics are determinant to the potential impact and intensity of different salinization processes. Less used parameters to characterize soils, such as exchangeable acidity, revealed to be of the utmost importance: a high value of this parameter may lead to an extreme reduction of pH, but also may counteract high levels of residual sodium carbonate in irrigation waters. Ultimately, however, even with leaching, the impacts of salinization, particularly to the soil structure, are mostly unavoidable once salts are introduced.

As a result, preventive or remediation measures are necessary. In this thesis a greater focus was given to phytotechnologies, either constructed wetlands or phytoremediation of soils. Their use was motivated by the recognition that these technologies are highly regarded as green and cheap alternatives compared to other, more traditionally used treatment systems, and present a lot of potential but are also poorly understood on a systemic basis, to the extent of being underused or even misused.

Considering this issue, no greater example can be found than the still controversial role of plants in constructed wetland systems: even though this technology is already applied for several decades in a variety of situations, the mechanisms and extent of the effect of plants in the treatment remains under debate. In the present study it was found that a surprising number of published articles fail to provide sufficient evidence of a significantly relevant impact of the presence of plants on the treatment efficiency of constructed wetlands. Although several shortcomings of experimental designs can also partly explain this phenomenon, specific experimental conditions (like lack of replication or unplanted controls, among others) enhance the potential for higher plant contribution, as well as higher efficiency of the constructed wetland system as a whole.

Partly based on identified conditions (such as low HRT, the use of hydroponic system to isolate the effect of the plants, etc.), this study tested the use of laboratory scale constructed wetlands for simultaneous removal of salt and nutrients. Several important aspects of their functioning were found, which are relevant for the application of constructed wetlands. For instance, the tested plants proved capable of significantly contribute to nutrient removal in highly saline conditions when planted in expanded clay and/or under hydroponic conditions.

However, the main objective of salt removal to prevent salinization, though technically possible, is unlikely to be of practical use considering the required retention time for significant removal. In fact, the use of a HRT of over 23 days in a wastewater treatment facility is not common practice in conventional activated sludge systems. Regardless, the tested plants demonstrated potential for salt phytoextraction and were subsequently used in soil phytoremediation were the longer exposure time was expected to increase the feasibility of the phytotechnology in question.

A preliminary, yet thoroughly done, analysis of the literature identified the main knowledge gaps on salt affected soil remediation in general and on salt phytoremediation in particular. Beyond the identification of several opportunities to enhance salt removal, as well as an analysis of the impact of climate change on remedial options, this initial review found that, under leaching conditions and for calcareous soils, chemical amendments and phytoremediation tend to have similar performance. However, little to no data was found for non-calcareous soils for both remediation techniques, and the unique potential of phytoremediation to work under non-leaching conditions was also underexplored.

As a result, remediation tests were designed to address these initially described shortcomings, particularly focusing on remediation options for non-calcareous soils. In terms of chemical amendments it was found that $CaCl_2$ and $CaSO_4$ have similar treatment efficiencies. Ultimately, however, the choice between these compounds will likely depend on compromises between cost and remediation time: while $CaCl_2$ is likely to be more costly, it is also faster in action due to higher solubility.

Phytoremediation tests with *Spartina maritima* and *Juncus maritimus* were designed to test more extreme conditions by focusing on its unique potential to avoid using leaching as part of the remedial process. Meant to test the limits of the technology, the obtained results were, unfortunately, inconclusive. Despite the existence of salt uptake, comparable with other plant species and studies, no noticeable impact of this technology on soil characteristics was found. A variety of hypothesis was postulated, but in the end, the effectiveness of the technology could not be objectively proven.

Specific recommendations for further work include the combined application of chemical amendments and phytoremediation, as well as the evaluation of the role of substrate in constructed wetlands and longer duration tests for phytoremediation.

While several other aspects could be highlighted as general recommendations, the most important aspect would be to improve the quality, as well as quantity of studies on phytotechnologies, in order to better understand their processes and limits. Phytotechnologies, in general, despite extensively researched, are yet to be widely applied in the field. The inherent biological variability of plant growth and response are partially responsible for this underutilization when choosing between phytoremediation and the more predictable chemical amendments, for example.

This aspect justifies the need to invest on improved experimental setups, with a strong statistical backbone, to provide more quality control and assurance to field engineers. For this purpose, this thesis provides several recommendations for improvement and where to lead future work with a focus on phytoremediation of salt affected soils.

8 Engineering implications

This study has several implications to applied engineering as a direct result of the obtained experimental data. It also hints at some other, potentially major implications, derived from the analysis of existent scientific literature and the integration of the obtained data within the experimental work referenced.

From the study of various salinization processes, it has become apparent that the analytical determination of a broader set of soil characteristics is required to more precisely assess soil salinization risks derived either from natural or anthropogenic occurring threats. The obtained results are more likely to have an impact on the field of agronomic engineering, particularly on the improved management of irrigation with poorer quality water to soils with different salinization susceptibility.

The literature analysis of constructed wetlands and the effect of plants to the treatment efficiency denote a lack of a statistical significance impact in most studies as well as a lack of standardization of experimental procedures. The ramifications of these findings to the design and management of future or existing engineered wetland systems is mostly unknown, as even statistical significant and positive results may or may not be relevant from an applied engineering perspective. As more scientific research is clearly needed, the main engineering implication that can be stated is for field engineers to exercise caution when proposing this technology and, whenever possible, to perform pilot *in situ* tests with unplanted controls and robust statistical analysis.

As a small part of the needed research effort to bridge the gaps referenced above, the lab scale constructed wetland tests performed in this thesis demonstrate a statistical significant effect of plants on treatment efficiency, either with or without a substrate, for nutrient removal at high salinity. Although preliminary, as few other studies have been performed under similar conditions, the potential for saline wastewater nutrient removal in CW is promising. Salt removal, on the other hand, is unlikely to have engineering implications in the future under realistic conditions of wastewater treatment.

Similar research gaps, albeit at a much lower extent, were also observed for phytoremediation of salt affected soils. The review of existing literature may help to clarify and better inform all stakeholders, including field engineers, on both the similar performance of phytoremediation when compared to chemical amendments, as well as novel aspects to enhance this technology.

Although in the case of soils, phytoextraction is not absolutely vital to the process, it may provide the potential to preclude leaching conditions. However, doubts persist on the feasibility and magnitude of salt phytoextraction. The phytoremediation tests conducted in this thesis were designed to isolate this specific removal mechanism, by testing the remediation of a saline-sodic non-calcareous soil under non-leaching conditions. However, the obtained results were inconclusive and therefore with no clear engineering implication.

