# Hydroponic cultivation of *Tetragonia decumbens* in seawater dilutions for commercial agriculture

**by**

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**DECLARATION**

I, Lufefe Ntoyaphi, affirm that all aspects of this dissertation/thesis are my own original work and have not previously been submitted for academic examination towards any qualification. Furthermore, it represents my personal opinions, not those of the Cape Peninsula University of Technology.

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| --- | --- |
| **Signed** | **Date** |

## ABSTRACT

The production of agricultural products is severely impacted by climate change, rising soil salinity, and developing freshwater shortages in many different nations, most notably South Africa. Soil salinization is a serious environmental threat to agricultural productivity and food security around the world. It impairs the structure of the soil, the availability of nutrients, and the growth of plants, resulting in lower agricultural yields. Freshwater shortages also represent a few of the agricultural factors impacting the production of agriculture and the availability of food in South Africa. It has been noted that water needs have been rising since the 1950s, yet the availability of freshwater has been steadily declining. In water-constrained regions, the development of agricultural irrigation competes with rising household and industrial requirements, which could result in excess water being transferred to high-priority sectors at the expense of agriculture. As food production and a lack of water develop, these complex changing factors put strain on agriculture in many areas where traditional water sources are used for irrigation. Therefore, investigation on dune spinach propagation, salinity stress, cultivation, and nutritional values is required.

The aim of this research is to examine the impact of diluted seawater and different pruning intervals on the nutritional profile, antioxidant capacity, and vegetative development of *T. decumbens* shoots grown hydroponically. *Tetragonia decumbens* cuttings were collected from a specific clone plant that was flourishing alongside the coastline at Granger Bay, Western Cape. A stem cutting propagation technique was used to root new plant material. Four identically designed Nutrient Film Technique (NFT) structures configured in a full block design were set up, with different diluted seawater concentrations (100% tap water/50L, 20% seawater/50L, and 40% seawater/50L) in each sump. Nutrifeed was essential for proper nutrition across all systems. Dune spinach plants grown within these circumstances had four different pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm cuts). The plant growth was measured with a tape measure, and the pruned fresh and dry plant components were weighed on a laboratory scale. The data obtained from shoot samples subjected to diluted seawater and different pruning intervals was statistically computed using a two-way examination of variance (ANOVA). The Fisher's least significant difference will be used to compare the significant differences between treatment means at $p \leq 0.05$ using MINI-TAB statistical software. The amount of chlorophyll in dune spinach plants was determined using a Konic-Minolta meter (SPAD-502). Dune spinach shoots were dried and tested for total flavonols, total polyphenols, ABTS, ferric reducing antioxidant power (FRAP), and DPPH to determine phytochemicals and antioxidants. The dried *T. decumbens* shoots were evaluated by using tests for crude proteins, crude fat content, ash content, moisture content, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), macro-elements (sodium, phosphorus,

magnesium, potassium, calcium, K/Ca+Mg, nitrogen), and micro nutrients (magnesium, zinc, copper and iron) to determine its nutritional values.

Plants cultivated in 20%SW with pruning intervals of 30 and 45 cm produced large quantities of both dry and fresh weights of dune spinach shoots at weeks 4 and 12, but at week 8 they started to decline. Increased polyphenol, flavonol, and FRAP capacity were observed in diluted seawater (20%SW) at pruning intervals of 15 and 30 cm. In contrast, the control shown enhances both DPPH and ABTS capacity. Macronutrients (magnesium, salt, and nitrogen), micronutrients (copper and zinc), and proximate components (ash, moisture, and crude protein) produced significant yields in plants cultivated in 20%SW with three different pruning intervals (15 cm, 30 cm, and 45 cm).

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## DEDICATION

**This work is dedicated to:**

- I humbly dedicate this piece of work to my loving parent, Nozipho Ntoyaphi, who has been a guardian in my life. Thank you for your endless love, belief, encouragement, and support on this journey.
- My late grandmother (Nozolile Ntoyaphi), whose strength and wisdom continue to guide me. May her soul continue to rest in peace.
- My family, who have been motivating me every day, Thank you so much for their endless guidance and support.

## STRUCTURE OF THE THESIS

The thesis is drafted differently from the alternative of a traditional format for a thesis. The article-format thesis examples of published, co-published, and/or “ready-for-publication” articles were prepared during candidature and apply to the format prescribed by CPUT for 100% master's studies, which complies with the following guidelines:

1. The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.

2. Chapters containing the journal articles form a coherent and integrated body of work that focuses on a single project or set of related questions or propositions. All journal articles form part of a sustained thesis with a coherent theme.

3. The study does not include work published prior to the commencement of the candidature.

4. The number of articles included depends on the content and length of each article and takes full account of the university’s requirements for the degree as well as the one article already published or “ready-for-publication” expected for a master’s degree in this discipline.

5. The thesis should be examined in the normal way and according to the normal requirements as set out by the “Guidelines for Examiners of Dissertations and Theses” (using Form HDC 1.7).

The thesis consists of the following chapters, which are concisely discussed as follows:

**Chapter One:** This chapter outlines the importance of the study, its aim, and the comprehensive list of specific objectives that guided the study.

**Chapter Two:** This chapter explores the potential of *T. decumbens* (Dune spinach) as a leafy vegetable. It also discusses its uses, propagation, distribution, the impact of the environment on growth, and potential hydroponic cultivation techniques that could be used commercially.

**Chapter Three:** This chapter evaluated the effect of diluted seawater and different pruning levels on the growth and chlorophyll content of *T. decumbens* (Aizoaceae). The research justification, materials and methods, results, and discussions are presented.

**Chapter Four:** This chapter evaluated the effect of diluted seawater and different pruning levels on the growth parameters of *T. decumbens* (Dune spinach) shoots. The research justification, materials and methods, results, and discussions are presented.

**Chapter Five:** This chapter evaluated the effect of diluted seawater and different pruning levels on the polyphenolic content and antioxidant capacity of *T. decumbens* (Dune spinach) shoots. The research justification, materials and methods, results, and discussions are presented.

**Chapter Six:** This chapter evaluated the effect of diluted seawater and different pruning levels on the nutrient content of *T. decumbens* (Dune spinach) shoots. The research justification, materials and methods, results, and discussions are presented.

**Chapter Seven:** General Discussion, Conclusions, and Recommendations. This section provides a general discussion that connects the previous sections and is followed by the study's results. Recommendations are given for future study and to present new research subjects.

**Chapter Eight:** References

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**GLOSSARY**

| **Terms/Acronyms/Abbreviations** | **Definition/Explanation** |
|---|---|
| °C | Degrees Celsius |
| ABTS | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) |
| ANOVA | Analysis of Variance |
| $CH_3CH_2OH$ | Ethanol |
| cm | Centimetres |
| CPUT | Cape Peninsula University of Technology |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl ethanol |
| DSW | Diluted seawater |
| EC | Electrical Conductivity |
| $FeCl_3 * 6 H_2O$ | Iron (III) chloride hexahydrate |
| FRAP | Ferric reducing antioxidant power |
| G | Gutter |
| $H_3PO_4$ | Phosphoric acid |
| HCl | Hydrochloric acid |
| $K_2S_2O_8$ | Potassium-Peroxodisulphate |
| KOH | Potassium hydroxide |
| L | Litres |
| LDPE | Low-Density Polyethylene |
| $Na_2CO_3$ | Sodium carbonate |
| NaCl | Sodium chloride |
| NFT | Nutrient Film Technique |
| pH | Potential Hydrogen |
| PVC | Polyvinyl Chloride |
| S | Sump |
| SWOT | Strengths, Weaknesses, Opportunities, and Threats |
| T | Treatment |

# CHAPTER ONE

# RESEARCH PROBLEM, AIMS, HYPOTHESES AND OBJECTIVES

## 1.1 RESEARCH PROBLEM

Climate change, increasing soil salinity, and emerging freshwater scarcity have severe impacts on agricultural production in many different countries, notably South Africa (Sogoni et al., 2021). Soil salinization is a serious environmental threat to agricultural productivity and food security around the world (Sahbeni *et al*., 2023). It impairs the structure of the soil, the availability of nutrients, and the growth of plants, resulting in lower agricultural yields (Wondim *et al*., 2020; Daba & Qureshi, 2021; Tarolli *et al*., 2024). Salt affects about 831 to 932 million hectares of land worldwide, producing 397 million hectares of saline and 434 million hectares of sodic soils (Haddadi *et al*., 2023). Therefore, investigation of halophytes that are capable of surviving in high salinity environments is required.

Freshwater shortages represent a few of the agricultural factors impacting the production of agriculture and the availability of food in South Africa (Ngxabi *et al*., 2021). It has been noted that water needs have been rising since the 1950s, yet the availability of freshwater has been steadily declining (Gleick, 2003). In water-constrained regions, the development of agricultural irrigation competes with rising household and industrial requirements, which could result in excess water being transferred to high-priority sectors at the expense of agriculture (Khondoker *et al*., 2023). As food production and a lack of water develop, these complex changing factors put strain on agriculture in many areas where traditional water sources are used for irrigation. Therefore, investigating new agricultural water sources is now required (Martínez-Alvarez *et al*., 2017; Shemer *et al*., 2023).

Hydroponics is a method of cultivating plants in a soilless environment using inert media or through their roots plunged in a solution that contains nutrients with no substrate (Tzortzakis *et al*., 2022; Serio *et al*., 2022; Othman *et al*., 2024; Sharma *et al*., 2024). Due to urbanisation and contemporary society, agricultural land is shrinking. To address this issue, new technologies such as hydroponic farming provide expanding channels to reduce water use for the cultivation of vegetables (Ravuri *et al*., 2024). The cultivation of crops in monitored conditions improves development, quality, cleanliness, and uniformity (Dsouza *et al*., 2024). Nonetheless, hydroponic cultivation of coastal plants has been suggested as potential substitutes since they are salt-tolerant, use a small amount of water, and can thrive in a salinity climate (Ventura *et al*., 2011; Ngxabi *et al*., 2021).

*Tetragonia decumbens* Mill. is one of the most underutilized salt-tolerant species in South Africa, with potential economic benefits (Sogoni *et al*., 2021). The leaves and soft stems have been used in a wide variety of ways, such as green food served in restaurants, consumed as spinach, cooked with other vegetables, eaten raw in salads, and have become popular in several cultures (Tembo-Phiri *et al*., 2019; Sogoni *et al*., 2021). However, its knowledge on propagation, chlorophyll content, nutritional values, salt tolerance, antioxidants, and phytochemicals could support its cultivation potential as a leafy vegetable crop. Therefore, the purpose of this study was to investigate the effects of diluted seawater (DSW) and different pruning intervals on the vegetative growth, chlorophyll content, nutritional values, antioxidants, and phytochemical potential of *T. decumbens* (dune spinach).

## 1.2 AIM

This study aims to investigate the effects of diluted seawater (100% tap water/50L, 20% SW/50L, and 40% SW/50L) and different pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm cut) on the chlorophyll content, vegetative growth, phytochemical and antioxidant potential, and nutrient content of *T. decumbens* in hydroponics in order to formulate a viable growth protocol for both home gardeners and potential commercial farmers.

## 1.3 HYPOTHESIS

It is hypothesized that low to moderate diluted seawater concentrations will increase the chlorophyll content, crop growth, nutrient uptake, antioxidant, and phytochemical content of *T. decumbens*. It is hypothesized that diluted seawater and different pruning intervals will positively affect the growth of *T. decumbens*. Additionally, high seawater concentrations will negatively affect the growth of *T. decumbens*, resulting in plant death.

## 1.4 OBJECTIVES

### 1.4.1 Main objective

The purpose of this research is to explore the vegetative growth and nutrient content of *T. decumbens* (dune spinach) in response to diluted seawater and different pruning intervals in order to develop a suitable hydroponic growth protocol.

### 1.4.2 Specific objectives

- To determine the chlorophyll content of *T. decumbens* in response to diluted seawater concentrations and different pruning intervals, to develop and implement the most efficient hydroponic growth protocol.
- To determine the vegetative growth of *T. decumbens* in response to diluted seawater and different pruning intervals to understand its salt tolerance and cultivation potential in hydroponics.
- To determine the aerial fresh and dry weight of *T. decumbens* in response to diluted seawater concentrations and different pruning intervals to understand its salt tolerance and cultivation potential in hydroponics.

