

DAAN LOUIS TEN BERGE

**OPTIMISATION OF AQUACULTURE
WASTEWATER BY HALOPHYTES CULTIVATION**



Faculty of Science and Technology

2024

DAAN LOUIS TEN BERGE

**OPTIMISATION OF AQUACULTURE
WASTEWATER BY HALOPHYTES CULTIVATION**

Mestrado em Aquacultura e Pescas

Especialidade em Aquacultura

Trabalho efetuado sob a orientação de:

Dr Cátia Andreia Lourenço Marques, IPMA/S2AQUAcoLAB

Dr Luísa Margarida Batista Custódio, CCMAR



Faculdade de Ciências e Tecnologia

2024

Optimisation of aquaculture wastewater by halophytes cultivation

Declaration of authorship

Declaro ser autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Daan Louis ten Berge

Copyright © Daan Louis ten Berge

A Universidade do Algarve tem o direito, perpétuo e sem limites geográficos, de arquivar e publicar este trabalho através de exemplares impressos reproduzidos em papel ou de forma digital, ou por qualquer outro meio conhecido ou que venha a ser inventado, de o divulgar através de repositórios científicos e de admitir a sua cópia e distribuição com objetivos educacionais ou de investigação, não comerciais, desde que seja dado crédito ao autor e editor.



Aerial view of the IPMA - Estação Piloto de Piscicultura de Olhão where this work was carried out.

Institutions where the work was carried out:

This work was funded and carried out at the IPMA – Estação Piloto de Piscicultura de Olhão (EPPO) using the available infrastructure and means. IPMA reserves the right to use all the information contained therein for the purpose of publications, reports of the funding entity and dissemination to the general public. Further biomass processing and characterisation were performed at facilities of CCMAR-Algarve, University of the Algarve, Faro, Portugal.

Acknowledgements

First and foremost, I would like to thank Ivo Monteiro for introducing me to this topic and giving me the opportunity and necessary help to create this thesis concept. Ivo has been my day-to-day contact person who has helped me to great extent in every aspect during this thesis project. Over the time period of my thesis, we have laughed a lot and became friends. He has been extremely attentive, and I really enjoyed working with him.

Moreover, I would like to thank my official supervisors, Luísa Custódio and Cátia Marques. When I was looking for a supervisor within the university, Luísa immediately accepted and offered to help me when I asked her if she could supervise me with my thesis. With the great knowledge that she has on halophytes, I could always ask her when I had any doubts. She has been extremely supportive and would always be a phone call away when I would be in need of advice. Cátia has helped me a lot with creating the concept and its details around the thesis project. We have had a good number of brainstorm meetings where both of us shared our thoughts to make this thesis as inclusive as can be. Therefore, once again, a big thanks to both of you, for taking time and energy to help me with this step in my life.

I also would like to thank my family, who have mentally supported me in any way they could. My mother particularly, who, as curious and caring as she can be, has helped me with transplanting procedures in the greenhouse. Also, a special thanks to my uncle Hein ten Berge, who has helped me in the final stages of the thesis by reviewing my work and sharing his opinion on parts to be improved.

Furthermore, I would like to thank my girlfriend Candela Garcia Pichel, who has helped me immensely throughout the degree, both professionally and personally, and whom without participating in this master's degree I would never have met, for which I am therefore very grateful. Additionally, I want to thank my friends Pedro Parente, João Rito, Tanguy Rabillé and Timon Rùth who have motivated and supported me always, and of which the latter has helped me through some rather harsh times with statistical analyses and more.

Finally, I would like to thank Elsa Cabrita, the director of the master program, for accepting me to the program and being one of the best teachers I have ever had. I am extremely grateful to have received the opportunity to have studied at the Universidade do Algarve, meeting people whom I can now call dear friends, and living on the beach in Praia de Faro, all whilst enriching my knowledge and experience by learning more about aquaculture. I can honestly say these have been some of the best two years of my life.

Abstract

This thesis represents a comprehensive assessment regarding the cultivation of halophyte *Salicornia ramosissima* within aquaculture effluent-based systems and use of different water sources commonly available at aquaculture farms as treatments. Halophytes, recognised for their ability to thrive in saline environments, are gaining popularity for their versatile applications including culinary uses and source of secondary compounds with biorefinery potential. The experimental study spanned 6 weeks in a greenhouse at the Aquaculture Research Station of Olhão, Portugal. The setup entailed two production systems: semi-hydroponics and substrate mixture system consisting of potting soil and mud from a semi-intensive fishpond. Four treatments were applied in both systems. These involved water sources containing salinities 10, 20, and 35 PSU, the latter sourced from a semi-intensive pond system. In these treatments, plants received supplementary fertiliser. Additionally, the 35 PSU treatment was tested without supplementary fertiliser. Other treatments included water from a nearby brackish well (20 PSU) and its mixture with freshwater (10 PSU). The study aimed to assess how the two production systems combined with varying salinities and nutrient levels affect the growth, biochemical properties and nutritional content of *S. ramosissima*. Results indicated that the mud-based substrate system consistently produced higher biomass across all salinities compared to the semi-hydroponics system, particularly at 35 PSU. In the semi-hydroponics system, plants performed better at moderate salinity (20 PSU), while performance was more restricted at lower and higher salinities. Additional fertiliser in the high salinities (35 PSU) did not seem to affect growth or nutritional properties of biomass. Secondary metabolites were generally higher in biomass without supplementary fertiliser. The mud-based system appeared more robust, indicating suitability for high salinity environments. These results indicate that *S. ramosissima* could be used to effectively optimise aquaculture wastewater and act as biofilter whilst producing valuable biomass in highly saline conditions, promoting circular economy.

Keywords: Halophytes; Aquaculture; Wastewater; *Salicornia ramosissima*; Circular Economy

Resumo

Esta tese explora a otimização do uso das águas residuais provenientes da aquacultura por meio do cultivo de halófitas, com ênfase na espécie *Salicornia ramosissima*. As halófitas são plantas que possuem a capacidade única de sobreviver e prosperar em ambientes de alta salinidade, o que as torna adequadas para serem cultivadas em sistemas de aquacultura e aproveitadas para o tratamento de efluentes salinos. Além disso, estas plantas têm atraído atenção crescente devido às suas múltiplas utilizações, como a aplicação na alimentação, utilização como biocombustíveis e pelo seu conteúdo em compostos bioativos com potencial na biotecnologia e na indústria farmacêutica. Este estudo foi realizado no contexto de uma economia circular, que visa a reutilização eficiente de recursos, minimizando resíduos e contribuindo para a sustentabilidade ambiental. A experiência foi realizada ao longo de seis semanas, numa estufa localizada na Estação Piloto de Piscicultura de Olhão, Portugal, e envolveu dois sistemas de produção distintos: um sistema semi-hidropônico e um sistema de substrato que consistia numa mistura de solo para vasos e lamas de uma piscicultura semi-intensiva. Ambos os sistemas foram irrigados com diferentes fontes de água, cada uma com níveis distintos de salinidade, com o objetivo de testar a performance de crescimento e o perfil e composição bioquímico da *S. ramosissima*, sob diferentes condições de cultivo. As águas utilizadas variaram entre 10, 20 e 35 Unidades Práticas de Salinidade (UPS), sendo a água com 35 UPS proveniente de efluentes da aquacultura. As plantas nesses tratamentos receberam fertilizantes suplementares. Além disso, as plantas com água de 35 UPS foram testadas com e sem a adição de fertilizantes suplementares, o que possibilitou a avaliação dos efeitos da fertilização em combinação com a salinidade no desenvolvimento das plantas. A *S. ramosissima* é uma planta halófitas conhecida pela sua tolerância à salinidade e pelas suas propriedades nutricionais e funcionais. Neste estudo, o principal objetivo foi avaliar como os dois sistemas de produção, aliados aos diferentes níveis de salinidade e fertilizante, influenciam o crescimento, as propriedades bioquímicas e o valor nutricional desta planta halófitas. Além de ser uma planta de interesse para o tratamento de efluentes, a *S. ramosissima* também apresenta valor comercial devido à sua aplicabilidade na alimentação e ao seu potencial como biofiltro em sistemas de aquacultura, especialmente em ambientes com alta salinidade. Os resultados experimentais indicaram que o sistema de substrato, composto por uma mistura de lamas de tanques de uma piscicultura semi-intensiva e solo para vasos, produziu, consistentemente, uma maior quantidade de biomassa em todos os níveis de salinidade quando comparado com o sistema semi-hidropônico. Este aumento na produção de biomassa foi particularmente evidente nos tratamentos com a salinidade de 35 UPS, onde as plantas demonstraram um crescimento mais robusto e uma maior acumulação de nutrientes essenciais. No sistema semi-hidropônico, as plantas tiveram um desempenho superior quando submetidas a uma salinidade moderada (20 UPS), enquanto que o crescimento foi limitado nas salinidades mais baixas (10 UPS) e nas mais elevadas (35 UPS). A adição de fertilizante nos tratamentos de alta salinidade (35 UPS) não pareceu afetar o crescimento ou as propriedades nutricionais da biomassa em ambos os sistemas de produção. A análise bioquímica revelou que as plantas cultivadas no sistema de substrato apresentaram níveis mais elevados de minerais, como cálcio, potássio e sódio, em comparação com as plantas cultivadas no sistema semi-hidropônico. Estes resultados sugerem que o sistema de substrato oferece melhores condições para a absorção e acumulação de minerais, particularmente em ambientes de alta salinidade. Além disso, a *S. ramosissima* demonstrou ser uma fonte rica em compostos bioativos, como fenóis, flavonoides e carotenoides, que possuem propriedades antioxidantes importantes e

são altamente valorizados na indústria alimentar e farmacêutica. Esses compostos têm o potencial de ser usados em formulações nutracêuticas e como ingredientes funcionais em alimentos, para além dos seus benefícios para a saúde humana, como a proteção contra o stresse oxidativo e a inflamação. Outro aspecto relevante deste estudo foi a avaliação das propriedades nutricionais da *S. ramosissima*. A planta mostrou um teor considerável de proteínas, o que a torna uma candidata promissora para uso como fonte alternativa de proteínas em áreas onde o cultivo de plantas convencionais é limitado pela salinidade do solo. A presença de proteína é um atributo de grande interesse, uma vez que a procura por fontes sustentáveis de proteína está em crescimento, e as halófitas, como a *S. ramosissima*, podem desempenhar um papel importante no preenchimento dessa necessidade. Este estudo também avaliou o teor de humidade, cinzas e compostos anti-nutricionais, como os taninos e os fitatos, que, embora possam interferir na absorção de certos minerais, não foram encontrados em níveis suficientemente elevados para representar uma preocupação nutricional significativa. Com base nos resultados obtidos, pode-se concluir que o sistema de substrato à base de lamas se mostrou mais adequado para o cultivo da *S. ramosissima* em condições de alta salinidade, devido à sua capacidade para promover um maior crescimento e uma melhor retenção de minerais. Por outro lado, o sistema semi-hidropônico exigiu uma maior atenção na gestão de nutrientes e de salinidade, uma vez que as plantas cultivadas nesse sistema demonstraram menor resistência em condições sem fertilizante adicional e maior salinidade. Assim, este estudo sugere que a integração do cultivo de *S. ramosissima* em sistemas de aquacultura, especialmente em regiões costeiras onde a salinidade do solo representa um desafio para a agricultura convencional, pode ser uma solução viável para o tratamento de efluentes ao mesmo tempo que há produção de biomassa de alto valor acrescentado. A produção desta planta nestes ambientes não só contribui para a melhoria da qualidade da água, removendo nutrientes em excesso, particularmente compostos nitrogenados, como também proporciona uma nova fonte de rendimento para os produtores de aquacultura, que podem comercializar a planta como um produto alimentar ou utilizar a biomassa para a extração de compostos bioativos. Além disso, a *S. ramosissima* também pode desempenhar um papel importante na mitigação dos impactos da mudança climática, uma vez que o seu cultivo requer menos água doce em comparação com plantas convencionais, tornando-a uma cultura atrativa em regiões onde a escassez de água é uma preocupação crescente. O seu uso em sistemas de produção integrados, como a aquaponia ou sistemas de aquacultura multi-trófica, favorece uma maior sustentabilidade ambiental e contribui para a transição para uma economia mais circular. Em suma, este trabalho demonstra que a *S. ramosissima* tem grande potencial para otimizar a utilização de efluentes de aquacultura, ao mesmo tempo que gera produtos de valor acrescentado, promovendo uma economia circular e sustentável. Os sistemas de produção com base em substratos à base de lamas oferecem as melhores condições para o cultivo desta halófitas em ambientes de alta salinidade, enquanto que o sistema semi-hidropônico pode ser viável com a gestão adequada de nutrientes. A implementação destes sistemas pode trazer benefícios significativos tanto para os produtores de aquacultura quanto para o meio ambiente, abrindo caminho para o desenvolvimento de práticas mais sustentáveis e rentáveis na produção de alimentos em regiões costeiras.

Palavras-chave: Halófitas; Aquicultura; Águas Residuais; *Salicornia ramosissima*; Economia Circular

Table of Contents

1	Introduction	1
1.1	Aquaculture wastewater	1
1.1.1	Aquaculture systems	2
1.1.2	Polyculture systems	3
1.2	Halophytes	4
1.2.1	Plant characteristics	5
1.2.2	Growing conditions	6
1.2.3	<i>Salicornia ramosissima</i>	7
1.3	Analytic techniques	7
1.3.1	Nutritional properties	7
1.3.2	Minerals	9
1.3.3	Bioactive compounds	9
1.3.4	Antinutritional properties	10
1.4	Objective	11
2	Materials and methods	12
2.1	Molecular identification of species	12
2.2	Experimental cultivation setup	12
2.2.1	Experimental production systems	13
2.2.2	Salinity treatments and nutrients	14
2.2.3	Experimental conditions	15
2.3	Biomass analysis and processing	16
2.3.1	Nutritional properties analysis	16
2.3.2	Mineral analysis	16
2.3.3	Bioactive compound analysis	16
2.3.4	Anti-nutritional properties analysis	17
2.4	Statistical analysis	18
3	Results	19
3.1	Plant biomass results	19
3.2	Proximate composition results	21
3.2.1	Moisture content	21
3.2.2	Ash content	22
3.2.3	Plant saltiness	24
3.2.4	Protein content	25
3.3	Mineral content results	27
3.3.1	Calcium (Ca)	32
3.3.2	Potassium (K)	32
3.3.3	Magnesium (Mg)	33
3.3.4	Sodium (Na)	34
3.3.5	Phosphorus (P)	34
3.3.6	Zinc (Zn)	35

3.3.7	Copper (Cu)	36
3.3.8	Nickel (Ni)	37
3.3.9	Lead (Pb).....	37
3.3.10	Manganese (Mn)	38
3.3.11	Chromium (Cr)	39
3.3.12	Iron (Fe)	40
3.4	Bioactive compounds results	41
3.4.1	Total phenolics content (TPC).....	41
3.4.2	Total flavonoid content (TFC)	42
3.4.3	Total liposoluble pigments content.....	44
3.4.4	Total tannins content (TTC)	47
4	Discussion	49
4.1	Biomass production implications	49
4.2	Proximate composition implications	52
4.3	Minerals implications	55
4.3.1	Macroelements	56
4.3.2	Microelements	58
4.4	Bioactive compound implications	59
4.5	Antinutritional properties implications	62
4.6	Trial effects	63
5	Conclusion and future recommendations	67
	References.....	68

Table of Figures

- Figure 2.1:** Experimental cultivation setup, where System 1 (left) indicates the semi-hydroponics system and System 2 (right) indicates the mud-based soil substrate system, with according salinity levels. “A” indicates the treatment with water of salinity 10 PSU, “B” of salinity 20 PSU, “C” and “D” of salinity 35 PSU. All treatments except “D” received additional supplementary fertiliser. 13
- Figure 2.2:** Practical overview of experimental cultivation setup, where the left picture indicates the semi-hydroponics system and the right picture indicates the mud-based soil substrate system..... 14
- Figure 3.1:** Fresh weight of harvested plant biomass (g) from a) first (week 3) and b) second (week 6) cutting, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn’s post-hoc test, $p < 0.05$). 19
- Figure 3.2:** Fresh plant weight (g) from plants at day 42 (end of trial), separated in a) edible (green, non-lignified) (cuttings excluded), b) non-edible (lignified stems) comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based system. No denotation means no differences. (Kruskal-Wallis; Dunn’s post-hoc test, $p < 0.05$). 20
- Figure 3.3:** Weight of total harvested plant biomass (g) from a) semi-hydroponics system and b) mud-based substrate system, comparing the edible biomass (first and second cutting and remaining edible biomass) (left, green bar) and non-edible biomass (stems) (right, orange bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at edible biomass. Low case letters indicate differences between non-edible biomass. No denotation means no differences. (Kruskal-Wallis; Dunn’s post-hoc test, $p < 0.05$). ...21
- Figure 3.4:** Moisture content (%) from a) first (week 3) and b) second (week 6) cutting and c) stems, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn’s post-hoc test, $p < 0.05$). 22
- Figure 3.5:** Ash content (%) from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences (Kruskal-Wallis; Dunn’s post-hoc test, $p < 0.05$).24
- Figure 3.6:** Saltiness of plants (mS/cm) from the second cutting (week 6), comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate

differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 25

Figure 3.7: Protein content (% DW) in a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 27

Figure 3.8: TPC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 42

Figure 3.9: TFC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 44

Figure 3.10: Total chlorophyll content of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 46

Figure 3.11: Carotenoids content of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 47

Figure 3.12: TTC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate production system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$). 48

Table of tables

Table 3.1: Mineral contents of biomass from the first cutting of <i>S. ramosissima</i> , comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).....	28
Table 3.2: Mineral contents of biomass from the second cutting of <i>S. ramosissima</i> , comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).....	29
Table 3.3: Mineral contents of biomass from the stems of <i>S. ramosissima</i> , comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).	30
Table 3.4: Mineral contents of biomass from the roots of <i>S. ramosissima</i> , comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).	31

Abbreviations

AOB: Ammonia-Oxidizing Bacteria

CEC: Cation Exchange Capacity

DW: Dry Weight

FC: Foliar Concentration

MP-AES: Microwave Plasma-Atomic Emission Spectrometer

ND: Nutrient Dilution

NOB: Nitrite-Oxidizing Bacteria

PSU: Practical Salinity Units

RAS: Recirculating Aquaculture System

ROS: Reactive Oxygen Species

SSP: Suspended Solid Particles

TFC: Total Flavonoid Content

TPC: Total Phenolic Content

TTC: Total Tannin Content

35 w/o: 35 PSU without additional fertiliser

1 Introduction

The global fisheries and aquaculture production in 2022 expanded to 223.2 million tonnes, a 4.4 percent increase from 2020. Production included 185.4 million tonnes of aquatic animals and 37.8 million tonnes of algae, and for the first time in history, aquaculture surpassed fisheries as the main producer of aquatic animals (FAO, 2024). Global aquaculture production reached record of 130.9 million tonnes, of which 94.4 million tonnes are aquatic animals, 51 percent of the total aquatic animal production. FAO states that aquaculture growth indicates its capacity to further contribute to meeting the rising global demand for aquatic foods, but future expansion and intensification must prioritise sustainability and benefit regions and communities most in need (FAO, 2017). Overexploitation of marine resources and overfishing are causing depletion of wild aquatic stocks (Bastardie et al., 2024; Mansfield, 2010). However, since these marine resources contain essential nutrients such as fatty acids and their necessity in diets, next to the general rising demand for aquatic foods, causes the imperative growth of aquaculture (FAO, 2024; Rocha et al., 2021). Hence, as aquaculture will see an intensification, algae and plants such as halophytes could become important in the biofiltering and remediation of effluents (Buhmann & Papenbrock, 2013b; Milhazes-Cunha & Otero, 2017). Continuous growing interest on low trophic species highlight the importance of increasing the knowledge on halophyte production (European Commission, 2022).

1.1 Aquaculture wastewater

The increase in aquaculture production is imperative. However, the by-product waste produced is directly proportional to the fish production in the aquaculture sector. Nitrogen and phosphorus are considered two main waste components from fish farming, having potential to pollute and cause severe environmental problems (Bhavsar et al., 2016; Brown et al., 1999; Zhang et al., 2023). Hence, the current animal aquaculture sector is facing challenges in the mitigation of the problems that can occur by the creation of wastewater from fish aquaculture. It is therefore necessary to establish wastewater treatment or purification systems before discharging back into the natural environment (Bhavsar et al., 2016). This step is an important measure for the conservation of the natural ecosystems (Tom et al., 2021).

1.1.1 Aquaculture systems

There are many different aquaculture systems for fish production. The most used are off-shore farming in cages, farming in semi-intensive ponds and intensive recirculating aquaculture systems (RAS) (Naylor et al., 2021; Simon & Michael, 2001). The wastewater produced by the offshore cages systems does not endure real wastewater treatment process, as waste is released directly into the natural ecosystems. Hence, important fish monitoring is essential to not pollute the natural environment more than necessary. By monitoring the fish, its feeding behaviour and the surroundings of the cages, it is possible to, by for instance adjusting feed quantities, reduce and keep environmental pollution to a minimum (FAO, 2007). Aquaculture cages also tend to provide positive effects, particularly to the local communities, as it increases feed availability and therefore biodiversity at the site (Sangirova et al., 2020).

For pond and RAS farming however, it is possible, and often legally obligated in the Mediterranean (IUCN, 2009), to use at least one form of wastewater treatment before discharging effluent streams back into the environment. Conventional semi-intensive pond farming systems often use settlement ponds, where suspended solid particles (SSP) and dissolved nutrients are removed from the water column before discharging the water back into the natural environment. This method of water treatment reduces the SSP by 60% at best and is relatively inconsistent (Castine et al., 2013). RAS on the other hand are intensive production systems that consequently create more waste. Hence, these systems tend to have highly efficient wastewater treatment systems in place, as the principle of recirculating water implies the need of cleaning the used water before using it again (Tom et al., 2021). This can only be possible with water purification steps before recirculating it back into the fish tanks. It is performed by using multiple filter steps, which usually involve both mechanical and biological filters (Orellana et al., 2014). The mechanical filter removes the SSP and the biological filter converts and reduces the nutrients present in the system. Biofilters play a crucial role in the nitrogen cycle, particularly in the conversion of toxic ammonia excreted by fish into less harmful compounds, such as nitrite and nitrate (Ruiz et al., 2020). This process is fundamental for preventing ammonia toxicity, a common challenge in intensive aquaculture systems. Biofilters harness the power of beneficial bacteria, particularly nitrifying bacteria, which adhere to various surfaces within the filter media, such as bio-balls (Hüpeden et al., 2020). These bacteria are naturally found and are able to form biofilms, creating a thriving microbial community (Hargreaves, 1998). The two key phases of the nitrogen cycle, ammonia

(NH₃) to nitrite (NO₂⁻) and nitrite to nitrate (NO₃⁻), are facilitated by different groups of nitrifying bacteria: Ammonia-Oxidizing Bacteria (AOB), which is the bacteria of the genus *Nitrosomonas* and Nitrite-Oxidizing Bacteria (NOB), the bacteria of the genus *Nitrobacter* (Ruiz et al., 2020). As these bacteria are naturally occurring in presence of high levels of nitrogen, the settlement ponds from the semi-intensive pond systems also tend to contain these in considerable levels in the sediment (Boyd, 2019). Therefore, the collected waste from pond systems and RAS, removed by the settlement pond or the filters in the RAS, has high concentrations of nutrients. Occasionally, this waste, also called sludge, is used for compost or treated to be able to use it on land for fertilising purposes (Turcios & Papenbrock, 2014).

1.1.2 Polyculture systems

Another alternative to wastewater treatment is aquaponics. Aquaponics is a food production system that incorporates conventional aquaculture systems or RAS, raising aquatic animals such as fish, prawns or shellfish in tanks with hydroponics, cultivating plants in water, in symbiosis where both products benefit from one another, as excretion products from one production can be re-used as raw material for another production (Lavaud et al., 2023; Martan, 2008; Rakocy et al., 2006). Although depending on quantity of nutrients present, plants tend to grow rapidly with the available dissolved nutrients that are excreted by fish directly or produced by the microbial breakdown of fish waste (Rakocy et al., 2006). Hydroponic cultivation systems make use of many different materials for substrates that the plants can use to establish their roots. Traditionally, mediums including vermiculite, saw dust, peat moss and sand were used. However, recently mediums such as rockwool, expanded clay pebbles and perlite are available as popular options for hydroponic systems (Sheikh, 2006). Aquaponics has an ancient history, where rice farmers in Thailand and South China would grow fish in their rice fields. The ancient Aztecs in Mexico would use similar principles as early as 1150–1350 BC (Okomoda et al., 2022). These polyculture farming systems have already existed in many far Eastern countries where fish, such as common carp and crucian carp, swamp eel, as well as pond snails, would be raised in the paddy fields (Binh & Phillips, 1997). However, since most agricultural crops are salt intolerant, it is challenging to create aquaponic systems in saline environments and marine aquaculture systems (Flowers, 2004).

An alternative approach to sustainable food production is Integrated Multi-Trophic Aquaculture (IMTA). IMTA is essentially an expanded version of aquaponics, as it combines

conventional aquaculture systems with the cultivation of multiple species from different trophic levels, such as fish, shellfish, seaweeds and/or halophytes, in a mutually beneficial arrangement (Chopin et al., 2012). In IMTA systems, nutrient-rich effluents from one species serve as nourishment for another, creating a balanced and resource-efficient ecosystem (Chopin et al., 2012). This integrated approach minimises environmental impact and enhances overall system resilience. Another popular wastewater effluent optimisation system is the use of constructed wetlands, which offer a natural and ecologically comprehensive method for treating aquaculture wastewater. In this system, popular with saline water environments, plants and microorganisms can work together to filter and purify aquaculture effluents (Vymazal, 2011). The plants absorb nutrients from the wastewater, providing a natural and sustainable solution for nutrient removal. Constructed wetlands are recognised for their efficiency in reducing pollutants and improving water quality, presenting a sustainable option for mitigating the environmental impact of aquaculture effluents (Vymazal, 2011). This ecological and sustainable approach aligns with the growing emphasis on environmentally friendly practices in aquaculture. Moreover, constructed wetlands have shown positive effects for growing the halophyte species *Salicornia*, using aquaculture effluents (Diaz et al., 2020; Lee et al., 2009; Turcios et al., 2021).

1.2 Halophytes

Halophytes are salt-tolerant plants able to complete their life cycle in soil or waters with high salinity, with some species, such as *Salicornia*, tolerating up to 1 M NaCl (approximately 58 PSU) (M. A. Khan et al., 2005; Lv et al., 2012). They comprise at most 2% of terrestrial plant species, but have independently evolved multiple times across unrelated plant families during the evolution of angiosperms (Bennett et al., 2013). This limited representation belies the large diversity of plant forms within this group, and intriguingly, halophytes have found versatile applications as vegetables, forage, and oilseed crops. Among the adaptations of halophytes to saline conditions is the accumulation and compartmentalisation of compatible solutes and ions, facilitating osmotic adjustments. Additionally, halophytes can regulate transpiration and accumulate essential nutrients, such as potassium (K), even in the presence of elevated sodium (Na) and chloride (Cl) concentrations (Flowers et al., 2010). Consequently, halophyte plants emerge as valuable sources of essential minerals. The presence of sodium, potassium, chloride, iron, calcium, magnesium and phosphorous highlights the mineral richness (Lopes et al., 2021). Moreover, halophytes possess

various nutritional and functional properties that make them appealing, such as low fat, adequate protein levels and the presence of secondary metabolites, such as phenolics (Barreira et al., 2017; El-Amier et al., 2021). Furthermore, contrary to glycophytes, halophytes can be cultivated in saline conditions, therewith reducing freshwater use and making them of particular importance to the issues of climate change and soil and water salinisation (Hameed et al., 2024).

1.2.1 Plant characteristics

Salicornia spp. generally grow in areas periodically flooded by salty or brackish water (Cárdenas-Pérez et al., 2022). They are characterised as succulent annual species with highly reduced leaves and racemose terminal inflorescences with a linear arrangement of flowers (Scott, 1977). *Salicornia* species are widely distributed across Eurasia, North America, and South Africa, comprising of approximately 25–30 species in the present context (Kadereit et al., 2007). However, the poorly characterised taxonomy of *Salicornia* spp. poses substantial challenges for its practical use as a crop, as *Salicornia* spp. is generally sold as mix of *Salicorniaceae* species due to incorrect taxonomical identification. The complex taxonomy makes the classification of species, microspecies, subspecies, varieties, and putative hybrids particularly difficult (Kadereit et al., 2007; Teege et al., 2011). Factors such as the scarcity of morphological characters, phenotypic plasticity, breeding systems, and hybridisations contribute to additional complexities. Therefore, it is extremely difficult to recognise whether plants occurring in the same area, sharing a similar morphology, have the same genotype. Among the many *Salicornia* species, *S. europaea* and *S. ramosissima* are two of the most identified in Portugal (Kadereit et al., 2007).