However, chemical amendments were also tested for the remediation of a non-calcareous saline sodic soil. Although limited by the necessity for leaching, the tests conducted revealed that both gypsum and calcium chloride could be effectively used, albeit with different desalinization rates. This indicates that gypsum is likely to remain effective even in non-calcareous soils, and the choice between gypsum and calcium chloride is dependent on remedial goals, particularly time and costs.

9 Annexes

A. Methods description for soil analysis

A.1. Texture

For texture determination, the NP EN 932-1 and 932-2 2002 Portuguese norms were followed for representative sample collection and sampling reduction. The sampling reduction was made with a sample divider (multiple slots type). The soil was poured into the central line of the sample divider and was collected into two recipients. The soil in one of them was rejected and the other was again poured into the sample divider repeatedly until reaching approximately 1 kg of final laboratory sample for analysis.

The soil was sieved according to ASTM norms. The fraction under 250 µm was analyzed by a laser diffraction particle size analyzer Mastersizer 2000. Ultrasound was used to disperse clay aggregates. In Figure 9.1, there is an example of the obtained distribution of particles under 250 µm after ultrasound application.

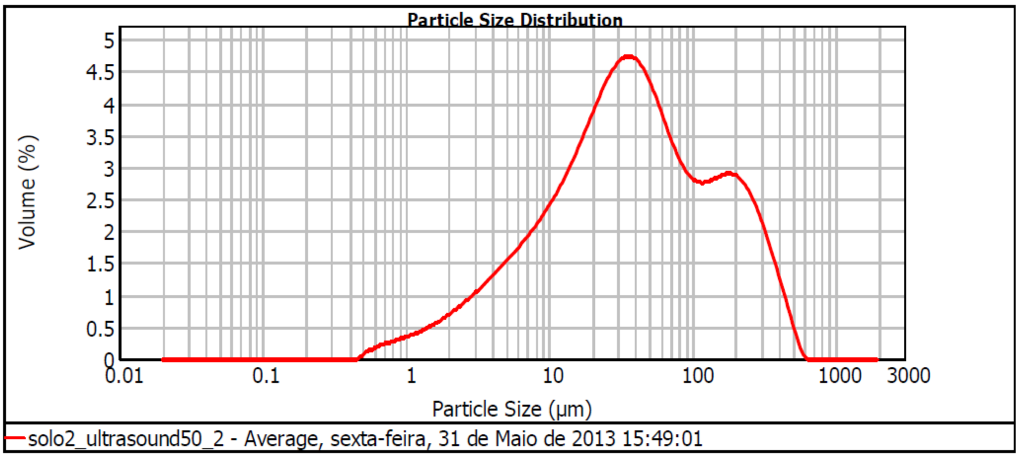
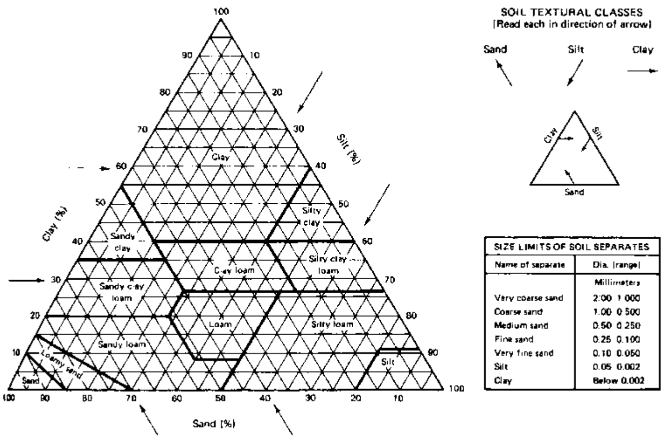


Figure 9.1 - Particle size distribution curve for soil particles under 250 μm

The results from sieving and particle size analysis were combined accordingly and the results are expressed in Table 9.1 for texture calculation (in duplicate).

Table 9.1 - Particle size distribution of two samples of the tested soil, respective average and standard deviation as well as percentage of silt, sand and clay (100% percentage excluding gravel) SD - Standard deviation.

	1	2	Average±SD
Gravel (g)	257.55	68.00	162.78±134.03
Sand (g)	1042.43	668.36	855.40±264.50
Silt (g)	313.04	252.94	282.99±42.50
Clay (g)	29.16	33.05	31.10±2.75
Sand (%)	75.29	70.03	72.66±3.71
Silt (%)	22.61	26.50	24.56±2.75
Clay (%)	2.11	3.46	2.78±0.96



SIZE LIMITS OF SOIL SEPARATES	
Name of separate	Dia. (range)
	Millimeters
Very coarse sand	2.00 1.000
Coarse sand	1.00 0.500
Medium sand	0.50 0.250
Fine sand	0.25 0.100
Very fine sand	0.10 0.050
Silt	0.05 0.002
Clay	Below 0.002

FIGURE 3–9 Guide for textural classification by the U.S. System for Texture Designations.

Figure 9.2 - Soil texture classification based on clay, silt and sand percentage, Source: https://www.nrcs.usda.gov

Based on these results and on Figure 9.2, the soil can be classified as loamy sand.

A.2. Lime content

Lime or carbonate content is an important parameter for salt affected soil remediation as it indicates the presence or absence of calcium ions that might become available for exchange with sodium if soil conditions change. For this parameter, a qualitative screening test was performed (U. S. Salinity Laboratory, 1954), based on the reaction of 3 N HCl with the carbonates of soil with 4 terms: no reaction, slightly, moderately or highly calcareous.

The procedure involves placing several grams of soil (in this case, exactly 5 g) and add sufficient water to saturate (in this case approximately 2 mL which is equal to the pore volume of 5 g of this particular soil) in order to displace the air present.

Afterwards, a few drops of 3 N HCl are added and the soil visually inspected for effervescence.

In this test, no effervescence or bubbles of any kind were observed after acid addition, further indicating that the organic and mineral soils are both non-calcareous.

A.3. Cation exchange capacity

Cation exchange capacity (CEC) refers to the total value of exchangeable cations in the soil. As many procedures have several problems with interferences, a more general protocol is often used that provides accurate cation exchange capacity results but not the value of individual cations. The following protocol is based on the work of Aprile and Lorandi (2012):

1) Add 2 g of soil and 10 mL of distilled water in a flask.
2) Mix thoroughly.
3) Add 0.5 mL of 1.5 g L-1 methylene blue solution to each soil sample.
4) Remove drop with glass rod into a filter paper.
5) Repeat procedure 3 and 4 until a blue halo appears (Figure 9.3).