- To determine the number of leaves and shoot length of *T. decumbens* in response to diluted seawater concentrations and different pruning intervals to develop and implement the most efficient hydroponic growth protocol.
- To determine phytochemical and antioxidant capacity of *T. decumbens* in response to diluted seawater concentrations and different pruning intervals to develop and implement the most efficient hydroponic growth protocol for phytochemicals and antioxidants.
- To determine the nutrient content of *T. decumbens* in response to diluted seawater concentrations and different pruning intervals to support its potential consumption and commercialization.

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# CHAPTER TWO

## AN OVERVIEW ON THE CULTIVATION, ORNAMENTAL, NUTRITIONAL POTENTIAL AND ENDORSMENT OF *TETRAGONIA DECUMBENS* MILL. AS A STAPLE VEGETABLE: A REVIEW

**An overview on the cultivation, ornamental, nutritional potential and endorsement of *Tetragonia decumbens* Mill. as a staple vegetable**

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## 2.1 Abstract

*Tetragonia decumbens* Mill. is a sprawling succulent shrub belonging to the Aizoaceae family. The species is drought-tolerant in the Mediterranean climate with mainly winter rain and grows in neutral to alkaline, well-drained soils. It is distributed along the coastal dunes from southern Namibia to the Eastern Cape. The species is commonly called dune spinach or duinespinasie (Afrikaans), which refers to the edible leaves that resemble commercial spinach. Historically, the Koi-San tribe along the Cape Coast of South Africa was the first community to use this edible plant. Today, the species has gained popularity as a leafy green vegetable foraged along the coast and destined for mainly exclusive restaurant menus. The leaves and soft stems are eaten raw or steamed, and they can also be cooked with other vegetables. Although there is no knowledge on commercial cultivation of the species, a community garden in Khayelitsha, in the Western Cape, is piloting the species for potential cultivation. A full understanding of the plant's cultivation requirements, drought tolerance, coastal adaptability, and product market potential is imperative to inherently develop and formulate a cultivation protocol for this wild species. This review aims to highlight and formulate selection and development advances, cultivation criteria, and a cultivation protocol to support the hydroponic cultivation of this species for commercial agriculture.

**Keywords:** Aizoaceae, climate-resilient crop, food and nutritional security, hydroponics, wild leafy vegetable.

## 2.2 Introduction

About 831 to 932 million hectares of land around the world are affected by salt, resulting in 397 million hectares of saline and 434 million hectares of sodic soils (Haddadi *et al*., 2023). In the agricultural industry, salt stress is one of the most significant elements that restricts crop development and production in arid areas. As a result, more than 1 billion people do not consistently have access to enough affordable, healthy, and nutritious food (da Silva *et al*., 2023; Khan *et al*., 2023). Furthermore, salt stress poses serious threats to more than 20% of the world's arable land, and in the United States, about 12 billion US dollars per year of agricultural losses are attributed to salinity, and this number is predicted to increase in the coming years (Singh, 2022a; Balasubramaniam *et al*., 2023).

By 2050, global food production is expected to rise by 70 to 100% to meet the needs of more than 8 billion people (Loconsole *et al*., 2019; Molotoks *et al*., 2021). However, environmental variables such as high temperatures, climate change, soil salinity, lack of freshwater, and soil degradation have detrimental impacts on the crop production and yield of significant crops such as rice, wheat, and maize to meet the basic dietary needs of communities (Raza *et al*., 2019; Malhi *et al*., 2021). The latest initiatives to adjust to such climatic circumstances include the utilisation of plants that tolerate salt with potential economic benefits such as animal fodder, medicine, landscaping, vegetables, grains, and fruit (Lu *et al*., 2021). The consumable fleshy parts of salt-tolerant species are valued for their salty taste as well as their elevated levels of antioxidants and important nutrients (Castañeda-Loaiza *et al*., 2020; Lombardi *et al*., 2022).

*Tetragonia decumbens* is one of the underutilized salt-tolerant species in South Africa, with potential economic benefits (Sogoni *et al*., 2021). The leaves and soft stems have been used in a wide variety of ways, such as green food served in restaurants, consumed as spinach, cooked with other vegetables, eaten raw in salads, and have become popular in several cultures (Tembo-Phiri, 2019; Sogoni *et al*., 2023). Nevertheless, there is still little information available addressing the cultivation and potential uses of this underutilised salt-tolerant species (Sogoni *et al*., 2022). Therefore, the main objective of this review is to highlight and formulate selection and development advances, cultivation criteria, and a cultivation protocol to support the hydroponic cultivation of this species for commercial agriculture with the purpose of increasing its availability on the market.

## 2.3 Materials and methods

Relevant literature was retrieved from the library database in the research unit of the Cape Peninsula University of Technology (CPUT). Search engines such as Google Scholar, ScienceDirect, Scopus, SpringerLink, Web of Science, and Wiley Online Library were used in retrieving the relevant literature on the botanical, ecological, cultivation, food, and medicinal uses of the species. Keywords such as wild edible plants, halophytes, hydroponic systems, and nutritional composition of leafy vegetables were used to capture data from all relevant articles. A SWOT (strengths, weaknesses, opportunities, and threats) analysis was used to critically assess the relevance of documented literature in the databases to determine the cultivation potential of the species.

### 2.4 Halophytes

Halophytes are characterized as plants that can survive and complete their life cycle in saline water or in soil with a salt concentration of 200 mM or more (Cortinhas *et al*., 2023). Halophytes have many diverse plant families that may survive in a variety of salty conditions, including coastal regions, inland deserts, salt flats, and steppes (Shabala, 2013; Tian *et al*., 2023). In many countries, these halophytes are characterized as plants that can be used to sustain life and satisfy human needs with their potential economic values such as greening and coastal protection, grain, vegetables, fruit, medicines, animal feed, and feedstock for biofuels (Ksouri *et al*., 2012), and they are thought to be an alternate to address issues related to food security, freshwater shortage, salinization, and varying diets for better nutrition (Hasnain *et al*., 2023). Halophytes are divided into two categories: (I) obligate and (II) facultative halophytes. Obligate halophytes can only thrive at moderate to high salinities and cannot grow at low salinities or in freshwater, i.e., some Chenopodiceae plant species (Hasanuzzaman *et al*., 2014). Facultative halophytes can thrive on salty soils, but their ideal environment is one where there is little to no salt present, i.e., some Poaceae plant species. Globally, the future is bright for numerous uses of halophytes, and they must continue to be cultivated for commercial agriculture as well as for human and animal consumption to minimize hunger and malnutrition.

### 2.5 Adaptive mechanisms of halophytes

Plants are frequently subjected to unfavourable conditions like abiotic stressors, which can sometimes drastically affect the physiological processes within the plant and reduce growth and productivity (Ranjan *et al*., 2023; Zarbakhsh & Shahsavar, 2023). However, some halophyte species have reportedly been found to exhibit a range of physiological traits that enable them to deal with abiotic stress (Shabala, 2013). Desert environments also pose a variety of environmental challenges to plants, such as high temperatures, salinity in the soil, and water stress due to a lack of precipitation (Naorem *et al*., 2023). To thrive in these environments, halophytes use a variety of physiological and biochemical strategies that support their growth, reproduction, and life cycle completion under high-saline conditions (Nikalje *et al*., 2018). These adaptive mechanisms include salt exclusion, salt excretion through specialised organs like salt glands or salt bladders, dilution of salt ions through succulence, ion homeostasis, osmotic adjustment, and ROS-detoxification, which support their growth, reproduction, and ability to complete their life cycle in high-saline conditions (Rahman *et al*., 2021).

## 2.6 Biochemical mechanisms of halophytes to thrive in saline environments

### 2.6.1 Osmotic adjustment

According to Haj-Amor *et al*. (2022), one of the challenges that plants encounter when grown in salinized soils is water loss as a result of lower osmotic pressure. In this circumstance, plants can adjust for variations in osmotic pressure with organic solutes to maintain cell volume and turgor. To ensure osmotic adjustment in the cells in a salt-caused osmotic environment, it is essential that $Na^+$ and $Cl^-$ are present in vacuoles, as well as $K^+$ and suitable organic solutes such as proline and carbohydrates in the cytoplasm (Munns *et al*., 2020). One of the primary methods by which halophytes tolerate salt is the accumulation of osmolytes in the cytoplasm for osmotic adjustment (Masouleh *et al*., 2020). Osmolytes are molecules of organic matter with low molecular weight and high solubility that remain ineffective even at high concentrations and do not interfere with the normal functioning of cells (Gustavs *et al*., 2010). Osmolytes play an essential role in maintaining the functioning of enzymes under stressful conditions as well as protecting subcellular structures from oxidative damage brought on by reactive oxygen species. Although it is believed that sodium is toxic to plants, halophytes can use it as an osmolyte. Proline, trehalose, sucrose, polyoles, glycine betaine, and proline betaine are a few of the crucial compatible solutes. Proline is an amino acid that develops in plant cytosols and serves as an osmoprotectant. It is an essential signalling molecule and an efficient way of stabilising and preserving proteins, proteinaceous enzymes, and membranes (Arif *et al*., 2020). It controls membrane proteins, ROS scavengers, and cell solute homeostasis to regulate plant metabolism while under stress. Numerous studies have shown that proline under salinity improves the antioxidant and water absorption systems and reduces the buildup of toxic ions (Ismail & Horie, 2017; Arif *et al*., 2020).

### 2.6.2 ROS-detoxification

Nearly all biological activities are regulated by reactive oxygen species (ROS), which are crucial for life (Huang *et al*., 2019). Production of ROS is essential for plant growth, abiotic stress responses, and immunological responses. Halophytes utilise efficient antioxidant defence mechanisms to shield their cells and tissues from oxidative stress, which is brought on by an excessive build-up of ROS in high salinity environments (Bose *et al*., 2014; Castro *et al*., 2021). These efficient antioxidant defence mechanisms include enzymatic antioxidants such as SOD, CAT, APX, glutathione reductase (GR), monodehydroascorbate reductase

(MDHAR), and dehydroascorbate reductase (DHAR), as well as non-enzymatic antioxidants like flavonoids and tocopherols to reduce ROS-induced damage (Czarnocka & Karpiński, 2018). The enhanced salt tolerance of certain halophytes coincides with their capacity to sustain ROS homeostasis through effective activation of their antioxidant system during salt stress (Wang *et al*., 2020; Altuntaş & Terzi, 2021; Yıldız & Terzi, 2021). In this process, halophytes regulate the amounts of endogenous phytohormones to control several defence processes, such as antioxidant defence (Wiszniewska *et al*., 2021).

### 2.6.3 Ion homeostasis

Salinity is one of the environmental stresses that create soil ionic imbalance, impairing mineral uptake and resulting in mineral deficiencies that further inhibit the physiology, root architecture, and growth of plants (Evelin *et al*., 2019; Gupta *et al*., 2020). In order to accommodate $Na^+$, $Cl^-$, and $K^+$ in the tissues of halophytes, several channels and transporters are actively involved in the process. It is generally known that the apoplastic and symplastic pathways are mostly used for $Na^+$ absorption from soils to roots (Arif *et al*., 2020; Rahman *et al*., 2021). Through the use of ion influx and compartmentalization to regulate ion homeostasis and equilibrium, the plant is able to support the correct development of plants under salinity. As a result, extra salt is either transported to the vacuole or sequestered in the old portion of plant tissue since plants cannot handle excessive quantities of salt in their cytoplasm. With the assistance of several pumps, $Na^+$ , which enters the soil through the root, travels to the cytoplasm and eventually to the vacuole. Prevention of salt from entering the roots and vacuole sequestration of salt ions are the primary techniques that halophytes use to avoid the accumulation of excess salt in their cytosols (Himabindu *et al*., 2016).