Moreover, *Salicornia* spp. are known and renowned in both the food and pharmaceutical industries for their bioactive compounds that offer health benefits. In Asia, *Salicornia* spp. have traditionally been used as a medicine for constipation, nephropathy, hepatitis, diarrhoea, obesity, diabetes and other disorders (Rhee et al., 2009). The bioactivities and chemical components reported in *Salicornia* spp. are primarily attributed to their diverse secondary metabolites. Phenolic compounds, including flavonoids and phenolic acids like caffeic acid, ferulic acid, and protocatechuic acid, are renowned for their biological activities such as antioxidant, anti-inflammatory, antidiabetic, and cytotoxic effects. Therefore, *Salicornia* spp. and its secondary metabolites have the potential to contribute significantly to health benefits such as protective effects against oxidative stress, cancer, and inflammation (Kim et al., 2021). Hence, these

compounds can serve as valuable secondary metabolites in pharmaceuticals or as nutraceuticals in food (Cárdenas-Pérez et al., 2021). Aside of that, *Salicornia* spp. holds significant commercial and culinary value, often used as a salt substitute. The generalisation of *Salicornia* consumption creates commercial interest in cultivating the species (Lima et al., 2020). *Salicornia* species, such as *S. bigelovii*, are reported to be cultivated in farms for commercial distribution mainly for biodiesel, animal feed, salt and oil extraction (Cybulska et al., 2014). Moreover, companies such as RiaFresh and Salivita, based in the Algarve region, Portugal, are cultivating halophytes, including *S. ramosissima*, in greenhouse and open-field conditions for commercial food-consumption purposes. When harvesting, edible fresh tips, also called shoots, are cut from the stem, which, depending on the conditions, can be done up to three times per plant. After harvesting, remaining lignified stem is left and often seen as waste, without economic value (Hulkko et al., 2022).

1.2.2 Growing conditions

Salicornia spp. requires salt for its optimal growth, yet the precise concentration required by the specific species tends to vary depending on variable greenhouse conditions such as temperature, day length, humidity and substrate (Singh et al., 2014). Previous work reported optimal temperature and salinity for growth of *S. ramosissima* between 25°C and 29°C, and around 171 mM NaCl (Mesa-Marín et al. (2019), although plants can tolerate different ranges. However, reported by earlier-mentioned study was that growth can be significantly inhibited at salinity levels exceeding 510 mM NaCl. Furthermore, irrigation can be performed with saline water and, particularly during germination and early growth stages, consistent moisture is crucial (Ozturk et al., 2018). However, *Salicornia* spp. seed germination decreases with the increase of salinity (Orlovsky et al., 2016). Furthermore, *Salicornia* spp. favour well-drained, sandy, or loamy soils. The plants are commonly found in coastal salt marshes where the soil is periodically submerged with seawater, providing a naturally saline environment (Cárdenas-Pérez et al., 2022; Mesa-Marín et al., 2019). Notably, the vegetative growth of *Salicornia* spp. significantly slows down after flower initiation, leading to a reduction in yield. This process also results in the production of undesired flowering on shoots, which hold no market value (Ozturk et al., 2018). The initiation of flowering is influenced by environmental factors such as day length, with longer days promoting flowering. Managing the timing of flowering through controlled environmental conditions can therefore be crucial in optimising yield and maintaining quality (Ventura et al., 2011).

1.2.3 *Salicornia ramosissima*

Salicornia ramosissima is a dicotyledonous edible halophyte, belonging to the Amaranthaceae family. *Salicornia* species are of particular interest as halophytes because they are emerging as multi-purpose plants with significant potential. Demonstrating adaptability for cultivation as a vegetable in saline conditions (Ventura et al., 2010, 2011), *S. ramosissima* has proven to be a valuable source of secondary compounds, such as phenolic compounds, including flavonoids, phenolic acids, liposoluble pigments and more (Buhmann & Papenbrock, 2013a; ElNaker et al., 2020). In terms of nutritional content, these plants typically exhibit higher ash content compared to other edible plants, as reported by Borah et al. (2009). Lopes et al. (2021) reported that on a dry weight (DW) basis, *S. ramosissima* consisted of 47.4% ash, 4.16% protein, 0.45% lipids, 48.0% carbohydrates and 10.4% crude fibre, with moisture content at of 85.62% % (g/100 g of fresh weight). The elevated ash content in *Salicornia* spp. is associated with the its overall mineral concentration, a consequence of their adaptation to saline environments, and allows it to effectively retain minerals (Díaz et al., 2013).

1.3 Analytic techniques

1.3.1 Nutritional properties

Proximate composition analysis, the foundation in nutritional assessment, involves the identification and quantification of macronutrient components including the levels of moisture, ash, crude protein, lipids and carbohydrates. Primary metabolites, such as carbohydrates, proteins, and lipids are essential for life functions including growth, development and reproduction of living cells (Zaynab et al., 2019). The assessments are generally performed using multiple possible techniques. The determination of moisture content involves the drying of the biomass. This is commonly performed by use of conventional methods such as a ventilated oven (Pereira et al., 2023). However, other technologies, including microwave and infrared drying, can contribute to faster analysis and reduced sample degradation (Łechtańska et al., 2015). Determining moisture content in *Salicornia* is crucial as this plant grows in saline environments which influences its water content (Lopes et al., 2023). Furthermore, understanding the moisture content is essential as it could impact the concentration of other components when expressed on a dry weight basis.

The determination of ash content relies on the incineration of dried samples. Recent approaches include dry ashing and wet ashing methods. Dry ashing, the incinerating of a sample

at high temperatures in for instance a muffle furnace in the absence of added liquids, is most used (Mukherjee, 2019; Pereira et al., 2023; Youssef et al., 2022). Wet ashing on the other hand, involves the use of mixing of a sample with a suitable acid or acid mixture such as nitric acid or perchloric acid, which allows the selective extraction of elements (Mukherjee, 2019). Ash content reflects the total mineral content of *Salicornia*, which is important given its strong ability to accumulate minerals due to its growth in saline environments (Lopes et al., 2023). Hence, ash analysis can prove useful for understanding the nutritional value of the plant and indicating the mineral contents.

Determining the crude protein is generally performed through nitrogen assessment. Commonly used methods to assess nitrogen content are Kjeldahl method (Licon, 2022) and Jones method (Jones, 1931). Where combustion-based methods by Jones are generally faster and more suitable for samples with lower expected nitrogen contents, Kjeldahl offers a traditionally, widely used and cost-effective solution by means of digestion of a sample in concentrated sulfuric acid. Once the nitrogen content is determined, it is multiplied by the corresponding factor to determine crude protein content. The factor depends on the analyses matrix, in which the Kjeldahl method uses factor 6.25 for plants. This factor assumes that proteins on average contain about 16% nitrogen ($1 / 0.16 = 6.25$). Protein content determination is important, as proteins are essential for human and animal nutrition. *Salicornia* spp. could, particularly in regions where conventional crops may struggle to grow due to saline soils, serve as an alternative protein source.

Regarding lipids and carbohydrates, lipids provide essential fatty acids and are a source of energy, though their total content is usually low in *Salicornia* species (Cárdenas-Pérez et al., 2021; Hulkko et al., 2022). In contrast, carbohydrate detection offers insights into its energy distribution and dietary fibre content, which could be useful for future applications of *Salicornia* due to its fibrous nature. For those interested in determining lipid content, extraction methods such as the Bligh and Dyer or Folch methods are generally used (Bligh & Dyer, 1959; Breil et al., 2017), followed by calculating the difference between the extracted components and the total weight for carbohydrates quantification (Pereira et al., 2023; Youssef et al., 2022). Additionally, more advanced techniques for carbohydrate analysis include high-performance liquid chromatography (HPLC) and enzymatic methods involving amylase and lactase (Yan, 2014).

1.3.2 Minerals

Minerals are essential for plant growth, but also human health. In plants, minerals are required to complete their life cycle, and they support key processes such as photosynthesis, enzyme function, and nutrient transport, ensuring optimal growth and resilience (Maathuis, 2009). On a nutritional level, minerals like calcium, iron, magnesium, and potassium are vital for bone health, immune function, and maintaining fluid balance (Gharibzahedi & Jafari, 2017). Edible plants rich in minerals can help address nutritional deficiencies, making their mineral content critical for both agriculture and human nutrition. For mineral composition analysis, spectroscopic methods are widely used. Dried biomass samples are first digested using acids such as nitric acid (Pereira et al., 2023), and afterwards analysed with the help of spectroscopic techniques such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS). These are notable for their precision and sensitivity (Geiger, 2004). Another method that can be used is the Microwave Plasma-Atomic Emission Spectrometer (MP-AES), which has the lower running costs and uses no flammable gases (Agilent, 2023). The mineral analysis for *Salicornia* spp. is specifically relevant and of particular interest due to its ability to accumulate minerals (Lopes et al., 2021).

1.3.3 Bioactive compounds

Secondary compounds, also known as secondary metabolites, are organic compounds that are produced by plants and other organisms which are not directly involved in the primary metabolic processes like growth, development, or reproduction. Unlike primary metabolites (carbohydrates, proteins, and lipids), which are necessary for basic life functions, secondary metabolites often serve ecological roles, such as defence against herbivores, attraction of pollinators, or interactions with other organisms (Demain & Fang, 2000; Zaynab et al., 2019). Phenolics and flavonoids are secondary metabolites synthesised by plants in response to various environmental stresses and play significant roles in plant defence mechanisms (Luna-Guevara et al., 2018). The determination of total phenolics contents (TPC) involves extracting phenolic compounds from plant material using suitable solvents such as methanol or ethanol. Spectrophotometric methods, such as the Folin-Ciocalteu (FC) assay, are commonly employed to quantify TPC based on the ability to reduce the FC reagent (Singleton & Rossi, 1965). Similarly, the assessment of total flavonoids content (TFC) follows an extraction process using appropriate solvents like methanol or acetone. The aluminium

chloride colorimetric method is often utilised to quantify flavonoids based on their ability to form complexes with aluminium chloride, which are measured spectrophotometrically (Chang et al., 2002). Both TPC and TFC provide data on antioxidant potential and bioactive compounds (Sánchez-Gavilán et al., 2021).

Next to phenolics and flavonoids, liposoluble pigments such as chlorophylls and carotenoids are additional components of the biochemical profile of *S. ramosissima*. Chlorophylls are responsible for the green shades, while carotenoids contribute to the yellow to red shades in the plant. Both pigments are recognised for their antioxidant properties (Britton, 1995). These compounds play crucial roles in photosynthesis and photoprotection against excess light and contribute to the plant's adaptation to saline environments (Niyogi, 1999). The extraction and quantification of carotenoids involve solvent extraction methods followed by spectrophotometric analysis or chromatography techniques like high-performance liquid chromatography (HPLC) (Rodríguez-Amaya, 2001).

1.3.4 Antinutritional properties

Antinutritional properties include a range of compounds that can interfere with the absorption of nutrients or affect digestive processes in animals. For plants, these compounds often serve as defence mechanisms against pests or environmental stress. However, for consumers, anti-nutritional factors such as phytates, oxalates, or tannins can reduce the bioavailability of important minerals like iron, calcium, and zinc (Salim et al., 2023). Understanding and managing these compounds in edible plants is important to maximise their nutritional benefits while minimising negative effects on human health. Phytic acid is a compound found in seeds and grains that can form non-soluble compounds with minerals such as calcium, magnesium, iron, and zinc. These compounds reduce the bioavailability of these essential minerals in the digestive tract and are therefore impacting their absorption (Reddy et al., 1982).

Tannins are a diverse group of polyphenolic compounds that have both anti-nutritional, but also beneficial effects. Tannins can bind to proteins and other organic compounds, potentially reducing their digestibility and nutritional value. In moderate amounts, tannins can provide health benefits, but in high concentrations can negatively impact nutrition and health (Chung et al., 1998; Hoque et al., 2024). Antinutritional properties are determined with methods such as methanol extraction and subsequent assessment of antinutritional properties, including for instance the

determination of phytic acid and tannins contents, and trypsin inhibition (Majik & Gawas, 2023; Youssef et al., 2022). The quantification of antinutritional properties such as phytic acid and tannins typically involve spectrophotometry methods, such as colorimetry after acid digestion (El-Amier et al., 2021; Youssef et al., 2022)

1.4 Objective

This study aims to explore new possibilities and create awareness in the progression to circular economy implemented food production systems. The main goal of this research is to investigate the potential of using the edible halophytic plant, *Salicornia ramosissima*, to effectively optimise aquaculture wastewater in distinct production systems. It evaluates how two production systems, semi-hydroponics and mud-based substrate, with supplementary fertiliser, under varying salinity levels from commonly available water sources at aquaculture farms, including aquaculture wastewater from a semi-intensive pond system (35 PSU), brackish well water (20 PSU), and its mixture with fresh water (10 PSU), influence *S. ramosissima* cultivation. Additionally, the aquaculture wastewater source (35 PSU), was also tested without supplementary fertiliser. To fulfil this, the following secondary goals will be addressed. (1) Determine how the semi-hydroponics and mud-based substrate systems affect growth of *S. ramosissima* under varying salinity and nutrient conditions (2) How such production systems and conditions alter the agronomic features, such as plant biomass, of *S. ramosissima*. (3) How such production systems and conditions impact the biochemical properties of produced edible and lignified biomass, including nutritional and antinutritional profile, and secondary bioactive metabolites content.

2 Materials and methods

The experimental cultivation trials were carried out in a greenhouse at Instituto Português do Mar e da Atmosfera (IPMA)/Estação Piloto de Piscicultura de Olhão (EPPO), Olhão, Portugal (37°2'0.041"N; 7°49'11.615"E) for 6 weeks, from March 2023 to April 2023. *Salicornia ramosissima* plants were obtained from RiaFresh, a company based in Faro, Portugal, and identity of species was confirmed by molecular methodologies, seen in 2.1 Molecular identification of species. The agronomic handling and transplanting was carried out as described by Buhmann et al. (2015). The biomass processing and characterisation was performed at the facilities of Centro de Ciências do Mar (CCMAR), University of the Algarve, Faro, Portugal.

2.1 Molecular identification of species

Two *Salicornia* samples were received from RiaFresh (designated individuals 1 and 2) and transferred into Eppendorfs with silica. Primers were specifically designed to identify the genus. Genomic DNA was extracted using a commercial kit (ISOLATE II Genomic DNA Kit, Bionline), following the supplier's recommendations. Molecular identification was performed using PCR to amplify a genomic region containing ITS1, the 5.8S gene, and ITS2 in the two samples, followed by sequencing of the PCR products. The PCR protocol included an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was carried out at 72°C for 10 minutes, and the reaction was held at 4°C indefinitely. The amplified products were sequenced, and the sequences were aligned with available sequences in public databases from the National Center for Biotechnology Information (NCBI) for comparison and species identification.

2.2 Experimental cultivation setup

A comparison of two aquaculture effluent-based production systems, semi-hydroponics and mud-based substrate, was made for *S. ramosissima*. Each system was irrigated with water from common aquaculture farm sources, including wastewater from a semi-intensive pond system, brackish water from a nearby well and freshwater. *S. ramosissima* plants were obtained and maintained in clusters,

where each cluster (6 cm x 6 cm) contained roughly 20-30 individual plants with an average height of 15 cm. Full experimental cultivation setup scheme can be seen in Figure 2.1.

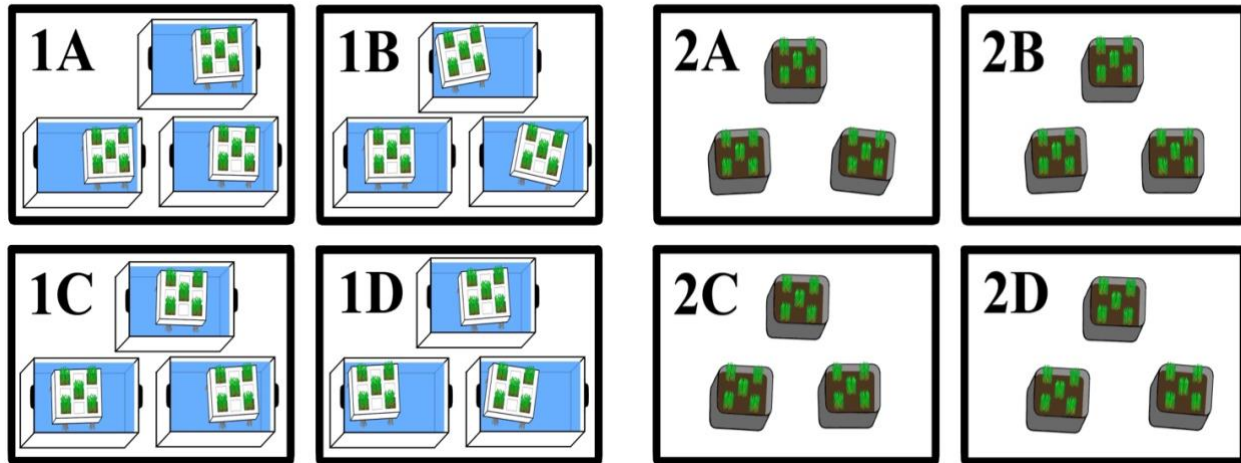


Figure 2.1: Experimental cultivation setup, where System 1 (left) indicates the semi-hydroponics system and System 2 (right) indicates the mud-based soil substrate system, with according salinity levels. “A” indicates the treatment with water of salinity 10 PSU, “B” of salinity 20 PSU, “C” and “D” of salinity 35 PSU. All treatments except “D” received additional supplementary fertiliser.

2.2.1 Experimental production systems

Two production systems for *S. ramosissima* were used. The first system was a semi-hydroponics system (Fig. 1A-D), where a platform of Styrofoam was created in which the plants were transplanted. This platform, containing the plants, was placed in a tray with dimensions of 60 x 35 x 7 cm (L x W x H), equalling approximately 15 L, in which the platform floated on top of the water of the designated treatments. The treatments are further elaborated on in chapter 2.2.2 Salinity treatments and nutrients. The Styrofoam platforms were square, with each platform containing nine empty slots of approximately 6 cm x 6 cm, arranged in three rows of three slots. Each replicate of each treatment had a platform that contained five clusters of plants, one placed in each corner and one in the middle, thus leaving four slots empty, one on each side.

The second system used a soil substrate (Fig. 1A-D) consisting of mud from the decantation pond of the semi-intensive pond system from the experimental fish farm at EPPO, mixed with potting soil (Gramoflor Profisubstrat, Germany), with a ratio of 1:2, respectively. The substrate mixture was added to 20L jerrycans that were cut in half to create two pots, with drainage holes in the bottom. Similarly to the semi-hydroponics system, each pot held five clusters of plants that

were positioned in the identical manner. This substrate system was designed to mimic effects of artificial wetlands, shown to be beneficial for salicornia cultivation on aquaculture effluents (Buhmann & Papenbrock, 2013b; Diaz et al., 2020). The practical setup can be seen in Figure 2.2.



Figure 2.2: Practical overview of experimental cultivation setup, where the left picture indicates the semi-hydroponics system and the right picture indicates the mud-based soil substrate system

2.2.2 Salinity treatments and nutrients

The water sources used included wastewater from a semi-intensive pond system, brackish water from a nearby located well and freshwater. The water source from the semi-intensive pond system had an average salinity of 35 Practical Salinity Units (PSU) and the water from the well had an average salinity of 20 PSU. Besides these two sources, a mixture of the brackish well water with fresh water, resulting in a salinity of 10 PSU, was used. This approximate salinity level was also used at RiaFresh, the supplier of the stock plants. Thus, a total of three different salinities were used in this experimental setup. Unfortunately, due to instrumental constraint, no nutrients could be analysed in the different water sources and mud from the settling pond. Therefore, the two water sources of salinities 10 PSU (the mixture), and 20 PSU (brackish well), respectively, were assumed to contain insufficient nutrients for plant growth, and were thus enriched by adding fertiliser (Folivex Crescimento 1L, Tradecorp, Spain) during the cultivation trial. The water source from the effluent of the semi-intensive pond, however, was expected to contain nutrients beneficent for plant growth (Castilla-Gavilán et al., 2024; Diaz et al., 2020). However, since the nutrients could not be measured, no data was available on precise quantities of available nutrients. Therefore, the water

source with 35 PSU from the effluent of the semi-intensive pond system was used with and without extra fertiliser. In summary, four different treatments were used: a mixture of fresh and brackish water from the well with added fertiliser (10 PSU), brackish water from the well with added fertiliser (20 PSU), semi-intensive pond system wastewater with added fertiliser (35 PSU) and semi-intensive pond system wastewater without added fertiliser (35 PSU). Each treatment was performed in triplicates.

2.2.3 Experimental conditions

Both production systems were run simultaneously. Plants were given two weeks to acclimatise to their treatment salinities without added nutrients, to reduce stress upon start of trial. Both cultivation systems were provided with equal supplies of water every 2-3 days. Plants with salinity treatments 10, 20 and 35 PSU were provided with fertiliser on day 14 and 28, respectively, by means of foliar application at a concentration of 3 L/ha. Moreover, two plant cuttings were harvested on day 22 and 40, respectively. Plants were cut at 15 cm above the substrate. Biomass from these cuttings were measured, weighed and frozen. On day 42, marking the end of the cultivation trial, plants were harvested entirely (partly lignified) and separated into green biomass, lignified stems and roots. Afterwards they were weighed, immediately frozen and kept at -20°C for further processing.

All biomass processing and characterisation analyses were, unless stated differently, carried out at facilities of CCMAR, University of Algarve, Faro, Portugal. Most solvents used were purchased from Sigma-Aldrich (Germany). Ethanol was purchased from AGA (Sweden), sulphuric acid from Merck (Germany) while other chemicals were provided by VWR (Belgium). The equipments used included a freeze-dryer (FreeZone, 6L) by Labonco (US), ultrasonic bath (Ultrasonic Cleaner USC-TH), a salinity meter (pHenomenal®) and an EZ Read 400 microplate reader supplied by Biochrom (Austria). Biomass was divided into 1) aerial edible parts (green biomass, tender shoots), 2) aerial nonedible parts (aerial organs, lignified), and 3) roots. The aerial edible parts comprised of two types of biomass: the biomass taken from the first cutting (week 3), and the final biomass from the second cutting (week 6) mixed with the green biomass separated from the cut plants at the end of the trial, which were weighed individually before mixing. All frozen biomass was dried by freeze drying and reduced to powder using a kitchen blender.

2.3 Biomass analysis and processing

2.3.1 Nutritional properties analysis

Freshly collected edible biomass was dried in a ventilated oven (105°C, 16h) to determine moisture content, which was performed at facilities EPPO. Furthermore, dried samples were analysed for ash content by incineration (515°C, 5h) in a muffle furnace. The salty flavour of the fresh plants was determined by macerating the samples with distilled water at a ratio of 1:10 (w/w) and measuring the electric conductivity (EC) using a conductivity meter (Hanna Instruments). The crude protein was assessed by total nitrogen (CHN Elemental Analyzer Vario EL III) and following macro Kjeldahl method where evaluated nitrogen was multiplied by 6.25. Results are expressed as g per 100 g of dry weight biomass (DW).

2.3.2 Mineral analysis

Dried biomass was assessed for mineral content: samples were digested on a Microwave Digestion System (Discover SP-D 80, CEM Corp., Matthews, USA) with 67% nitric acid (4 min. ramp temperature to 200°C, hold for 3 min), diluted with ultra-pure water and analysed with a Microwave Plasma-Atomic Emission Spectrometer (MP- AES; Agilent 4200 MP-AES, Agilent, Victoria, Australia), as described by Pereira et al. (2023). Results were conveyed as mg/g DW or mg/kg DW.

2.3.3 Bioactive compound analysis

Methanol extracts were prepared by mixing dried samples with equal parts of methanol and acetone 40 mL/g (1:20:20, w/v/v). Solutions were placed in an ultrasonic bath at room temperature (RT) for 30 minutes. Afterwards, the extracts were filtered (Whatmann n°. 4), the solvent removed in a rotary evaporator, and the dried extract resuspended in methanol at a concentration of 10 mg/mL.

2.3.3.1 Phenolics

Total phenolics content (TPC) was determined in the prepared extracts by the Folin-Ciocalteu (FC) assay, as described by Velioglu et al. (1998) and adapted to a 96-well plate. Extracts (5 µL) were mixed with 100 µL of FC reagent (diluted 1:10, v/v) and 100 µL of 75 g/L sodium carbonate solution. An incubation time of 90 minutes in the dark at RT was used. Absorbance was measured

at 725 nm. A calibration curve was generated using gallic acid standards (1 – 0.001 mg/mL), resulting in the equation $y = 1,2943x + 0.0385$ ($R^2 = 0.9988$). Results were calculated and expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.3.3.2 Flavonoids

Total flavonoid content (TFC) was determined in the prepared extracts according to the method described by Pirbalouti et al. (2013), adapted for a 96-well plate format. Extracts (50 μ L) were mixed with 50 μ L of 2% aluminium chloride-ethanol ($AlCl_3$ -EtOH) solution, incubated for 10 minutes, and measured at 420 nm. A calibration curve was generated using quercetin standards (1 – 0.001 mg/mL), resulting in the equation $y = 5.3918x + 0.1643$ ($R^2 = 0.9859$). Results were calculated and expressed as mg of quercetin equivalents per gram of dry weight (mg QUE/g DW).

2.3.3.3 Liposoluble pigments

Carotenoids, chlorophyll a and chlorophyll b were determined in the prepared extracts according to methods described by Wellburn (1994) for a 96-well plate. The absorbances were measured at 470, 653 and 666 nm. Following equations were used to make the calculations, expressed in mg/g:

$$\text{Chlorophyll a (C}_a\text{)} (\mu\text{g/mL}) = 16.72A_{666} - 9.16A_{653}$$

$$\text{Chlorophyll b (C}_b\text{)} (\mu\text{g/mL}) = 34.09A_{653} - 15.28A_{666}$$

$$\text{Carotenoids (C}_{X+C}\text{)} (\mu\text{g/mL}) = (1000A_{470} - 1.63C_a - 104.96C_b) / 221$$

2.3.4 Anti-nutritional properties analysis

2.3.4.1 Tannins

Total tannins content (TTC) was determined in the prepared extracts following the colorimetric method of 4-dimethylaminocinnamaldehyde hydrochloric acid (DMACA) described by Li et al. (1996) and adapted by Zou et al., (2011) for a 96-well plate. Extracts (10 μ L) were mixed with 200 μ L of DMACA (1% in MeOH) and 100 μ L of hydrogen chloride (HCl, 37%), incubated for 15 minutes, and measured at 640 nm. A calibration curve was generated using catechin standards (1 – 0.001 mg/mL), resulting in the equation $y = 1,0504x + 0,0747$ ($R^2 = 0,9817$). Results were expressed as mg of catechin equivalents per gram of dry weight (mg CAT/g DW).

2.4 Statistical analysis

The assays were conducted in triplicates, and results were presented as means of replicates and standard deviation (mean \pm SD). The data were subjected to statistical analysis Kruskal-Wallis and Dunn's post-hoc test to determine the significance of differences between treatments. The composition of water used in the respective experimental treatments was statistically regarded as a categorical factor, rather than quantitative, because water differed not only by salinity but also by origin (semi-intensive pond or brackish well plus fresh water) and, at salinity 35 PSU, by the presence or absence of added fertiliser. Nevertheless, the water qualities are referred to by their corresponding salinity level, for sake of brevity. All statistical analyses were performed by use of the statistical program SPSS, software version 29.0.2.0 (20) (Armonk, NY: IBM Corp). A level of $p < 0.05$ was considered to be significant.

3 Results

3.1 Plant biomass results

Fresh plant biomass weight from the first (a) and second (b) cutting is shown in Figure 3.1. The cultivation system using the substrate mix of mud and potting soil is referred to as the mud-based system. Comparing the two production systems in the first cutting, the mud-based system provided overall significantly higher biomass. No significant differences between salinities were found in the biomass from the first cutting, for either of the two systems. However, when comparing the production systems at respective salinity levels, the mud-based system showed significantly higher biomass production in salinity 10 and salinity 35 without added fertiliser (w/o), compared to the semi-hydroponics system. In the second cutting, the two production systems only showed significant difference in salinity 20, where the semi-hydroponics system resulted in higher biomass production. Within the semi-hydroponics system, salinity 20 produced significantly more biomass than salinity 10 and 35 w/o. In the mud-based system, salinity 10 produced significantly lower biomass than salinities 35 and 35 w/o.