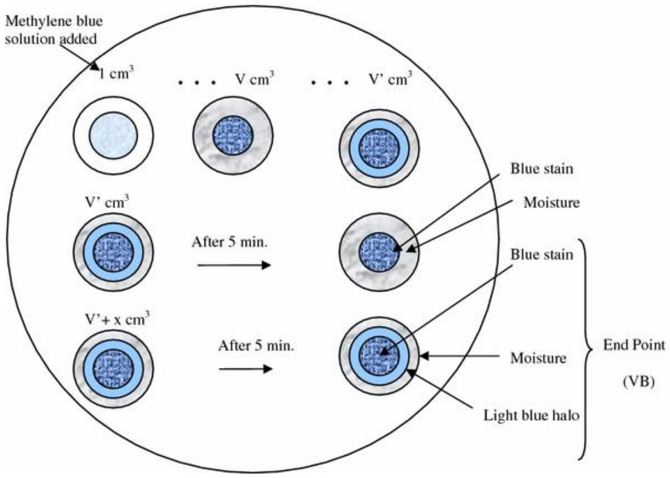


Figure 9.3 - Blue halo end point representation.

Calculate CEC with the following equation:

$$CEC = (V * C * 100) / m$$

In which V is volume of methylene blue solution used in mL, C is the molar concentration of the solution in M and m is the mass of soil in kg and CEC is in mmol kg^{-1} (divide by 10 to get meq per 100 g). The results are expressed in Table 9.2:

Table 9.2 - Cation exchange capacity by methylene blue adsorption - test results in methylene blue added volume for both organic and mineral soil.

	Sample	soil mass (kg)	Volume added (mL)	CEC (in mmol kg)	CEC (in meq per 100 g)
Organic soil	1	0.00204	2.0	393.3	39.3
	2	0.00203	2,2	434.8	43.5
	3	0.00206	2.2	428.5	42.8
Mineral soil	1	0.00101	4.7	1861.38	186.14
	2	0.00103	4.5	1747.57	174.76
	3	0.00101	4.5	1782.18	178.22

The tested CEC is 41.0±1.8 meq per 100 g for organic soil (Figure 9.4) and 179.7±4.8 meq per 100 g for mineral soil.

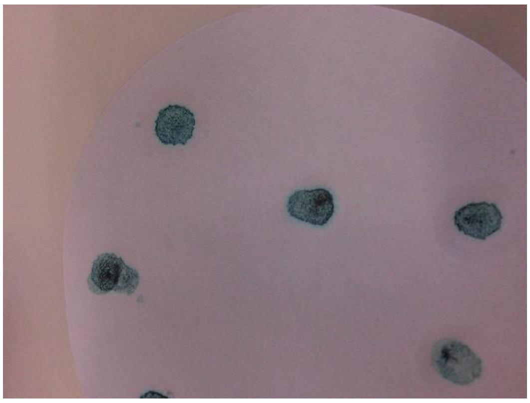


Figure 9.4 –Example of an end point of cation exchange capacity test in filter paper

A.4. Loss on ignition

Loss on ignition (LOI) tests can be used to assess organic and carbonate content of a soil. In this study, these tests were performed in accordance to Heiri et al. (2001).

Soil was dried at 105ºC and weighted in the crucible. It was then placed in triplicate into the muffle at 650ºC for 2 hours let to cool down and weighted again. The soils were reintroduced into the muffle at 900ºC for 1 hour, let to cool and weighted a final time (Table 9.3).

Table 9.3 - Results of LOI tests and respective conversions to organic and inorganic carbon for organic soil only

	Sample1	Sample2	Sample3
Crucible weight	16.0563	15.5637	16.8571
Crucible + 105ºC dry soil	26.0535	26.2954	26.1512
Dry soil	9.9972	10.7317	9.2941
Crucible + 650ºC (2 hours) soil	25.5996	25.86	25.4489
Organic carbon	0.4539	0.4354	0.7023
Crucible + 900ºC (1 hour) soil	25.5545	25.8244	25.3963
Inorganic carbon	0.0451	0.0356	0.0526
Total carbon	0.499	0.471	0.7549
% Organic carbon	4.54027	4.05714	7.55641
% Inorganic carbon	0.45113	0.33173	0.56595
% Organic matter	7.80927	6.97828	12.997
Carbonate content g	0.06134	0.04842	0.07154
Carbonate content g kg	6.1336	4.8416	7.1536
Carbonate %	0.61336	0.48416	0.71536

Conversion factors (OC – Organic carbon, IC – Inorganic carbon):

OM = 1.72 * OC

Carbonate content = 1.36 * IC

OM % average = 9.26±3.26 %

Carbonate % = 0.60±0.12%

A.5. Bulk density and particle density tests

The method used to estimate soil particle density was adapted from NP EN 1097-6 2003 (Annex A).

The soil sampling was done according to NP EN 932-1 and its reduction according to NP EN 932-2, involving the use of a sample divider (multiple slots type) to reduce the samples to the appropriate size of around 0.5 kg after sieving the soil, to limit the size of the aggregates to between 0.063 to 31.5 mm.

As the maximum aggregate size was approximately 8 mm, a minimum of 0.5 kg of reduced soil was prepared as indicated by the norm.

The two pycnometer used were calibrated by weighting both with and without water (Table 9.4), with the following results (considering temperature of 19.4ºC and corresponding water density of 0.9984 kg L^{-1}):

Table 9.4 - Pycnometer volume calibration test results.

Pycnometer	Dry mass (g)	Mass with tap water (g)	Volume (L)	Volume (L)
1	333	1532	1.200	1.200±0.048%
		1531	1.200	
		1531	1.200	
2	341	1498	1.159	1.155±0.050%
		1497	1.158	
		1498	1.159	

As the standard deviation is below 0.1% the procedure was continued.

It was assumed that temperature did not fluctuate significantly and so no water bath was used.

The soil samples were inserted into each pycnometer and weighted (M2). Tap water at approximately 20ºC was added slowly, initially to saturate the soil and then to fill the pycnometer up to 20 mm below the funnel.

After allowing the soil to settle for 20 minutes, additional water was added. Care was taken to avoid air bubbles. More water was added up to the meniscus and the assembly was weighted for a last time (M3).