## 2.7 Physiological mechanisms of halophytes to thrive in saline environments

### 2.7.1 Succulence

An increase in the water content of tissue is referred to as succulence. It typically goes hand in hand with the accumulation of osmotically active solutes, which preserves cell turgor pressure and thus minimises the effect of harmful ions (Nikalje *et al*., 2018). In saline environments, halophytes use succulence mechanisms for ion homeostasis and the accumulation of osmoprotectants to sustain cell turgor pressure (Mumtaz *et al*., 2021; Kaleem *et al*., 2022). Succulent leaves are good at maintaining osmotic balance, and they contain a greater number of large-sized mitochondrion than other types of leaves, which helps them fulfil the energy needs of their cells to enable ion compartmentalization and sequestration. As a result, the development of succulent leaves is regarded as a characteristic that allows for salt tolerance. Certain halophytes that use succulence mechanisms to thrive in saline environments through the buildup of excess salts in their leaves and stems while retaining an increase in photosynthetic productivity include *Zygophyllum xanthoxylum, Sesuvium portulacastrum, Puccinnellia nuttalliana, Beta vulgaris* susp. *Maritima, Cochlearia pyrenaica, Salsola drummondii, Achras sapota, Sarcocornia fruticosa, Salicornia herbacea, Suaeda maritima,* and *Arthrocnemum macrostachyum* (García-Caparrós *et al*., 2017; Elnaggar *et al*., 2020).

### 2.7.2 Salt excretion

Through a distinctive epidermal structure known as salt glands, recretohalophytes directly release noticeably larger salt ion contents on the leaf surface for dealing with maintenance of cellular ion homeostasis, causing them to be preferable to other kinds of halophytes and non-halophytes (Lu *et al*., 2021). Excess ions are able to reach salt glands more rapidly when transported by transpiration since it contains a layer of cuticle within the salt glands, and ions surrounding the leaves are able to reach the salt glands by means of the symplastic transport pathway (Leng *et al*., 2018; Shelake *et al*., 2022). Since the ions are released by the secretory pores through a convoluted transport mechanism between the salt glands individual cells, secretion serves as an active defence mechanism for plant tissues against harmful ions (Shelake *et al*., 2022). As the ions are excreted throughout this process, water is also eliminated, and due to the fact that salt glands cause salt crystals to form on the surface of leaves, plants may be in danger of serious injury from dehydration (Lu *et al*., 2021). According to Li *et al*. (2020), the majority of the ions secreted by salt glands during this process are inorganic, including $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, and $Zn^{2+}$. However, certain unfamiliar ions, including $Cl^-$, $Br^-$, $I^-$, $SO_4^{2-}$, $PO_4^{3-}$, and $NO_3^-$ can also be secreted during this process. Heavy metal contamination can also be decreased by using plants that can secrete salt to extract heavy metals, including cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), aluminium (Al), and iron (Fe), from soils (Wilson *et al*., 2017). Salt glands and bladders also perform a fundamental role in maintaining ion balance, osmotic pressure stability, and enhancing plant salt tolerance (Sogoni *et al*., 2023). Nonetheless, the number of cells that collectively make up salt glands varies, and they have distinct structural traits in various halophytes species (Leng *et al*., 2018; Kuster *et al*., 2020; Lu *et al*., 2021; Zhao *et al*., 2022).

### 2.7.3 Salt exclusion

Certain halophytic plants use root system-induced ultrafiltration mechanisms to filter out excess salts (Rahman *et al*., 2021). To transport $Na^+$ from the soil to the plant root cells during salt exclusion, two important mechanisms, such as symplastic and apoplastic, are used. In this process, apoplastic barriers are installed at the base of the roots to increase bypass flow resistance, which results in the efficient removal of salts from the roots and a decreased accumulation of harmful ions in the aboveground shoots through the transpiration stream

(Hussain *et al*., 2015; Hussain *et al*., 2023). During salt exclusion, two unique cell barrier layers called the root endodermis and exodermis have highly specialised tasks that are associated with two distinct cell wall properties, such as casparian band and suberin lamella. The suberin lamella, which mainly consists of long-chain fatty acids, pervaded the entire exodermis/endodermis cell wall, forming a hydrophobic barrier that plays a role in ion and water uptake regulation and is critical in minimising direct transportation of water and ions through the apoplast to the endodermal protoplasts (Wang *et al*., 2020; Cui *et al*., 2021; Zhang *et al*., 2021). The casparian band is a paracellular lignin buildup in the cell walls of the endodermis and exodermis and pushes all apoplastic transport into the tightly controlled symplastic system (Cui *et al*., 2021).

## 2.8 Potential uses of halophytes

### 2.8.1 Vegetable crops

There are numerous halophytes that can be consumed as vegetables, and in regions where only saline waters and soils are available, they are used as substitute crops (Liu & Wang, 2021). These halophytic plants can be cultivated in saline soils; however, they are also capable of growing even in environments that are not affected by salinity (Aslam *et al*., 2011). Halophytes are regarded as healthy greens and are abundant in vitamins, protein, and some vital trace elements (Li *et al*., 2020). For example, *Chenopodium quinoa* is one of the wild vegetable crops that have been used for human consumption (Angeli *et al*., 2020; Pathan & Siddiqui, 2022). This amaranthaceae species has a high concentration of phytochemicals that are good for human health, such as vitamins, proteins, fiber, minerals, phenolics, and other necessary trace elements (Graf *et al*., 2015; Pathan & Siddiqui, 2022). Moreover, *Crithmum maritimum* (sea fennel) is a perennial halophyte that is used for both food and non-food purposes (Kraouia *et al*., 2023). In Europe, it is still consumed as a leafy vegetable with secondary metabolites that serve as osmolytes (Renna, 2018). The purpose of osmolytes is to protect the cellular machinery and stop oxidative stress from damaging cells (Sharma *et al*., 2019). The roots, leaves, seeds, and fruits of *Crithmum maritimum* contain a variety of bioactive compounds that have potential uses as essential fatty acids, fragrances, medications, antimicrobials, and insecticides (Montesano *et al*., 2018; Atia *et al*., 2011). In China, *Suaeda salsa* L. is one of the halophytes that is used as a vegetable crop. It is rich in vitamins, antioxidants, nutritional fibre, minerals, and flavonoids and is a high-quality vegetable (Li & Song, 2019). Below are some of the most commonly consumed halophyte species around the world (see Table 2.1).

**Table 2.1:** The scientific names, plant parts used, and economic benefits of halophytes used as possible vegetable crops.

| S/N | Plant species | Family | Plant parts used | Economic uses | Reference |
|---|---|---|---|---|---|
| 1. | *Atriplex hortensis* | Amaranthaceae | Leaves | Pot herb. Used in green salads | (Kumorkiewicz-Jamro *et al*., 2023; Sai Kachout *et al*., 2023) |
| 2. | *Atriplex patula* | Amaranthaceae | Leaves, stems, and seeds | Cooked or raw used as spinach. Seeds consumed after cooking | (Chetina *et al*., 2023) |
| 3. | *Atriplex prostrata* | Amaranthaceae | Leaves | Cooked and use as a spinach substitute | (Lombardi *et al*., 2022; Koop-Jakobsen & Dolch, 2023) |
| 4. | *Beta maritima* | Amaranthaceae | Young shoots and roots | Eaten raw, cooked or prickled | (Bouchmaa *et al*., 2022; Puccinelli *et al*., 2023) |
| 5. | *Cakile maritima* | Brassicaceae | Stems, flower buds, roots and immature seeds | Stems, flower buds and immature seeds are consumed as raw or cooked | (Ksouri *et al*., 2007; Ellouzi *et al*., 2011; Ben Amor *et al*., 2020; Belghith *et al*., 2022) |
| 6. | *Capparis spinosa* | Capparidaceae | Leaves and seeds | Pricked and used as salt. Used in salads, pasta, and meat dishes | (Sun *et al*., 2023) |
| 7. | *Chenopodium album* | Amaranthaceae | Leaves, flower buds, and young shoots | Boiled and used as a salad; Eaten raw | (Poonia & Upadhayay, 2015; Chamkhi *et al*., 2022) |
| 8. | *Chenopodium quinoa* | Amaranthaceae | Leaves, young stems and seeds. | Consumed in as raw. Seeds are cooked or used as vegetable oil | (Graf *et al*., 2015; Angeli *et al*., 2020; Parvez *et al*., 2020; Pathan & Siddiqui, 2022) |

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|---|---|---|---|---|---|
| 9. | *Cichorium spinosum* | Asteraceae | Leaves | Young, freshly picked leaves are frequently fried or salted and sometimes are eaten as a bitter meal with vinegar and olive oil. | (Petropoulos *et al*., 2017) |
| 10. | *Crithmum maritimum* | Apiaceae | Leaves and seeds. | Consumed as vegetable after cooking or raw | (Atia *et al*., 2011; Gnocchi *et al*., 2022) |
| 11. | *Cynara cardunculus* var. *altilis* | Asteraceae | Leaves | | (Ferioli & D'Antuono, 2022; Mandim *et al*., 2023) |
| 12. | *Halimione portulacoides* | Amaranthaceae | Leaves | Eaten raw in salads or cooked | (Lombardi *et al*., 2022) |
| 13. | *Inula crithmoides* | Asteraceae | Young leaves | Consumed as either cooked, raw or pickled | (Rodrigues *et al*., 2023a; Rodrigues et al., 2023b) |
| 14. | *Lepidium latifolium* | Brassicaceae | Leaves | Cooked or candied as vegetable. | (Ali *et al*., 2023; Nezhadasad *et al*., 2023) |
| 15. | *Mesembryanthemum crystallinum* | Aizoaceae | Leaves, fruits and stems | Leaves are crushed and used as soap substitute. Boiled and eaten as a spinach. | (Cosentino *et al*., 2010; Oh *et al*., 2015; Guan *et al*., 2020; Mohamed *et al*., 2021; Cebani *et al*., 2023) |
| 16. | *Plantago coronopus* | Plantaginaceae | Leaves and flowered parts | Leaves are used to make medicine and are sometimes used an Italian salad. | (Ceccanti, *et al*., 2022a; Ceccanti *et al*., 2022b) |
| 17. | *Plantago lanceolata* | Plantaginaceae | Leaves and seeds. | Consumed as raw or cooked. Seeds are grounded into powder and mixed with flour | (Abate *et al*., 2022; Sanna *et al*., 2022) |

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|---|---|---|---|---|---|
| 18. | *Portulaca oleracea* | Portulacaceae | Leaves, stems, and seeds. | Consumed as raw or cooked. Used as a salt substitute | (Montoya-García *et al*., 2023; Srivastava *et al*., 2023) |
| 19. | *Prosopis glandulosa* | Fabaceae | Pods and seeds | Can make sweet flour that can mixed with water to make a drink. | (Starns *et al*., 2022; Singh & Pareek, 2023) |
| 20. | *Salicornia* spp. and *Sarcocornia* spp. | Amaranthaceae | Young shoots, leaves, and seeds. | Consumed in salad ingredients, raw, and pickled. Vegetable oil | (Im *et al*., 2003; Ventura & Sagi, 2013; Patel, 2016) |
| 21. | *Salsola soda* | Amaranthaceae | Young shoots and leaves | Consumed as raw, cooked, and used as a salt substitute | (Bañuelos *et al*., 2022; Yasseen & Al-Thani, 2022) |
| 22. | *Sesuvium portulacastrum* | Aizoaceae | Stems and leaves | Cooked and eaten as vegetable. | (Kulkarni *et al*., 2022; Wang *et al*., 2022; Alsherif *et al*., 2023) |
| 23. | *Suaeda fruticose* | Amaranthaceae | Seeds | Can be used as a salt substitute. Used as an edible oil. | (Ayaz *et al*., 2022; Zaier *et al*., 2022) |
| 24. | *Suaeda maritima* | Amaranthaceae | Young leaves | Used as vegetable and salad. | (Ghosh *et al*., 2022) |
| 25. | *Suaeda nudiflora* | Amaranthaceae | Leaves | Used in salad; prickled; consumed as a vegetable | (Ghosh *et al*., 2022; Joshi *et al*., 2023) |
| 26. | *Suaeda salsa* | Amaranthaceae | Leaves and seeds | Seeds used for oil production. Consumed as cooked or uncooked | (Guo *et al*., 2018; Li & Song, 2019) |