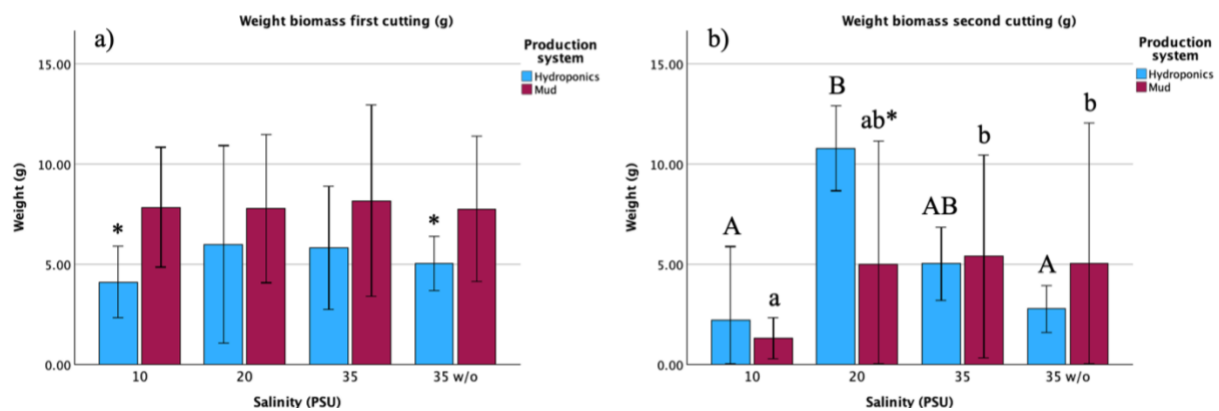


Figure 3.1: Fresh weight of harvested plant biomass (g) from a) first (week 3) and b) second (week 6) cutting, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

After the first and second cutting, the weight of the remaining edible biomass (green) and non-edible biomass (lignified aerial parts), were compared between the two production systems and different salinities (Figure 3.2). Figure 3.2a indicates the weight of the edible biomass and Figure 3.2b the weight of the non-edible biomass. Comparing the overall productivity between production systems revealed that the mud-based system produced significantly more non-edible biomass, while no significant differences were observed regarding the final edible biomass. In the cultivation system using the mixture of mud with potting soil, the remaining final edible biomass at salinities 35 and 35 w/o was significantly higher than at salinity 20. Remaining non-edible biomass in the semi-hydroponics system was significantly lower than in the mud-based substrate system at all salinities. Also, the plants at salinities 35 and 35 w/o in the semi-hydroponics system produced significantly higher biomass than at salinity 20. The mud-based system produced more non-edible biomass at salinity 35 than at salinities 10 and 20.

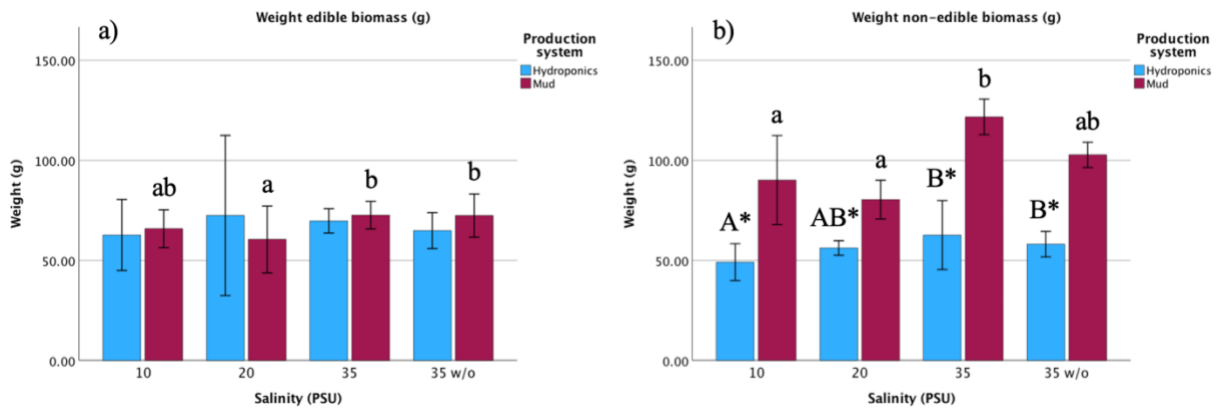


Figure 3.2: Fresh plant weight (g) from plants at day 42 (end of trial), separated in a) edible (green, non-lignified) (cuttings excluded), b) non-edible (lignified stems) comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

Total edible biomass, including first and second cutting and remaining edible biomass after cuttings, was compared with the non-edible biomass of the stems (Figure 3.3). The total edible biomass obtained in the semi-hydroponics system (a) was significantly higher in salinity 35 compared to salinity 10. In the mud-based system (b) both salinities 35 produced significantly

more biomass than at salinity 10. The non-edible biomass in the semi-hydroponics system was significantly higher at both salinities 35 than at salinity 10. In the mud-based substrate system, non-edible biomass production was higher at salinity 35 than at salinities 10 and 20.

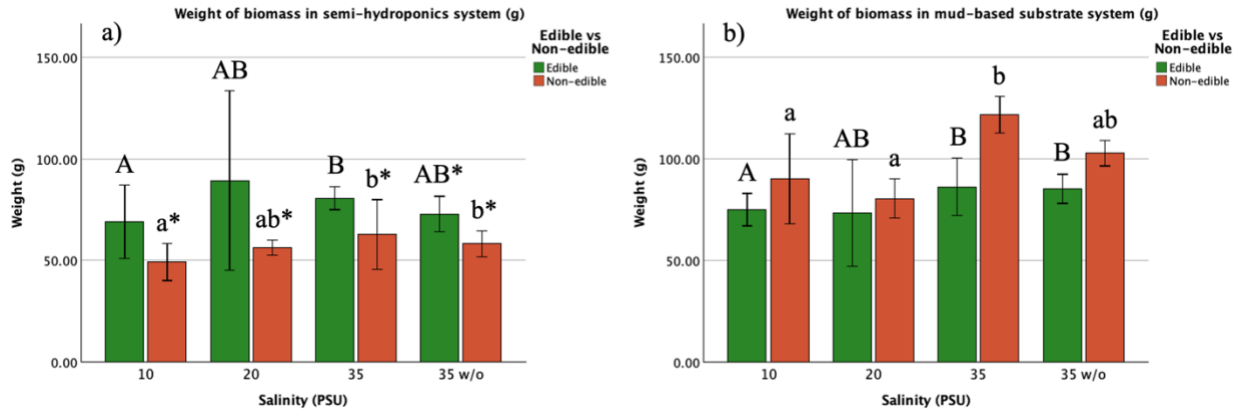


Figure 3.3: Weight of total harvested plant biomass (g) from a) semi-hydroponics system and b) mud-based substrate system, comparing the edible biomass (first and second cutting and remaining edible biomass) (left, green bar) and non-edible biomass (stems) (right, orange bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at edible biomass. Low case letters indicate differences between non-edible biomass. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.2 Proximate composition results

3.2.1 Moisture content

Moisture content (Figure 3.4) was determined for the biomass of the first (a) and second (b) cuttings and the stems (c). Overall, significantly higher moisture content across all salinities was observed in biomass from the mud-based system than from the first cutting and stems. Biomass from the semi-hydroponics system had a significantly lower moisture content in all salinities than those from plants grown in the mixture of mud with potting soil.

In the mud-based system of the second cutting, significant differences were found between salinities. Biomass cultivated at salinity 20 in the mud-based system had significantly higher moisture content than at salinities 35 and 35 w/o. Moreover, significant differences were found when comparing the different production systems, as biomass obtained in the mud-based substrate system in salinity 20 had a lower moisture content than the biomass from semi-hydroponics

system. Additionally, biomass from salinity 35 w/o in the semi-hydroponics system also had a lower moisture content.

The moisture content of the stems biomass showed significant differences in both production systems and salinities. In the mud-based system, biomass from salinity 20 had higher moisture content than biomass from salinities 35 and 35 w/o. Moreover, the moisture content in stems was significantly lower at all salinities in the semi-hydroponics system.

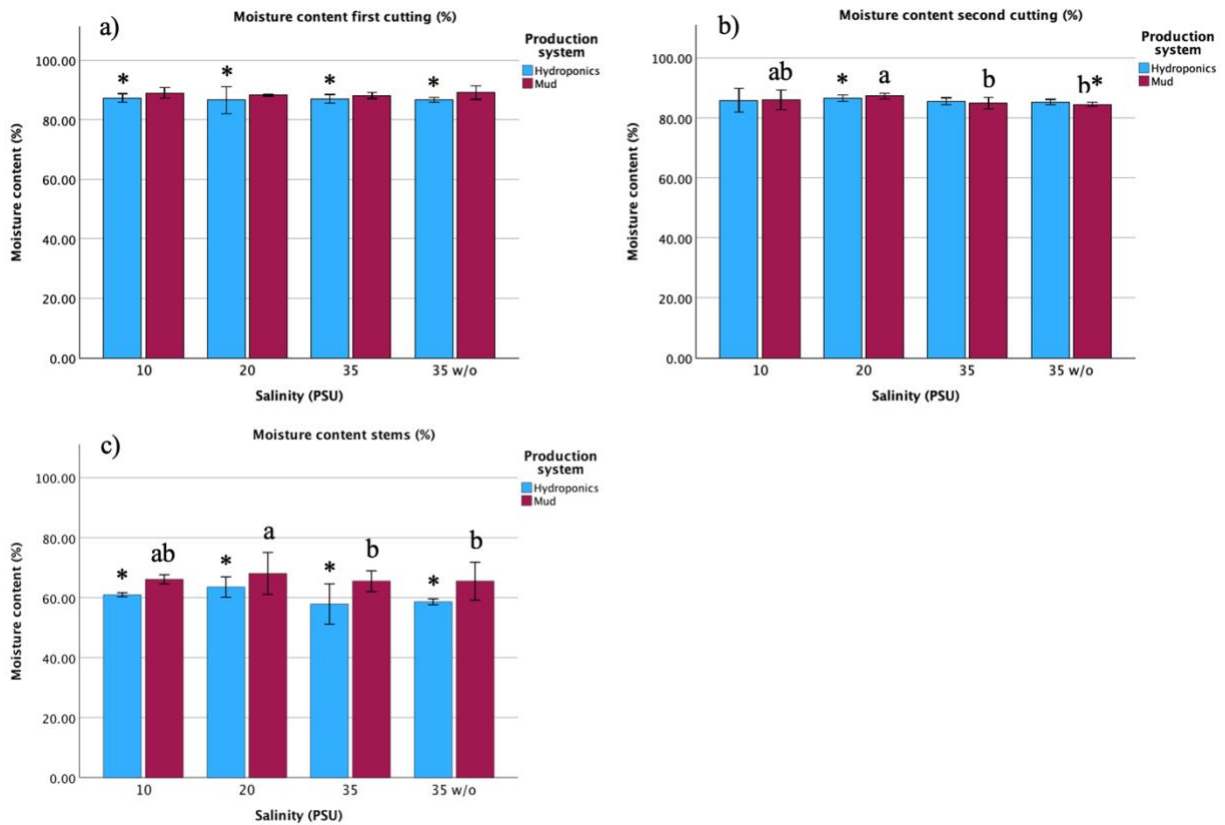


Figure 3.4: Moisture content (%) from a) first (week 3) and b) second (week 6) cutting and c) stems, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.2.2 Ash content

The ash content (Figure 3.5) was determined in biomass from the first cutting (a), second cutting (b), stems (c) and roots (d). Comparing the overall productivity between production systems, the

biomass of the first cutting and the roots from the mud-based system had significantly higher ash levels. In the biomass of the first cutting, both the semi-hydroponics system and the mud-based system had significantly higher ash content in the salinities 35 and 35 w/o when compared with salinity 10. Moreover, when comparing production systems in the biomass of the first cutting, biomass at both salinities 35 had significantly lower ash content in the semi-hydroponics system.

In the semi-hydroponics system, biomass from the second cutting indicated that plants cultivated at salinities 20 and 35 had significantly higher ash content than those from salinity 10. In the mud-based substrate system, biomass from plants cultivated at both salinities 35 presented significantly higher ash content than those from salinity 10. A significantly lower ash content was detected in biomass from the second cutting of plants cultivated in the semi-hydroponics system when compared to those in the mud-based system, except for salinity 20.

Biomass from the stems of plants cultivated in the semi-hydroponics system at salinity 35 had significantly higher ash content than those from salinity 10. In the mud-based system, however, biomass from both salinity 35 and 35 w/o had higher ash content than biomass from salinity 10. Overall, stems from the mud-based system had significantly lower ash content than those from the semi-hydroponics system.

In the biomass of the roots, no differences were found between salinities in the semi-hydroponics system. In the mud-based system, however, plants cultivated at salinity 35 w/o showed significantly higher ash content than those from salinities 10 and 20. Furthermore, a comparison of root biomass between the two production systems revealed that the ash content in the semi-hydroponic system was lower than in the mud-based system at both salinity levels 35.

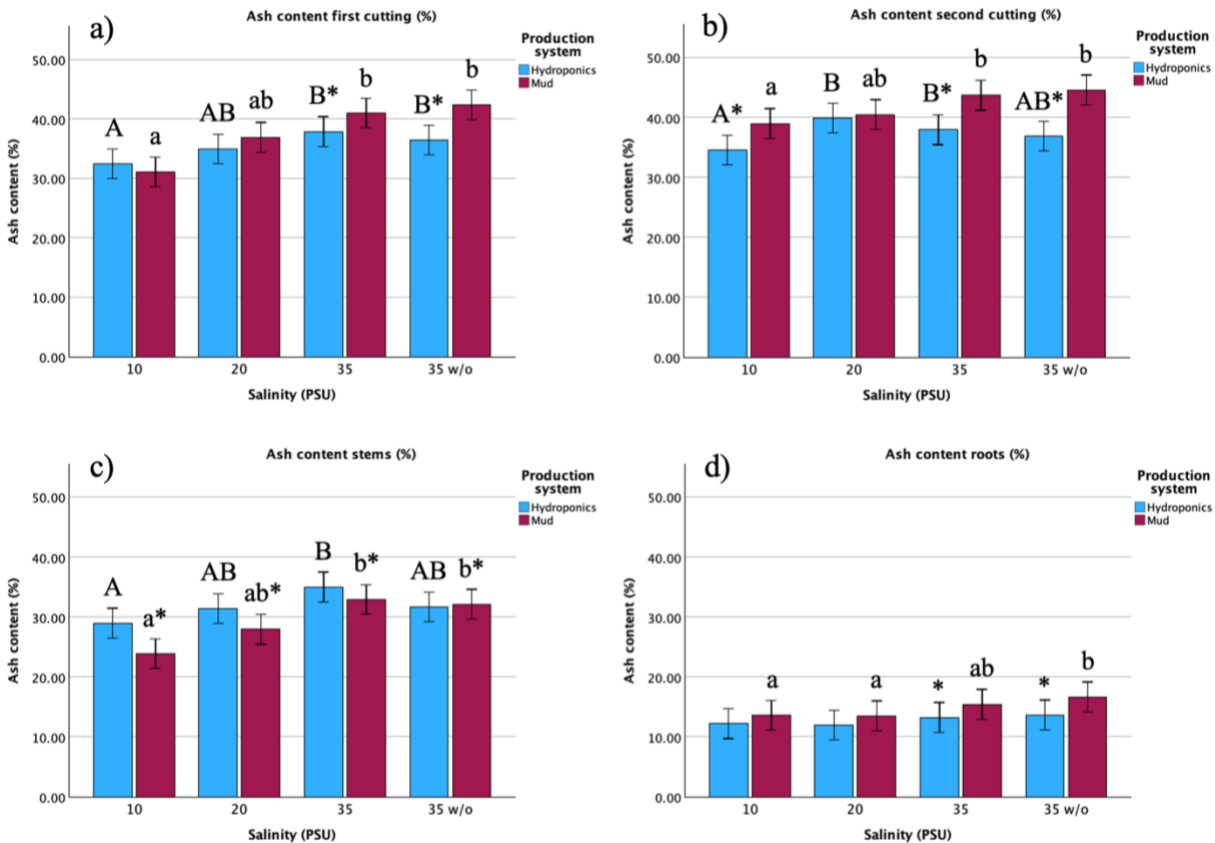


Figure 3.5: Ash content (%) from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.2.3 Plant saltiness

The salty flavour test, a measure to indicate salt concentrations in solutions, was only performed on the biomass of the second cutting (Figure 3.6). No significant differences were found between the overall production systems. However, significant differences between production systems were found at salinities 35 and 35 w/o, indicating lower saltiness in the semi-hydroponics system. Between salinities, the salty flavour of biomass from salinity 10 was significantly lower than those from salinities 35 and 35 w/o, in both systems. Moreover, in the semi-hydroponics system, saltiness in biomass from salinity 20 was lower than at salinity 35.

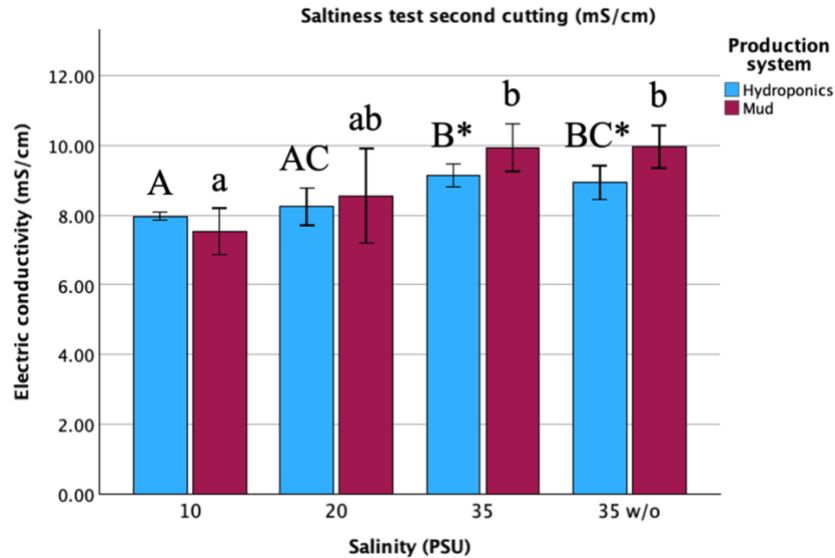


Figure 3.6: Saltiness of plants (mS/cm) from the second cutting (week 6), comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.2.4 Protein content

The protein content (Figure 3.7) was measured for the first cutting (a), second cutting (b), stems (c) and roots (d). Comparing the overall productivity between production systems, in the second cutting and in the roots biomass, biomass from the mud-based system had significantly higher protein levels. Furthermore, in the first cutting, protein content in biomass from the semi-hydroponics system at salinity 20 was significantly higher than in those from salinities 35 and 35 w/o. Additionally, biomass from salinity 10 also showed higher protein content than those from salinity 35 w/o. In the mud-based system, similar to the semi-hydroponics system, biomass from salinity 20 had higher protein content than those from both salinities 35. Moreover, comparing production systems in the biomass of the first cutting, biomass from salinities 10, 35 and 35 w/o had higher protein content in the semi-hydroponics system than in the mud-based system.

In the second cutting, protein content of biomass obtained in the semi-hydroponics system was significantly higher at salinity 20 than both salinities 35. In the mud-based system, biomass from salinity 20 had significantly higher protein content than biomass from salinity 35. Comparing production systems in the biomass of the second cutting, biomass from the semi-hydroponics

system had overall lower protein content at all salinities than biomass from plants grown in the mud-based substrate system

Regarding stems, biomass from plants cultivated in salinity 20 of the semi-hydroponics system had a higher protein content than those from salinity 35. In the mud-based system, biomass at salinity 20 had significantly higher protein content than at both salinities 35. Moreover, the comparison between production systems in the stems showed that the protein content was significantly lower in the semi-hydroponics system at salinity 20.

In the roots, the semi-hydroponics system at salinity 10 resulted in biomass with a significantly higher protein content than biomass from salinity 35. In the mud-based system, a higher protein content was observed in plants cultivated at salinities 20 and 35 compared to plants cultivated at salinity 35 w/o. Comparing roots obtained in both production systems, observed was that the protein content was significantly lower in biomass from the semi-hydroponics system at salinity 35.

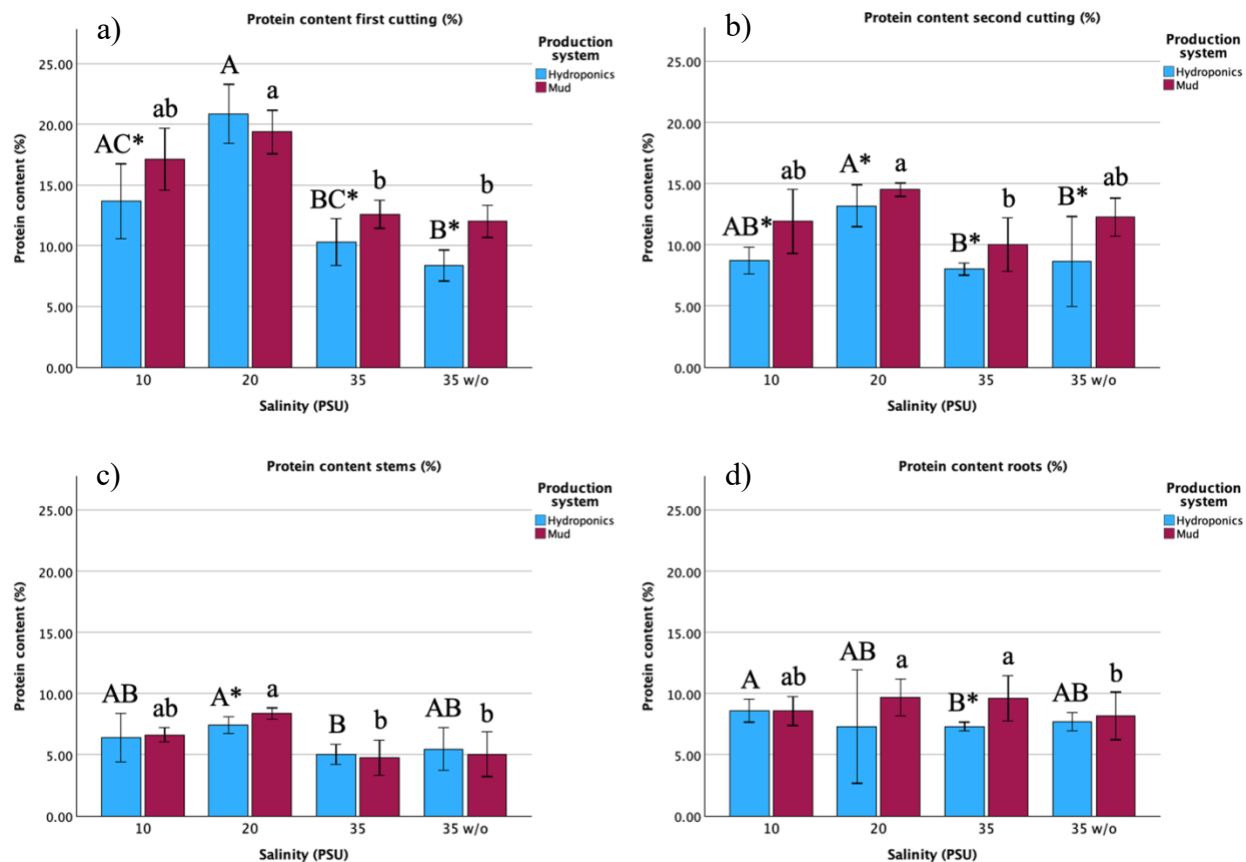


Figure 3.7: Protein content (% DW) in a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 3$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.3 Mineral content results

Mineral content was determined using dilutions from the stock solution. All minerals, apart from sodium and phosphorus, were determined by use of a single dilution. Values from minerals sodium and phosphorus were determined using two or more dilutions. If not mentioned specifically in text, dilution of 1:10 was used. Tables of full overview of mineral contents in first cutting, second cutting, stems and roots biomass can be seen in Table 3.1, Table 3.2, Table 3.3 and Table 3.4, respectively. Minerals selenium (Se), cadmium (Cd) and cobalt (Co) were not detected in any of the biomasses.

Table 3.1: Mineral contents of biomass from the first cutting of *S. ramosissima*, comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).

Minerals/ Salinity	Hydroponics				Mud			
	10 PSU	20 PSU	35 PSU	35 PSU w/o	10 PSU	20 PSU	35 PSU	35 PSU w/o
Ca (mg/g)	6.44 ± 0.39*	6.53 ± 0.13*	6.35 ± 0.33	6.70 ± 0.16	10.80 ± 2.14 ^a	10.72 ± 0.20 ^{ac}	5.71 ± 0.20 ^{b*}	5.85 ± 0.17 ^{bc*}
K (mg/g)	12.52 ± 0.44 ^{A*}	16.91 ± 0.52 ^B	14.67 ± 0.85 ^{AB*}	16.98 ± 0.09 ^B	16.18 ± 0.60 ^a	18.10 ± 0.31 ^b	18.34 ± 0.36 ^b	17.07 ± 0.63 ^{ab}
Mg (mg/g)	7.01 ± 0.30 ^{A*}	7.42 ± 0.24 ^{AB*}	6.84 ± 0.29 ^{A*}	8.87 ± 0.11 ^{B*}	12.03 ± 3.96 ^{ab}	16.04 ± 0.73 ^a	9.81 ± 0.28 ^b	9.78 ± 0.44 ^b
Na (mg/g)	198.55 ± 3.46 ^{AB}	230.30 ± 17.30 ^A	42.81 ± 19.31 ^{B*}	38.08 ± 2.11 ^{B*}	47.88 ± 3.88 ^{b*}	62.47 ± 3.88 ^{ab*}	248.29 ± 27.24 ^a	266.37 ± 0.59 ^a
P (mg/g)	2.24 ± 0.16 ^A	2.22 ± 0.19 ^{A*}	1.55 ± 0.02 ^{B*}	1.64 ± 0.15 ^{AB}	1.76 ± 0.04 ^{ab*}	4.18 ± 0.61 ^a	1.71 ± 0.11 ^{ab}	1.28 ± 0.09 ^{b*}
Se (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Zn (µg/g)	31.02 ± 0.85 ^{AB}	44.41 ± 1.12 ^A	22.78 ± 0.49 ^{B*}	21.49 ± 2.18 ^{B*}	34.58 ± 2.76 ^{ab}	42.83 ± 0.40 ^{a*}	27.84 ± 0.29 ^b	28.39 ± 1.06 ^b
Cd (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Cu (µg/g)	9.50 ± 1.28 ^{AB}	12.48 ± 1.25 ^A	7.66 ± 0.95 ^{AB}	4.88 ± 0.33 ^{B*}	7.55 ± 0.61 ^{ab*}	9.14 ± 1.14 ^{a*}	6.99 ± 0.89 ^b	8.24 ± 0.55 ^{ab}
Ni (µg/g)	0.50 ± 0.07 ^A	0.80 ± 0.12 ^B	0.38 ± 0.14 ^A	0.63 ± 0.06 ^{AB}	0.26 ± 0.23 ^a	0.62 ± 0.04 ^{b*}	0.42 ± 0.01 ^{ab}	0.46 ± 0.08 ^{ab*}
Co (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Pb (µg/g)	8.50 ± 0.36 ^A	8.28 ± 0.27 ^{AB}	6.86 ± 0.42 ^{BC}	6.55 ± 0.31 ^{C*}	6.57 ± 0.41 ^{a*}	7.06 ± 0.33 ^{ab*}	7.37 ± 0.37 ^{ab}	7.41 ± 0.47 ^b
Mn (µg/g)	359.70 ± 6.20 ^A	349.74 ± 30.57 ^{AB}	234.78 ± 27.87 ^{BC}	220.22 ± 11.16 ^{C*}	360.38 ± 11.28 ^a	258.62 ± 3.27 ^{bc*}	240.83 ± 5.30 ^b	281.45 ± 8.35 ^{ac}
Cr (µg/g)	0.65 ± 0.02 ^A	0.64 ± 0.11 ^A	0.39 ± 0.15 ^{B*}	0.53 ± 0.06 ^{AB}	0.66 ± 0.01 ^a	0.39 ± 0.01 ^{b*}	0.58 ± 0.04 ^{ac}	0.46 ± 0.03 ^{bc}
Fe (µg/g)	194.11 ± 3.92 ^{A*}	136.40 ± 2.83 ^{AB}	113.31 ± 13.30 ^{BC*}	87.56 ± 8.09 ^{C*}	204.5 ± 2.51 ^a	127.69 ± 2.38 ^{b*}	144.02 ± 5.99 ^{ab}	126.98 ± 2.26 ^b

nd – Not detected. Results are shown in mean ± SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, p < 0.05).