The results are as follow (Table 9.5):

Table 9.5 - Weight of pycnometer without soil and water (M1) with soil (M2) and with soil and water (M3) and respective volume.

	M1 (g)	M2 (g)	M3 (g)	V (mL)
1	333	1193	1991	1200
2	341	1008	1851	1155

Density was calculated from the following equation:

$$\rho_S = \frac{M2 - M1}{[V - (M3 - M2)]/\rho_w}$$

In which ρ_s and ρ_w represent the density of soil particles and water, respectively, and V the volume of the pycnometer.

The density result for pycnometer nº 1 is 2.136 and for nº2 is 2.134 kg L^{-1} with an average result of 2.135±0.001 kg L^{-1} (less than 0.05% difference). However, the result should be expressed as 2.14 kg L^{-1}.

Bulk density

Bulk density was calculated using a graduated beaker of 100 mL, dropping the soil freely up to 100 mL, and weighed (Table 9.6).

Table 9.6 - Bulk density calculation based on the weight of a 100 mL graduated beaker with and without soil without soil compaction.

Without soil (g)	With soil (g)	Difference	Bulk density
121.88	232.45	110.57	1.1057
120.92	231.76	110.84	1.1084
112.36	221.34	108.98	1.0898

Bulk density is 1.101 kg L or 1101 kg m^3.

By the formula St = 100 (1- (bulk density / particle density)) the % apparent porosity is 48%. Pore volume is therefore 0.48 * total soil volume.

A.6. Exchangeable acidity determination

Exchangeable acidity is important to evaluate how the soil may react to a cation excess that creates an imbalance on cation exchange sites. There are several methods but considering the pH of the soil it was chosen to use the 1 M KCl method.

The method is a simplified version from Jones (2001), whereas a 1 M KCl of 25 mL is added to a 10 g of soil and mixed thoroughly. This slurry is allowed to stand for 30 minutes before being filtered.

Subsequently, the resulting filtrate is titrated with 4 or 5 drops of phenolphtalein solution and 0.1 M of NaOH to the first permanent pink end point.

The resulting number of meq of used NaOH is equivalent to exchangeable acidity (divided by mass of soil for standardization). Results can be seen in Table 9.7 for both soils.

Table 9.7 - Exchangeable acidity results for organic and mineral soil after titration. RSD – Relative standard deviation.

	Volume NaOH	meq NaOH	meq exchangeable acidity per 100 g	Average	%RSD
Organic	0.2	0.02	0.2	0.25	28.28427
	0.3	0.03	0.3		
Mineral	1.8	0.18	1.8	1.85	3.822199
	1.9	0.19	1.9		

A.7. Heavy metals content

An estimate of heavy metals content in the soil was obtained (Table 9.8) with the use of a Portable Analytical X-Ray Dispersive Energy Fluorescence Spectrometer (Innov-X System) in two different soil samples.

Table 9.8 - Estimated heavy metal content in soil samples based on X-ray dispersive energy fluorescence.

Metals (in mg kg^{-1})	Sample 1	Sample 2		Sample 1	Sample 2
Ti	2185±243	1721±246	Cu	< 101	< 102
Mn	138±22	184±24	Co	< 173	< 117
Fe	12858±158	13196±164	Ni	< 32	< 33
Cu	25±7	27±7	Hg	< 9	3±3
Zn	116±6	110±6	Se	< 3	< 3
As	19±4	31±4	Mo	< 8	8±3
Pb	171±6	136±6	Ag	< 34	< 34
Rb	134±3	164±3	Cd	< 40	< 40
Zr	199±3	107±3	Sn	< 66	< 66
Ba	< 397	<424	Sb	< 70	< 7

The values are not particularly high for any metal with the exception of iron. However, these are well within excepted values for this type of soil and do not present any risk.

A.8. Electrical conductivity of soil determination

Electrical conductivity (EC) can be measured in soil water extracts in 2 different ways: EC 1:2 and 1:5, in a soil weight to water weight basis, or saturated soil paste (ECe).

Saturated soil paste (ECe) is obtained by adding small quantities of water to the soil until the end point is visually obtained (no running water, no hardset, reflection of light, paste flow and other visual clues).

Procedure according to Jones (2001) and U.S. Salinity Laboratory (1954):

1 – Collect a 200-250 g soil sample air-dried and passed through a 2 mm sieve. Take a subsample (25 g) to determine moisture content (in a tared can with lid) weight before and after dry at 105ºC.

2 - Add deionized water to the soil sample (initially large amounts, followed by small increments) while stirring with a spatula. From time to time, tap the 250 mL beaker on the workbench until saturation is reached. At saturation, the soil paste should glistens as it reflects light, flows slightly when tipped and the paste slides freely off the spatula. Register the amount of water added. If more soil is needed for the paste, register the weight of the additional soil.

(**NOTE**: in some hydrophobic tests it is preferable to add soil to the water).

3 - Let the sample stand for 1 hour.

4 - Transfer the soil paste to a Buchner funnel with filter paper in place and apply vacuum.

5 - If **gypsum** is present allow the paste to stand several hours before extraction.

6 - From the solution obtained by filtering, determine the conductivity by the use of an electrode.

7 – Obtain the value of saturation percentage by drying the paste in an oven at 60ºC and using the following equation: SP = (loss in weight on drying x 100) / weight of the soil after drying).

The ECe, tends to be about 1/2 of the concentration of the soil solution at the upper end of the field-moisture range and about 1/4 the concentration that the soil solution would have at the lower, dry end of the field-moisture range.

The salt-dilution effect that occurs in fine-textured soils, because of their higher moisture retention, is thus automatically taken into account.

Soil water extractions (ratios 1:1; 1:2; 1:5)

Procedure (adapted from (Jones, et al 2001; U. S. Salinity Laboratory, 1954).

1 - Weight 20 g (1:1) or 10 g (1:2) or 5 g (1:5) of soil into a test tube or small container and add 20 mL of water, stir thoroughly, and allow to settle for 30 min.

2 – Filtrate the solution and measure the conductivity

(**NOTE:** to increase accuracy add more soil, e.g, instead of 5 g of soil to 20 mL of water, add 10 g to 50 mL of water, maintain the 1:5 ratio).

These ratios can be converted back to ECe through the following relationships: 1:1 – 100/SP; 1:2 - 2 * 100/SP; 1:5 – 5 * 100/SP.