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|---|---|---|---|---|---|
| 27. | *Tetragonia decumbens* | Aizoaceae | Leaves and stems | Fermented, pickled, and used in stews and soups; Served raw in green salads, or cooked with other vegetables. | (Nkcukankcuka *et al*., 2021; Sogoni *et al*., 2021; Nkcukankcuka *et al*., 2022; Sogoni *et al.,* 2022; Sogoni *et al*., 2023) |
| 28. | *Tetragonia tetragonioides* | Aizoaceae | Leaves | Cooked or prickled. Eaten like spinach | (Atzori *et al*., 2020) |
| 29. | *Trachyandra ciliata* | Asphodelaceae | Flower stalk | Steamed or boiled, cooked in a stew | (De Vynck *et al*., 2016; Ngxabi *et al*., 2021) |
| 30. | *Trachyandra divaricata* | Asphodelaceae | Young flower | Used as vegetable | (Bulawa *et al*., 2022; Tshayingwe *et al*., 2023) |
| 31. | *Tribulus terrestris* | Zygophyllaceae | Leaves, stems, and fruits. | Consumed raw or cooked | (Šalamon *et al*., 2016) |
| 32. | *Zostera marina* | Zosteraceae | Seeds | Raw or cooked. | (Panta *et al*., 2014) |
| 33. | *Zygophyllum album* | Zygophyllaceae | Seeds | Vegetable oil | (Abdelhameed *et al*., 2022; Hadjadj *et al*., 2022) |
| 34. | *Zygophyllum fabago* | Zygophyllaceae | Flower buds | Prickled and consumed as vegetable oil | (Abbasvand & Hassannejad, 2023; Shahrajabian & Sun, 2023) |

### 2.8.2 Uses of halophytes in bioremediation

According to Pande *et a*l. (2020), bioremediation is the process of converting environmental pollutants into less harmful forms by using living organisms, primarily bacteria. It uses microbial, fungal, or plant enzymes to restore the natural ecosystem damaged by pollutants to its initial condition. In general, there are primarily two types of bioremediation technologies: (I) in situ and (II) ex situ. In ex situ bioremediation, contaminants are handled inside the controlled environment after being dug up or pumped out of the original site, whereas in situ bioremediation treats the polluted material at the original location (Azubuike *et al*., 2016). During bioremediation, microbes are required to enzymatically attack the contaminants and transform them into nontoxic compounds for bioremediation to be efficient (Bhandari *et al*., 2021). However, bioremediation, revegetation, and reclamation of such salt-affected areas have been recommended using halophytes, and they can absorb salt and lower EC levels if cultivated prior to planting conventional crops, i.e., *Atriplex numularia, Suaeda nudiflora*, *Suaeda fructicosa, Haloxylon recurvum*, *Sesuvium sesuviodes*, and *Trianthema triquatra* (Qadir *et al*., 2008; Munir *et al*., 2022; Barbafieri *et al*., 2023; Sordes *et al*., 2023).

### 2.8.3 Halophytes as medicinal plants

Traditional medicines are regarded as very important components of healthcare for marginalized, uneducated, and rural populations in developing countries (Chebii *et al*., 2020; Geck *et al*., 2020). Halophytes have the potential to be grown not solely for food and fuel but also for medicinal use in the treatment of various illnesses in rural and tribal communities (Lopes *et al*., 2023; Mohammed *et al*., 2023). For example, *T. tetragonioides* (New Zealand spinach) provides medicinal benefits for gastrointestinal disorders such as stomach cancer, gastritis, gastric ulcers, and acid excess (Lee *et al*., 2018). Moreover, the leaves, stems, roots, seeds, and flowers of other halophytes are used to make medicines by grinding them into a powder, juicing them, or chopping them up into pastes (Mohammed *et al*., 2023). In China, *Apocynum venetum* L. is one of the halophytes that was used for herbal medicine, and the leaves were used to medicate hypertension, dizziness, insomnia, neurasthenia, and heart failure (Xie *et al*., 2012; Jiang *et al*., 2019). *Mesembryanthemum crystallinum* is also a common annual halophyte with sprawling stems; it contains flavonoids with beneficial nutritional and health properties (Li & Song, 2019; Zhang *et al*., 2019). The flavonoids in this species act as natural antioxidants, strengthen the immune system and metabolism, and delay the ageing process in people (Zhang *et al*., 2019).

*Tetragonia decumbens* has not been used for medicinal purposes; however, it has been reported to have phytochemical activities that can be used for medicine (Sogoni *et al*., 2023). The potential medicinal benefits of *T. decumbens* will benefit both the herbal medicinal industry and the agricultural sector in saline land regions and coastal regions around the world, including South Africa.

## 2.9 Taxonomic review of *T. decumbens*

### 2.9.1 Evolutionary origin

According to Heyligers (1999), *T. decumbens* was first known as *Tetragonia nigrescens* var. *maritima*, and later, as extensive research was done with regards to the plant, it was concluded that *T. decumbens* was misidentified as *Tetragonia nigrescens* var. *maritima*. On November 11$^{th}$, 1916, *Tetragonia decumbens* was first discovered in New South Wales near Maroubra Bay, Sydney, by an author named A.A. Hamilton. The species was further discovered in February 1919 at Camp Cove at South Head, at the entrance to Sydney Harbour, and again collected at Maroubra in 1933 and 1955. Another collection of *T. decumbens* occurred in 1993 close to Birubi Point in Anna Bay, near the northeast of Stockton, where it was still found in 1998.

Nowadays, *T. decumbens* is a member of the Aizoaceae family that is widely found along the coastline of Namibia, from the southern part of the country to the Eastern Cape, and it is used as an edible halophyte (De Vynck *et al*., 2016; Klak *et a*l., 2017). The leaves and soft stems of this plant can be used like spinach, served in raw green salads, or cooked with other vegetables (Van Wyk, 2011; Nkcukankcuka *et al*., 2021). *T. decumbens* has been fermented, pickled, blended into stews and soups, and is very delicious when stir-fried; sometimes it blends excellently with butter beans, sun-dried tomatoes, mushrooms, and grain salads (Sogoni, 2020; Sogoni *et al*., 2022). Currently, this species does not only show potential as an edible vegetable; it also has some potential uses, such as animal fodder, and can be used in soil bioremediation.

### 2.9.2 Family and close relatives of *T. decumbens*

**Table 2.2:** The scientific names, plant families, distributions, and economic benefits of close relatives of *T. decumbens.*

| Plant species | Family | Distribution | Economic value | Reference |
|---|---|---|---|---|
| *Tetragonia tetragonioides* | Aizoaceae | Distributed along the coasts of Australia, New Zealand, and Tasmania but also in Argentina and Asian countries. | Used as a vegetable or in salads. Used for medicinal purposes. | (Atzori *et al*., 2020; Bekmirzaev *et al*., 2020). |
| *Tetragonia fruticosa* | Aizoaceae | *Tetragonia fruticose* grows from Namaqualand to the Eastern Cape, mostly along the coast, on granite and sandstone slopes that are arid or semi-arid. | It is a valuable fodder plant. It can be consumed as a vegetable and its young shoots should be boiled till soft, then chopped and served with butter, salt, and pepper. | (Manning, 2013) |
| *Mesembryanthemum crystallinum* | Aizoaceae | Grows in a variety of environments in southern and northern Africa as well as southern Europe. It can be found in the Eastern, Western, and Northern Cape provinces of South Africa. | The leaves contain some medical applications and can be crushed to make another use for soap. They are capable of being pickled or served as a garnish on food, and they are suitable for consumption as an alternative for spinach. | (Cebani *et al*., 2023; Mndi *et al*., 2023) |
| *Mesembryanthemum cordifolium f. variegata* | Aizoaceae | It can be found in coastal areas of KwaZulu-Natal and the Eastern Cape. | It can be used as a poultice, and anti-inflammatory in medicine. Also used as a charm for luck and love. Makes a mild enema for infants of the Zulus, and black powder is used for vaccinations and to protect against witchcraft. Used to relieve joint pains with burned stems and leaves. | (Lee & Nam, 2023) |

### 2.9.3 Ecological and geographical distribution of *T. decumbens*

The *Tetragonia* plant family, Aizoaceae (fig-marigold), belongs to the order of Caryophyllales, comprising about 135 genera and about 1800 species that are distributed in both tropical and subtropical regions and arid zones around the world (Tembo-Phiri, 2019). *T. decumbens* is a low-growing perennial plant found on coastal sand dunes from Southern Namibia to the Eastern Cape (South Africa), stretching from the borders on the West Coast to the Kei River on the East, where it receives winter rainfall (Forrester, 2004; De Vynck *et al*., 2016; Van Wyk & Gericke, 2017).

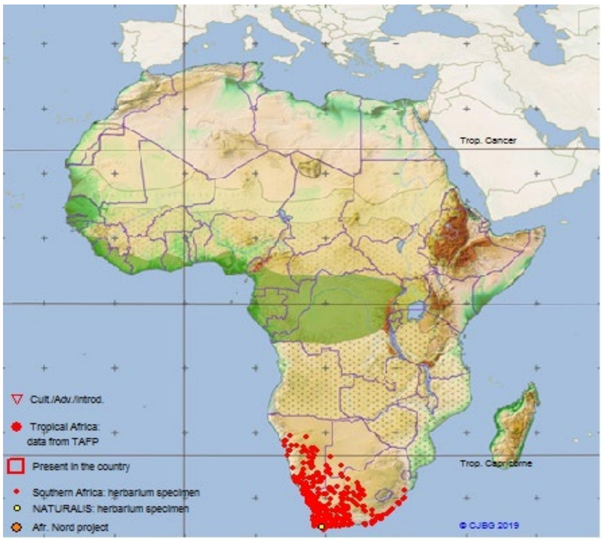


**Figure 2.1:** *Tetragonia decumbens* map of geographic distribution (Adapted from: http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=48025).

### 2.10 Botanical description of *Tetragonia decumbens*

*Tetragonia decumbens* consists of about 67 species. The name is derived from the Latin word *Tetragonus*, which refers to the fruit and indicates that it is four-angled, and *decumbens*, which means horizontal with the tip pointing upwards (Forrester, 2004). *Tetragonia decumbens* is a common underutilized wild edible vegetable in South Africa and is commonly known as dune spinach, duinespinasie, kinkelbos, and klapperbrak (Afrikaans) (Tembo-Phiri, 2019). *Tetragonia decumbens* is a wild edible vegetable crop that is characterized by lance-shaped, sessile, dark green, glistening leaves with stems that are woody at the base and rather rigid, long fibrous roots and runners with hairy leaves, as shown in figures 2.3 (Manning, 2013; Snijman, 2013). This species starts flowering from spring to early summer (August to November) and bears yellow flowers that survive dry summer months and re-sprout when exposed to water, as displayed in figure 2.3 (Manning, 2013; Snijman, 2013). The seeds are different-sized and have a hard brown outer layer with distinct rigid wings when dry, as displayed in figure 2.4. *Tetragonia decumbens* thrives well in a wide range of environments, such as sand dunes on the coast, dry, rocky, light, and heavy-textured soils, soils deficient in nutrients with minimal water needs, and coastline sea spray, as displayed in figure 2.2 (Tembo-Phiri, 2019; Sogoni, 2020; Nkcukankcuka *et al*., 2021). The species is recognized for its high nutritional value, drought-resistant traits, and crucial function in sand dune stabilization, serving as a seed trap and producing organic matter, enabling the dune to become a welcoming environment for various plants (Van Wyk, 2011; De Vynck *et al*., 2016).

**Figure 2.2:** *Tetragonia decumbens* growing on Strand beach, Western Cape, South Africa ( Adapted from: https://www.researchgate.net/figure/Salt-laden-spray-hovering-over-coastal-dunes-and-strandveld-thickets-in-the-background_fig5_40106511).

**Figure 2.3:** Dark green leaves and yellow flowers of *T. decumbens* (Adapted from: https://www.ukwildflowers.com/Web_pages/tetragonia_decumbens_sea_spinach.htm).