Table 3.2: Mineral contents of biomass from the second cutting of *S. ramosissima*, comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).

Minerals/ Salinity	Hydroponics				Mud			
	10 PSU	20 PSU	35 PSU	35 PSU w/o	10 PSU	20 PSU	35 PSU	35 PSU w/o
Ca (mg/g)	5.17 ± 0.10 ^A	4.89 ± 0.07 ^{AB}	4.55 ± 0.06 ^{B*}	4.67 ± 0.11 ^{B*}	5.48 ± 0.30 ^{ab}	4.74 ± 0.23 ^a	5.99 ± 0.39 ^b	5.33 ± 0.16 ^{ab}
K (mg/g)	15.39 ± 0.40 ^A	19.58 ± 0.14 ^B	19.38 ± 0.24 ^{AB}	19.88 ± 0.47 ^B	15.38 ± 1.26 ^a	17.21 ± 1.08 ^{ab*}	19.03 ± 1.16 ^b	17.38 ± 0.29 ^{ab*}
Mg (mg/g)	8.36 ± 0.28 ^{A*}	9.31 ± 0.20 ^B	8.28 ± 0.16 ^{A*}	8.76 ± 0.18 ^{AB*}	9.30 ± 0.25 ^a	10.21 ± 0.66 ^{ab}	12.80 ± 0.65 ^b	12.49 ± 0.34 ^b
Na (mg/g)	149.26 ± 23.81 ^{B*}	234.52 ± 25.61 ^{AB}	141.23 ± 13.82 ^{B*}	332.74 ± 26.16 ^A	266.73 ± 19.29 ^b	279.29 ± 16.89 ^b	294.59 ± 15.18 ^{ab}	332.33 ± 6.23 ^a
P (mg/g)	2.13 ± 0.05 ^{AB*}	2.20 ± 0.01 ^{B*}	1.96 ± 0.03 ^{AC*}	1.83 ± 0.07 ^C	6.47 ± 0.22 ^a	5.78 ± 0.36 ^{ab}	2.47 ± 0.01 ^{bc}	2.03 ± 0.25 ^c
Se (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Zn (µg/g)	26.31 ± 0.69 ^{AB*}	36.07 ± 0.56 ^{A*}	24.92 ± 0.15 ^B	26.20 ± 0.35 ^{AB}	36.97 ± 0.57 ^{ab}	44.71 ± 1.40 ^a	25.85 ± 3.77 ^b	25.94 ± 4.36 ^b
Cd (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Cu (µg/g)	6.93 ± 0.49 ^{A*}	9.70 ± 0.14 ^{B*}	9.54 ± 0.44 ^{AB}	9.70 ± 1.01 ^{AB}	11.64 ± 0.16 ^{ab}	15.52 ± 1.51 ^b	10.40 ± 0.61 ^{ac}	8.65 ± 0.31 ^{c*}
Ni (µg/g)	0.78 ± 0.14 ^{A*}	1.53 ± 0.17 ^{B*}	1.04 ± 0.07 ^{AB}	1.16 ± 0.12 ^{AB}	1.48 ± 0.14 ^{ab}	1.90 ± 0.12 ^a	1.05 ± 0.02 ^b	1.13 ± 0.07 ^b
Co (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Pb (µg/g)	7.12 ± 0.23 [*]	7.34 ± 0.34	7.10 ± 0.33	7.59 ± 0.58	7.58 ± 0.08	7.15 ± 0.19	7.55 ± 0.24	7.25 ± 0.35
Mn (µg/g)	171.64 ± 3.37 ^{AB*}	182.21 ± 6.73 ^A	136.76 ± 4.29 ^{B*}	169.75 ± 5.57 ^{AB}	200.07 ± 4.64 ^a	188.69 ± 3.95 ^{ab}	173.71 ± 2.12 ^{bc}	144.14 ± 8.40 ^{c*}
Cr (µg/g)	0.58 ± 0.02 ^{B^{C*}}	1.32 ± 0.10 ^{A*}	0.35 ± 0.04 ^{B*}	0.99 ± 0.03 ^{AC*}	2.09 ± 0.06 ^{ab}	3.99 ± 0.14 ^a	1.70 ± 0.05 ^b	2.16 ± 0.20 ^{ab}
Fe (µg/g)	71.97 ± 2.85 ^{AB*}	80.95 ± 2.91 ^{A*}	67.49 ± 6.31 ^{B*}	68.17 ± 0.93 ^{B*}	233.48 ± 2.46 ^{ab}	302.74 ± 2.80 ^a	224.97 ± 8.81 ^{ab}	171.32 ± 6.86 ^b

nd – Not detected. Results are shown in mean ± SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, p < 0.05).

Table 3.3: Mineral contents of biomass from the stems of *S. ramosissima*, comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).

Minerals/ Salinity	Hydroponics				Mud			
	10 PSU	20 PSU	35 PSU	35 PSU w/o	10 PSU	20 PSU	35 PSU	35 PSU w/o
Ca (mg/g)	6.01 ± 0.64 ^{A*}	6.80 ± 0.36 ^{AB*}	6.77 ± 0.13 ^{AB*}	6.92 ± 0.24 ^{B*}	7.97 ± 0.73	8.00 ± 0.28	7.69 ± 0.42	8.19 ± 0.10
K (mg/g)	18.13 ± 1.76 ^A	19.68 ± 0.71 ^{AB}	20.65 ± 0.26 ^B	20.38 ± 0.52 ^{AB}	17.91 ± 2.25	16.05 ± 0.37 [*]	16.24 ± 0.58 [*]	16.46 ± 0.14 [*]
Mg (mg/g)	8.47 ± 0.52 ^{A*}	9.45 ± 0.36 ^{AB*}	10.19 ± 0.15 ^{B*}	9.95 ± 0.28 ^{AB*}	10.96 ± 0.90 ^a	11.77 ± 0.94 ^{ab}	12.44 ± 0.55 ^{ab}	12.80 ± 0.27 ^b
Na (mg/g)	276.96 ± 44.99 ^A	203.70 ± 9.15 ^B	253.11 ± 18.69 ^{AB}	n/a	n/a	n/a	n/a	n/a
P (mg/g)	1.75 ± 0.03 ^{AB}	1.88 ± 0.06 ^B	1.67 ± 0.04 ^{AC}	0.88 ± 0.05 ^{C*}	1.04 ± 0.02 ^{a*}	1.65 ± 0.32 ^b	1.21 ± 0.05 ^{ac*}	1.33 ± 0.04 ^{bc}
Se (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Zn (µg/g)	31.22 ± 0.64 ^{AB*}	37.38 ± 1.52 ^{A*}	31.32 ± 1.71 ^{AB}	27.63 ± 2.59 ^{B*}	39.20 ± 3.82 ^{ab}	47.98 ± 5.15 ^a	33.72 ± 5.07 ^b	32.86 ± 0.02 ^b
Cd (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Cu (µg/g)	6.67 ± 0.82 ^{AB*}	7.30 ± 1.45 ^{A*}	7.02 ± 0.28 ^{A*}	5.11 ± 0.22 ^{B*}	14.86 ± 0.13 ^{ab}	16.88 ± 1.02 ^a	12.01 ± 0.68 ^b	11.89 ± 1.02 ^{bc}
Ni (µg/g)	0.77 ± 0.21 ^{A*}	0.88 ± 0.05 ^{A*}	1.40 ± 0.07 ^B	0.94 ± 0.04 ^{AB}	2.24 ± 0.03 ^{ab}	3.49 ± 0.17 ^b	0.89 ± 0.13 ^{ac*}	0.53 ± 0.04 ^{c*}
Co (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Pb (µg/g)	5.46 ± 0.73	5.68 ± 0.87	6.33 ± 0.63	6.09 ± 0.19 [*]	6.11 ± 0.42 ^{ac}	4.83 ± 0.17 ^a	7.19 ± 0.20 ^b	6.86 ± 0.17 ^{bc}
Mn (µg/g)	66.59 ± 3.60 ^{AB*}	70.65 ± 4.05 ^A	58.49 ± 1.15 ^B	67.5 ± 1.13 ^{AB}	74.75 ± 0.65 ^a	73.90 ± 1.77 ^a	48.00 ± 0.83 ^{b*}	54.42 ± 0.45 ^{ab*}
Cr (µg/g)	1.02 ± 0.08 ^{A*}	0.98 ± 0.06 ^{A*}	2.85 ± 0.07 ^{B*}	1.39 ± 0.02 ^{AB*}	6.24 ± 0.06 ^{ab}	8.17 ± 0.33 ^b	3.49 ± 0.12 ^{ac}	2.81 ± 0.04 ^c
Fe (µg/g)	60.51 ± 0.29 ^{A*}	67.97 ± 2.52 ^{B*}	67.72 ± 0.57 ^{B*}	61.59 ± 2.2 ^{AB*}	755.92 ± 17.14 ^a	908.02 ± 25.00 ^b	725.23 ± 57.64 ^a	857.54 ± 38.69 ^{ab}

nd – Not detected, and n/a – Not attached. Results are shown in mean ± SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, p < 0.05).

Table 3.4: Mineral contents of biomass from the roots of *S. ramosissima*, comparing the semi-hydroponics system (left) and mud-based substrate system (right) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser).

Minerals/ Salinity	Hydroponics				Mud			
	10 PSU	20 PSU	35 PSU	35 PSU w/o	10 PSU	20 PSU	35 PSU	35 PSU w/o
Ca (mg/g)	4.21 ± 0.16 ^{A*}	5.30 ± 0.24 ^{B*}	4.76 ± 0.36 ^{AB}	5.38 ± 0.09 ^B	5.65 ± 0.50 ^{ab}	7.00 ± 0.21 ^a	4.50 ± 0.07 ^b	4.25 ± 0.41 ^{b*}
K (mg/g)	13.11 ± 0.56 ^{AB*}	7.54 ± 0.42 ^{A*}	15.41 ± 1.21 ^{B*}	15.28 ± 0.19 ^{B*}	15.01 ± 1.26 ^{ab}	11.70 ± 0.38 ^b	20.51 ± 0.42 ^a	19.52 ± 1.48 ^a
Mg (mg/g)	4.68 ± 0.16 ^A	5.00 ± 0.30 ^{AB}	5.33 ± 0.45 ^B	4.97 ± 0.11 ^{AB}	5.33 ± 0.51	5.48 ± 0.19	5.42 ± 0.10	5.16 ± 0.42
Na (mg/g)	80.90 ± 7.21 ^A	69.55 ± 3.67 ^{AB*}	87.83 ± 2.29 ^{A*}	24.48 ± 0.43 ^{B*}	78.34 ± 5.69 ^b	89.08 ± 2.72 ^{ab}	104.52 ± 2.18 ^a	89.33 ± 1.68 ^{ab}
P (mg/g)	7.27 ± 0.34 ^A	5.90 ± 0.53 ^{AB*}	2.47 ± 0.01 ^{B*}	5.09 ± 0.56 ^{AB*}	7.81 ± 0.28 ^a	8.13 ± 0.51 ^{ab}	9.11 ± 0.51 ^b	8.76 ± 0.20 ^{ab}
Se (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Zn (µg/g)	79.68 ± 1.70 ^{A*}	112.03 ± 1.56 ^{AB}	115.40 ± 3.92 ^B	119.58 ± 0.95 ^B	87.55 ± 1.01 ^a	100.27 ± 1.47 ^{b*}	96.25 ± 0.92 ^{ab*}	83.73 ± 2.61 ^{a*}
Cd (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Cu (µg/g)	22.32 ± 0.68 ^{A*}	27.90 ± 0.67 ^{AC*}	45.96 ± 1.36 ^{B*}	37.43 ± 0.57 ^{BC}	49.31 ± 0.71 ^{ab}	48.68 ± 1.25 ^{ab}	50.19 ± 0.30 ^a	37.93 ± 0.31 ^b
Ni (µg/g)	5.41 ± 0.07 ^{BC*}	11.43 ± 0.08 ^A	7.36 ± 0.41 ^{AB}	4.67 ± 0.05 ^C	6.70 ± 0.10 ^{ab}	7.48 ± 0.18 ^{a*}	6.71 ± 0.05 ^{ab*}	2.64 ± 0.02 ^{b*}
Co (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Pb (µg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Mn (µg/g)	40.68 ± 0.82 ^{AB*}	51.25 ± 1.53 ^{B*}	32.06 ± 1.79 ^{C*}	34.96 ± 0.36 ^{AC*}	64.85 ± 1.10 ^{ab}	64.36 ± 2.34 ^{ab}	54.81 ± 0.73 ^a	70.55 ± 0.82 ^b
Cr (µg/g)	2.40 ± 0.10 ^{A*}	14.84 ± 0.50 ^B	9.05 ± 0.54 ^{BC}	6.76 ± 0.06 ^{AC*}	11.29 ± 0.36 ^{ac}	7.60 ± 0.32 ^{b*}	9.11 ± 0.34 ^{ab}	18.37 ± 0.49 ^c
Fe (µg/g)	499.28 ± 16.73 ^{A*}	699.98 ± 2.52 ^{AC*}	883.03 ± 35.15 ^{B*}	730.17 ± 1.07 ^{BC*}	1773.64 ± 45.76 ^{ac}	1176.48 ± 16.61 ^b	1297.52 ± 70.42 ^{ab}	2322.17 ± 66.44 ^c

nd – Not detected. Results are shown in mean ± SD (n = 3). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, p < 0.05).

3.3.1 Calcium (Ca)

No significant differences were observed in the Ca content of biomass from the first cutting between salinities, in the semi-hydroponics system. In the mud-based system, however, biomass from salinity 35 showed a significantly higher level of Ca, when compared to biomass at salinities 10 and 20. Additionally, biomass collected from plant grown at salinity 35 w/o also showed a significantly higher Ca content than biomass at salinity 10. Across the production systems, biomass from salinities 10 and 20 showed significantly lower Ca content in the semi-hydroponics system, whereas at both salinity levels of 35, Ca content was significantly lower in the mud-based system.

In the second cutting, salinity 10 exhibited significantly higher Ca content compared to both salinity 35 levels in the semi-hydroponics system. In the mud-based system, salinity 35 had significantly higher Ca content than salinity 20. Additionally, when comparing production systems, biomass at both salinity 35 levels showed significantly lower Ca content in the semi-hydroponics system compared to the mud-based system.

In the stem biomass, salinity 35 w/o had higher Ca content compared to salinity 10 in the semi-hydroponics system, while the mud-based system showed no significant differences in Ca levels between salinities. When comparing production systems, salinities 10, 35, and 35 w/o all exhibited lower Ca content in the semi-hydroponics system than in the mud-based system.

Finally, regarding the root biomass, salinities 20 and 35 w/o had significantly lower Ca content compared to salinity 10 in the semi-hydroponics system. In the mud-based system, salinity 20 showed significantly higher Ca content than both salinity 35 levels. Comparing the two production systems, Ca content in salinities 10 and 20 was significantly lower in the semi-hydroponics system compared to the mud-based system. However, for salinity 35 w/o, the semi-hydroponics system exhibited significantly higher Ca level.

3.3.2 Potassium (K)

In the first cutting, the K levels in biomass from salinities 20 and 35 w/o were significantly higher than from salinity 10. In the mud-based system, biomass from salinity 20 and 35 had higher K content than salinity 10. Overall, the K levels in biomass from the semi-hydroponics system were significantly lower than in the mud-based system.

Similar to the first cutting, in the second cutting biomass from salinities 20 and 35 w/o had significantly higher K content than salinity 10, in the semi-hydroponics system. In the mud-based

system, salinity 35 had significantly higher K content than salinity 10. Comparing production systems, biomass in salinities 20 and 35 w/o showed significant lower K content compared to the semi-hydroponics system.

Regarding stems, biomass from salinity 35 in the semi-hydroponics system was richer in K compared with biomass from salinity 10. No significant differences were found between salinities in the K content of biomass from the mud-based system. The mud-based system, however, had a significantly lower K content in salinities 20, 35 and 35 w/o, when compared to the semi-hydroponics.

Finally, for roots, biomass from both salinities 35 had higher K content than biomass from salinity 20, in the semi-hydroponics system. The mud-based system showed a similar tendency, where both salinities 35 had higher content than both salinity 20. Biomass from all salinities of the semi-hydroponics system had significantly lower K content than the mud-based system.

3.3.3 Magnesium (Mg)

In the first cutting, the Mg content was significantly higher in salinity 35 w/o than salinities 10 and 20, in the semi-hydroponics system. In the mud-based system, biomass from salinity 20 had significantly higher Mg content compared to both salinities 35. Moreover, in the semi-hydroponics system of the first cutting all salinities provided biomass with a significant lower Mg content than the mud-based system.

In the second cutting, biomass from the semi-hydroponics system at salinity 20 had significantly higher Mg content than salinities 10 and 35. In the mud-based system, both salinities 35 provided significantly higher Mg content compared to salinity 10. When comparing production systems, biomass from salinities 10, 35 and 35 w/o from the semi-hydroponics system had significantly lower Mg content than the mud-based system.

In the biomass of the stems, the semi-hydroponics system at salinity 35 resulted in a higher Mg content than salinity 10. In the mud-based system, biomass from salinity 35 w/o had higher Mg content than salinity 10. Comparing production systems, biomass from all salinities of the semi-hydroponics system had lower Mg content than the mud-based system.

Regarding roots biomass of the roots from the semi-hydroponics system, salinity 35 resulted in a higher Mg level than salinity 10. No significant differences were found between salinities in the mud-based system, and none between production systems either.

3.3.4 Sodium (Na)

In the first cutting, the Na content was significantly higher in salinity 20 than both salinities 35 in the semi-hydroponics system. Moreover, salinities 35 of the mud-based system resulted in a higher Na content than salinity 10. Comparing production systems, a lower Na content was observed in biomass from salinities 10 and 20 from the mud-based system and in both salinities 35 for the semi-hydroponics system.

Biomass from the second cutting showed a significantly higher Na content at salinity 35 w/o of the semi-hydroponics system compared to salinities 10 and 35. In the mud-based system, a significantly higher Na content was observed for salinity 35 w/o when compared to salinities 10 and 20. Comparing production systems, a significantly lower Na content at salinities 10 and 35 was observed in the semi-hydroponics system, when compared to the mud-based system.

The Na level analysis in the biomass of the stems could only be performed for plant biomass cultivated at salinities 10, 20 and 35 of the semi-hydroponics system. Nonetheless, these results showed that Na content was significantly higher in salinity 10 than in salinity 35.

The biomass of the roots was analysed with dilution 1:100 for salinity 35 w/o. All the other biomass salinity treatments were analysed with dilution 1:1000. A significantly higher Na content was detected in the roots biomass from salinity 35 when compared to salinities 10 and 35 w/o in the semi-hydroponics system. Biomass from salinity 35 in the mud-based system had a higher Na content than from salinity 10. Overall, a significantly lower Na content was observed in biomass from the semi-hydroponics system in salinities 20, 35 and 35 w/o.

3.3.5 Phosphorus (P)

The P content in the first cutting was higher in the semi-hydroponics system for salinities 10 and 20, when compared to salinity 35. In the mud-based system, biomass from salinity 20 had a significant higher P content than salinity 35 w/o. Comparing production systems, biomass from salinities 20 and 35 of the semi-hydroponics system, and salinities 10 and 35 w/o the mud-based system, had significantly lower P content.

In the second cutting, biomass from the semi-hydroponics system in salinity 20 had a higher P content, than both salinities 35. Moreover, biomass from salinity 10 was richer than biomass from salinity 35 w/o. In the mud-based system, salinity 10 had significantly higher P content compared to both salinities 35. Also, salinity 20 had significantly higher content than

salinity 35 w/o. When comparing production systems, salinities 10, 20 and 35 showed lower P content in the semi-hydroponics system than the mud-based system.

Regarding stems biomass, the P content was significantly higher in salinities 10 and 20 when compared with salinity 35 w/o. Additionally, biomass from salinity 20 had a higher content of P than salinity 35. In the mud-based system, salinity 20 showed significantly higher P content compared to salinities 10 and 35, and at salinity 35 w/o it observed a higher phosphorus content than at salinity 10. The comparison between production systems showed that salinities 10 and 35 had significantly lower P content in the mud-based system, and salinity 35 w/o significantly lower content in the semi-hydroponics system.

The roots biomass showed significantly higher P content at salinity 10 of the semi-hydroponics system when compared to salinity 35. Significantly higher P content in the mud-based system was found at salinity 35 than at salinity 10. The comparison of both production systems showed that salinities 20, 35 and 35 w/o had significantly lower phosphorus content than those of the mud-based system.

3.3.6 Zinc (Zn)

Zinc content in the first cutting of both the semi-hydroponics system and mud-based system was significantly higher at salinity 20 than at both salinities 35. Comparing both production systems, significantly lower content at salinity 20 of the mud-based system and at salinity 35 of the semi-hydroponics system was found.

In the second cutting, biomass from the semi-hydroponics system had a higher Zn content at salinity 20 than at salinity 35. In the mud-based system, biomass from salinity 20 had significantly higher Zn content than both salinities 35. Furthermore, when comparing production systems, biomass from salinities 10 and 20 of the semi-hydroponics system had significantly lower Zn content than those of the mud-based system.

The stems biomass from salinity 20 had significantly higher Zn content than from salinity 35 w/o in the semi-hydroponics system. The mud-based substrate system resulted in biomass richer in Zn at salinity 20, than at salinity 35 and 35 w/o. The comparison between production systems showed that the semi-hydroponics system had lower Zn content than the mud-based system at salinities 10, 20 and 35 w/o.

The analysis of roots biomass indicated that both salinities 35 showed significantly higher Zn content than salinity 10 in the semi-hydroponics system. The mud-based system provided higher Zn contents at salinity 20 compared to salinities 10 and 35 w/o. The comparison of the two production systems in the roots showed significantly lower Zn content at salinity 10 in the semi-hydroponics system, and at salinities 20, 35 and 35 w/o in the mud-based system.

3.3.7 Copper (Cu)

The copper content in biomass from the first cutting was significantly higher at salinity 20 than at salinity 35 w/o in the semi-hydroponics system. In the mud-based system, biomass from salinity 20 had a significantly higher Cu content than salinity 35. When comparing both production systems, it was observed that salinities 10 and 20 of the mud-based system resulted in biomass with significantly lower Cu content than the same salinities in the semi-hydroponics system.

The analysis of the second cutting showed significantly higher Cu content at salinity 20 than salinity 10 in the semi-hydroponics system. The mud-based system had significantly higher Cu content at salinity 20 compared to both salinities 35. Moreover, salinity 10 had significantly higher Cu content than salinity 35 w/o. The comparison between production systems showed significantly lower Cu levels at salinities 10 and 20 in the semi-hydroponics system and salinity 35 w/o in the mud-based system.

The analysis of stems biomass indicated significantly higher Cu content at salinities 20 and 35 compared to salinity 35 w/o in the semi-hydroponics system. Biomass from the mud-based system at salinity at 20 had a significantly higher Cu content at both salinities 35. Furthermore, the comparison between production systems indicated that in the semi-hydroponics system biomass from all salinities had significantly lower Cu content than in the mud-based system.

The analysis of the roots biomass showed significantly higher Cu content at salinity 35 than at salinities 10 and 20, in the semi-hydroponics system. Aside of that, salinity 35 w/o also had significantly higher content than salinity 10. In the mud-based system, salinity 35 had significantly higher Cu content than salinity 35 w/o. Moreover, the comparison between production systems showed that salinities 10, 20 and 35 from the semi-hydroponics system had significantly lower Cu content than those of the mud-based system.

3.3.8 Nickel (Ni)

Nickel content in the first cutting was significantly higher at salinity 20 than at salinities 10 and 35 in the semi-hydroponics system. The analysis of the mud-based substrate system indicated that the Ni content at salinity 20 was significantly higher than at salinity 10. The comparison between production systems showed that salinities 20 and 35 w/o in the mud-based system had significantly lower Ni content than those in the semi-hydroponics system.

The second cutting showed significantly higher Ni content at salinity 20 than at salinity 10 from the semi-hydroponics system. In the mud-based system, Ni content at salinity 20 was significantly higher than at both salinities 35. Moreover, comparing of the production systems, both salinities 10 and 20 on the semi-hydroponics system had significantly lower Ni content than on the mud-based system.

The analysis of stems biomass indicated that the Ni content was significantly higher at salinity 35 than salinities 10 and 20 in the semi-hydroponics system. In the mud-based system, Ni content was significantly higher at salinity 20 than both salinities 35. Additionally, salinity 10 also showed higher content than salinity 35 w/o. Comparing production systems, the semi-hydroponics system had significantly lower Ni content at salinities 10 and 20 than in the mud-based system. For salinities 35 and 35 w/o, this was reversed. In these salinities the mud-based system provided significantly lower Ni content than the semi-hydroponics system.

The biomass of the roots had a significantly higher Ni content in salinity 20 compared to salinities 10 and 35 w/o in the semi-hydroponics system. Additionally, biomass from salinity 35 also had higher Ni content than salinity 35 w/o. The mud-based system had significantly higher content in salinity 20 than salinity 35 w/o. Comparing production systems, salinity 10 in the semi-hydroponics system showed lower nickel content than the mud-based system. For salinities 20, 35 and 35 w/o, the mud-based system provided significantly lower Ni content than the semi-hydroponics system.

3.3.9 Lead (Pb)

Lead content in the first cutting showed significances in both production systems. In the semi-hydroponics system, salinity 10 had higher Pb content than both salinities 35, and salinity 20 had also higher content than salinity 35 w/o. In the mud-based substrate system, salinity 35 w/o had significantly higher Pb content than salinity 10. Comparing both production systems, salinities 10

and 20 had lower Pb content in the mud-based system and salinity 35 w/o had lower Pb content in the semi-hydroponics system.

In the second cutting, no significant differences were found between salinities in the production systems. Between production systems however, salinity 10 of the semi-hydroponics system had significantly lower Pb content than the mud-based system.

The stems biomass showed no significant differences between salinities in the semi-hydroponics system. The mud-based system however, showed that salinity 35 had significantly higher Pb content than salinities 10 and 20, and salinity 35 w/o had also higher Pb content than salinity 20. Comparing production systems, the semi-hydroponics system had significantly lower Pb content in salinity 35 w/o than the mud-based system. Furthermore, the roots biomass had no values detected.

3.3.10 Manganese (Mn)

Manganese content in the first cutting showed that biomass from salinity 10 of the semi-hydroponics system had significantly higher Mn content than both salinities 35, and that those from salinity 20 had also higher content than salinity 35 w/o. In the mud-based system, biomass from salinity 10 had significantly higher Mn content than salinities 20 and 35, and salinity 35 w/o also had higher content than salinity 35. Comparing production systems, salinity 20 in the mud-based system and salinity 35 w/o in the semi-hydroponics system showed significantly lower Mn content.

Biomass from the second cutting at salinity 20 of the semi-hydroponics system had significantly higher Mn content than salinity 35. In the mud-based system, salinity 10 had significantly higher content compared to both salinities 35. Additionally, biomass at salinity 20 also had higher Mn content than salinity 35 w/o. Comparing both production systems in the second cutting, salinities 10 and 35 of the semi-hydroponics system, and salinity 35 w/o of the mud-based system had significantly lower manganese content.

Regarding stems, a significantly higher Mn was observed in salinity 20 than salinity 35 in the semi-hydroponics system. Moreover, in the mud-based system, salinities 10 and 20 had significantly higher content than salinity 35. When comparing the two production systems, the semi-hydroponics system had significantly lower Mn content in salinities 10, 35 and 35 w/o than the mud-based system.

Roots biomass had significantly higher content in salinity 20 than both salinities 35 in the semi-hydroponics system. Additionally, biomass from salinity 10 also had higher Mn content than salinity 35. The mud-based system had significantly higher content in salinity 35 w/o compared to salinity 35. The comparing of production systems indicated that all salinities of the semi-hydroponics system had lower Mn content than those of the mud-based system.

3.3.11 Chromium (Cr)

Chromium content in the first cutting was significantly higher in salinities 10 and 20 of the semi-hydroponics system than in salinity 35. In the mud-based system, biomass from salinity 10 had significantly higher Cr content than salinities 20 and 35 w/o. Additionally, biomass from salinity 35 also had higher content than salinity 20. Comparing both production systems, biomass from salinity 20 in the mud-based system, and biomass from salinity 35 in the semi-hydroponics system, had significantly lower Cr content.