A.9. Soil contamination tests

The basis for soil saline contamination in this study is the input of dissolved salts in the soil through a concentrated saline solution of known concentration. In order not to use excessive water during contamination, which could be disruptive to the soil structure, half saturation and therefore double NaCl concentration were applied for each soil. Initial tests aimed to increase ECe value to 10 dS m^{-1} in the organic soil, equivalent to 6.4 g L^{-1} NaCl. Therefore a solution of 12.8 g L^{-1} of volume equivalent to 16.5% of soil weight was used. After contamination, soil is air dried at 60° C for over 8 hours, after which extractions (saturation paste, 1:2 or 1:5) are prepared and filtered and EC measured.

Initial tests failed to provide accurate and reproducible levels of EC due to 3 important factors:

- The soil water extracts (1:2 and 1:5) were not properly mixed, limiting the water contact with the soil.

- The extracts were not filtrated. Although not indicated in many different sources, filtration is necessary, in particular for soils with high organic matter and clay, as the suspended solids influence the conductivity measure (in tested cases, up to 2 times lower conductivity levels were measured in the unfiltered supernatant than after filtration). The filters used in these tests had a porosity of 42 μm and were made of cellulose.

- Initial tests were done in a volume by volume basis as indicated by some sources. However, although the interference in this particular case cannot be pinpointed, this method is not the most adequate as soil bulk density can be sufficiently higher than water density to create important differences.

After correcting for these parameters several independent tests resulted in an average ECe (after EC 1:5 conversion to ECe) of 9.87 ± 0.39 dS m^{-1} (n = 8), very close to the intended 10 dS m^{-1}. ECe was also obtained through saturated soil paste resulting in an average of 9.65 ± 0.52 dS m^{-1}, close to the previously calculated EC 1:5. More tests were subsequently performed, in order to confirm the conversion factor between EC 1:5 and ECe at different salinities. The conversion factor was tested at 10, 20 and 30 dS m^{-1} and the conversion factor was always maintained throughout.

Afterwards, a solution with 6 dS m^{-1}, intended to increase EC value by 3 dS m^{-1} was used to test for the possibility of incremental EC increase resulting in an average increase of 2.835 dS m^{-1}, close to the objective of 3 dS m^{-1}.

Several other tests were performed to attempt to see the effect of the fraction above and below 2 mm to the overall salinity. Although the fraction above 2 mm had widely different salinities values when isolated (from 0.269 up to 0.747), its percentage did not contribute to significant changes to justify sieving.

A final test was performed to test the effect of prolonged contact of the solution before soil drying. It was observed that it did not produced any significant effect.

A.10. Sodium determination by ion-selective electrode

Several test runs were necessary to correctly calibrate the sodium electrode. Only one successful example is described here, randomly chosen, as different experimental setups required different calibration curves.

The following solutions were prepared:

Primary solution: 14.61 g NaCl to 250 mL = 1 M NaCl.

ISA solution: 20 g NH_4Cl + 27 mL NH_4OH (28-30% v/v) in 100 mL;

For each dilution, 0.5 mL ISA, before dilution is completed,

Dilution #1 - 1.25 mL of primary solution diluted in 25 mL = 50 mM

Dilution #2 - 2.5 mL of primary solution diluted in 25 mL = 100 mM

Dilution #3 - 3.75 mL of primary solution diluted in 25 mL = 150 mM

Dilution #4 - 5 mL of primary solution diluted in 25 mL = 200 mM

Results presented are two calibration curves from the two electrodes of sodium from HANNA ref nº FC 300 B Na^+ (Figure 9.5).

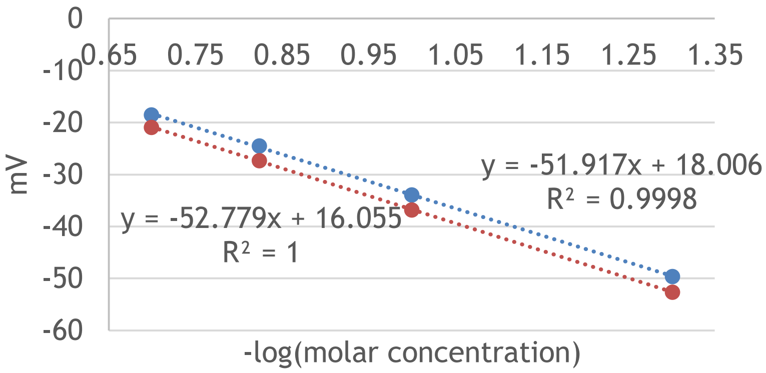


Figure 9.5 - Example of calibration curve for sodium electrode

Important notes

- The ISA solution provided by the manufacturer does not seem to be in proper condition and the use of a freshly prepared solution yielded better results. Also, the proportion of ISA to solution should be the one referenced above, ignoring the manufacturer's manual. This was later confirmed by the manufacturer itself, and new manuals reflect this.

- 15 minutes for less concentrated and 10 minutes for more concentrated samples / calibrating solutions, are required for appropriate response.

A.11. Calcium and magnesium determination

For calcium and magnesium determination the following preparation procedure test following EPA method # 130.2 Hardness, Total (mg/L as $CaCO_3$) (Titrimetric, EDTA) was used:

Solution 1: 1) 1.17772 g of disodium EDTA.$2H_2O$ + 0.820 g of $MgSO_4.7H_2O$ added to 50 mL;

2) 16.9002 g of NH_4Cl + 144 mL NH_4OH in 200 mL

The first solution was added to the second and made into a solution of 250 mL with expiration time of one month more or less.

Solution 2: 0.10 g methyl red in a 100 mL vessel – solution of indicator, no indication of expiration date.

Solution 3: 3.7231 g of disodium EDTA in 1 L. Resistance in time, variable.

Solution 4: 105 mL of conc NH_4OH in 500 mL – NH_4OH 3 N

Solution 5: 35 mL of conc NH_4OH in 500 mL - NH_4OH 1 N

Solution 6: It was assumed that $CaCO_3$ < 30 µm anhydrous was appropriate and therefore 1.0005 g of $CaCO_3$ was used + drops of 6N HCl + boiling for a 10 minutes + drops of methyl red solution + adjust to orange / weak yellow with 6N HCl and NH_4OH.

Indicator: 99.67 g of NaCl + 0.5023 g of Black T mixed.

Standardization titration procedure - 50 mL of distilled water + 10 mL of Ca standard (solution 6) + 1 mL of buffer solution (solution 1) + small scoop of indicator + titration with solution 3. N = 0,2 / mL EDTA

To test a sample add 1 to 2 mL of buffer solution and a small scoop of indicator and titrate with solution nº3.