**Figure 2.4:** *Tetragonia decumbens* brown dry seeds (Tembo-Phiri, 2019).

## 2.11 Endorsement of *T. decumbens* as a vegetable

### 2.11.1 Economic potential of its commercial cultivation

Halophytes are prospective new vegetable species that can be grown in greenhouses, which can provide high-quality products all year-round while using fewer resources like water, fertiliser, and human labour (Lombardi *et al*., 2022). However, as the farming industry is endangered by rising saline levels in the soil, hydroponic cultivation of salt-tolerant vegetable crops must be developed to replace existing crop plants and ensure global food and nutrition security (Ngxabi *et al*., 2021). Hydroponics is a technique for growing crops without soil through the use of mineral nutrient solutions (Ngxabi *et al*., 2021). It is gaining popularity since it is a safe, simple procedure, and there is no risk of soil-borne disease, bug, or pest infection to the crops (Sharma *et al*., 2018). This eliminates the need for pesticides and the negative effects they have. *Tetragonia decumbens* is one of the underutilized edible wild vegetables in South Africa, and although this plant has never been cultivated, it has been tested in a community garden in Khayelitsha, Cape Town, South Africa, along with other promising winter rainfall crops, for eventual commercial cultivation (Tembo-Phiri, 2019). However, according to the study conducted by Nkcukankcuka *et al*. (2021), *T. decumbens* can be cultivated in hydroponics under different fertigation regimes. For the cultivation of crops in greenhouses, numerous soilless growing techniques have been successfully developed and tested (Lombardi *et al*., 2022). However, the Nutrient Film Technique (NFT) is considered the easiest and most economical method to grow *T. decumbens* at high crop densities. When cultivating *T. decumbens* in hydroponics, it is necessary to keep the pH between 5.5 and 6.5 and the electrical conductivity at 3.38, 2.34, and 1.10 ds m-1(S), respectively (Nkcukankcuka *et al*., 2021). Some of the key benefits of hydroponic cultivation over soil agriculture is that plants grow more quickly and produce more food because of improved mineral nutrition and water absorption (Lombardi *et al*., 2022).

### 2.11.2 Ornamental uses of *T. decumbens*

Ornamental plants are any plant species or varieties that bring aesthetic value, enhance the environment, or boost the quality of our lives (Viljoen *et al*., 2021). Recent research has examined the effective use of native plants in landscaping in accordance with agronomic, political, social, cultural, and environmental needs (Rohal *et al*., 2023; Vasilescu *et al*., 2023). The ability of these native plants to respond to both biotic and abiotic stress plays a more significant role in their survival than their aesthetic features, and there has been an interest in them for the purposes of repairing damaged landscapes, reducing erosion, and enhancing the aesthetic value of different places (Leotta *et al*., 2023). Halophytes can be used for revegetation or soil preservation in landscaping, and there is an increasing desire for plants that require minimal maintenance and water use (Nikalje *et al*., 2018). *Tetragonia decumbens* is one of the halophytes that respond to both biotic and abiotic stress and has gained popularity as a sand dune stabilizer, serving as a seed trap, producing organic matter, and enabling the dune to become a welcoming environment for various plants (Forrester, 2004; De Vynck *et al*., 2016; Van Wyk & Gericke, 2017).

Certain halophytes, such as *Carpobrotus edulis, Mesembryanthemum crystallinum*, and *Mesembryanthemum nodiflorum*, are used as groundcovers (Nikalje *et al*., 2018). These kinds of plants can be considered because of their extremely beautiful flowering, even for a short amount of time. Different regions across the world are developing intensive research programmes to identify ornamental halophytes suitable for arid environments (Liu & Wang, 2021; Garcia-Caparros *et al*., 2023). Currently, there is no evidence for ornamental uses of *T. decumbens*, and intensive research on potential ornamental uses of this species is required as it shows some of the characteristics of groundcovers.

### 2.11.3 *Tetragonia decumbens* as food sources

*Tetragonia decumbens* has been recorded as a traditional wild edible species with a rich source of macro and micronutrients (Pereira *et al*., 2022). However, due to its salt content and crunchiness on the leaves and soft stems, it has been used in a wide variety of foods, such as green food served in restaurants, consumed as spinach, cooked with other vegetables, and eaten raw in salads. It has also become popular in several cultures, as shown in figures 5 and 6 (Tembo-Phiri, 2019). Additionally, *T. decumbens* has been fermented, pickled, blended into stews and soups, and is very delicious when stir-fried; sometimes it blends excellently with butter beans, sun-dried tomatoes, mushrooms, and grain salads (Rusch, 2016; Sogoni, 2020). The species has a dull flavour and a grainy texture when cooked and is transformed into an exquisite meal when combined with butter and *Oxalis pescaprae* (Tembo-Phiri, 2019). The mildly salty, juicy leaves and stems of other halophytes, such as *Soutslaai,* can be eaten both raw and cooked (Nkcukankcuka *et al*., 2022). Furthermore, *Tetragonia tetragonioides,* a close relative of *T. decumbens* is used in food and salads in Brazil and commercially cultivated in Australia (Cecílio Filho *et al*., 2017). Even though *T. decumbens* is native to South Africa, it remains underutilized as a commercial crop. However, at a community garden in Khayelitsha, Cape Town, it was tested as a prospective commercial crop with many other potential winter rainfall crops (Tembo-Phiri, 2019). Therefore, a proper investigation of its nutritional benefits is needed to encourage its consumption as an underutilized edible leafy vegetable crop.

**Figure 2.5:** Oep ve Koep's Sandveld restaurant dumplings with dune spinach (Tembo-Phiri, 2019).

**Figure 2.6:** Leaves of *T. decumbens* and pickled veldkool (Rusch, 2016).

**Table 2.3:** Consumption uses of fresh, cooked, and dried leaves of *T. decumbens* for potential marketing.

| Type | Products | References |
|---|---|---|
| Fresh leaves and stems | Used in green salad. Eaten raw | (De Vynck *et al*., 2016; Rusch, 2016; Sogoni *et al*., 2023) |
| Cooked leaves | Stew, soup, stir fry. Used as spinach | (Nkcukankcuka *et al*., 2021; Nkcukankcuka *et al*., 2022; Sogoni *et al*., 2023) |
| Fresh leaves and stems | Animal fodder | (Bekmirzaev *et al*., 2020) |
| Fresh leaves and stems | Medicines (Phytochemicals-polphenols) | (Sogoni *et al*., 2021) |
| Leaves, stems, and flowers | Ornamental purpose | (Forrester, 2004; Van Wyk & Gericke, 2017) |

### 2.11.4 *Tetragonia decumbens* used as animal fodder

Halophytes have been used as a substitute for animal fodder in saline environments, and they have economic benefits in medicine, landscaping, vegetables, grains, and fruit (Ksouri *et al.*, 2007; Ksouri *et al.*, 2012; Panta *et al.*, 2014; Nikalje *et al.*, 2018; Zhang *et al.*, 2019). Insufficient amounts of water and salinity in agricultural soils are common challenges across African nations, and climate change is predicted to make these issues substantially worse in the coming years (Badri & Ludidi, 2020). In Africa, there has been a persistent problem with livestock feed shortages, particularly during the dry seasons. Therefore, halophytes with potential as fodder may be used in regions where seasonal feed shortages are significant threats to effective animal husbandry (Badri & Ludidi, 2020; Singh *et al*., 2022b). The effective use of these plants as animal feed further depends upon their biomass production, nutritional content, and voluntary consumption of feed, i.e., *Pennisetum clandestinum* (Norman *et al*., 2013). The expansion of animal farming might be considerably aided by halophytes with potential for animal fodder, which could also significantly increase the nutritional value and quality of cattle and sheep (Hasnain *et al*., 2023). Since halophytes thrive in a variety of environments with varying salinities and climatic circumstances, they can be regarded as an alternative to address issues relating to animal feed during the dry season. Therefore, the cultivation of halophytes with potential human and animal consumption still needs to be addressed.

## 2.12 A climate-adapted environment for cultivating a commercial crop

*Tetragonia decumbens* can be propagated by means of pulling up branches from the sand and separating pieces with roots attached as cuttings (Manning, 2013; Nkcukankcuka *et al*., 2021). The cuttings can be planted directly into sandy, well-drained soil, and the soil should be kept slightly moist enough to allow the plants to establish their roots (Forrester, 2004; Rusch, 2016). According to the study conducted by Sogoni *et al*. (2022), sand and peat-and-sand mixtures have the greatest cutting rates of survival, root number, root length, and number of leaves for this species. Substrates such as vermiculite, perlite, and coco peat had lower root development. The availability of nutrients, water, and oxygen on the growing substrate has a few aspects that impact how well cuttings root (Bhardwaj, 2014; Rajkumar *et al*., 2017). Rooting hormone is also used to increase the chances of cuttings rooting. The roots will usually develop quickly and be stronger when propagated using the rooting hormone. In areas suffering from the negative effects of drought, high temperatures, soil salinity, and sodicity, wild vegetables have enormous commercial potential (Laurie *et al*., 2017). *Tetragonia decumbens* can be effectively cultivated through rooted stem cuttings (Rusch, 2016). Overharvesting of *T. decumbens* might occur from propagation methods that include collecting mature runners from the sand with roots attached as cuttings (Sogoni, 2020). As a result, an appropriate and efficient propagation protocol emphasizing cultivation techniques for *T. decumbens* is required to encourage the large-scale production of this green vegetable crop for commercial agriculture (Table 2.4).

**Table 2.4:** Vegetative propagation techniques and growth conditions of *T. decumbens* for cultivation practices.

| **Activity** | **Cultivation practice** | **References** |
|---|---|---|
| Seeds | Coastal plant seeds are best sown in a free-draining seed mixture. Tiny seeds should be put into seed trays after being combined with dry, clean sand. Carefully apply a layer of coarse river sand over the seeds. As soon as seeds are the right size, they should be pricked out. Large seeds can be sowed individually and coated with coarse river sand. Keep moist but not too much as the seed may rot. | (Dixon, 2020) |
| Cuttings | Nodal stem cutting of a mature mother stock plant along the coast in summer. Growth regulator hormone powder (Dynaroot no. 1). Coarse river sand or 1:1 sand: peat or 1:1 perlite: peat or 1:1:1 perlite: peat: vermiculite used in hydroponics. Automatic nozzle sprayers for intermittent spray, heating beds. Average humidity of 60 – 70%. Temperature kept between 21 - 26°C (day) and 12 - 18°C (night). | (Rusch, 2016; Nkcukankcuka *et al*., 2021; Sogoni *et al*., 2022) |
| Growth conditions, Pests and diseases | Tolerate high temperature in full sun and drought conditions. Tolerate environmental control temperature of 21 - 26°C (day) and 12 - 18°C (night) in hydroponics. Leaves occasionally attached by insects and snails. Trampling also causes damage. | (Forrester, 2004; Sogoni *et al*., 2022) |
| Medium, Planting, Fertilizing | Sandy, well-drained soil, and the soil should be kept slightly moist. Grow up to 1 m long. Found in dunes and on both light and heavy-textured soils. In hydroponics pH and EC maintained at 4.5 – 6.5 and 3.38 – 1.10 dS $m^{-1}$ respectively. Nutrifeed is also used as a fertilizer in hydroponics. | (Tembo-Phiri, 2019; Nkcukankcuka *et al*., 2021) |
| Pollinators, Fruiting, Harvesting. | Open pollinated by a wide variety of insects | (Forrester, 2004) |

## 2.13 SWOT analysis of *Tetragonia decumbens* for commercial production

*Tetragonia decumbens* has been documented as a nutritious edible wild vegetable with low maintenance that can tolerate drought conditions. A community garden in Khayelitsha, Western Cape, has piloted the commercial cultivation of *T. decumbens* to advance its cultivation and promote its consumption as food (Nkcukankcuka *et al*., 2021). The leaves of this species are used in green salads, stews, soups, and stir fries (Van Wyk, 2011; De Vynck *et al*., 2016). With its use as a vegetable crop, *T. decumbens* has the potential to become one of the most lucrative wild edible vegetable crops for its nutritional and medicinal properties. *Tetragonia decumbens* may be commercially grown, and more people would be aware of it, which would benefit both local and international markets (Table 2.5).