Biomass from the second cutting at salinity 20 of the semi-hydroponics system had significantly higher Cr content than salinities 10 and 35, and salinity 35 w/o had higher content than salinity 20. In the mud-based system, biomass from salinity 20 had significantly higher Cr content than salinity 35. Moreover, the comparison of both production systems indicated that all salinities of the semi-hydroponics system had significantly lower Cr content than those of the mud-based substrate system.

The stems biomass had significantly higher Cr content in salinity 35 than salinities 10 and 20 in the semi-hydroponics system. In the mud-based system, biomass from salinity 20 had significantly higher content than both salinities 35, and salinity 10 also had higher content than salinity 35 w/o. Comparing both production systems, biomass from all salinities in the semi-hydroponics system had significantly lower Cr content than the mud-based system.

Regarding roots, biomass from salinity 20 of the semi-hydroponics system had significantly higher Cr content than salinities 10 and 35 w/o, where salinity 35 had also higher content than salinity 10. In the mud-based system, biomass from salinity 35 w/o had significantly higher Cr content than salinities 20 and 35, where salinity 10 also had higher content than salinity 20. When comparing both production systems, observed was that biomass from salinities 10 and 35 w/o of the semi-hydroponics system and salinity 20 of the mud-based system had significantly lower Cr content.

3.3.12 Iron (Fe)

Iron content in the first cutting from salinity 10 of the semi-hydroponics system was significantly higher than both salinities 35, while biomass from salinity 20 had also higher Fe content than salinity 35 w/o. In the mud-based system, biomass from salinity 10 had significantly higher Fe content than salinities 20 and 35 w/o. When comparing both production systems, the semi-hydroponics system had significantly lower Fe content in salinities 10, 35 and 35 w/o, and the mud-based system had significantly lower content in salinity 20.

In the second cutting, biomass from the semi-hydroponics system at salinity 20 had significantly higher Fe content than both salinities 35. In the mud-based system, biomass from salinity 20 had significantly higher content than salinity 35 w/o. Comparing both production systems, biomass produced in the semi-hydroponics system had lower Fe content in all salinities compared to the mud-system.

Stems biomass had significantly higher Fe content in salinities 20 and 35 than salinity 10 in the semi-hydroponics system. In the mud-based system, biomass from salinity 20 had significantly higher Fe content than salinities 10 and 35. Moreover, comparing of both production systems showed that biomass from the semi-hydroponics system had lower Fe content in all salinities compared to the mud-system.

Biomass of the roots had significantly higher Fe content in salinity 35 than salinities 10 and 20 in the semi-hydroponics system. Aside of that, salinity 35 w/o also had significantly higher content than salinity 10. Furthermore, the mud-based system at salinity 35 w/o resulted in biomass with a significantly higher Fe content than salinities 20 and 35. Additionally, biomass from salinity 10 also had higher content than salinity 20. Comparing both production system, the semi-hydroponics system produced biomass with a lower Fe content in all salinities compared to the mud-system.

3.4 Bioactive compounds results

3.4.1 Total phenolics content (TPC)

TPC (Figure 3.8) was determined for the first cutting (a), second cutting (b), stems (c) and roots (d). Comparing overall productivity between production systems, the biomass from the second cutting had significantly higher TPC in the semi-hydroponics system than in the mud-based system. In the semi-hydroponics system, biomass from salinities 10 and 35 w/o had significantly higher TPC than salinities 20 and 35. Biomass from the mud-based system at salinities 10, 20 and 35 w/o had significantly higher TPC than the salinity 35. Furthermore, comparing production systems from the biomass of the first cutting showed that lower TPC was observed in biomass from the first cutting in the mud-based system at salinity 35.

In the second cutting, the TPC of biomass from salinity 10 was significantly higher than salinity 35 w/o in the semi-hydroponics system. Moreover, no differences were found when comparing production systems in the second cutting.

In the stems, biomass from salinity 35 w/o had significantly higher TPC than salinities 20 and 35 in the semi-hydroponics system. In the mud-based system, biomass from salinity 10 had significantly higher TPC than salinities 35 and 35 w/o. Additionally, significant differences were found between the production systems in the stems. These included lower TPC at salinity 10 in the semi-hydroponics system, and lower TPC at both salinities 35 in the mud-based system.

Finally, in the biomass of the roots, the semi-hydroponics system showed significant differences between salinities, where salinities 10 and 35 had significantly higher TPC than salinities 20 and 35 w/o. Moreover, comparing overall production systems in the roots biomass showed that salinity 20 in the semi-hydroponics system provided a significantly lower TPC.

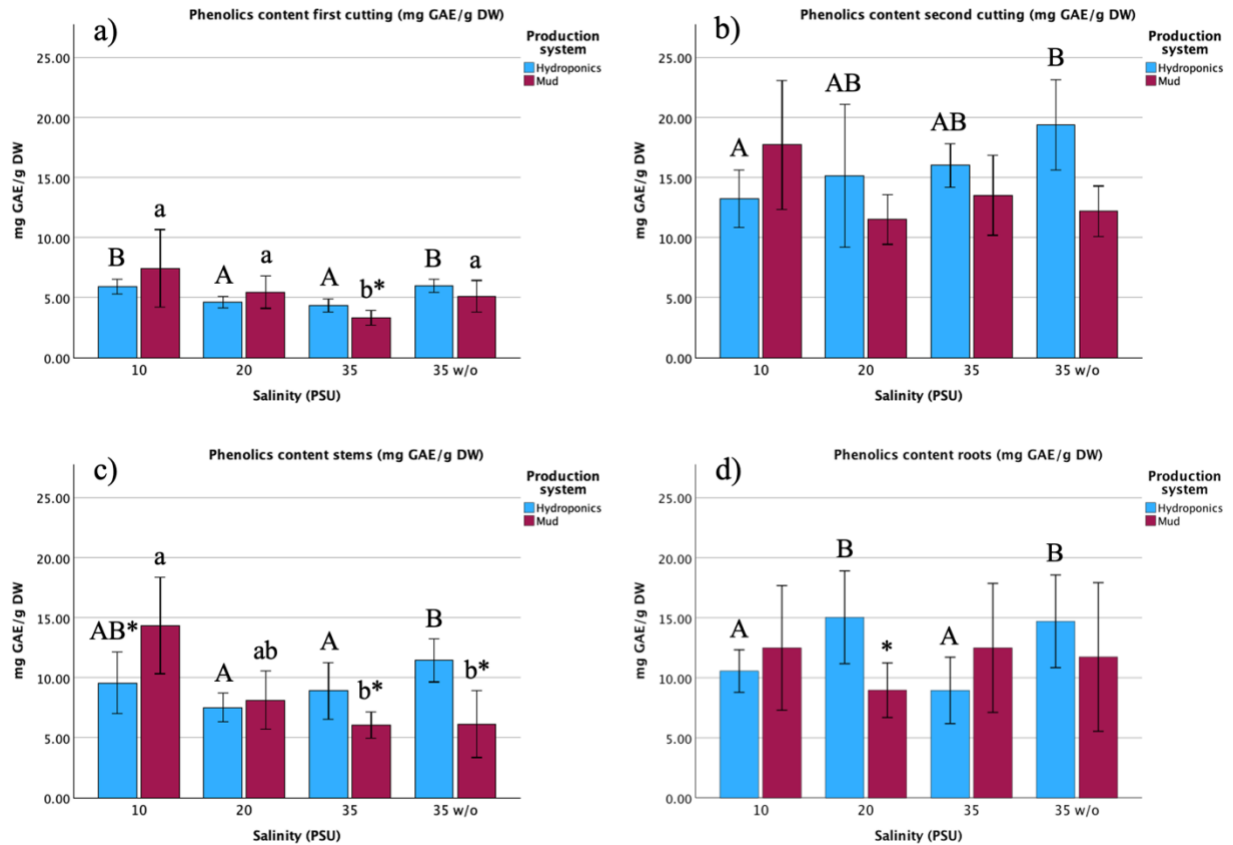


Figure 3.8: TPC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.4.2 Total flavonoid content (TFC)

TFC (Figure 3.9) was determined for the first cutting (a), second cutting (b), stems (c) and roots (d). Comparing overall productivity between production systems, both the first and second cuttings showed significant differences in the production system. In the first cutting, the mud-based system had higher TFC, while in the second cutting, the semi-hydroponics system had higher TFC. In the biomass of the first cutting of the semi-hydroponics system, salinity 20 had higher TFC than salinities 35 and 35 w/o. Moreover, biomass from salinity 10 showed significantly higher TFC when compared to salinity 35 w/o. In the mud-based system, biomass from salinity 10 had significantly higher TFC than salinities 20 and 35. Aside of that, biomass from salinity 35 w/o also

showed higher TFC than salinity 20. Comparing production systems in the first cutting showed lower TFC at salinities 10, 35 and 35 w/o in the semi-hydroponics system and at salinity 20 in the mud-based system

In the second cutting, biomass from salinities 35 and 35 w/o had higher TFC than salinity 10 in the semi-hydroponics system. Additionally, biomass cultivated at salinity 35 w/o also had higher TFC than salinity 20. Comparing production systems in the second cutting showed lower TFC at salinity 10 in the semi-hydroponics system and at salinities 20, 35 and 35 w/o in the mud-based system

Stems biomass from the semi-hydroponics system salinity 35 w/o had higher TFC compared to salinities 20 and 35. Additionally, biomass from salinity 10 also showed higher content compared to salinity 20. Moreover, biomass cultivated at salinity 10 in the mud-based system had significantly higher TFC than the others. Comparing production systems, stems had lower TFC at salinity 10 in the semi-hydroponics system and at salinity 35 w/o in the mud-based system

The roots biomass from salinity 20 in the semi-hydroponics system showed significantly higher TFC than in salinities 10 and 35. Additionally, biomass from salinity 35 w/o also had higher content compared to salinity 35. In the mud-based system, biomass cultivated at salinity 20 had higher TFC than those at salinities 10, 35 and 35 w/o. Additionally, biomass cultivated at salinity 35 had higher TFC than at salinity 10. Comparison between production systems in the roots showed lower TFC at salinities 10 and 35 w/o in the semi-hydroponics system.

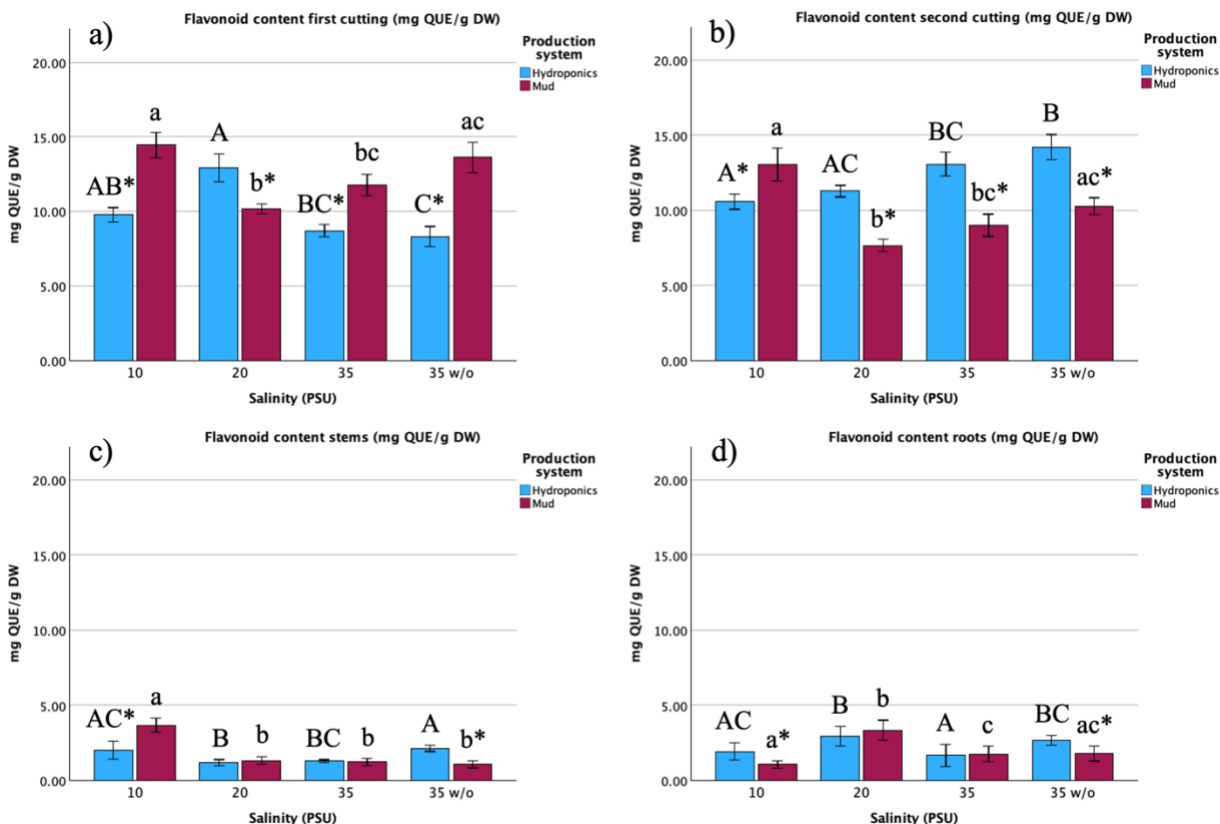


Figure 3.9: TFC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.4.3 Total liposoluble pigments content

3.4.3.1 Total chlorophyll content

The total chlorophyll content (Fig. 3.10) was determined in biomass from the first cutting (a), second cutting (b), stems (c) and roots (d). Comparing overall productivity between production systems, biomass from the first and second cutting, stems and roots resulted in significantly higher chlorophyll content in the mud-based system than in the semi-hydroponics system. In the first cutting, the semi-hydroponics system at salinity 20 resulted in a significantly higher chlorophyll content than those from salinities 10, 35 and 35 w/o. Additionally, biomass from salinity 10 had higher chlorophyll content than salinity 35 w/o. In the mud-based system, biomass from salinity

10 had higher chlorophyll content than those from salinities 20 and 35. Additionally, biomass from salinity 35 w/o also had higher chlorophyll content than salinity 35. Comparing production systems in the first cutting, lower chlorophyll content was observed in biomass from salinities 10, 35 and 35 w/o in the semi-hydroponics system and from salinity 20 in the mud-based system.

In the second cutting, the semi-hydroponics system at salinity 20 produced biomass with significantly higher chlorophyll content than those from salinities 10 and 35. Additionally, biomass from salinity 35 w/o had higher chlorophyll content than from salinity 10. In the mud-based system, biomass from at salinity 10 had significantly higher chlorophyll content than both salinities 35. Additionally, biomass from salinity 20 had higher chlorophyll content than from salinity 35 w/o. Comparing production systems in the second cutting showed lower chlorophyll content at salinities 10 and 35 in the semi-hydroponics system and at salinity 20 in the mud-based system.

In the stems, the semi-hydroponics system at salinities 10, 20 and 35 w/o produced biomass with significantly higher levels of chlorophylls than salinity 35. In the mud-based system, chlorophyll content from stems biomass at salinity 10 was significantly higher than at salinities 20, 35 and 35 w/o. Additionally, chlorophyll content from stems biomass at salinities 20 and 35 w/o was significantly higher than at salinity 35. Overall comparison between production systems in the stems showed that biomass from the semi-hydroponics system had lower chlorophyll content than those from the mud-based system, in all salinities.

Regarding roots, the semi-hydroponics system showed that biomass from salinities 20 and 35 w/o had significantly higher chlorophyll content than those from salinity 10. Additionally, biomass from salinity 35 w/o was also significantly higher than from salinity 35. In the mud-based system, biomass from salinity 20 had significantly higher chlorophyll content than salinities 10 and 35 w/o. Additionally, biomass from salinity 35 had significantly higher chlorophyll content than salinity 35 w/o. Comparing production systems in the roots showed significantly lower chlorophyll content at salinities 10, 20 and 35 in the semi-hydroponics system and at salinity 35 w/o in the mud-based system.

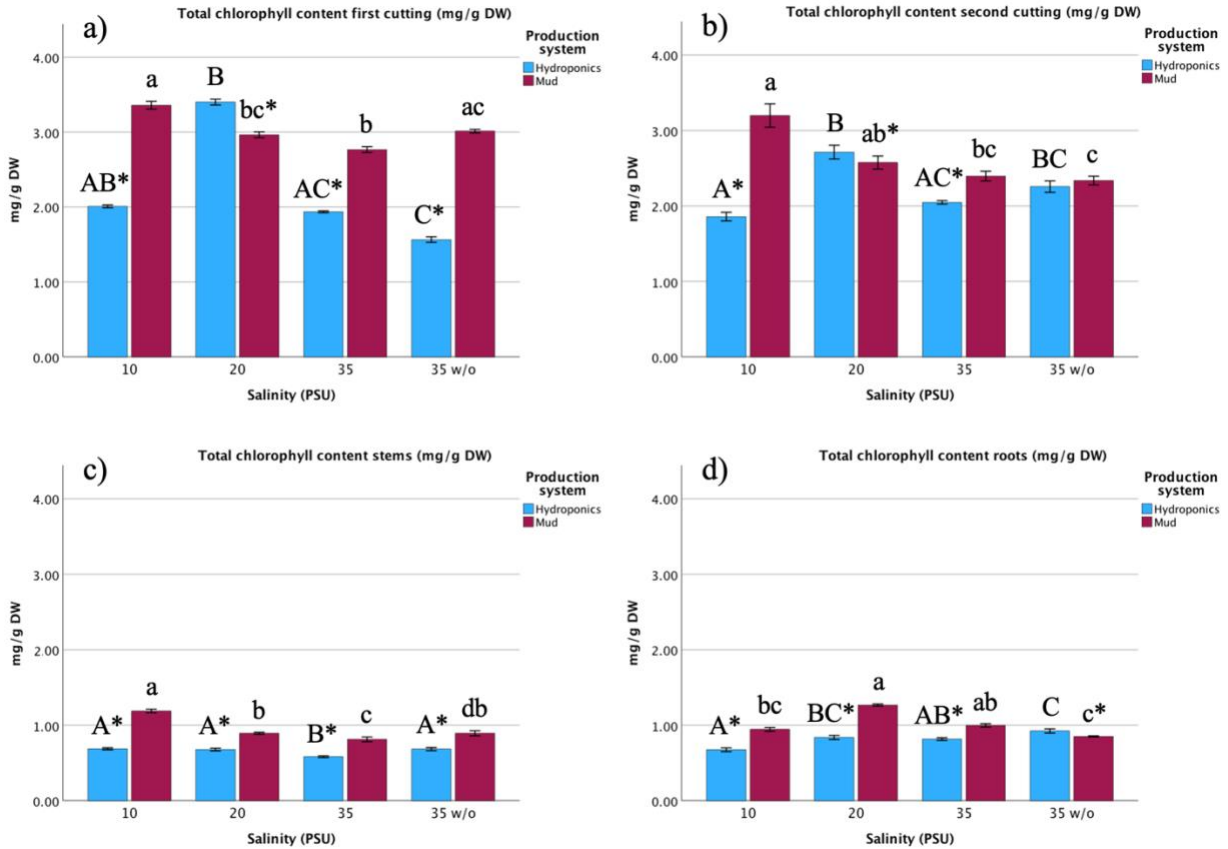


Figure 3.10: Total chlorophyll content of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.4.3.2 Carotenoids content

Carotenoids were calculated for all biomasses (Fig. 3.11), but were only detected in biomass from salinities 10 and 35 w/o, in the semi-hydroponics system for the first cutting. In the first cutting, the carotenoids content in biomass at salinity 10 was significantly higher than from biomass at salinity 35 w/o. In the second cutting, carotenoids were only detected in biomass at salinities 10, 20 and 35 from the semi-hydroponics system. In the semi-hydroponics system of the second cutting, biomass at salinity 20 was richer in carotenoids when compared with salinities 10 and 35.

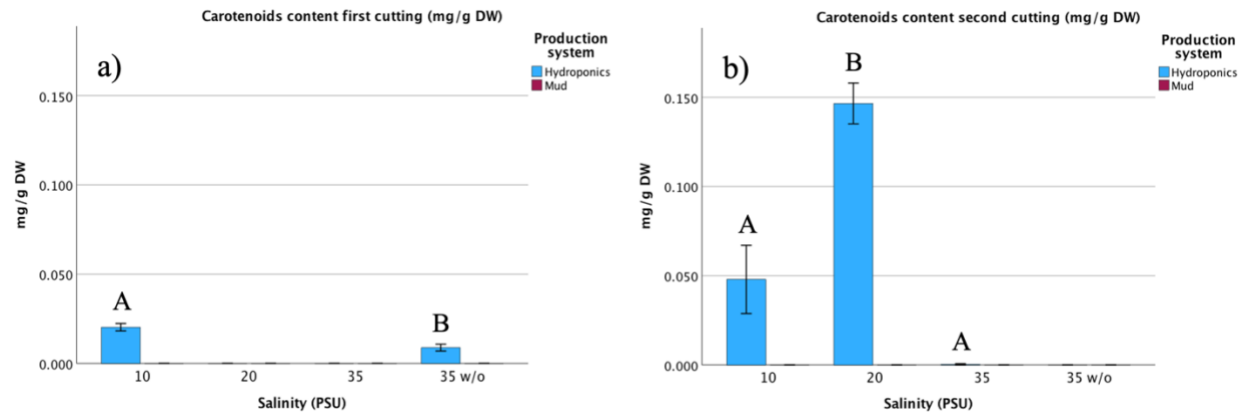


Figure 3.11: Carotenoids content of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD ($n = 6$). Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

3.4.4 Total tannins content (TTC)

The TTC (Fig. 3.12) was determined in biomass from first cutting (a), second cutting (b), stems (c) and roots (d). Comparing the overall productivity between production systems, the TTC of biomass from mud-based system was significantly higher in the first cutting and the stems biomass. In the first cutting, biomass from the semi-hydroponics system at salinity 20 showed higher TTC when compared to biomass at salinities 35 and 35 w/o. Additionally, biomass at salinity 10 showed higher TTC than at salinity 35 w/o. In the mud-based system, biomass at salinity 10 had increased TTC compared with plants cultivated at salinities 20 and 35. Comparing production systems in the first cutting showed lower TTC at salinities 10 and 35 w/o in the semi-hydroponics system and at salinity 20 in the mud-based system.

In the second cutting, biomass from the semi-hydroponics system at salinity 20 was richer in TTC than from salinities 10 and 35 w/o. In the mud-based system, biomass cultivated at salinity 10 had significantly higher TTC than all other salinities. Comparing production systems in the second cutting showed lower chlorophyll content at salinity 10 in the semi-hydroponics system and at salinity 20 in the mud-based system.

Regarding the biomass of the stems, the semi-hydroponics system showed that biomass cultivated at salinity 10 had higher TTC than at both salinities 35. Additionally, biomass cultivated at salinity 20 also had higher TTC than at salinity 35. The mud-based system, similarly to the

second cutting, showed higher TTC in biomass cultivated at salinity 10 than at the other salinities. Overall comparison between production systems in the stems showed that biomass from the semi-hydroponics system had lower TTC at salinities 10 and 35 than those at the mud-based system.

For roots biomass obtained from plants cultivated in the semi-hydroponics system, higher TTC was observed in biomass at salinity 35 w/o than at salinity 35. In the mud-based system, biomass cultivated at salinity 20 showed significantly higher TTC than those from both salinities 35. Comparison between production systems in the roots showed that biomass from the semi-hydroponics system had lower TTC at salinity 20 than at the mud-based system.

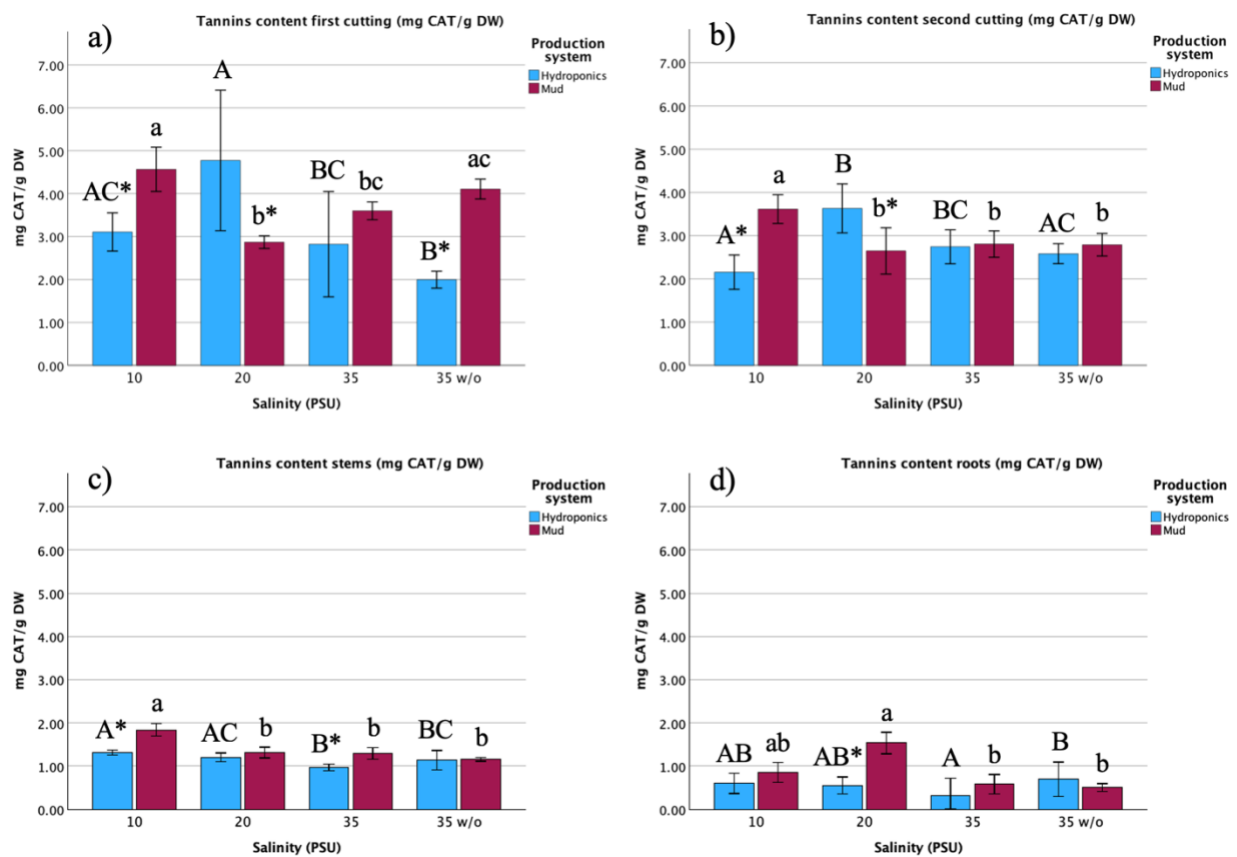


Figure 3.12: TTC of methanol extracts made from biomass of *S. ramosissima* from a) first (week 3) and b) second (week 6) cutting, c) stems and d) roots, each comparing the semi-hydroponics system (left, blue bar) and mud-based substrate system (right, red bar) at different salinities (PSU) 10, 20, 35 and 35 w/o (without added fertiliser). Results are shown in mean \pm SD (n = 6). Asterisk denotes significant differences between semi-hydroponics and mud-based substrate production systems, placed on the lower value. Capital letters indicate differences between salinities at semi-hydroponics system. Low case letters indicate differences between mud-based substrate production system. No denotation means no differences. (Kruskal-Wallis; Dunn's post-hoc test, $p < 0.05$).

4 Discussion

The present study has evaluated the influence of cultivating *S. ramosissima* in a semi-hydroponics system and a mud-based substrate system using water sources commonly available at aquaculture farms. Water used for plant irrigation included wastestreams from semi-intensive aquaculture fishponds, water from a brackish well and freshwater, with their respected different salinities of 10, 20 and 35 PSU. Plants irrigated with the different water sources were supplied with supplementary fertiliser. Additionally, plants irrigated with the water source of the wastestreams from the semi-intensive pond (35 PSU) were also tested without supplementary fertiliser. Analysed parameters included biomass production and biochemical properties of the produced edible and lignified biomass, including its nutritional and antinutritional profiles, as well as the levels of bioactive compounds and antioxidant properties.