Calculations: $Ca^{2+} + Mg^{2+}$ (in meq L^{-1}) = (V_{EDTA} * N_{EDTA} * 1000) / V_{sample}

This method was used to test several different samples throughout this study with results varying with experimental condition. However, organic compounds can interfere with the determination and so an additional screening test for the organic soil was performed prior to any experiment.

For this test, four samples of soil were used to make a 1:5 soil water extraction for the determination of $Ca^{2+} + Mg^{2+}$ of non-contaminated soil.

One sample did not receive any treatment while the other three samples were oven dry at 650ºC for 2 hours and then removed, air cooled and added 20 mL of HCl 1 N during 1 day (in accordance with Eaton et al. (2005).

The resulting solution was properly neutralized with NaOH 1 N and, along with the untreated solution, was titrated with EDTA, following the same procedure above. Samples 1 to 3 represent the treated extracts, while sample 0 represents the untreated sample (Table 9.9).

Table 9.9 - Results of calcium and magnesium of treated (1 through 3) and untreated soil (0) samples to eliminate organic interference.

Sample #	mL of EDTA		Average	Conversion to equivalent concentration
1	0,40	0,39	0,395	5,93 mmol
2	0,48	0,48	0,48	7,20 mmol
3	0,42	0,40	0,41	6,15 mmol
0	0,43	0,45	0,44	6,60 mmol

Average treated result is 6.43±0.62 mmol while untreated is 6.60±0.21 mmol. This indicates that the organic matter present does not create any interference on the used method.

A.12. Chloride determination by ion-selective electrode

Only one successful example for chloride electrode calibration is described here, randomly chosen as different experimental setups required different calibration curves.

The following solutions were prepared:

Primary solution: 14.61 g NaCl to 250 mL = 1 M NaCl.

ISA solution: provided by the manufacturer

For each dilution, 0.5 mL ISA, before dilution is completed,

Dilution #1 - 1.25 mL of primary solution diluted in 25 mL = 50 mM

Dilution #2 - 2.5 mL of primary solution diluted in 25 mL = 100 mM

Dilution #3 – 5 mL of primary solution diluted in 25 mL = 200 mM

Dilution #4 – 7.5 mL of primary solution diluted in 25 mL = 300 mM

Results of the calibration curve from the chloride electrode (HANNA HI 4107 Chloride combination electrode) can be found in Figure 9.6.

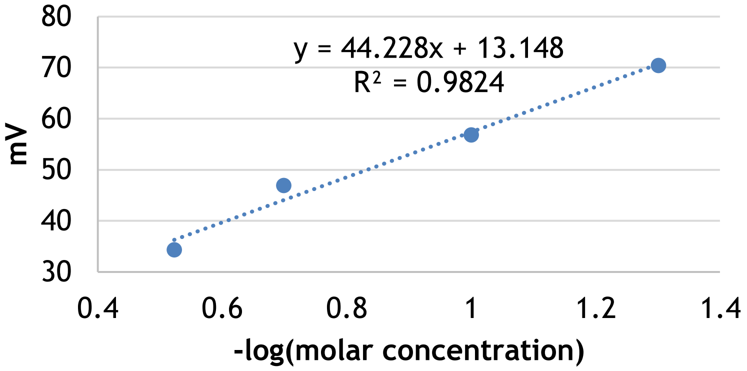


Figure 9.6 - Example of calibration curve for chloride electrode

A.13. Ammonia determination by ion-selective electrode

Only one successful example for the ammonia electrode calibration is described here, randomly chosen, as different experimental setups required different calibrations curves.

The following solutions were prepared:

Primary solution: 1.147 g NH_4Cl = 1.147 / 53.491 = 0.021443 mol, = 0.3 g N in 100 mL (3000 mg L of NH_4^+-N)

ISA solution: provided by the manufacturer;

For each dilution, 0.5 mL ISA, immediately before testing,

Dilution #1 - 1 mL of primary solution diluted in 25 mL = 1.2 mg L N

Dilution #2 - 1.5 mL of primary solution diluted in 25 mL = 1.8 mg L N

Dilution #3 – 2.5 mL of primary solution diluted in 25 mL = 3.0 mg L N

An example calibration curve can be found for the ammonia electrode (Thermo Scientific 9512HPBNWP high performance ammonia electrode) in Figure 9.7.

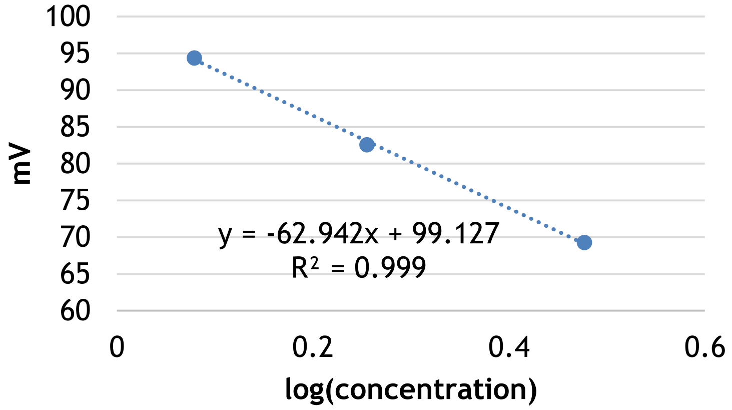


Figure 9.7 - Example of calibration curve for ammonia electrode

A.14. References

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B. Example of statistical analysis performed

In this appendix, an example of data analysis is demonstrated for a specific data set. In this case the results obtained in constructed wetlands tests with expanded clay at HRT of 4 days with ammonia, the second repetition, were chosen as an example.

The applied statistical analysis and explanation concern basic concepts for hypothesis testing with low number of replicates, and apply for independent samples only (Field, 2009) (Figure 9.8).