**Table 2.5:** Strength, Weaknesses, Opportunities and Threats in propagation, commercial cultivation, and uses of *T. decumbens*.

| Factors | Strengths | Weaknesses | Opportunities | Threats |
|---|---|---|---|---|
| **Natural distribution** | Tolerate high temperatures. Drought tolerant plant. Stabilize sand dunes. Act as a seed-trap. Enables other species to grow on the dunes. Trap sand from blowing. Tolerate salinity and sodicity. | Unexplored possibilities as food, medicine, and animal fodder. Searched for by populations. Unable to access seeds and propagation material. | Will addresses significant concerns on food, nutrition insecurity and poverty. | Overexploitation. Foraged by many populations. Lack of legal protection. Biodiversity loss. Migration of people from rural areas to coastal regions. |
| **Species/ Cultivars** | Tolerate salinity and sodicity. Less pests and diseases. Drought tolerant. Tolerate high temperatures. | Unavailability of improved cultivars. Few variations documented. No phenology studies documented. | Can be used as food source, medicinal purpose, ornamental use and animal fodder. | Plant extinction. Urbanisation and not sustainable harvested. Migration of people from rural areas to coastal regions. Urbanisation. |
| **Propagation** | Easy to propagate. Require little watering. Tolerate high temperature. Tolerate wide range of soils. Few pests and diseases. Documented information on vegetative propagation and cultivation. | Lack of tested information on propagation. Lack of agronomic skills and knowledge. Limited knowledge on the plant. Low market value. | Increase cultivation method. Gain new market potential on the nutritional and health properties. Gain popularity in local and international markets. Increase the economy. Provide opportunities for employment. | Limited information of propagation and cultivation techniques. Limited propagation and cultivation interest. Biodiversity loss. |
| **Cultivation** | Easily cultivated. Few pests and diseases. Tolerate high temperature. Tolerate wide range of soils. Require little watering. Tolerate drought. Ability to be cultivated in poor soils. Practice not seasonal. Tolerate salinity and sodicity. | Failure to establish cultivation protocols, and lack of information on productivity. Limited knowledge on the plant. Low market value. Lack of evidence in cultivation and marketing. Lack of information on harvesting. Very low yield. | Protocols for cultivation growth. Development of new cultivation methods. Minimize poverty, food and nutrition insecurity. Gain new market potential on the nutritional and health properties. Increase healthy food supply. Provide opportunities for employment. | Limited information on cultivation methods. Lack of commercialization and lack of enthusiasm in cultivation. Land use competition. Limited propagation and cultivation interest. Biodiversity loss. Land degradation. |
| **Uses** | Well documented uses on medicine, ornamental, leaf consumption, and animal fodder. High nutrient contents and health enhancing compounds. Easy to cook. | Little information of commercial value. Lack of customer recognition on the species. Little evidence on economic, medicinal, and ornamental uses. Non- availability to public. Lack of marketing. Low consumption value. | To promote food security and increase the nutritional value of food. Gain popularity in local and international markets. Increase the economy of a country. High foreign invested enterprise. High chances of becoming staple food in dominant populations. | Nutrients loss on cultivated plants. High market competition. Lack of legal protection. Alterations in consumer choices and tastes. Low public awareness. |

## 2.14 Conclusion

*Tetragonia decumbens* Mill., commonly called dune spinach, is a promising but underrated halophyte with enormous potential for culinary and commercial uses. It belongs to the Aizoaceae family and may be grown in dry and coastal areas due to its exceptional resistance to salt and drought. Its importance as a nutrient-dense leafy vegetable is illustrated by its historical use by the Koi-San tribes and its present appeal in upscale cuisine. This revies identifies a number of critical areas of interest for dune spinach future growth, especially with regards to its possible commercial application and cultivation. Dune spinach is a notable option for hydroponic system due to its tolerance to salty and drought conditions. This might reduce soil-related issues and improve the growing conditions of this particular species. Harnessing the full potential of the species requires a well-designed cultivation technique that takes into account its distinct requirements for managing nutrients, water, and soil. Because of its unique characteristics, the plant can survive in harsh conditions because to its physiological and biochemical processes, which include osmotic adjustment, ROS-detoxification, and succulence. It is an important resource for food security because of these processes, which also increase its nutritional content and resistance. Beyond its usage in food preparation, Tetragonia decumbens has a variety of other uses, including as possible involvement in soil bioremediation and animal feed. In order to create efficient culture techniques and determine the market potential, further study is necessary as commercial cultivation is still mainly unknown.

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# CHAPTER THREE

# THE EFFECT OF DILUTED SEAWATER AND DIFFERENT PRUNING INTERVALS ON CHLOROPHYLL CONTENT OF *TETRAGONIA DECUMBENS* MILL. IN HYDROPONICS

**Effect of diluted seawater and different pruning intervals on the chlorophyll content of *Tetragonia decumbens* Mill. in hydroponics**

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## 3.1 Abstract

Chlorophyll is an essential pigment found in plants, algae, and some microorganisms. It is crucial for photosynthesis as it absorbs light energy and converts it into chemical energy. This study investigated how diluted seawater (DSW) and different pruning intervals affected the chlorophyll content in *T. decumbens* leaves over a 12-week period. The experiment tested various seawater dilutions (100% tap water/50L, 20% seawater/50L, and 40% seawater/50L) and pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm cuts). Results showed that both the diluted seawater and different pruning treatments had a significant impact on chlorophyll *a*, chlorophyll *b*, and total chlorophyll levels. Specifically, lower salinity (20%SW) and pruning at 15 cm or 45 cm improved chlorophyll content, suggesting better photosynthetic efficiency and stress tolerance. These results highlight the value of tailored agricultural practices in optimizing chlorophyll levels to boost plant health and productivity.

**Keywords:** chlorophyll, photosynthesis, stress tolerance, salinity stress, *T. decumbens*.

## 3.2 Introduction

Many environmental stressors, such as abiotic and biotic factors, have detrimental effects on plant growth and development, reducing their yield (Ashapkin *et al*., 2020; Elateeq *et al*., 2023). Chlorophyll is a rectangular, strongly connected tetrapyrrole molecules that are produced by plants, algae, and certain microorganisms (Perez-Galvez et al., 2018). Chlorophyll is common in green fruits and vegetables, which constitute an essential component of our diet and play an essential function in photosynthesis (Helena *et al*., 2023). Increased levels of chlorophyll in fruit and vegetable crops have been observed to boost the biological functions and nutritional value. In plants, chlorophyll is the most significant kind of green pigment, and an increase in this pigment enhances the ability of the plant to absorb light, which is necessary for photosynthesis, the process by which plants produce glucose and oxygen from carbon dioxide in the atmosphere and water they take from the soil (Fu *et al*., 2021; Wang *et al*., 2020; Mndi *et al*., 2023).

There are several types of chlorophyll, namely chlorophyll *a*, chlorophyll *b*, chlorophyll *c,* chlorophyll *d*, and chlorophyll *e* (Björn *et al*., 2009). Although there are various forms of chlorophyll, mainly two forms are more common in green plants: chlorophyll *a* and chlorophyll *b* (Wang *et al*., 2023). In plants, chlorophyll *a* appears to be the most prevalent kind of green pigment, and it captures light successfully in the red and blue sections of the spectrum, showing maximum absorbance between 430 and 662 nanometres, respectively (Zhu *et al*., 2017; Durrett & Welti, 2021). Its chemical composition consists of a connected hydrocarbon tail called a phytol and a porphyrin ring containing a core magnesium ion. The phytol tail is comprised of multiple isoprenoid units, while the porphyrin ring is formed by four nitrogen-containing groups known as pyrrole (Helena *et al*., 2023). Another kind of chlorophyll that may be present in plants, algae, and some microorganisms is called chlorophyll *b*. It possesses a slightly different porphyrin ring, and its chemical composition is comparable to the structure of chlorophyll *a*. Due to this variation, chlorophyll *b* absorbs light in the blue-green part of the spectrum, reaching its peak around 453 nanometres (Helena *et al*., 2023). Chlorophyll *b* further serves as a shield for chlorophyll *a* against excessive light; however, it is also involved in photosynthesis.

Salinity stress, on the other hand, provides substantial difficulties to plants by altering photosynthetic enzymes and nutrient intake, accelerating ageing processes, and lowering chlorophyll and carotenoid levels (Yang *et al*., 2020; Yang *et al*., 2021; Al-Gaadi *et al*., 2024). These effects differ based on the plant type and its natural resistance to salt stress (Gong *et al*., 2018). When subjected to 0-500 mM sodium chloride, salt-resistant plants like *Thellungiella halophila* showed greater or constant chlorophyll content, but salt-sensitive plants like *Arabidopsis thaliana* exhibited reduced chlorophyll content (Stepien & Johnson, 2009). A decrease in photosynthetic pigments under salt levels can be attributed to stimulation of the chlorophyll-degrading enzymes such as chlorophyllase and ROS generation (Abdelaal *et al*., 2019; Hasanuzzaman *et al*., 2020; El-Banna *et al*., 2022), higher susceptibility of pigment-protein complexes to deprivation (Siddiqui *et al*., 2020), and the chloroplast ultrastructure (Farouk & Arafa, 2018). Moreover, high salinity levels can further cause damage to the thylakoid membrane (Hamouda *et al*., 2023), resulting in reduced carbon dioxide absorption (Yusuf *et al*., 2024), rapid closure of stomatal pores, and the inhibition of enzymes needed to trigger dark-reaction (Zahra *et al*., 2022).

Nonetheless, pruning is one of the most significant procedures as it enhances the growth, appearance, and vegetative development of crops (Zhang *et al*., 2022). It also allows for the effective intake of nutrients, the rejuvenation of edible portions, and the strategic positioning of the plants to receive light (Pangesti *et al*., 2020; Kadlec *et al*., 2022). During the process of pruning, when branches are trimmed, their capacity to absorb sunlight increases in the vegetative tissues of the unpruned branches to offset the reduction in photosynthesis in the leaves of the pruned parts, and this allows more light to enter through the canopy (Desotgiu *et al*., 2012). Although pruning itself does not directly affect chlorophyll levels, it can indirectly influence chlorophyll dynamics through its effects on light exposure, photosynthetic efficiency, stress response, and regrowth to optimize chlorophyll production and distribution within a plant, ultimately supporting its growth, health, and vitality. Consequently, pruning combined with diluted seawater may enhance the development of *T. decumbens* shoots. Therefore, the purpose of the present study was to assess how *T. decumbens* chlorophyll contents were affected by diluted seawater and different pruning intervals.

## 3.3 Materials and methods

### 3.3.1 Experimental location

This research was carried out at the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa (33°55'56" S and 18°38'25" E). The research project was conducted in an experimental greenhouse with temperature regulation of 21–26 ºC during the day and 12–18 ºC at night. The average relative humidity was maintained at 60%.

### 3.3.2 Plant material and experimental description

*Tetragonia decumbens* cuttings were collected on February 27, 2023, from a specific clone plant that was flourishing alongside the coastline at Granger Bay campus CPUT, Western Cape. A stem cutting propagation technique was used to root new plant material, as reported by (Sogoni *et al.*, 2021). A total of one hundred and ten plants were propagated with rooting powder (Dynaroot™ No. 1 with active ingredient 0.1 % IBA) in polyethylene seedling flat trays filled with a ratio of 50:50 sand/peat. The trays were placed in the propagation greenhouse on heated beds with intermitted mist irrigation. Seventy cuttings were selected out the total number of rooted and moved to a shaded area for five weeks to allow them to acclimatise. Thereafter the rooted cuttings were transplanted into 12.5 cm black plastic pots filled with Consul silica sand. Only healthy plants were selected and arranged in a randomised block design of three treatments and a control with three replicates per treatment totalling forty-eight plants.

### 3.3.3 Nutrient solution

Nutrifeed™ fertiliser from Starke Ayres in Cape Town, South Africa was used as source of aqueous solution in a hydroponic set up. The Nutrifeed contained the following ingredients: 65 g/kg Nitrogen (N), 27 g/kg Phosphorus (P), 130 g/kg Potassium (K), 70 mg/kg Calcium (Ca), 20 mg/kg Copper (Cu), 1500 mg/kg Iron (Fe), 10 mg/kg Molybdenum (Mo), 22 mg/kg Magnesium (Mg), 240 mg/kg Manganese (Mn), 75 mg/kg Sulphur (S), 240 mg/kg Boron (B), and mg/kg Zinc (Zn). For the first three weeks before the experiment started the system operated using tap water only to ensure that plants adapt to their new environment. Thereafter the Nutrifeed solution was prepared at a concentration of 1:500 (1 kg/500 litres, or approximately 10 g per 5 L) in each hydroponic reservoir.