4.1 Biomass production implications

The overall increased biomass productivity in the mud-based system for first cutting could be due to higher quantity of nutrients present in the substrate. Although not specifically quantified due to instrumental constraint, it was assumed that the mixture of potting soil and mud from the decantation pond had a higher nutrient content, and therefore provided a higher nutrient load than the semi-hydroponics system. Biomass from the second cutting showed a significant increase at salinity 20 in the semi-hydroponics system. However, no such favourable effect of salinity 20 was found in the first cutting and in the mud-based system, nor has there been found any research to elaborate on this, therefore the relationship remains unclear. Furthermore, the reduced biomass productivity observed at the lowest salinity used, 10 PSU, is contrary to other findings by other authors on related species, such as Mesa-Marín et al. (2019), that reported that the optimal salinity for *S. europaea* cultivation was 171 mM NaCl (approximately 10 PSU), and by Cárdenas-Pérez et al. (2022), also on *S. europaea*, where 200-400 mM NaCl (approximately 11 to 23 PSU) was considered optimal. Research by Khan et al. (2001) on *S. rubra* provided similar results, in accordance with the previously mentioned studies. Other halophytes however, such as *Haloxylon recurvum* (Khan et al., 2000), *Sarcocornia natalensis* (Naidoo & Rughunanan, 1990) and *Allenrolfea occidentalis* (Gul et al., 2000), had reported optimum growth observed at salinities

between 200 to 600 mM NaCl, converting to approximately 11.7 to 35 PSU respectively. The large range in salinities can be attributed to the different treatments and conditions used, such as cultivation systems, plant densities and plant species.

Furthermore, the earlier mentioned potential effect of higher nutrient content in the substrate of the mud-based system compared to the semi-hydroponics system can be seen also by the overall higher biomass production in the mud-based system. Moreover, at salinity 35, both systems showed no significant difference in biomass production with or without the added supplementary fertiliser. Particularly the biomass production in the mud-based system provided very similar results when looking at the effect of supplementary fertiliser. Additionally, the general increase in productivity of edible and non-edible biomass after cuttings at salinity 35, compared to salinities 10 and 20, is contrary to earlier reports by Mesa-Marín et al. (2019) and Cárdenas-Pérez et al. (2022), on *S. ramosissima* and *S. europaea*, respectively. The higher production of non-edible biomass in the mud-based system compared to the semi-hydroponics system is possibly due to the increased availability of nutrients in the substrate, since nutrient availability plays an important role in the lignin biosynthetic pathway (Liu et al., 2018). Moreover, research highlights that soil microbial communities, which have been reported to increase by addition of fish effluent fertilisation (Fruscella et al., 2023), can influence lignin content by affecting the nutrient delivery to plants (Bennett et al., 2015). When carbon is abundant relative to other nutrients, it can promote lignin biosynthesis. This suggests that higher nutrient levels, particularly nitrogen, which is expected to be more abundant in the mud-based system due to the used potting soil and aquaculture sludge (Zhang et al., 2023), could enhance lignin production in *Salicornia* species by providing the necessary resources for the metabolic pathways involved in lignin synthesis. Aside of nutrient availability, the density of the soil substrate can also impact plant growth and nutrient uptake. Higher soil density may enhance root penetration and stability, potentially leading to the improved nutrient acquisition. This is particularly relevant in saline environments where *Salicornia* spp. thrives. For instance, *S. brachiata* has been shown to exhibit significant biomass production in coastal saline soils, indicating that optimal soil conditions can enhance its growth and possibly lignin content (Rathore et al., 2016).

Furthermore, total biomass in the semi-hydroponics system showed that more edible biomass was produced than non-edible biomass. Yet, the total biomass of the semi-hydroponics system was lower than biomass of the mud-based substrate system. Even though total biomass in

the mud-based system was higher, the edible biomass in both systems was similar, indicating that the main difference was in a higher non-edible content in the mud-based system. Moreover, edible biomass did not seem to decrease with the increase of salinity, demonstrating beneficial prospects for potential implementation of these cultivation systems in aquaculture settings where water with salinity of 35 PSU is mostly available. In a hydroponics setting, however, additional fertiliser may be beneficial to promote growth.

Total edible biomass obtained at the end of the experiment, including both cuttings and remaining edible biomass, considering the growing area of 6 cm x 6 cm per cluster of plants, resulted in an average biomass production of 103 g/m² per day from plants cultivated in the semi-hydroponics system, with a highest value recorded of 118 g/m² per day at salinity 20. Plants cultivated in the mud-based substrate system had an average edible biomass production of 106 g/m² per day, with highest recorded values in salinity 35 with additional fertiliser of 114 g/m² per day. Plant biomass in the semi-hydroponics system showed high production in salinity 20, while the salinities 10 and 35 with and without additional fertilisation were significantly lower. Plant biomass from the mud-based system showed higher production in salinities 35 with and without additional fertiliser, with differences to the lower salinities 10 and 20 being much lower compared to the semi-hydroponics system. Comparing observed values to those of other research is not straightforward, as cuttings in this study were performed at precisely 15 cm above the substrate, while several other studies performed cuttings on new harvestable edible shoots (Castilla-Gavilán et al., 2024; Lima et al., 2020). Therefore, the cuttings performed by these studies was based on what they considered was harvestable size depending on the plants, and not always at the same height. Thus, potentially more edible plant biomass could have been harvested by performing cuttings in this study by use of similar methods. However, cuttings performed at 15 cm above substrate was decided to avoid harvesting too much woody material and to ensure re-growth after cutting (Díaz et al., 2013). Moreover, it would obtain more reliable and consistent data, and not necessarily focus on achieving maximum harvested biomass output.

Nonetheless, Castilla-Gavilán et al. (2024) cultivated *S. ramosissima* cultured with RAS effluents in the time period between July and September, and observed average highest harvested biomass per month in their Deep Water Culture system of 2.5 kg/m², converted to approximately 116.67 g/m² per day. Their highest recorded value however, was observed to be 320 g/m² per day. These values are considerably higher than those obtained in this study, especially considering the

harvesting of solely fresh tips compared to the total edible biomass in this study. Nevertheless, their study also reported that the treatment that showed the highest recorded biomass production, had a subsequent decrease in biomass production (25–250 g/m² per day) in later stages of the experimental period, indicating the variability in biomass production during the life stages of *Salicornia*. Moreover, research by (Lima et al., 2020) on the cultivation of *S. ramosissima* also showed a decrease in productivity with the increase of salinity. Whilst these observations are in line with those of the semi-hydroponics system in this study, biomass obtained from plants in the mud-based system in contrast, had increased biomass output in the higher salinities.

4.2 Proximate composition implications

High moisture content leads to more fleshy tips, and more tender and succulent texture, as preferred in fresh salads or as a vegetable side dish (Ventura et al., 2011). In this work, and as expected, moisture was the major parameter, with average values of 86% and 87% for the semi-hydroponics and mud-based systems, respectively. The highest moisture content, 87%, was observed in biomass from plants cultivated at salinity 10, from the first cutting in the semi-hydroponics system and 89% in salinity 35 without additional fertiliser from the first cutting in the mud-based system. These values were in line with previous reported values of 86% for wild (Lopes et al., 2021) and 90% for cultivated *S. ramosissima* at a salinity of 35 mM NaCl (approximately 2 PSU) (Lima et al., 2020).

The moisture content of the biomass from the first cutting was higher in the mud-based system. However, this difference was not observed in the second cutting, as moisture content in the second cutting showed a reduction, particularly in the higher salinities. The reduced moisture content in particularly the higher salinities in the second cutting, could be a consequence of the increased osmotic stress caused by increase of salinity, which decreases water uptake by the cells (Lima et al., 2020). Hence, due to the longer cultivation time and potential induced stress by the performing of the cutting, the increase of salinity could have caused this reduction of moisture in biomass with higher salinity treatments.

Furthermore, the higher levels of moisture content observed in the mud-based system could be due to the enhanced water retention capacity and nutrient availability in the substrate, which may have provided a more stable environment for the plants initially. Mud-based systems typically have better nutrient cycling and water-holding capacity, which could lead to better initial moisture

content in the plants (Cárdenas-Pérez et al., 2022). However, after the first cutting, the plants might have experienced stress that could affect their water uptake and retention mechanisms due to changes in root function (Barros et al., 2015) and, therefore, affect the moisture content of plants cultivated in the mud-based system with the second cutting. This variability could be due to adaptation and recovery processes, which can differ depending on the growing medium (Cárdenas-Pérez et al., 2022). Moreover, several studies indicate that halophytes such as *Salicornia* spp. show an increase in synthesis of secondary metabolites compounds with antioxidant properties to decrease production of Reactive Oxygen Species (ROS), when under stress due to salt, as this can help the plant retain water in its cells, preventing excessive loss of moisture, leading to higher moisture content (Rahmani et al., 2022; Türkan & Demiral, 2009; Ventura & Sagi, 2013). Furthermore, the increase in temperature over the course of the next three weeks after the first cutting could have led to lower moisture content in the second cutting (Hamed et al., 2013).

The stems biomass from plants grown in the mud-based system showed higher moisture content when compared to the semi-hydroponics system, thus suggesting that plants in this system can retain more water. This could be due to the nutrient availability, which directly influences the osmotic potential of *Salicornia* spp., which is critical for water uptake. Research by Lopes et al. (2023) indicates that *S. ramosissima* exhibits high moisture content, which is essential for diluting the high concentrations of NaCl present in their tissues. This dilution is necessary to mitigate osmotic stress, thereby facilitating water uptake under saline conditions. Moreover, the presence of potential beneficial microorganisms in the rhizosphere in the mud-based system could alter nutrient availability and consequently, water uptake. Mesa-Marín et al. (2020) found that inoculation of *S. ramosissima* with plant growth-promoting bacteria increased water use efficiency (WUE) and water content. This indicates that microbial interactions can enhance nutrient absorption, which in turn supports better water management under stress conditions.

The ash content represents the inorganic residue remaining after the organic matter has been completely burned off and consists primarily of minerals. The ash levels were high, especially in biomass from higher salinities, similar to the results reported by Lopes et al. (2021), who analysed *S. ramosissima* plants from wild populations in salt marshes. Halophytic plants generally have high ash content, as this relates to the saline environment in which they naturally grow and their earlier mentioned mechanisms of tolerance to salt stress (Lopes et al., 2023). These halophytes are required to adjust the water potential of their tissues to a lower level than the water

in the soil in order to grow in saline environments and to ensure water uptake. Decreased turgor pressure, decreased cell size and osmotic processes are methods that are commonly used by these halophytes to adjust water potential in their tissues. In *S. ramosissima*, it is mainly performed due to osmosis, by accumulation of inorganic ions, mostly sodium and chloride, in cell vacuoles (Katschnig et al., 2013). To protect metabolic processes from the potential harmful effects of high inorganic ion concentrations, halophytes maintain elevated levels of compatible organic solutes within their cytoplasm. The means by which minerals are stored and distributed in these plants, especially in *Salicornia* spp. is heavily shaped by both external environmental factors such as soil and water properties, pollutants and climate, and internal factors, including the developmental stage of the plant and the specific tissues involved (Díaz et al., 2013; Flowers & Colmer, 2008). Hence, the noticeable higher ash content in biomass from salinity 35 in the two cuttings is likely due to the higher NaCl level in the used irrigation water.

The higher NaCl contents for these treatments are somehow confirmed by the salty flavour test performed on the biomass of the second cutting, which indicates that both 35 salinities show a significant increase of saltiness compared to the lower salinities. Salty flavour test was only performed on the plant biomass from the second cutting. The test was not performed in the biomass of the first cutting due to reduced availability of biomass. Biomass of stems and roots had no significant use for this analysis, as these would not be for human consumption. Reported values of 10.1 dS/m at a salinity of 200 mM NaCl (approximately 11.7 PSU) and 11.9 dS/m at 350 mM NaCl (approximately 20 PSU) by Lima et al. (2020) are slightly higher than observed in this research. Nevertheless, the trend of increasing salty flavour with the increase of salinity levels is very similar. Additionally, similar trends were reported in halophytes *I. crithmoides* and *M. nodiflorum* (Lima et al., 2021).

In terms of protein content, the relatively higher content of protein in the biomass of the first cutting compared to the second cutting could be due to the stress induced by performing of the cuttings. This induced stress could have similar effects as saline stress, which has been shown to down regulate the energy metabolism and protein synthesis (Bandeagh & Taylor, 2020). Under stress, plants can shift their metabolic focus from protein synthesis to the production of ATP and amino acids. This shift enables cellular functions that are critical for stress response, such as osmoregulation and stress tolerance (Ferreira et al., 2023). Moreover, the generally higher protein content in the mud-based system compared to the semi-hydroponics system is likely due to the

higher nitrogen availability in the substrate of the mud-based system (Soto-Sierra et al., 2018). Furthermore, the recorded protein content, 11.5% DW in the semi-hydroponics system and 13.7 % DW in the mud-based system, is substantially higher than those reported by Barreira et al. (2017) and Lopes et al. (2021), of 5.20 % (DW) and 4.16% (DW), respectively. Particularly in the first and second cutting, in the edible parts, the protein content was considerably higher. Lima et al. (2020) reported that the protein content in higher salinities, 350 mM NaCl and 465 mM NaCl (approximately 20 and 27 PSU, respectively) is not affected much compared to the lower salinities. Results in this study showed that protein content reduced with the higher salinities of 35 PSU. Therefore, these results suggest that protein content tends to be highest at moderate salinities of around 20-27 PSU, after which they seem to decrease with higher salinities. Finally, the observed average protein levels of 12.6 % (DW) indicates a protein content of 1.7 grams at a consumption of 100g fresh tips. Highest protein content of 2.8 grams was recorded in the first cutting at salinity 20 in the semi-hydroponics system. Although not particularly high, it suggests a potential for using *Salicornia* in more plant-based diets. The serious detrimental environmental effects of animal protein consumption (Rust et al., 2020) could thereby be mitigated by moving towards a more plant-based diet.

4.3 Minerals implications

The most abundant macro minerals in the tested biomass were the macronutrients Na, K, Mg, Ca and P, while the major micronutrients were Mn and Fe. The use of potting soil together with the mud from the decantation pond as a substrate for the mud-based system likely resulted in the general higher mineral content due to higher nutrient availability. Singh et al. (2014), cultivating multiple *Salicornia* species in hydroponics and sand-substrate conditions, reported similar results and indicated that hydroponic systems, in absence of solid substrates, prevent plants from forming micro-environments, therewith reducing the ability to take up micronutrients. Moreover, Mesa-Marín et al. (2020) reported that presence of potential beneficial microorganisms in the rhizosphere of the substrate could alter nutrient availability and consequently, nutrient uptake. This could have caused the generally higher mineral content in the mud-based system.

Nevertheless, the different dilution factors used in the assessment of sodium and phosphorus may have resulted in slightly altered outcomes than when a single dilution factor would have been used. Additionally, the significant differences observed between the Na values

between treatments, particularly in the first cutting, indicate there may have been a technical problem in the analysing process. The critical review on MP-AES by (Balaram, 2020) stated that analyses of biomass with high salinity levels, particularly for trace elements, is very challenging due to matrix interferences from its high dissolved salt content. For instance, the presence of high concentrations of Na or other ions in the sample matrix might interfere with the emission signal, making it difficult to quantify Na accurately. These matrix effects could significantly degrade the quality of the signal (Balaram & Sawant, 2020). Hence, these potential matrix interferences could result in artificially low sodium concentrations in higher salinity samples. Therefore, the obtained values of Na and P, particularly Na, are to be interpreted with caution due to the mentioned different dilution factors used and analysing issues.

4.3.1 Macroelements

The level of the major macromineral detected, Na, tended to naturally increase by the increase of salinity, particularly in the second cutting, and the highest value was recorded at 35 salinities. The semi-hydroponics system of the first cutting indicated large decrease in Na levels. However, this is likely related to the analysis problems mentioned above. Biomass from the mud-based system generally had higher Na levels than the semi-hydroponics system. Soil particles, particularly clay and organic matter, have a high cation exchange capacity (CEC), which allows them to retain positively charged ions, including sodium (Na⁺) (Weil & Brady, 2017). This could have likely caused the higher Na levels in plants cultivated in the mud-based system. The World Health Organisation (WHO) recommends a maximum Na intake of 2000mg per day. Hence, due care must be taken in order not to exceed the maximum allowed daily dose recommended by WHO. Although observed Na levels were high, with highest Na content recorded at 332.74 mg/100 g DW, these values are still below daily recommended intake considering a consumption of 100 g of fresh tips. However, with the high fluctuation in values, the analysis problems should be considered, and results on Na levels are yet to be defined and confirmed.

The Ca content in the shoots from first and second cutting generally reduced with the increase of salinity. Particularly in the first cutting of the mud-based system, Ca content reduced by nearly 50% when salinity increased from 10 and 20 PSU to 35 PSU. In the second cutting, however, these large differences were not noted. Research by Flowers & Colmer (2008) observed that concentrations of macronutrients generally, in contrary to Na, tend to decrease with the

increase of salinity, likely as a result of inhibition of uptake due to competition by excessive Na present. Although this is in accordance with observed Ca and P levels, K and Mg levels did not follow this decrease tendency. Additionally, the biomass content in Ca, K and Mg in salinity 35 is lower than in salinity 35 without additional fertiliser in the semi-hydroponics system, where in the mud-based system the opposite trend was found. This indicates that the use of the two different production systems, together with the addition of fertiliser, seems to affect uptake of said minerals.

While direct causes remain unclear, one possible explanation is nutrient dilution (ND). ND occurs when plant tissues exhibit lower concentrations of essential elements as plant biomass increases. This is often accompanied by a decline in concentrations of sugars, amino acids, and minerals (Kaspari & Welti, 2024). Factors such as climate conditions or CO₂ enrichment can drive this phenomenon, leading to increased biomass while diluting nutrient content (Welti et al., 2020). In the semi-hydroponics system, the reduced mineral content in salinity 35, compared to the treatment without fertiliser, could be linked to greater plant biomass production, leading to ND. Additionally, van Grinsven et al. (2022) highlighted the importance of optimising fertiliser use to sustain high yields without compromising nutrient density, as excessive or imbalanced fertiliser use may boost plant growth but inadvertently lead to nutrient dilution, similar to what might have occurred in the semi-hydroponics system.

Another possible cause could have been nutrient availability. Fertiliser provided in salinities 10, 20 and 35 contained nutrients, in particular N, P and K. Salinity 35 therefore naturally had more nutrient availability than salinity 35 without added fertiliser. Hence, enhancing nutrient availability and promoting better uptake of, in case the mud-based system, Ca, K, and Mg. This could be due to the higher quantity of organic matter in the mud-based system and other minerals to hold nutrients more effectively, releasing them over time and ensuring more consistent absorption by the plants. In the semi-hydroponics system however, especially with high salinity stress, earlier mentioned competition for uptake of minerals increases and could cause plants to promote nutrient uptake (Flowers & Colmer, 2008). Nutrients supplied by the addition of fertiliser, such as N and P, will therefore be more readily available in salinity 35 of the semi-hydroponics system, resulting possibly in their lower content of Ca, K and Mg compared to salinity 35 without added fertiliser. Nevertheless, more research regarding nutrient dynamics in *Salicornia* spp. is required to interpret the direct causes of these differences.

Furthermore, resulting macroelement minerals were significantly higher than those reported by Lopes et al. (2021), who analysed wild *S. ramosissima* plants. However, research by (Díaz et al., 2013) reported relatively similar results of Mg and Ca in their cultivation of *S. bigelovii* in highly saline soil (28.6 dS/m), although observed Ca levels in their study were generally marginally elevated and Mg slightly reduced. Moreover, apart from K and Fe, analysed wild *S. ramosissima* plants by Barreira et al. (2017) showed similar levels to those observed in this study. The Na content observed in this work was generally much higher than those obtained in the above-mentioned studies. Lima et al. (2020) indicated similar results and observed an increase of Na with the increase of salinity in cultivated *S. ramosissima*, which appeared to stagger at 275 mM NaCl (approximately 16 PSU). This trend of Na levels remaining consistent at higher salinities was not observed in the results of this study. Additionally, the values recorded were significantly higher than those reported on *S. ramosissima* by Lima et al. (2020). However, the differences in Na observed in this research and those reported by above-mentioned studies are likely due to the analysing issues for Na. Moreover, the macroelements in the biomass of stems and roots showed similar patterns across the two cuttings. Notably, the roots had a significantly higher P content. This is consistent with the primary role of *Salicornia* spp. roots in nutrient absorption from the soil, particularly P. Accordingly, Brown et al. (1999) reported that halophytes tend to accumulate high quantity of phosphorus in roots, compared to other parts of the plants.

4.3.2 Microelements

Similarly to the macroelements, the microelements of the first and second cutting generally followed a decrease of microelement content with the increase of salinity, similar to observations by Flowers & Colmer (2008). Research by Lima et al. (2020) cultivated *S. ramosissima* in multiple salinities and indicated a similar increase of microelement levels with the increase of salinity to moderate levels of 275 mM NaCl (approximately 16 PSU), after which levels started to decrease with higher salinity levels of 465 mM NaCl (approximately 27 PSU).

Metals such as Fe and Mn presented high levels in the aboveground tissues. Research shows that Fe and Mn usually present higher translocation factors, as they are important for photosynthesis, particularly in electron transport and enzyme activation, and are therefore generally present in aboveground tissues at higher concentrations (El Amine et al., 2023). Aside of that, Nikalje et al. (2017) reported that *Salicornia* spp. through their accumulation mechanism

absorb toxic salt ions and rapidly and translocate them towards aerial parts such as leaves. These ions are sequestered into vacuoles to avoid accumulation in the cytoplasm. Similar to salt ions, these plants have been reported to accumulate (toxic) metal ions in the aerial parts (Nikalje & Suprasanna, 2018). The high Mn levels showed a highest recorded concentration of 0.36 mg/g DW observed at salinity 10 in the mud-based system from the first cutting. However, despite these elevated levels, consuming 100 g of fresh tips would remain below recommended daily intake of 2 mg by the WHO. The *Salicornia* spp. appear to be resistant to high levels of Mn and reports of elevated concentrations in *Salicornia* are frequently found in the literature (Ventura & Sagi, 2013).

Moreover, the Commission Regulation (EU) 2023/915 indicated that metals Pb, Cd, Hg, As and Sn have a maximum level intake. Pb and Cd, analysed and quantified in this study, have maximum legislated values of 0.3 mg/kg WW for Pb and 0.1 mg/kg WW for Cd. Although no values for Cd were detected, Pb values were with averagely 0.91 mg/kg WW in the semi-hydroponics system and 0.93 mg/kg WW in the mud-based system above the described maximum levels. Their relatively high Pb levels are curious, as they were present in both systems, and all three water sources, including the mixture of freshwater with brackish well water (10 PSU) and brackish well water (20 PSU). Hence, this indicates that the high Pb values cannot strictly be attributed to the aquaculture wastewater and mud. Nevertheless, with regards to the use of aquaculture wastewater for *Salicornia* irrigation, careful consideration is required. Aquaculture wastewater in many cases has been reported to contain metals (Emenike et al., 2022). This naturally highly depends on the location of the aquaculture farm, their methods and materials, including fish feed, they use. The strong ability of *Salicornia* to accumulate metals such as Zn, Cr, Pb, Ni and Cd (Caetano et al., 2008) should not be underestimated and could become problematic for commercial purposes when cultivated at polluted locations.

4.4 Bioactive compound implications

Phenolic compounds have many advantages for consumers, such as anti-aging, anti-inflammatory, antioxidant and antiproliferative agents (Lin et al., 2016). Moreover, relevant antioxidant enzymes are able to counter oxidants. Polyphenols, particularly flavonoids, phenolic acids, and tannins, play a crucial role in inhibiting α -glucosidase and α -amylase, two key enzymes responsible for breaking down carbohydrates into glucose (Reetu et al., 2023). Plant-based polyphenols and polyphenol-rich foods influence the metabolism of carbohydrates and fats, helping to reduce high blood sugar,

abnormal lipid levels, and insulin resistance. They also enhance the function of β -cells, boost insulin production, improve fat metabolism, and alleviate oxidative stress, as well as stress-related signalling and inflammation. Additionally, polyphenolic compounds may help prevent long-term diabetes complications such as cardiovascular disease, neuropathy, kidney damage, and vision problems (Moo-Huchin et al., 2015; Shukitt-Hale et al., 2008). Hence, adequate levels of phenolic compounds in the edible biomass of *S. ramosissima* proves useful. Production of phenolic compounds can be induced by stress, such as salinity, drought, UV radiation and more (Alhdad et al., 2013). In controlled and covered growing places such as greenhouses, plants are shielded and protected from some of the extreme environmental conditions to which they would be exposed in the wild. These conditions, usually presenting (abiotic) stress factors to the plants, are considered to be responsible for the production of secondary metabolites such as the phenolic compounds (Lima et al., 2020). During abiotic stress conditions, the biosynthesis of most phenolic compounds such as phenolic acids, flavonoids, lignin, and tannins increases and thus help the plant to withstand these environmental constraints (Reetu et al., 2023). Therefore, *S. ramosissima* plants collected from wild and cultivated in controlled environments may present very different levels of secondary metabolites (Antunes et al., 2021; Castañeda-Loaiza et al., 2020).

In this work, the TPC of biomass from the first cutting of plants cultivated in salinities 10 and 20 was lower than those reported for the same species, cultivated in greenhouse conditions under similar salinities (Lima et al., 2020). However, the TPC of biomass from those salinities in the second cutting were higher. The TPC results of all samples were generally lower than the values reported by Correia et al. (2022) on cultivated *S. ramosissima* supplied by RiaFresh. However, reported values ranging between 8.34 and 15.02 GAE/g DW by Silva et al. (2021), also for *S. ramosissima*, but from wild samples, were more similar to those observed in this study. Yet, in this research larger margins in TPC levels were obtained between first and second cutting. Additionally, TPC of both cuttings were generally higher in the semi-hydroponics system compared to the mud-based system. Research shows that *Salicornia* species increase their phenolic content with rising salinity, reaching a maximum threshold after which plants may either lose the capacity to maintain phenolic production or redirect their energy resources (Lima et al., 2020; Ventura et al., 2011, 2015). Slama et al. (2017) suggest that at elevated salinity levels, plants may shift their energy allocation toward other protective mechanisms, such as osmolyte production or salt repulsion, to combat salinity stress. While a decline in total phenolic content (TPC) and total flavonoid content

(TFC) due to increased salinity is often observed in mud-based systems, this trend is not as pronounced in semi-hydroponics systems. It is important to recognise that the synthesis, accumulation, and degradation of secondary metabolites are influenced by several factors, including soil and water composition, UV radiation, and the plant's life stage (Castagna et al., 2022; Lopes et al., 2023). Moreover, TPC was significantly higher in the second cutting, likely due to the stress induced by the first cutting. As a response to physical damage, reactive oxygen species (ROS) are produced, and antioxidants, including phenolic compounds, can control the levels of ROS (Alhdad et al., 2013).

Very similar TPC and TFC were observed from plants of the second cutting. However, these similarities were not seen in the first cutting. Plants from the first cutting showed lower TPC, while TFC was higher. These differences between first and second cutting in TPC and TFC were likely due to earlier mentioned performed cutting and responses to stressors. Flavonoids play a more immediate role in protecting cells against UV damage and oxidative stress and might therefore be produced at a different rate compared to phenolics in the first cutting (Manzoor et al., 2023). This could result in more variability between TPC and TFC at the first cutting. Nevertheless, TFC results in the two cuttings were generally higher than the values reported by De Souza et al. (2018) on cultivated *S. neei*. It is, however, challenging to directly compare TFC results with other studies, as extraction protocols for TFC are often slightly modified, leading to variations in methodology. Furthermore, TPC in the stems is high, especially when compared to TFC and TTC, in the biomass from the stems. Stems require a high number of phenolic compounds, especially those involved in lignin biosynthesis. As a complex phenolic polymer, lignin provides rigidity, structural support and helps regulate water transport (Liu et al., 2018). Since stems are highly lignified tissues, and lignification leads to a higher accumulation of phenolic compounds, it results in high TPC in the stems compared to lower TFC and TTC in the stems.