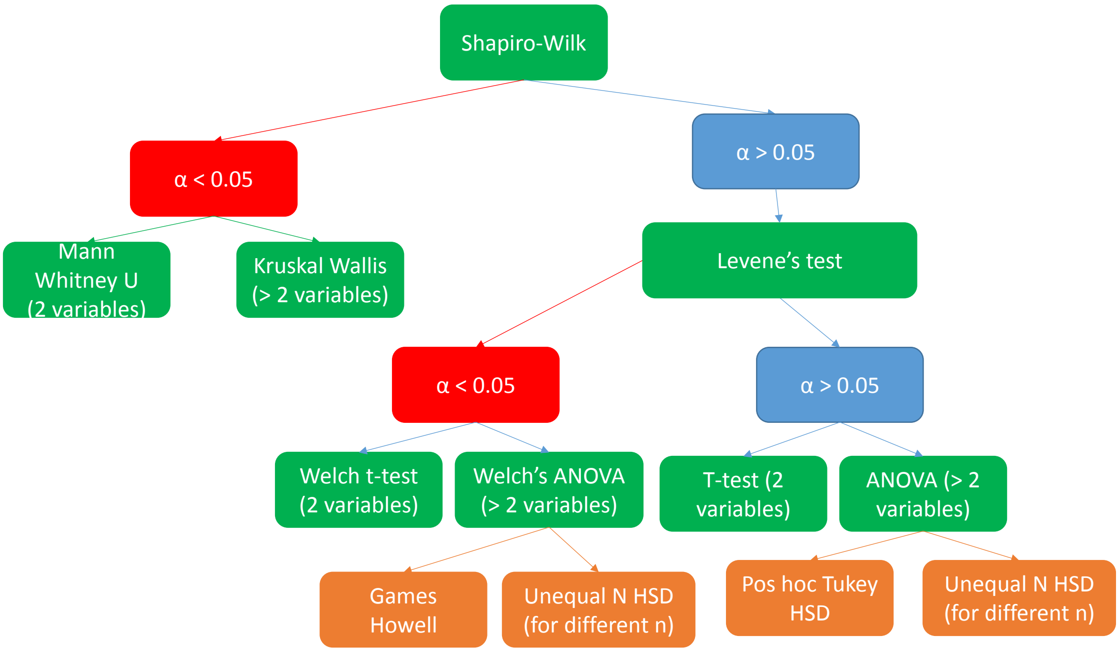

Figure 9.8 - Schematic demonstration of the sequence of statistical tests performed.

Initial tests to assess whether the distribution of individual sets of replicates is normally distributed are required. This is important to define whether parametric or non-parametric tests are to be used. In the case of this study, most experimental setups use triplicate microcosms. For such low number of replicates, the most appropriate normality test is Shapiro-Wilk: not only is one of the most robust tests, but also it can be applied to only 3 replicates.

If the obtained p value is below the threshold alpha value of 0.05, then non-parametric statistics has to be employed. Depending on the number of variables, these can be Mann Whitney U (comparing only two variables) or Kruskal-Wallis (comparing more than two variables).

If, however, the obtained p value is higher than the threshold alpha value, then further tests are required. The more commonly used t-test (for two variables) or ANOVA (more than two variables) not only require normal distribution but also variance homogeneity. For this purpose, the Levene's test can be used and only if the p value is higher than the alpha value of 0.05, these two tests can be applied. If, on the other hand, the p value of Levene's test is below 0.05, than the Welch t-test or Welch's ANOVA test should be used instead.

Furthermore, both ANOVA and Welch's ANOVA tests simply indicate whether there is a statistically significant difference among all the variables tested (3 or more) but they do not provide information on any specific interactions (e.g whether variable 1 is different from 3 or 2 different from 1, etc). Post hoc analyses are required, but depend on the type of ANOVA being used: for Welch's ANOVA, Games Howell post hoc should be used, while for ANOVA post hoc Tukey, for example, could be used. Furthermore, Unequal N HSD should be used in both ANOVA when the number of replicates is not similar among different variables.

These post hoc tests are the preferred method of analysis as, unlike several t-tests on the ANOVA results, post hoc tests correct for family-wise error-rate.

Based on the above procedure, the raw results (Table 9.10) are initially tested by Shapiro-Wilk (Table 9.11).

Table 9.10 - Raw results from the 12 different microcosms of experimental setup, coded by parameter.

	EC	pH	CaMg	Na	NO3-N	NH4-N	Cl	SAR
1	14.56	7.528	10.6	4831.383	127.2543	18.44599	5393.77	91.24429
2	14.72	7.608	11.6	4590.637	117.6705	19.29607	5536.378	82.87644
3	16.11	7.525	9.6	4433.394	173.4394	15.69358	6074.659	87.9808
4	15.87	7.555	12.2	5462.558	200.6878	12.66594	5568.577	96.16197
5	16.45	7.43	19	5232.52	461.5435	16.73365	5536.378	73.81103
6	17.95	7.508	21.2	5227.52	254.7313	14.10303	6253.375	69.80957
7	16.87	8.1	6.2	5714.42	54.94012	43.29558	6271.533	141.1118
8	18.01	7.822	9.2	5695.165	124.5657	36.81403	6569.341	115.4515
9	18.68	7.884	5.8	6383.683	62.45029	36.30496	7002.054	162.9838
10	16.47	7.56	8.6	5631.631	67.29673	4.352698	5816.116	118.0789
11	16.51	7.452	8.6	5469.73	100.6137	16.06869	5649.897	114.6843
12	16.37	7.497	8.8	5370.978	49.55239	4.671782	5883.965	111.3267

Table 9.11 - Shapiro-Wilk normality test for different experimental setups (1-3; 4-6; 7-9; 10-12 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively). Red indicates non-normal distributions.

Parameter / Code	Shapiro-Wilk - W	p result
EC 1-3	0.8265	0.1795
EC 4-6	0.9388	0.5225
EC 7-9	0.978	0.7158
EC 10-12	0.9423	0.5367
pH 1-3	0.7771	0.0609
pH 4-6	0.97799	0.7284
pH 7-9	0.9072	0.4088
pH 10-12	0.9908	0.8168
CaMg 1-3	1	-
CaMg 4-6	0.9199	0.4520
CaMg 7-9	0.8369	0.2059
CaMg 10-12	0.75	0.0000
Na 1-3	0.9855	0.7698
Na 4-6	0.7659	0.0356
Na 7-9	0.771	0.0469
Na 10-12	0.9808	0.7346
NH4-N 1-3	0.915	0.4348
NH4-N 4-6	0.9721	0.6795
NH4-N 7-9	0.8043	0.1248
NH4-N 10-12	0.7704	0.0457
Cl 1-3	0.8988	0.3818
Cl 4-6	0.7836	0.0759
Cl 7-9	0.9888	0.7971
Cl 10-12	0.9444	0.5454
SAR 1-3	0.9841	0.7587
SAR 4-6	0.8609	0.2700
SAR 7-9	0.9979	0.9122
SAR 10-12	1	0.9940

Based on the results of Table 9.11, the parameters sodium, calcium + magnesium and ammonia do not follow a normal distribution and therefore should be tested with non parametric tests, more specifically, with Kruskal-Wallis, since 4 different experimental setups are involved.