### 3.3.4 Hydroponic design

The hydroponic system was constructed in accordance with (Bulawa *et al.*, 2022) and built with four identical Nutrient Film Technique (NFT) systems. Each system was constructed on a wire mesh square table (2.5 m) to give stability and flat surface. A 50 L low-density polyethylene (LDPE) reservoir and a single submersible water pump capable of producing 2000 litres per hour (1 x 2000 L/h) were present in each of these Nutrient Film Technique (NFT) systems, which were referred to as table 1 to 4 (T1-T4). The low-density polyethylene (LDPE) reservoir served as a container for the nutritional solution that would be utilised in the experiment. The submersible water pump was designed to generate a nutrient solution and transport it from the reservoir to the gutters. White polyvinyl chloride (PVC) square gutters that were 1.36 metres long were used to construct the hydroponic system and were labelled G1, G2, G3, and G4. Each white polyvinyl chloride (PVC) square gutter was covered with black plastic polyethylene sheets to prevent algae growth and three holes per gutter were also created to accommodate 12.5 cm plastic pots. A 50 L low-density polyethylene (LDPE) reservoir, 1 x 2000 l/h submersible water pump, 20 mm low-density polyethylene (LDPE) irrigation pipe, 20 mm elbow irrigation fittings, 20 mm flow regulators, 20 mm T-piece irrigation fittings, and 20 mm end cap irrigation fittings were all used during the construction of each system. Each gutter on each system had 3 pots with a consul silica sand medium. For preventing leaks in the system, a silicon glue (PVC) was used to seal the two components firmly together and when inserting irrigation fittings to the polyvinyl chloride (PVC) square gutters. A portable digital electrical conductivity (EC) metre (Hanna Instruments®TM HI 98312) that has been calibrated was used to measure the electrical conductivity (EC) in the nutritional solution on a daily basis and a calibrated hand-held digital pH metre (Eurotech®TM pH 2 pen) was used to measure the pH in the nutritional solution on a daily basis.  The pH of the nutritional solution was increased with potassium hydroxide [KOH] and reduced with phosphoric acid [$H_3PO_4$]. The pH and electrical conductivity (EC) were maintained at 4.5, to 6.5, and 3.38 to 1.10 ds m -1 (S) respectively.

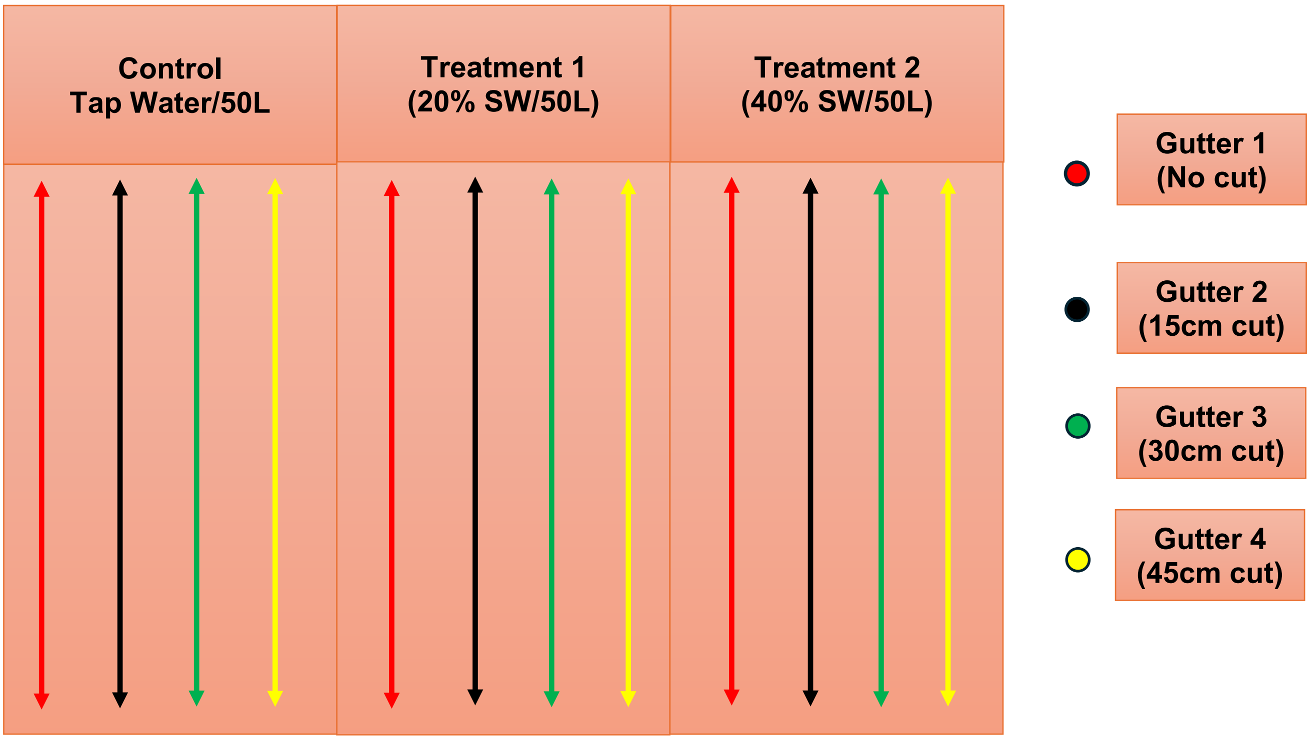


**Figure 3.1:** Layout and random design of the experiment showing the seawater dilution treatments and pruning cuts applied.

### 3.3.5 Treatments

#### 3.3.5.1 Seawater treatments

Seawater was collected from Granger Bay, Cape Town and diluted with tap water to prepare three diluted seawater treatments and clean tap water as the control. All the sumps (S2, S3, and S4) with seawater treatments were refilled every week (Friday) to avoid build-up of algae. The dilutions were as follows:

- S1 Sump 1 = clean tap water (control)
- S2 Sump 2 = 20% SW/50L (Treatment 1)
- S3 Sump 3 = 40% SW/50L (Treatment 2)

#### 3.3.5.2 Pruning treatments

At the beginning of the experiment (week one), all plants were placed in the gutters and pruned back to 15 cm. This was done to ensure uniform height in all plants. Thereafter plants were pruned again at weeks 4 and 8 to three different heights as follows:

- G1 Gutter 1 = No cut (S1G1, S2G1, and S3G1)
- G2 Gutter 2 = 15 cm (S1G2, S2G2, and S3G2)
- G3 Gutter 3 = 30 cm (S1G3, S2G3, and S3G3)
- G4 Gutter 4 = 45 cm (S1G4, S2G4, and S3G4)

At week 12, no pruning occurred, however, all plant samples were harvested and prepared for analysis. At weeks 4 and 8, all the unpruned plants were allowed to grow throughout the experiment without being pruned, hence they are represented with "NA" on the tables. "NA" means the plants were not analysed at that particular stage.

### 3.3.6 Extraction of chlorophyll

Shoots from plants subjected to diluted seawater and pruning were harvested after 4, 8 and 12 weeks of growth to assess the chlorophyll content. The method for estimating chlorophyll within samples was outlined by (Abbas *et al.*, 2023). Using a mortar and pestle, 1 g of fresh leaves was ground into a fine pulp with 20 millilitres of 80% acetone. It was then centrifuged between 5000 and 10000 rpm for 5 minutes. The 50 millilitre flask was filled with the supernatant. After centrifuging the residue for 5 minutes at 5000 to 10000 rpm using 20 millilitres of 80% acetone, the supernatant was poured into the same container. The procedure was repeated until the residue became colourless. Moreover, 80% acetone was used to clean the inner part of the mortar and pestle, and the clear washings were gathered in a glass beaker. The volume was increased to 100 millilitres using 80% acetone. Moreover, 80% acetone was used to clean the inner part of the mortar and pestle, and the clear washings were gathered in a glass beaker. The volume was increased to 100 millilitres using 80% acetone. For every plant sample, this procedure was repeated. The absorption of the extraction solution was measured at 645, 663, and 652 nm in comparison to the solvent (80% acetone).

**Calculations**

The following equation was used to calculate the concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll (Hanafy *et al*., 2021; Sahu *et al*., 2023):

$$\text{Total chlorophyll} = [20.2(A_{645}) - 8.02(A_{663})] \times V/(W)$$

$$\text{Chlorophyll } a = [12.7(A_{663}) - 2.69(A_{645})] \times V/(W)$$

$$\text{Chlorophyll } b = [22.9(A_{645}) - 4.68(A_{663})] \times V/(W)$$

**Where:**

A= absorbance at specific wavelengths

V= final volume of chlorophyll extract in 80% acetone

W= fresh weight of tissue extracted

### 3.3.7 Statistical analysis

The chlorophyll data obtained from shoot samples subjected to diluted seawater and different pruning intervals was statistically computed using a two-way examination of variance (ANOVA). The Fisher's least significant difference will be used to compare the significant differences between treatment means at $p \leq 0.05$ using MINI-TAB statistical software.

## 3.4 Results

### 3.4.1 Effect of diluted seawater and different pruning intervals on the chlorophyll content of *T. decumbens*.

#### 3.4.1.1 Effect of diluted seawater and different pruning intervals on chlorophyll *a*

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on chlorophyll *a* in *T. decumbens* shoots (Table 3.1). Plants grown under 20%DSW recorded the highest chlorophyll content (565.12 ug/g), while the control recorded the lowest chlorophyll content (294.98 ug/g). The highest mean value was significantly higher than other treatments, including the control.

**Pruning (Week 4):** Different pruning intervals had a significant effect on chlorophyll *a* in the shoots of *T. decumbens*. The application of 45 cm pruning intervals resulted in high chlorophyll *a* content, while 15 cm pruning in the control treatment resulted in low chlorophyll *a* content.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals significantly affected the chlorophyll *a* content.

**Diluted seawater (Week 8):** Diluted seawater showed a significant effect ($p \leq 0.05$) on chlorophyll *a* in *T. decumbens* shoots (Table 3.2). Plants cultivated under control treatment recorded the highest chlorophyll content (390.02 ug/g), while plants cultivated under 20%DSW recorded the lowest chlorophyll content (244.00 ug/g). The lowest mean value was significantly lower than other treatments but did not differ significantly from the control treatment with a 30 cm pruning interval (250.16 ug/g).

**Pruning (Week 8):** Table 3.2 shows a significant effect on chlorophyll *a* in *T. decumbens* shoots. The plants subjected to 30 cm pruning intervals recorded the highest mean value of chlorophyll *a*. The lowest mean value of chlorophyll *a* was recorded in plants subjected to 15 cm and 30 cm in 20%DSW treatment.

**Diluted seawater interaction with pruning (Week 8):** A significant impact in chlorophyll *a* was observed in the interaction of diluted seawater and different pruning intervals in the shoots of *T. decumbens*.

**Diluted seawater (Week 12):** Diluted seawater (DSW) had a significant effect ($p \leq 0.05$) on chlorophyll *a* in *T. decumbens* shoots (Table 3.3). Plants cultivated under 20%DSW recorded the highest mean value (298.07 ug/g) of chlorophyll *a* and this was comparable with the chlorophyll obtained in plants cultivated under 20%DSW with a 45 cm pruning interval (297.01 ug/g). The control treatment recorded the least chlorophyll *a* content (96.35 ug/g).]

**Pruning (Week 12):** Different pruning intervals showed a significant impact on chlorophyll *a* content in the shoots of *T. decumbens*. The application of 15 cm pruning intervals recorded the highest mean values of chlorophyll *a*, while plants without pruning in the control recorded the lowest mean value of chlorophyll *a*.

**Diluted seawater interaction with pruning (Week 12):** During this week, the interaction of diluted seawater and different pruning intervals had no significant effect on chlorophyll *a*.

#### 3.4.1.2 Effect of diluted seawater and different pruning intervals on chlorophyll *b*

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on chlorophyll *b* in *T. decumbens* shoots (Table 3.1). The plants cultivated under 20%DSW recorded the highest chlorophyll *b* content (431.06 ug/g), while the control treatment recorded the lowest chlorophyll *b* content (198.60 ug/g). The lowest mean value was significantly lower than other treatments but did not differ from plants subjected to 40%DSW with 45 cm pruning interval (213.50 ug/g).