Photosynthetic pigments, including chlorophyll a and b and carotenoids, are the pigments used for photosynthesis, the capturing of light and converting it into chemical energy (Krayem et al., 2021). They are important in the prevention and combating of oxidative stress in the photosystem, called photooxidative stress (De Souza et al., 2018). These pigments are valued for their colour and roles in health, nutrition, pharmaceuticals, cosmetics, and biotechnology (Qaisar et al., 2019). In this work, the highest levels of chlorophylls were detected in biomass from the lower salinities, particularly for the mud-based system. The chlorophyll content of biomass from

salinity 10 of the semi-hydroponics system, however, was relatively low, with samples from the mud-based system having generally a higher chlorophyll content. This is in line with results reported by other authors, analysing cultivated *S. neei* (De Souza et al., 2018), wild *S. europaea* (Cárdenas-Pérez et al., 2022) and wild *S. prostata* (Akcin & Yalçın, 2015). Interestingly, in the first and second cutting, chlorophyll content at salinity 20 in the semi-hydroponics system showed a spike in content. This may be explained by the lower nutrient availability combined with a stimulation of chlorophyll synthesis in moderate salinities, as reported by study on wild *S. prostata* (Akcin & Yalçın, 2015). Moreover, low levels of chlorophylls were detected in biomass from salinity 10 in the semi-hydroponics system. This likely due to the low salinity levels or low nutrient availability, or its combined effect, as potentially salinity 10 does not provide sufficient salinity stress and/or affects nutrient uptake to trigger chlorophyll synthesis. However, this is speculative, and more research is required to understand these effects. Furthermore, biomass from cuttings presented similar levels of pigments. Chlorophyll content in non-edible biomass, stems and root, was much lower as expected, because the shoots are the primary sites for photosynthesis, since they contain a higher quantity of chloroplasts.

Carotenoids were not detected in biomass from the mud-based system. Overall, higher levels were observed in biomass from the lower salinities, which is in line with results reported by Aghaleh et al. (2009). Moreover, results are similar to those observed for total chlorophylls, where total carotenoids decreased with the increasing salinity. As mentioned earlier with TPC, the plants likely shift their energy towards other protective mechanisms to protect against the higher salinities (Slama et al., 2017).

4.5 Antinutritional properties implications

In the green shoots of the first and second cutting, tannins, similar to other secondary metabolites, play a significant role in defending against herbivores, pathogens, and environmental stressors. Tannins can act as deterrents to herbivores and protect the plant from microbial infections (Barbehenn & Peter Constabel, 2011). Although tannins were present in the stems and roots, they are generally, like other phenolic compounds, more involved in the photosynthetic active tissues such as the shoots, and even more abundant in vulnerable parts of plants, such as new leaves and flowers (Tong et al., 2022). Lima et al. (2020) reported an increase of TTC with the increase of salinity with cultivated *S. ramosissima*, with highest levels (32.5 mg CE/g DW) recorded at their

maximum salinity of 465 mM NaCl (approximately 27 PSU). Their lowest value (22.1 mg CE/g DW) was recorded at lowest salinity of 35 mM NaCl (approximately 2 PSU). TTC levels in plants observed in this study tended to increase from salinity 10 to salinity 20, after which they decreased again in the higher salinities. Although TTC levels were significantly lower than those observed by (Lima et al., 2020), the trend where TTC increases by increase of salinity suggests that TTC levels present were highest at around 20-27 PSU, after which they seem to decrease. Additionally, Hulkko et al. (2023) reported to have had no values for TTC detected on *S. ramosissima*, both cultivated in organic open-field conditions and from wild. Furthermore, regarding antinutritional effects, review by Hoque et al. (2024) states that regular tannin consumption of 1.5 to 2.5 g is healthy and has no negative effects. Observed values for TTC, 0.42 mg CE/g FW for the average TTC of total edible biomass and 0.64 mg CE/g FW for highest recorded TTC at salinity 20 of the semi-hydroponics system from the first cutting, are relatively low. Regarding TTC, consumption of 100g of fresh tips would therefore not show any negative antinutritional effects and would be considered healthy.

4.6 Trial effects

The findings from this research provide insight into how the two production systems, semi-hydroponics and mud-based substrate, could favour the production of *S. ramosissima*. It demonstrates an overview where the production systems together with the use of different salinities as treatments provide insight into how and in what best way such a cultivation system could be implemented in for instance aquaculture fish farms. The concept of implementing such a cultivation system in aquaculture farms would optimise general productivity, reduce freshwater scarcity and improve circular economy, as it would have the potential to reduce pollution by optimising aquaculture wastewater. Research by Cárdenas-Pérez et al. (2021) also stresses this by noting that the importance of *Salicornia* not only lies in its potential biomass use for consumption as vegetable, bioprocessing into products such as bioethanol, or the oil content of seeds, but also in its response to watering and capacity to survive and reproduce in high salinity environments.

S. bigelovii has been used globally to help restore local ecosystems, demonstrating effective nutrient removal as a biofilter. Additionally, constructed wetlands have shown positive effects for *Salicornia* spp. using aquaculture effluent (Diaz et al., 2020; Lee et al., 2009; Turcios et al., 2021). Constructed wetlands were developed by Shpigel et al. (2013) with *S. bigelovii*, demonstrating an

uptake of nitrogen by the plants of approximately 60% of total dissolved nitrogen. Moreover, Diaz et al. (2020) also reported high nitrogen removal efficiency, next to significant biomass growth, of *S. neei*. Moreover, research by Castilla-Gavilán et al. (2024) demonstrated positive results by growing *S. ramosissima* in two distinct hydroponic systems with RAS effluents, indicating that the experimental systems minimised waste and maximised resources utilisation. These characteristics make *Salicornia* a suitable candidate as a biofilter to recycle water and nutrients contained in effluent streams from marine aquaculture, and as a phytoremediator of saline soils polluted with heavy metals (Khalilzadeh et al., 2021; Mesa-Marín et al., 2020).

The results from this study demonstrate that over the course of the six weeks cultivation trial, all plants in all treatments showed consistent growth, indicating the resilience and adaptability of *S. ramosissima* to high saline environments, typically found in aquaculture settings. The addition of fertiliser in salinity to the 35 PSU treatment resulted in higher biomass weight in both production systems, yet these differences were not significant. Although the mud-based system produced significantly more non-edible biomass than in the semi-hydroponics system, edible biomass production was similar. In fact, salinity 35 without additional fertiliser had significantly higher edible biomass in the mud-based system. Thus, the addition of fertiliser in the mud-based system did not seem to affect edible biomass production. This suggests that the addition of supplementary fertiliser is not strictly necessary in the mud-based system.

In contrast, the semi-hydroponics system showed a greater dependency on additional fertiliser at salinity 35 PSU. Without fertiliser, the semi-hydroponics system showed reduced nutritional quality, particularly in terms of ash content, protein, and bioactive compounds like TFC. Nutrient deficits in this system appeared to limit the ability of the plants to thrive in these highly saline conditions (35 PSU). However, when fertiliser was added, the semi-hydroponics system showed improvements in biomass and biochemical composition, although still remaining below those found in mud-based system. Hence, when opting for a hydroponic system, special attention should be given to providing plants with appropriate nutrient availability.

In most cases, the mud-based system provided more commercially beneficent results than the semi-hydroponics system. Among those are plant weight, protein content, liposoluble pigments, ash and mineral content, showing augmented content, particularly when looking at the 35 PSU salinities. Bioactive compounds such as TPC and TFC however, generally showed slightly

higher contents in the semi-hydroponics system. Moreover, the increase of salinity quite clearly caused the decrease protein and most minerals, apart from Na.

Due to the low sample sizes and sometimes high standard deviations, it is difficult to derive significant conclusions regarding treatment effects on biomass weights and some of the performed mineral and biochemical analyses. A larger sample size would be recommended for future research to reduce measurement error. A higher number of treatment replicates would give higher statistical power and thus make outcomes more reliable. Moreover, for forthcoming studies the statistical Scheirer–Ray–Hare test should be considered, instead of the Kruskal-Wallis test, in order to gain more reliable insights regarding the small sample size and non-parametric distribution. Presenting an extension of the Kruskal-Wallis test, the Scheirer–Ray–Hare test is essentially a non-parametric version of a two-way ANOVA, allowing for interaction effects between factors and better accounting for low sample sizes.

Moreover, considered should be that plants used were already approximately 15 cm when bought. For a full overview on the potential of *S. ramosissima* in these conditions, seeds should be germinated in the same conditions. Furthermore, for future research, considered should be performing of cuttings at a lower height than 15 cm. This would be more comparable to commercial methods and would likely alter biomass production results, as more biomass would be harvested by each cutting. Additionally, to fully comprehend what affect the treatments with higher salinities have on the growth and biochemical properties of *S. ramosissima*, a longer cultivation trial would be recommended for future research, as six weeks is not enough to demonstrate how these plants are affected by these treatments on a potential full cultivation season. Such extended-duration trials could shine light on the total number of harvests attainable in practice, which is obviously of economic value. Moreover, in order to fully grasp the effect the aquaculture wastewater has on the *S. ramosissima* plants, future research should investigate the use seawater as another treatment, next to the aquaculture wastewater treatments (35 PSU) and compare outcomes. Additionally, nutrients present in the aquaculture wastewater and mud should be analysed to provide a more comprehensive view on their potential. Moreover, finding optimal fertiliser ranges for *Salicornia* spp., particularly *S. ramosissima*, would benefit cultivation, as it would enable to maximise plant performance without inhibition or nutrient imbalances.

Lastly, exploring options to use wasteful, non-edible parts, stems and roots could be well-suited for future research. Although findings in this study concluded that beneficial parts of the plants are mostly in the edible tips, the non-edible stems and roots in many cases still contained valuable resources. Mineral content in the stems was consistently present, though not in the highest concentrations. Aside of that, protein content in the stems and roots was relatively low compared to the fresh tips. Yet, with averages of between 5 to 10 % DW protein did contain considerable contents and should therefore not be disregarded. Non-edible biomass however, demonstrated low bioactive compound levels, particularly TFC. Although, TPC did in fact provide considerable contents in the non-edible biomass. Consequently, the use and/or processing of these non-edible parts, stems and roots, could be interesting for future research. Barreto et al. (2024) reported no significant differences in performance when wheat meal of the diets of juvenile European seabass (*Dicentrarchus labrax*) was replaced with up to 10% of non-edible by-products of *S. ramosissima*. Additionally, Belal & Al-Dosari, (1999) replaced fish meal in their feed for Nile tilapia (*Oreochromis niloticus*) by *S. bigelovii*, whole plant biomass. They reported that *Salicornia* meal could replace up to 40% of the fish meal in *O. niloticus* feeds without affecting their growth or body composition. The results of these studies demonstrate the potential of valorisation for the non-edible biomass of *Salicornia* species.

5 Conclusion and future recommendations

This study provides a comprehensive overview on the potential for cultivation of *Salicornia ramosissima* in two production systems under varying salinity levels from water sources including aquaculture wastewater from a semi-intensive pond system, brackish well water and fresh water. Addition of fertiliser in the high salinity treatments (35 PSU) did not seem to affect growth or nutritional properties of biomass in both production systems. Secondary metabolites however, tended to be higher in biomass cultivated without supplementary fertiliser. The mud-based substrate system consistently produced higher biomass, particularly at the highest salinity levels (35 PSU), compared to the semi-hydroponics system. It also showed enhanced biochemical properties, including ash, mineral and protein content, aside of high accumulation of secondary metabolites. These outcomes indicate that the mud-based substrate system supports *S. ramosissima* growth effectively in highly saline conditions, likely due to higher nutrient availability. The semi-hydroponics system showed higher sensitivity to salinity. While moderate salinities (20 PSU) induced better growth, both lower (10 PSU) and higher salinities (35 PSU) led to slightly reduced performance. The slightly reduced performance of the semi-hydroponics system highlights the importance of appropriate nutrient supplementation. These findings suggest that the mud-based substrate system tends to be more robust and better suited for environments with fluctuating or high salinity, while the semi-hydroponics system may require more careful management of salinity levels and nutrient inputs to ensure its consistent productivity. Future research recommendations include the use of larger sample sizes, germination and growing of seeds, cutting of shoots similar to methods of commercial farms, longer cultivation trial such as a full cultivation season, analysing of nutrients present in both wastewater and mud from settling pond and lastly, further investigation into non-edible biomass use for fish feed additives. In conclusion, the use and integration of the halophyte *S. ramosissima* as an effective component to valorise aquaculture effluents shows promise. It has the potential to promote circular economy and to reduce freshwater use whilst valorising aquaculture effluents as a biofilter and produce valuable crop biomass.

6 References

- Aghaleh, M., Niknam, V., Ebrahimzadeh, H., & Razavi, K. (2009). Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. *Biologia Plantarum*, 53(2), 243–248. <https://doi.org/10.1007/s10535-009-0046-7>
- Agilent. (2023). *Elemental Analysis using ICP-OES, Flame AAS or MP-AES*. Agilent. <https://www.agilent.com/en/product/atomic-spectroscopy/icp-oes-vs-flame-aas-vs-mp-aes#>
- Akcin, A., & Yalçın, E. (2015). Effect of salinity stress on chlorophyll, carotenoid content, and proline in *Salicornia prostrata* Pall. and *Suaeda prostrata* Pall. Subsp. Prostrata (Amaranthaceae). *Brazilian Journal of Botany*, 39. <https://doi.org/10.1007/s40415-015-0218-y>
- Alhdad, G. M., Seal, C. E., Al-Azzawi, M. J., & Flowers, T. J. (2013). The effect of combined salinity and waterlogging on the halophyte *Suaeda maritima*: The role of antioxidants. *Environmental and Experimental Botany*, 87, 120–125. <https://doi.org/10.1016/j.envexpbot.2012.10.010>
- Antunes, M., Gago, C., Guerreiro, A., Sousa, A., Julião, M., Miguel, M., Faleiro, M., & Panagopoulos, T. (2021). Nutritional Characterization and Storage Ability of *Salicornia ramosissima* and *Sarcocornia perennis* for Fresh Vegetable Salads. *Horticulturae*, 7(1), 6. <https://doi.org/10.3390/horticulturae7010006>
- Balaram, V. (2020). Microwave plasma atomic emission spectrometry (MP-AES) and its applications – A critical review. *Microchemical Journal*, 159, 105483. <https://doi.org/10.1016/j.microc.2020.105483>
- Balaram, V., & Sawant, S. S. (2020). Indicator Minerals, Pathfinder Elements, and Portable Analytical Instruments in Mineral Exploration Studies. *Microchemical Journal*, 159, 105483. <https://doi.org/10.1016/j.microc.2020.105483>
- Bandehagh, A., & Taylor, N. L. (2020). Can Alternative Metabolic Pathways and Shunts Overcome Salinity Induced Inhibition of Central Carbon Metabolism in Crops? *Frontiers in Plant Science*, 11, 1072. <https://doi.org/10.3389/fpls.2020.01072>
- Barbehenn, R. V., & Peter Constabel, C. (2011). Tannins in plant–herbivore interactions. *Phytochemistry*, 72(13), 1551–1565. <https://doi.org/10.1016/j.phytochem.2011.01.040>
- Barreira, L., Resek, E., Rodrigues, M. J., Rocha, M. I., Pereira, H., Bandarra, N., Da Silva, M. M., Varela, J., & Custódio, L. (2017). Halophytes: Gourmet food with nutritional health benefits? *Journal of Food Composition and Analysis*, 59, 35–42. <https://doi.org/10.1016/j.jfca.2017.02.003>
- Barreto, A., Couto, A., Jerónimo, D., Laranjeira, A., Silva, B., Nunes, C., Veríssimo, A. C. S., Pinto, D. C. G. A., Dias, J., Pacheco, M., Costas, B., & Rocha, R. J. M. (2024). *Salicornia ramosissima* Biomass as a Partial Replacement of Wheat Meal in Diets for Juvenile European Seabass (*Dicentrarchus labrax*). *Animals*, 14(4), Article 4. <https://doi.org/10.3390/ani14040614>
- Barros, J., Serk, H., Granlund, I., & Pesquet, E. (2015). The cell biology of lignification in higher plants. *Annals of Botany*, 115(7), 1053–1074. <https://doi.org/10.1093/aob/mcv046>
- Bastardie, F., Salvany, L., Cooper, A. M., & Carvalho, N. (2024). A roadmap to reduce the risk of overexploiting EU marine living resources in a changing ocean. *Frontiers in Marine Science*, 11. <https://doi.org/10.3389/fmars.2024.1352500>

- Belal, I. E. H., & Al-Dosari, M. (1999). Replacement of Fish Meal with *Salicornia* Meal in Feeds for Nile Tilapia *Oreochromis niloticus*. *Journal of the World Aquaculture Society*, 30(2), 285–289. <https://doi.org/10.1111/j.1749-7345.1999.tb00877.x>
- Bennett, A. E., Grussu, D., Kam, J., Caul, S., & Halpin, C. (2015). Plant lignin content altered by soil microbial community. *New Phytologist*, 206(1), 166–174. <https://doi.org/10.1111/nph.13171>
- Bennett, T. H., Flowers, T. J., & Bromham, L. (2013). Repeated evolution of salt-tolerance in grasses. *Biology Letters*, 9(2), 20130029. <https://doi.org/10.1098/rsbl.2013.0029>
- Bhavsar, D. O., Pandya, H. A., & Jasrai, Y. T. (2016). *Aquaculture and Environmental Pollution: A Review Work*. 2, 40–45.
- Binh, C. T., & Phillips, M. J. (1997). Integrated shrimp-mangrove farming systems in the Meicong delta of Vietnam. *Aquaculture Research*.
- Bligh, E. G., & Dyer, W. J. (1959). A Rapid Method Of Total Lipid Extraction And Purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917. <https://doi.org/10.1139/o59-099>
- Borah, S., Baruah, A. M., Das, A. K., & Borah, J. (2009). Determination of Mineral Content in Commonly Consumed Leafy Vegetables. *Food Analytical Methods*, 2(3), 226–230. <https://doi.org/10.1007/s12161-008-9062-z>
- Boyd, C. E. (2019). *What is the role of bacteria in aquaculture ponds?* Responsible Seafood Advocate. <https://www.globalseafood.org/advocate/what-is-the-role-of-bacteria-in-aquaculture-ponds/>
- Breil, C., Abert Vian, M., Zemb, T., Kunz, W., & Chemat, F. (2017). “Bligh and Dyer” and Folch Methods for Solid–Liquid–Liquid Extraction of Lipids from Microorganisms. Comprehension of Solvation Mechanisms and towards Substitution with Alternative Solvents. *International Journal of Molecular Sciences*, 18(4), 708. <https://doi.org/10.3390/ijms18040708>
- Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, 9(15), 1551–1558. <https://doi.org/10.1096/fasebj.9.15.8529834>
- Brown, J. J., Glenn, E. P., Fitzsimmons, K. M., & Smith, S. E. (1999). Halophytes for the treatment of saline aquaculture effluent. *Aquaculture*, 175(3), 255–268. [https://doi.org/10.1016/S0044-8486\(99\)00084-8](https://doi.org/10.1016/S0044-8486(99)00084-8)
- Buhmann, A., Hellmueller, L., & Bosshart, L. (2015). *Popular Culture and Communication Practice*. 34(3), 43.
- Buhmann, A., & Papenbrock, J. (2013a). An economic point of view of secondary compounds in halophytes. *Functional Plant Biology*, 40(9), 952. <https://doi.org/10.1071/FP12342>
- Buhmann, A., & Papenbrock, J. (2013b). Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. *Environmental and Experimental Botany*, 92, 122–133. <https://doi.org/10.1016/j.envexpbot.2012.07.005>
- Caetano, M., Vale, C., Cesário, R., & Fonseca, N. (2008). Evidence for preferential depths of metal retention in roots of salt marsh plants. *The Science of the Total Environment*, 390, 466–474. <https://doi.org/10.1016/j.scitotenv.2007.10.015>
- Cárdenas-Pérez, S., Piernik, A., Chanona-Pérez, J. J., Grigore, M. N., & Perea-Flores, M. J. (2021). An overview of the emerging trends of the *Salicornia* L. genus as a sustainable crop. *Environmental and Experimental Botany*, 191, 104606. <https://doi.org/10.1016/j.envexpbot.2021.104606>

- Cárdenas-Pérez, S., Rajabi Dehnavi, A., Leszczyński, K., Lubińska-Mielińska, S., Ludwiczak, A., & Piernik, A. (2022). *Salicornia europaea* L. Functional Traits Indicate Its Optimum Growth. *Plants*, *11*(8), Article 8. <https://doi.org/10.3390/plants11081051>
- Castagna, A., Mariottini, G., Gabriele, M., Longo, V., Souid, A., Dauvergne, X., Magné, C., Foggi, G., Conte, G., Santin, M., & Ranieri, A. (2022). Nutritional Composition and Bioactivity of *Salicornia europaea* L. Plants Grown in Monoculture or Intercropped with Tomato Plants in Salt-Affected Soils. *Horticulturae*, *8*, 828. <https://doi.org/10.3390/horticulturae8090828>
- Castañeda-Loaiza, V., Oliveira, M., Santos, T., Schüler, L., Lima, A. R., Gama, F., Salazar, M., Neng, N. R., Nogueira, J. M. F., Varela, J., & Barreira, L. (2020). Wild vs cultivated halophytes: Nutritional and functional differences. *Food Chemistry*, *333*, 127536. <https://doi.org/10.1016/j.foodchem.2020.127536>
- Castilla-Gavilán, M., Muñoz-Martínez, M., Zuasti, E., Canoura-Baldonado, J., Mondoñedo, R., & Hachero-Cruzado, I. (2024). Yield, nutrients uptake and lipid profile of the halophyte *Salicornia ramosissima* cultivated in two different integrated multi-trophic aquaculture systems (IMTA). *Aquaculture*, *583*, 740547. <https://doi.org/10.1016/j.aquaculture.2024.740547>
- Castine, S., McKinnon, A., Paul, N., Trott, L., & De Nys, R. (2013). Wastewater treatment for land-based aquaculture: Improvements and value-adding alternatives in model systems from Australia. *Aquaculture Environment Interactions*, *4*(3), 285–300. <https://doi.org/10.3354/aei00088>
- Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis*, *10*(3).
- Chopin, T., Cooper, J., Reid, G. K., Cross, S., & Moore, C. (2012). Open-water Integrated Multi-Trophic Aquaculture: Environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. *Reviews in Aquaculture*, *4*, 209–220. <https://doi.org/10.1111/j.1753-5131.2012.01074.x>
- Chung, K.-T., Wei, C.-I., & Johnson, M. G. (1998). Are tannins a double-edged sword in biology and health? *Trends in Food Science & Technology*, *9*(4), 168–175. [https://doi.org/10.1016/S0924-2244\(98\)00028-4](https://doi.org/10.1016/S0924-2244(98)00028-4)
- Correia, A., Silva, A. M., Moreira, M. M., Salazar, M., Švarc-Gajić, J., Brezo-Borjan, T., Cádiz-Gurrea, M. D. L. L., Carretero, A. S., Loschi, F., Dall'Acqua, S., Delerue-Matos, C., & Rodrigues, F. (2022). *Salicornia ramosissima*: A New Green Cosmetic Ingredient with Promising Skin Effects. *Antioxidants*, *11*(12), 2449. <https://doi.org/10.3390/antiox11122449>
- Cybulska, I., Chaturvedi, T., Brudecki, G. P., Kádár, Z., Meyer, A. S., Baldwin, R. M., & Thomsen, M. H. (2014). Chemical characterization and hydrothermal pretreatment of *Salicornia bigelovii* straw for enhanced enzymatic hydrolysis and bioethanol potential. *Bioresource Technology*, *153*, 165–172. <https://doi.org/10.1016/j.biortech.2013.11.071>
- De Souza, M. M., Mendes, C. R., Doncato, K. B., Badiale-Furlong, E., & Costa, C. S. B. (2018). Growth, Phenolics, Photosynthetic Pigments, and Antioxidant Response of Two New Genotypes of Sea Asparagus (*Salicornia neei* Lag.) to Salinity under Greenhouse and Field Conditions. *Agriculture*, *8*(7), Article 7. <https://doi.org/10.3390/agriculture8070115>

- Demain, A. L., & Fang, A. (2000). The Natural Functions of Secondary Metabolites. In A. Fiechter (Ed.), *History of Modern Biotechnology I* (Vol. 69, pp. 1–39). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-44964-7_1
- Díaz, F. J., Benes, S. E., & Grattan, S. R. (2013). Field performance of halophytic species under irrigation with saline drainage water in the San Joaquin Valley of California. *Agricultural Water Management*, *118*, 59–69. <https://doi.org/10.1016/j.agwat.2012.11.017>
- Díaz, M. R., Araneda, J., Osses, A., Orellana, J., & Gallardo, J. A. (2020). Efficiency of *Salicornia neei* to Treat Aquaculture Effluent from a Hypersaline and Artificial Wetland. *Agriculture*, *10*(12), 621. <https://doi.org/10.3390/agriculture10120621>
- El Amine, B., Mosseddaq, F., Naciri, R., & Oukarroum, A. (2023). Interactive effect of Fe and Mn deficiencies on physiological, biochemical, nutritional and growth status of soybean. *Plant Physiology and Biochemistry*, *199*, 107718. <https://doi.org/10.1016/j.plaphy.2023.107718>
- El-Amier, Y., Soufan, W., Almutairi, K., Zaghloul, N., & Abd-ElGawad, A. (2021). Proximate Composition, Bioactive Compounds, and Antioxidant Potential of Wild Halophytes Grown in Coastal Salt Marsh Habitats. *Molecules*, *27*(1), 28. <https://doi.org/10.3390/molecules27010028>
- ElNaker, N. A., Yousef, A. F., & Yousef, L. F. (2020). A review of *Arthrocnemum* (Arthrocaulon) *macrostachyum* chemical content and bioactivity. *Phytochemistry Reviews*, *19*(6), 1427–1448. <https://doi.org/10.1007/s11101-020-09686-5>
- Emenike, E. C., Iwuozor, K. O., & Anidiobi, S. U. (2022). Heavy Metal Pollution in Aquaculture: Sources, Impacts and Mitigation Techniques. *Biological Trace Element Research*, *200*(10), 4476–4492. <https://doi.org/10.1007/s12011-021-03037-x>
- European Commission. (2022). *Towards a Strong and Sustainable EU Algae Sector*. FAO. <https://doi.org/10.4060/cb5670en>
- FAO. (2007). *Cage Aquaculture: Regional Reviews and Global Overview*. Food & Agriculture Org.
- FAO. (2017). *The future of food and agriculture: Trends and challenges*. Food and Agriculture Organization of the United Nations.
- FAO. (2024). *The State of World Fisheries and Aquaculture 2024*. FAO. <https://doi.org/10.4060/cd0690en>
- Ferreira, M. J., Sierra-Garcia, I. N., Cremades, J., António, C., Rodrigues, A. M., Pinto, D. C. G. A., Silva, H., & Cunha, Â. (2023). Biostimulation of *Salicornia europaea* L. crops with plant growth-promoting bacteria in laboratory and field conditions: Effects on growth and metabolite profile. *Journal of Applied Microbiology*, *134*(3), lxad036. <https://doi.org/10.1093/jambio/lxad036>
- Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*, *55*(396), 307–319. <https://doi.org/10.1093/jxb/erh003>
- Flowers, T. J., & Colmer, T. D. (2008). Salinity tolerance in halophytes. *New Phytologist*, *179*(4), 945–963. <https://doi.org/10.1111/j.1469-8137.2008.02531.x>
- Flowers, T. J., Galal, H. K., & Bromham, L. (2010). Evolution of halophytes: Multiple origins of salt tolerance in land plants. *Functional Plant Biology*, *37*(7), 604. <https://doi.org/10.1071/FP09269>
- Fruscella, L., Kotzen, B., Paradelo, M., & Milliken, S. (2023). Investigating the effects of fish effluents as organic fertilisers on onion (*Allium cepa*) yield, soil nutrients, and soil microbiome. *Scientia Horticulturae*, *321*, 112297. <https://doi.org/10.1016/j.scienta.2023.112297>