For the remaining parameters, a Levene's test is required to assess variance (Table 9.12).

Table 9.12 - Levene's test results for several parameters.

	Levene's Test for Homogeneity of Variances (Statistical tests 26 Jan 2016) Effect: "Variables" Degrees of freedom for all F's: 3, 8			
	MS Effect	MS Error	F	p
EC	0.33	0.12	2.719583	0.114657
pH	0.00	0.00	2.872190	0.103475
Cl	29000.77	18659.14	1.554239	0.274328
SAR	134.32	50.82	2.643058	0.120824

All the parameters tested have p value higher than 0.05 and therefore are cleared to be tested under ANOVA tests. The following parameters are tested with ANOVA + post hoc Tukey (EC, Table 9.13 ; pH, Table 9.14; Cl, Table 9.15 and SAR, Table 9.16) and the remaining with Kruskal-Wallis (Ca^{2+}+Mg^{2+}, Table 9.17; Na^{+}, Table 9.18 and NH_4^+-N, Table 9.19).

Table 9.13 - Tukey HSD results for different variables for EC (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Tukey HSD test; variable EC (Statistical tests 26 Jan 2016) Approximate Probabilities for Post Hoc Tests Error: Between MS = .68037, df = 8.0000				
Variables	1 15.130	2 16.757	3 17.853	4 16.450
1		0.151272	0.015829	0.278168
2	0.151272		0.416378	0.966762
3	0.015829	0.416378		0.236813
4	0.278168	0.966762	0.236813	

Table 9.14 - Tukey HSD results for different variables for pH (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Tukey HSD test; variable pH (Statistical tests 26 Jan 2016) Approximate Probabilities for Post Hoc Tests Error: Between MS = .00761, df = 8.0000				
Variables	1 7.5537	2 7.4977	3 7.9353	4 7.5030
1		0.858839	0.003153	0.889967
2	0.858839		0.001405	0.999847
3	0.003153	0.001405		0.001507
4	0.889967	0.999847	0.001507	

Table 9.15 - Tukey HSD results for different variables for Cl^- (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Tukey HSD test; variable Cl (Statistical tests 26 Jan 2016) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1106E2, df = 8.0000				
Variables	1 5668.3	2 5786.1	3 6614.3	4 5783.3
1		0.970961	0.033822	0.972869
2	0.970961		0.062067	1.000000
3	0.033822	0.062067		0.061177
4	0.972869	1.000000	0.061177	

Table 9.16 - Tukey HSD results for different variables for SAR (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Tukey HSD test; variable SAR (Statistical tests 26 Jan 2016) Approximate Probabilities for Post Hoc Tests Error: Between MS = 199.22, df = 8.0000				
Variables	1 87.367	2 79.928	3 139.85	4 114.70
1		0.914273	0.008194	0.160693
2	0.914273		0.003769	0.065027
3	0.008194	0.003769		0.207683
4	0.160693	0.065027	0.207683	

Table 9.17 - Kruskal Wallis results for different variables for $Ca^{2+}Mg^{2+}$ (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Depend.: CaMg	Multiple Comparisons p values (2-tailed); CaMg (Statistical tests 26 Jan 2016) Independent (grouping) variable: Variables Kruskal-Wallis test: H (3, N= 12) =9.494737 p =.0234			
	1 R:8.0000	2 R:11.000	3 R:3.0000	4 R:4.0000
1		1.000000	0.536576	1.000000
2	1.000000		0.039470	0.104503
3	0.536576	0.039470		1.000000
4	1.000000	0.104503	1.000000	

Table 9.18 - Kruskal Wallis results for different variables for Na^+ (1; 2; 3; 4 - refer to *Spartina maritima*, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Depend.: Na	Multiple Comparisons p values (2-tailed); Na (Statistical tests 26 Jan 2016) Independent (grouping) variable: Variables Kruskal-Wallis test: H (3, N= 12) =9.974359 p =.0188			
	1 R:2.0000	2 R:5.3333	3 R:11.000	4 R:7.6667
1		1.000000	0.013407	0.325473
2	1.000000		0.325473	1.000000
3	0.013407	0.325473		1.000000
4	0.325473	1.000000	1.000000	

Table 9.19 - Kruskal Wallis results for different variables for NH_4^+-N (1; 2; 3; 4 - refer to *Spartina* maritima, *Juncus maritimus*, unplanted control and *Arundo donax* microcosms, respectively).

Depend.: NH4-N	Multiple Comparisons p values (2-tailed); NH4-N (Statistical tests 26 Jan 2016) Independent (grouping) variable: Variables Kruskal-Wallis test: H (3, N= 12) =8.435897 p =.0378			
	1 R:7.3333	2 R:4.6667	3 R:11.000	4 R:3.0000
1		1.000000	1.000000	0.846190
2	1.000000		0.188703	1.000000
3	1.000000	0.188703		0.039470
4	0.846190	1.000000	0.039470	

Based on these results the following final result Table can be constructed (Table B.11):

Table 9.20 - Synthetic saline wastewater final values for salts and ammonia-nitrogen after treatment in simulated constructed wetlands with expanded clay only (control) and planted with three different plants, after a 4 days retention time. Different letters in the same column indicate statistically different results between treatments for each parameter per retention time, i.e, results that share at least one letter are not statistically different.

Treatment	EC ($dS\ m^{-1}$)	pH	Cl^- ($mg\ L^{-1}$)	$Ca^{2+}+Mg^{2+}$ ($meq\ L^{-1}$)	Na^+ ($mg\ L^{-1}$)	SAR	NH_4^+-N ($mg\ L^{-1}$)
S. maritima	15.1±0.7^b	7.55±0.04^b	5668±293^b	10.6±0.8^{ab}	4618±164^b	87±3^b	17.8±1.5^{ab}
J. maritimus	16.8±0.9^{ab}	7.50±0.05^b	5786±331^{ab}	17.5±3.8^b	5308±110^{ab}	80±12^b	14.5±1.7^{ab}
A. donax	16.5±0.1^{ab}	7.50±0.04^b	5783±98^{ab}	8.7±0.1^{ab}	5491±107^{ab}	115±3^{ab}	8.4±5.5^b
Unplanted	17.9±0.8^a	7.94±0.12^a	6614±300^a	7.1±1.5^a	5931±320^a	140±19^a	38.8±3.2^a

B.1. References

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