- Geiger, C. A. (2004). An introduction to spectroscopic methods in the mineral sciences and geochemistry. In G. Papp, T. G. Weiszbürg, A. Beran, & E. Libowitzky (Eds.), *Spectroscopic methods in mineralogy* (pp. 1–42). Mineralogical Society of Great Britain and Ireland. <https://doi.org/10.1180/EMU-notes.6.1>
- Gharibzahedi, S. M. T., & Jafari, S. M. (2017). The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends in Food Science & Technology*, *62*, 119–132. <https://doi.org/10.1016/j.tifs.2017.02.017>
- Gul, B., Weber, D. J., & Khan, M. A. (2000). Effect of salinity and planting density on physiological responses of *Allenrolfea occidentalis*. *Western North American Naturalist*, *60*.
- Hamed, K. B., Ellouzi, H., Talbi, O. Z., Hessini, K., Slama, I., Ghnaya, T., Bosch, S. M., Savouré, A., & Abdelly, C. (2013). Physiological response of halophytes to multiple stresses. *Functional Plant Biology*, *40*(9), 883. <https://doi.org/10.1071/FP13074>
- Hameed, A., Hussain, S., Rasheed, A., Ahmed, M. Z., & Abbas, S. (2024). Exploring the Potentials of Halophytes in Addressing Climate Change-Related Issues: A Synthesis of Their Biological, Environmental, and Socioeconomic Aspects. *World*, *5*(1), Article 1. <https://doi.org/10.3390/world5010003>
- Hargreaves, J. A. (1998). Nitrogen biogeochemistry of aquaculture ponds1. *Aquaculture*, *166*(3), 181–212. [https://doi.org/10.1016/S0044-8486\(98\)00298-1](https://doi.org/10.1016/S0044-8486(98)00298-1)
- Hoque, M. B., Tanjila, M. J., Hosen, Md. I., Hannan, Md. A., Haque, P., Rahman, Md. M., & Hasan, T. (2024). A comprehensive review of the health effects, origins, uses, and safety of tannins. *Plant and Soil*. <https://doi.org/10.1007/s11104-024-06768-7>
- Hulkko, L. S. S., Rocha, R. M., Trentin, R., Fredsgaard, M., Chaturvedi, T., Custódio, L., & Thomsen, M. H. (2023). Bioactive Extracts from *Salicornia ramosissima* J. Woods Biorefinery as a Source of Ingredients for High-Value Industries. *Plants*, *12*(6), 1251. <https://doi.org/10.3390/plants12061251>
- Hulkko, L. S. S., Turcios, A. E., Kohnen, S., Chaturvedi, T., Papenbrock, J., & Thomsen, M. H. (2022). Cultivation and characterisation of *Salicornia europaea*, *Tripolium pannonicum* and *Crithmum maritimum* biomass for green biorefinery applications. *Scientific Reports*, *12*(1), Article 1. <https://doi.org/10.1038/s41598-022-24865-4>
- Hüpeden, J., Wemheuer, B., Indenbirken, D., Schulz, C., & Spieck, E. (2020). Taxonomic and functional profiling of nitrifying biofilms in freshwater, brackish and marine RAS biofilters. *Aquacultural Engineering*, *90*, 102094. <https://doi.org/10.1016/j.aquaeng.2020.102094>
- IUCN (Ed.). (2009). *Aquaculture: Responsible practices and certification*. IUCN, in collaboration with the Spanish Ministry of the Environment and Rural and Marine Affairs, and the European Federation of Aquaculture Producers (FEAP).
- Jones, D. B. (1931). Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. *Circular*, *183*, 16–21.
- Kadereit, G., Ball, P., Beer, S., Mucina, L., Sokoloff, D., Teege, P., Yaprak, A. E., & Freitag, H. (2007). A taxonomic nightmare comes true: Phylogeny and biogeography of glassworts (*Salicornia* L., Chenopodiaceae). *TAXON*, *56*(4), 1143–1170. <https://doi.org/10.2307/25065909>
- Kaspari, M., & Welte, E. A. R. (2024). Nutrient dilution and the future of herbivore populations. *Trends in Ecology & Evolution*, *39*(9), 809–820. <https://doi.org/10.1016/j.tree.2024.05.001>

- Katschnig, D., Broekman, R., & Rozema, J. (2013). Salt tolerance in the halophyte *Salicornia dolichostachya* Moss: Growth, morphology and physiology. *Environmental and Experimental Botany*, 92, 32–42. <https://doi.org/10.1016/j.envexpbot.2012.04.002>
- Khalilzadeh, R., Pirzad, A., Sepehr, E., Khan, S., & Anwar, S. (2021). The *Salicornia europaea* potential for phytoremediation of heavy metals in the soils under different times of wastewater irrigation in northwestern Iran. *Environmental Science and Pollution Research International*, 28(34), 47605–47618. <https://doi.org/10.1007/s11356-021-14073-4>
- Khan, M. A., Ungar, I. A., & Showalter, A. M. (2005). Salt Stimulation and Tolerance in an Intertidal Stem-Succulent Halophyte. *Journal of Plant Nutrition*, 28(8), 1365–1374. <https://doi.org/10.1081/PLN-200067462>
- Khan, M., Gul, B., & Weber, D. (2001). Effect of salinity on the growth and ion content of *Salicornia rubra*. *Communications in Soil Science and Plant Analysis*, 32, 2965–2977. <https://doi.org/10.1081/CSS-120000975>
- Khan, M., Ungar, I., & Showalter, A. (2000). Effects of sodium chloride treatments on growth and ion accumulation of the halophyte *Haloxylon recurvum*. *Communications in Soil Science and Plant Analysis - COMMUN SOIL SCI PLANT ANAL*, 31, 2763–2774. <https://doi.org/10.1080/00103620009370625>
- Kim, S., Lee, E.-Y., Hillman, P. F., Ko, J., Yang, I., & Nam, S.-J. (2021). Chemical Structure and Biological Activities of Secondary Metabolites from *Salicornia europaea* L. *Molecules (Basel, Switzerland)*, 26(8), 2252. <https://doi.org/10.3390/molecules26082252>
- Krayem, M., Khatib, S. E., Hassan, Y., Deluchat, V., & Labrousse, P. (2021). In search for potential biomarkers of copper stress in aquatic plants. *Aquatic Toxicology*, 239, 105952. <https://doi.org/10.1016/j.aquatox.2021.105952>
- Lavaud, R., Ullman, D. S., Venolia, C., Thornber, C., Green-Gavrielidis, L., & Humphries, A. (2023). Production potential of seaweed and shellfish integrated aquaculture in Narragansett Bay (Rhode Island, U.S.) using an ecosystem model. *Ecological Modelling*, 481, 110370. <https://doi.org/10.1016/j.ecolmodel.2023.110370>
- Łechtańska, J. M., Szadzińska, J., & Kowalski, S. J. (2015). Microwave- and infrared-assisted convective drying of green pepper: Quality and energy considerations. *Chemical Engineering and Processing: Process Intensification*, 98, 155–164. <https://doi.org/10.1016/j.cep.2015.10.001>
- Lee, C., Fletcher, T. D., & Sun, G. (2009). Nitrogen removal in constructed wetland systems. *Engineering in Life Sciences*, 9(1), 11–22. <https://doi.org/10.1002/elsc.200800049>
- Li, Y.-G., Tanner, G., & Larkin, P. (1996). The DMACA-HCl Protocol and the Threshold Proanthocyanidin Content for Bloat Safety in Forage Legumes. *Journal of the Science of Food and Agriculture*, 70(1), 89–101. [https://doi.org/10.1002/\(SICI\)1097-0010\(199601\)70:1<89::AID-JSFA470>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-0010(199601)70:1<89::AID-JSFA470>3.0.CO;2-N)
- Licon, C. (2022). Proximate and Other Chemical Analyses. In *Encyclopedia of Dairy Sciences* (pp. 521–529). Elsevier. <https://doi.org/10.1016/B978-0-12-818766-1.00344-5>
- Lima, A. R., Castañeda-Loaiza, V., Salazar, M., Nunes, C., Quintas, C., Gama, F., Pestana, M., Correia, P. J., Santos, T., Varela, J., & Barreira, L. (2020). Influence of cultivation salinity in the nutritional composition, antioxidant capacity and microbial quality of *Salicornia ramosissima* commercially produced in soilless systems. *Food Chemistry*, 333, 127525. <https://doi.org/10.1016/j.foodchem.2020.127525>
- Lima, A. R., Gama, F., Castañeda-Loaiza, V., Costa, C., Schüller, L. M., Santos, T., Salazar, M., Nunes, C., Cruz, R. M. S., Varela, J., & Barreira, L. (2021). Nutritional and Functional

- Evaluation of *Inula crithmoides* and *Mesembryanthemum nodiflorum* Grown in Different Salinities for Human Consumption. *Molecules (Basel, Switzerland)*, 26(15), 4543. <https://doi.org/10.3390/molecules26154543>
- Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. *Molecules*, 21(10), 1374. <https://doi.org/10.3390/molecules21101374>
- Liu, Q., Luo, L., & Zheng, L. (2018). Lignins: Biosynthesis and Biological Functions in Plants. *International Journal of Molecular Sciences*, 19(2), Article 2. <https://doi.org/10.3390/ijms19020335>
- Lopes, M., Roque, M. J., Cavaleiro, C., & Ramos, F. (2021). Nutrient value of *Salicornia ramosissima*—A green extraction process for mineral analysis. *Journal of Food Composition and Analysis*, 104, 104135. <https://doi.org/10.1016/j.jfca.2021.104135>
- Lopes, M., Sanches-Silva, A., Castilho, M., Cavaleiro, C., & Ramos, F. (2023). Halophytes as source of bioactive phenolic compounds and their potential applications. *Critical Reviews in Food Science and Nutrition*, 63(8), 1078–1101. <https://doi.org/10.1080/10408398.2021.1959295>
- Lopes, M., Silva, A. S., Séndon, R., Barbosa-Pereira, L., Cavaleiro, C., & Ramos, F. (2023). Towards the Sustainable Exploitation of Salt-Tolerant Plants: Nutritional Characterisation, Phenolics Composition, and Potential Contaminants Analysis of *Salicornia ramosissima* and *Sarcocornia perennis* alpini. *Molecules*, 28(6), Article 6. <https://doi.org/10.3390/molecules28062726>
- Luna-Guevara, Ma. L., Luna-Guevara, J. J., Hernández-Carranza, P., Ruíz-Espinosa, H., & Ochoa-Velasco, C. E. (2018). Phenolic Compounds: A Good Choice Against Chronic Degenerative Diseases. In *Studies in Natural Products Chemistry* (Vol. 59, pp. 79–108). Elsevier. <https://doi.org/10.1016/B978-0-444-64179-3.00003-7>
- Lv, S., Jiang, P., Chen, X., Fan, P., Wang, X., & Li, Y. (2012). Multiple compartmentalization of sodium conferred salt tolerance in *Salicornia europaea*. *Plant Physiology and Biochemistry: PPB / Société Française de Physiologie Végétale*, 51, 47–52. <https://doi.org/10.1016/j.plaphy.2011.10.015>
- Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology*, 12(3), 250–258. <https://doi.org/10.1016/j.pbi.2009.04.003>
- Majik, M. S., & Gawas, U. B. (2023). Recent advances in extraction of natural compounds. In *New Horizons in Natural Compound Research* (pp. 17–33). Elsevier. <https://doi.org/10.1016/B978-0-443-15232-0.00010-2>
- Mansfield, B. (2010). “Modern” industrial fisheries and the crisis of overfishing. In *Global Political Ecology*. Routledge.
- Manzoor, M. A., Sabir, I., Shah, I., Riaz, M., Ur Rehman, S., Song, C., Li, G., Malik, M., Ashraf, G., Haider, M., Cao, Y., & Abdullah, M. (2023). Flavonoids: A review on biosynthesis and transportation mechanism in plants. *Functional & Integrative Genomics*, 23. <https://doi.org/10.1007/s10142-023-01147-4>
- Martan, E. (2008). *Polyculture of Fishes in Aquaponics and Recirculating Aquaculture*.
- Mesa-Marín, J., Pérez-Romero, J. A., Mateos-Naranjo, E., Bernabeu-Meana, M., Pajuelo, E., Rodríguez-Llorente, I. D., & Redondo-Gómez, S. (2019). Effect of Plant Growth-Promoting Rhizobacteria on *Salicornia ramosissima* Seed Germination under Salinity,

- CO₂ and Temperature Stress. *Agronomy*, 9(10), Article 10. <https://doi.org/10.3390/agronomy9100655>
- Mesa-Marín, J., Pérez-Romero, J. A., Redondo-Gómez, S., Pajuelo, E., Rodríguez-Llorente, I. D., & Mateos-Naranjo, E. (2020). Impact of Plant Growth Promoting Bacteria on *Salicornia ramosissima* Ecophysiology and Heavy Metal Phytoremediation Capacity in Estuarine Soils. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.553018>
- Milhazes-Cunha, H., & Otero, A. (2017). Valorisation of aquaculture effluents with microalgae: The Integrated Multi-Trophic Aquaculture concept. *Algal Research*, 24, 416–424. <https://doi.org/10.1016/j.algal.2016.12.011>
- Moo-Huchin, V. M., Moo-Huchin, M. I., Estrada-León, R. J., Cuevas-Glory, L., Estrada-Mota, I. A., Ortiz-Vázquez, E., Betancur-Ancona, D., & Sauri-Duch, E. (2015). Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. *Food Chemistry*, 166, 17–22. <https://doi.org/10.1016/j.foodchem.2014.05.127>
- Mukherjee, P. K. (2019). Qualitative Analysis for Evaluation of Herbal Drugs. In *Quality Control and Evaluation of Herbal Drugs* (pp. 79–149). Elsevier. <https://doi.org/10.1016/B978-0-12-813374-3.00004-1>
- Naidoo, G., & Rughunanan, R. (1990). Salt Tolerance in the Succulent, Coastal Halophyte, *Sarcocornia natalensis*. *Journal of Experimental Botany*, 41. <https://doi.org/10.1093/jxb/41.4.497>
- Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, 591(7851), 551–563. <https://doi.org/10.1038/s41586-021-03308-6>
- Nikalje, G. C., Nikam, T. D., & Suprasanna, P. (2017). Looking at Halophytic Adaptation to High Salinity Through Genomics Landscape. *Current Genomics*, 18(6), 542–552. <https://doi.org/10.2174/1389202918666170228143007>
- Nikalje, G. C., & Suprasanna, P. (2018). Coping With Metal Toxicity – Cues From Halophytes. *Frontiers in Plant Science*, 9, 777. <https://doi.org/10.3389/fpls.2018.00777>
- Niyogi, K. K. (1999). Photoprotection Revisited: Genetic and Molecular Approaches. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50(1), 333–359. <https://doi.org/10.1146/annurev.arplant.50.1.333>
- Okomoda, V. T., Oladimeji, S. A., Solomon, S. G., Olufeagba, S. O., Ogah, S. I., & Ikhwanuddin, M. (2022). Aquaponics production system: A review of historical perspective, opportunities, and challenges of its adoption. *Food Science & Nutrition*, 11(3), 1157–1165. <https://doi.org/10.1002/fsn3.3154>
- Orellana, J., Waller, U., & Wecker, B. (2014). Culture of yellowtail kingfish (*Seriola lalandi*) in a marine recirculating aquaculture system (RAS) with artificial seawater. *Aquacultural Engineering*, 58, 20–28. <https://doi.org/10.1016/j.aquaeng.2013.09.004>
- Orlovsky, N., Japakova, U. N., Zhang, H. F., & Volis, S. (2016). Effect of salinity on seed germination, growth and ion content in dimorphic seeds of *Salicornia europaea* L. (Chenopodiaceae). *Plant Diversity*, 38. <https://doi.org/10.1016/j.pld.2016.06.005>
- Ozturk, M., Altay, V., Orçen, N., Yaprak, A. E., Tuğ, G. N., & Güvensen, A. (2018). A Little-Known and a Little-Consumed Natural Resource: *Salicornia*. In M. Ozturk, K. R. Hakeem, M. Ashraf, & M. S. A. Ahmad (Eds.), *Global Perspectives on Underutilized Crops* (pp. 83–108). Springer International Publishing. https://doi.org/10.1007/978-3-319-77776-4_3

- Pereira, C. G., Neng, N. R., & Custódio, L. (2023). From Threat to Opportunity: Harnessing the Invasive *Carpobrotus edulis* (L.) N.E.Br for Nutritional and Phytotherapeutic Valorization Amid Seasonal and Spatial Variability. *Marine Drugs*, 21(8), 436. <https://doi.org/10.3390/md21080436>
- Pirbalouti, A. G., Setayesh, M., Siahpoosh, A., & Mashayekhi, H. (2013). Antioxidant activity, total phenolic and flavonoids contents of three herbs used as condiments and additives in pickles products. *Herba Polonica*, 59(3), 51–62. <https://doi.org/10.2478/hepo-2013-0016>
- Qaisar, U., Afzal, M., & Tayyeb, A. (2019). Commercial Applications of Plant Pigments. *International Journal of Biotech Trends and Technology*, 9(3), 18–22. <https://doi.org/10.14445/22490183/IJBTT-V9I3P604>
- Rahmani, R., Arbi, K. E., Aydi, S. S., Hzami, A., Tlahig, S., Najjar, R., Aydi, S., & Debouba, M. (2022). Correction: Biochemical composition and biological activities of *Salicornia europaea* L. from southern Tunisia. *Journal of Food Measurement and Characterization*, 16(6), 5077–5077. <https://doi.org/10.1007/s11694-022-01622-9>
- Rakocy, J. E., Masser, M. P., & Losordo, T. M. (2006). *Recirculating Aquaculture Tank Production Systems: Aquaponics—Integrating Fish and Plant Culture*.
- Rathore, A. P., Chaudhary, D. R., & Jha, B. (2016). Biomass production, nutrient cycling, and carbon fixation by *Salicornia brachiata* Roxb.: A promising halophyte for coastal saline soil rehabilitation. *International Journal of Phytoremediation*, 18(8), 801–811. <https://doi.org/10.1080/15226514.2016.1146228>
- Reddy, N. R., Sathe, S. K., & Salunkhe, D. K. (1982). Phytates in Legumes and Cereals. In *Advances in Food Research* (Vol. 28, pp. 1–92). Elsevier. [https://doi.org/10.1016/S0065-2628\(08\)60110-X](https://doi.org/10.1016/S0065-2628(08)60110-X)
- Redondo-Gómez, S., Mateos-Naranjo, E., Figueroa, M. E., & Davy, A. J. (2010). Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*: Salt stimulation in an extreme halophyte. *Plant Biology*, 12(1), 79–87. <https://doi.org/10.1111/j.1438-8677.2009.00207.x>
- Reetu, Tomar, M., Kumar, M., & Seva Nayak, D. (2023). Role of Phenolic Metabolites in Salinity Stress Management in Plants. In R. Lone, S. Khan, & A. Mohammed Al-Sadi (Eds.), *Plant Phenolics in Abiotic Stress Management* (pp. 353–368). Springer Nature. https://doi.org/10.1007/978-981-19-6426-8_16
- Rhee, M., Park, H.-J., & Cho, J. (2009). *Salicornia herbacea*: Botanical, chemical and pharmacological review of halophyte marsh plant. *Journal of Medicinal Plants Research*, 3, 548–555.
- Rocha, C. P., Pacheco, D., Cotas, J., Marques, J. C., Pereira, L., & Gonçalves, A. M. M. (2021). Seaweeds as Valuable Sources of Essential Fatty Acids for Human Nutrition. *International Journal of Environmental Research and Public Health*, 18(9), 4968. <https://doi.org/10.3390/ijerph18094968>
- Rodriguez-Amaya, D. B. (2001). *A Guide to Carotenoid Analysis in Foods* (p. 64). ILSI Human Nutrition Institute.
- Ruiz, P., Vidal, J. M., Sepúlveda, D., Torres, C., Villouta, G., Carrasco, C., Aguilera, F., Ruiz-Tagle, N., & Urrutia, H. (2020). Overview and future perspectives of nitrifying bacteria on biofilters for recirculating aquaculture systems. *Reviews in Aquaculture*, 12(3), 1478–1494. <https://doi.org/10.1111/raq.12392>
- Rust, N. A., Ridding, L., Ward, C., Clark, B., Kehoe, L., Dora, M., Whittingham, M. J., McGowan, P., Chaudhary, A., Reynolds, C. J., Trivedy, C., & West, N. (2020). How to transition to

- reduced-meat diets that benefit people and the planet. *The Science of the Total Environment*, 718, 137208. <https://doi.org/10.1016/j.scitotenv.2020.137208>
- Salim, R., Nehvi, I. B., Mir, R. A., Tyagi, A., Ali, S., & Bhat, O. M. (2023). A review on anti-nutritional factors: Unraveling the natural gateways to human health. *Frontiers in Nutrition*, 10, 1215873. <https://doi.org/10.3389/fnut.2023.1215873>
- Sánchez-Gavilán, I., Ramírez Chueca, E., & De La Fuente García, V. (2021). Bioactive Compounds in *Sarcocornia* and *Arthrocnemum*, Two Wild Halophilic Genera from the Iberian Peninsula. *Plants*, 10(10), 2218. <https://doi.org/10.3390/plants10102218>
- Sangirova, U., Khafizova, Z., Yunusov, I., Rakhmankulova, B., & Kholiyorov, U. (2020). The benefits of development cage fish farming. *E3S Web of Conferences*, 217, 09006. <https://doi.org/10.1051/e3sconf/202021709006>
- Scott, A. J. (1977). Reinstatement and revision of Salicorniaceae J. Agardh (Caryophyllales). *Botanical Journal of the Linnean Society*, 75(4), 357–374. <https://doi.org/10.1111/j.1095-8339.1977.tb01493.x>
- Sheikh, B. A. (2006). Hydroponics: Key to sustain agriculture in water stressed and urban environment. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences*, 22(2), 53–57.
- Shpigel, M., Ben-Ezra, D., Shauli, L., Sagi, M., Ventura, Y., Samocha, T., & Lee, J. J. (2013). Constructed wetland with *Salicornia* as a biofilter for mariculture effluents. *Aquaculture*, 412–413, 52–63. <https://doi.org/10.1016/j.aquaculture.2013.06.038>
- Shukitt-Hale, B., Lau, F. C., & Joseph, J. A. (2008). Berry Fruit Supplementation and the Aging Brain. *Journal of Agricultural and Food Chemistry*, 56(3), 636–641. <https://doi.org/10.1021/jf072505f>
- Silva, A. M., Lago, J. P., Pinto, D., Moreira, M. M., Grosso, C., Cruz Fernandes, V., Delerue-Matos, C., & Rodrigues, F. (2021). *Salicornia ramosissima* Bioactive Composition and Safety: Eco-Friendly Extractions Approach (Microwave-Assisted Extraction vs. Conventional Maceration). *Applied Sciences*, 11(11), Article 11. <https://doi.org/10.3390/app11114744>
- Simon, F.-S., & Michael, J. P. (2001). *Aquaculture Systems and Species*. FAO. <https://www.fao.org/3/AB412E/ab412e07.htm>
- Singh, D., Buhmann, A. K., Flowers, T. J., Seal, C. E., & Papenbrock, J. (2014). *Salicornia* as a crop plant in temperate regions: Selection of genetically characterized ecotypes and optimization of their cultivation conditions. *AoB PLANTS*, 6, plu071. <https://doi.org/10.1093/aobpla/plu071>
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>
- Slama, I., M'Rabet, R., Ksouri, R., Talbi, O., Debez, A., & Abdelly, C. (2017). Effects of salt treatment on growth, lipid membrane peroxidation, polyphenol content, and antioxidant activities in leaves of *Sesuvium portulacastrum* L. *Arid Land Research and Management*, 31(4), 404–417. <https://doi.org/10.1080/15324982.2017.1329759>
- Soto-Sierra, L., Stoykova, P., & Nikolov, Z. L. (2018). Extraction and fractionation of microalgae-based protein products. *Algal Research*, 36, 175–192. <https://doi.org/10.1016/j.algal.2018.10.023>

- Teege, P., Kadereit, J. W., & Kadereit, G. (2011). Tetraploid European *Salicornia* species are best interpreted as ecotypes of multiple origin. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 206(10), 910–920. <https://doi.org/10.1016/j.flora.2011.05.009>
- Tom, A. P., Jayakumar, J. S., Biju, M., Somarajan, J., & Ibrahim, M. A. (2021). Aquaculture wastewater treatment technologies and their sustainability: A review. *Energy Nexus*, 4, 100022. <https://doi.org/10.1016/j.nexus.2021.100022>
- Tong, Z., He, W., Fan, X., & Guo, A. (2022). Biological Function of Plant Tannin and Its Application in Animal Health. *Frontiers in Veterinary Science*, 8, 803657. <https://doi.org/10.3389/fvets.2021.803657>
- Turcios, A. E., Miglio, R., Vela, R., Sánchez, G., Bergier, T., Włodyka-Bergier, A., Cifuentes, J. I., Pignataro, G., Avellan, T., & Papenbrock, J. (2021). From natural habitats to successful application—Role of halophytes in the treatment of saline wastewater in constructed wetlands with a focus on Latin America. *Environmental and Experimental Botany*, 190, 18. <https://doi.org/10.1016/j.envexpbot.2021.104583>
- Turcios, A., & Papenbrock, J. (2014). Sustainable Treatment of Aquaculture Effluents—What Can We Learn from the Past for the Future? *Sustainability*, 6(2), 836–856. <https://doi.org/10.3390/su6020836>
- Türkan, I., & Demiral, T. (2009). Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, 67(1), 2–9. <https://doi.org/10.1016/j.envexpbot.2009.05.008>
- van Grinsven, H. J. M., Ebanyat, P., Glendining, M., Gu, B., Hijbeek, R., Lam, S. K., Lassaletta, L., Mueller, N. D., Pacheco, F. S., Quemada, M., Bruulsema, T. W., Jacobsen, B. H., & ten Berge, H. F. M. (2022). Establishing long-term nitrogen response of global cereals to assess sustainable fertilizer rates. *Nature Food*, 3(2), 122–132. <https://doi.org/10.1038/s43016-021-00447-x>
- Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *Journal of Agricultural and Food Chemistry*, 46(10), 4113–4117. <https://doi.org/10.1021/jf9801973>
- Ventura, Y., Eshel, A., Pasternak, D., & Sagi, M. (2015). The development of halophyte-based agriculture: Past and present. *Annals of Botany*, 115(3), 529–540. <https://doi.org/10.1093/aob/mcu173>
- Ventura, Y., & Sagi, M. (2013). Halophyte crop cultivation: The case for *Salicornia* and *Sarcocornia*. *Environmental and Experimental Botany*, 92, 144–153. <https://doi.org/10.1016/j.envexpbot.2012.07.010>
- Ventura, Y., Wuddineh, W. A., Ephrath, Y., Shpigel, M., & Sagi, M. (2010). Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. *Scientia Horticulturae*, 126(3), 395–401. <https://doi.org/10.1016/j.scienta.2010.07.015>
- Ventura, Y., Wuddineh, W. A., Myrzabayeva, M., Alikulov, Z., Khozin-Goldberg, I., Shpigel, M., Samocha, T. M., & Sagi, M. (2011). Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. *Scientia Horticulturae*, 128(3), 189–196. <https://doi.org/10.1016/j.scienta.2011.02.001>
- Vymazal, J. (2011). Constructed Wetlands for Wastewater Treatment: Five Decades of Experience. *Environmental Science & Technology*, 45(1), 61–69. <https://doi.org/10.1021/es101403q>
- Weil, R., & Brady, N. (2017). *The Nature and Properties of Soils. 15th edition.*

- Wellburn, A. R. (1994). The Spectral Determination of Chlorophylls *a* and *b*, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *Journal of Plant Physiology*, 144(3), 307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2)
- Welti, E. A. R., Roeder, K. A., de Beurs, K. M., Joern, A., & Kaspari, M. (2020). Nutrient dilution and climate cycles underlie declines in a dominant insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America*, 117(13), 7271–7275. <https://doi.org/10.1073/pnas.1920012117>
- Yan, X. (2014). High Performance Liquid Chromatography For Carbohydrate Analysis. *Amway Corporation*.
- Youssef, S., Custódio, L., Rodrigues, M. J., Pereira, C. G., Calhelha, R. C., Pinela, J., Barros, L., Jekő, J., Cziáky, Z., & Ben Hamed, K. (2022). Nutritional anti-nutritional chemical composition and antioxidant activities of the leaves of the sea cliff dwelling species *Limonium spathulatum* (Desf.) Kuntze. *Frontiers in Plant Science*, 13, 979343. <https://doi.org/10.3389/fpls.2022.979343>
- Zaynab, M., Fatima, M., Sharif, Y., Zafar, M. H., Ali, H., & Khan, K. A. (2019). Role of primary metabolites in plant defense against pathogens. *Microbial Pathogenesis*, 137, 103728. <https://doi.org/10.1016/j.micpath.2019.103728>
- Zhang, B., Bian, Y., Chen, J., Zhang, Z., Sun, S., Yang, F., Lei, Z., & Huang, W. (2023). Nitrogen reclamation from aquaculture wastewater as potential fish feed additives via bacterial and algal-bacterial granular sludge systems. *Bioresource Technology Reports*, 24, 101609. <https://doi.org/10.1016/j.biteb.2023.101609>
- Zou, Y., Chang, S. K. C., Gu, Y., & Qian, S. Y. (2011). Antioxidant Activity and Phenolic Compositions of Lentil (*Lens culinaris* var. Morton) Extract and Its Fractions. *Journal of Agricultural and Food Chemistry*, 59(6), 2268–2276. <https://doi.org/10.1021/jf104640k>