**Pruning (Week 4):** Table 3.1 showed a significant effect on chlorophyll *b* content in the shoots of *T. decumbens*. The application of 45 cm pruning intervals resulted in the highest chlorophyll *b* content. The lowest chlorophyll *b* content was recorded in 15 cm pruning intervals in the control treatment, and these were comparable to plants subjected to 45 cm pruning intervals in 40%DSW treatment.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had no significant effect on chlorophyll *b* in the shoots of *T. decumbens*.

**Diluted seawater (Week 8):** Diluted seawater (DSW), had a significant effect ($p \leq 0.05$) on chlorophyll *b* in *T. decumbens* shoots (Table 3.2). The plants cultivated under the control recorded the highest chlorophyll content (309.08 ug/g), while 20%DSW recorded the lowest chlorophyll content (202.45 ug/g). The lowest mean value was significantly lower than other treatments including the control.

**Pruning (Week 8):** Different pruning intervals showed a significant increase on chlorophyll *b* content in the shoots of *T. decumbens* (Table 3.2). The application of 30 cm pruning intervals recorded the highest mean value of chlorophyll *b.* The lowest mean values of chlorophyll *b* were recorded in 20%DSW treatment with 15 cm and 30 cm pruning intervals.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals had no significant effect on chlorophyll *b* in the shoots of *T. decumbens*.

**Diluted seawater (Week 12):** Diluted seawater (DSW) showed a significant effect ($p \leq 0.05$) on chlorophyll *b* in *T. decumbens* shoots (Table 3.3). The plants cultivated under 20%DSW recorded the highest mean value (172.94 ug/g) of chlorophyll *b* but did not differ significantly from the control with a 15 cm pruning interval (170.00 ug/g). The control also recorded the least chlorophyll *b* content (55.68 ug/g) and did not differ significantly from 20%DSW without a pruning interval (81.27 ug/g).

**Pruning (Week 12):** Table 3.3 shows a significant effect on chlorophyll *b* in *T. decumbens* shoots. The application of 15 cm pruning intervals resulted in the highest chlorophyll *b* content in both the control and 20%DSW treatment. The plants without pruning intervals resulted in the lowest chlorophyll *b* content in both the control and 20%DSW treatment.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and different pruning intervals showed no significant effect on chlorophyll *b* in the shoots of dune spinach.

#### 3.4.1.3 Effect of diluted seawater and different pruning intervals on total chlorophyll

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on total chlorophyll in *T. decumbens* shoots (Table 3.1). The plants cultivated under 20%DSW recorded the highest total chlorophyll content (996.05 ug/g), while the control treatment recorded the lowest total chlorophyll content (406.06 ug/g). The highest mean value was significantly higher than all other treatments.

**Pruning (Week 4):** Different pruning intervals showed a significant impact on the total chlorophyll content of *T. decumbens* shoots. The application of 45 cm pruning intervals recorded the highest total chlorophyll content, while 15 cm pruning intervals recorded the lowest total chlorophyll content (Table 3.1).

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals showed a significant effect on the total chlorophyll content in the shoots of *T. decumbens*.

**Diluted seawater (Week 8):** Diluted seawater showed a significant effect ($p \leq 0.05$) on total chlorophyll in *T. decumbens* shoots (Table 3.2). The plants subjected under the control treatment recorded the highest total chlorophyll content (699.08 ug/g), while the plants subjected under the 20%DSW recorded the lowest total chlorophyll content (447.00 ug/g). The lowest mean value was significantly lower than other treatments but did not differ significantly from the 20%DSW with a 30 cm pruning interval (452.05 ug/g).

**Pruning (Week 8):** Table 3.2 showed a significant effect on the total chlorophyll content in *T. decumbens* shoots. The plants subjected to 30 cm pruning intervals resulted in the highest total chlorophyll content. The lowest total chlorophyll content was recorded in 15 cm and 30 cm in 20%DSW treatment.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals showed a significant effect on the total chlorophyll content in the shoots of *T. decumbens*.

**Diluted seawater (Week 12):** Table 3.3 showed a significant effect ($p \leq 0.05$) on the total chlorophyll content in *T. decumbens* shoots. The plants cultivated under 20%DSW recorded the highest mean value (471.06 ug/g) of total chlorophyll and were comparable to those cultivated under 20%DSW with a 45 cm pruning interval (454.01 ug/g). The control treatment recorded the lowest total chlorophyll content (151.99 ug/g) and was significantly lower than all other treatments.

**Pruning (Week 12):** The application of different pruning intervals showed a significant effect on the total chlorophyll content in the shoots of *T. decumbens*. The plants subjected to 15 cm pruning intervals recorded the highest mean value of total chlorophyll and was comparable to plants subjected to 45 cm pruning intervals in 20%DSW treatment. The lowest mean value was recorded in plants without pruning intervals in the control treatment.

**Diluted seawater interaction with pruning (Week 12):** During week 12, the interaction of diluted seawater and different pruning intervals showed no significant effect on the total chlorophyll content in the shoots of *T. decumbens*.

**Table 3.1: The effect of diluted seawater and different pruning intervals on chlorophyll content of *T. decumbens* shoots (Week 4).**

| Seawater | Pruning level | Chlorophyll *a* (ug/g) | Chlorophyll *b* (ug/g) | Total Chlorophyll (ug/g) |
|---|---|---|---|---|
| Control | No cut | NA | NA | NA |
| | 15 cm | 294.98 ± 2.24g | 198.60 ± 0.95e | 471.06 ± 21.00d |
| | 30 cm | 438.12 ± 7.62cd | 316.02 ± 11.04b | 406.06 ± 13.08e |
| | 45 cm | 468.42 ± 7.00b | 309.27 ± 4.86b | 454.08 ± 17.07de |
| 20% | No cut | NA | NA | NA |
| | 15 cm | 373.07 ± 12.08f | 242.45 ± 4.45d | 616.00 ± 17.00c |
| | 30 cm | 418.08 ± 28.07de | 271.03 ± 22.01c | 689.09 ± 50.08b |
| | 45 cm | 565.12 ± 7.50a | 431.06 ± 12.05a | 996.05 ± 19.08a |
| 40% | No cut | NA | NA | NA |
| | 15 cm | 418.89 ± 5.24de | 250.08 ± 13.06cd | 669.05 ± 18.07bc |
| | 30 cm | 453.38 ± 3.56bc | 263.66 ± 2.03cd | 716.84 ± 5.58b |
| | 45 cm | 400.46 ± 0.50ef | 213.50 ± 0.44e | 613.79 ± 0.93c |
| Two-way ANOVA F- Statistics | | | | |
| Seawater | | 15.29* | 35.24* | 160.66* |
| Pruning | | 1430.33* | 731.64* | 783.65* |
| Seawater*Pruning | | 31.89* | 41.85* | 48.79* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed.

**Table 3.2: The effect of diluted seawater and different pruning intervals on chlorophyll content of *T. decumbens* shoots (Week 8).**

| Seawater | Pruning level | Chlorophyll *a* (ug/g) | Chlorophyll *b* (ug/g) | Total Chlorophyll (ug/g) |
|---|---|---|---|---|
| Control | No cut | NA | NA | NA |
| | 15 cm | 306.29 ± 6.52c | 235.28 ± 5.58c | 541.04 ± 11.02c |
| | 30 cm | 390.02 ± 12.09a | 309.08 ± 16.01a | 699.08 ± 28.03a |
| | 45 cm | 355.06 ± 6.56b | 264.85 ± 9.51bc | 619.07 ± 15.09b |
| 20% | No cut | NA | NA | NA |
| | 15 cm | 244.00 ± 13.06d | 203.02 ± 13.02d | 447.00 ± 26.07d |
| | 30 cm | 250.16 ± 7.80d | 202.45 ± 5.98d | 452.05 ± 13.06d |
| | 45 cm | 324.04 ± 16.06c | 274.02 ± 17.02b | 598.04 ± 33.08bc |
| 40% | No cut | - | - | - |
| | 15 cm | - | - | - |
| | 30 cm | - | - | - |
| | 45 cm | - | - | - |
| Two-way ANOVA F- Statistics | | | | |
| Seawater | | 70.16* | 19.12* | 40.92* |
| Pruning | | 517.30* | 286.58* | 400.87* |
| Seawater*Pruning | | 18.72* | 12.66* | 15.57* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed. (-) = Death of plants.

**Table 3.3: The effect of diluted seawater and different pruning intervals on chlorophyll content of *T. decumbens* shoots (Week 12).**

| Seawater | Pruning level | Chlorophyll *a* (ug/g) | Chlorophyll *b* (ug/g) | Total Chlorophyll (ug/g) |
|---|---|---|---|---|
| Control | No cut | 96.35 ± 0.85e | 55.68 ± 2.35d | 151.99 ± 3.12f |
| | 15 cm | 244.03 ± 13.02c | 170.00 ± 14.05a | 414.02 ± 27.06b |
| | 30 cm | 202.86 ± 5.52d | 158.08 ± 12.04ab | 361.06 ± 17.00cd |
| | 45 cm | 193.49 ± 1.54d | 128.21 ± 6.63c | 321.61 ± 5.74de |
| 20% | No cut | 196.45 ± 1.22d | 81.27 ± 0.46d | 277.65 ± 1.19e |
| | 15 cm | 298.07 ± 14.00a | 172.94 ± 7.02a | 471.06 ± 21.00a |
| | 30 cm | 272.99 ± 5.03b | 133.75 ± 8.87bc | 406.06 ± 13.08bc |
| | 45 cm | 297.01 ± 10.07ab | 157.07 ± 7.81ab | 454.01 ± 17.06ab |
| 40% | No cut | - | - | - |
| | 15 cm | - | - | - |
| | 30 cm | - | - | - |
| | 45 cm | - | - | - |
| | | Two-way ANOVA F- Statistics | | |
| Seawater | | 197.94* | 61.45* | 63.84* |
| Pruning | | 87.37* | 52.32* | 76.70* |
| Seawater*Pruning | | 4.15ns | 4.13ns | 4.04ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed. (-) = Death of plants.

### 3.5 Discussion

Chlorophyll, a complex green pigment found in plants is essential in photosynthesis because it absorbs light energy and converts it into chemical energy (Björn *et al*., 2009). This crucial pigment not only gives plants their distinctive green colour, but it also plays a critical function in capturing and harnessing solar energy to sustain the metabolic processes required for growth and development. Salinity stress, on the other hand, provides substantial difficulties to plants by altering photosynthetic enzymes and nutrient intake, accelerating ageing processes, and lowering chlorophyll and carotenoid levels (Yang *et al*., 2020; Yang *et al*., 2021; Al-Gaadi *et al*., 2024). These effects differ based on the plant type and its natural resistance to salt stress (Gong *et al*., 2018). When subjected to 0-500 mM sodium chloride, salt-resistant plants like *Thellungiella halophila* showed greater or constant chlorophyll content, but salt-sensitive plants like *Arabidopsis thaliana* exhibited reduced chlorophyll content (Stepien & Johnson, 2009).

In the present study, chlorophyll content was used as a biochemical indicator to assess the ability of *T. decumbens* to tolerate salt. Throughout the experiment, the amount of chlorophyll in each treatment fluctuated. Diluted seawater (DSW), pruning intervals, and their interaction significantly affected the chlorophyll content of *T. decumbens* on weeks 4 and 8. However, on week 12, only diluted seawater and pruning that significantly affected the chlorophyll content of *T. decumbens*. Low salinity levels (20% SW) at 15 cm and 45 cm pruning levels showed increased chlorophyll levels on weeks 4 and 12 when compared to the control treatment. These findings are consistent with those of (Parvin *et al*., 2015) and (Sogoni *et al*., 2021), who reported a significant reduction in total chlorophyll content at high levels of salinity and a slight rise at low to moderate salinity levels. Likewise, (Ngxabi *et al*., 2021) reported a similar trend, indicating a significant increase in chlorophyll content at low salinity levels in *Trachyandra ciliata*. Furthermore, chlorophyll content reduced as salt levels increased in this experiment, as demonstrated by the 40%SW treatment at week 4. These findings are consistent with those of (Farouk *et al*., 2020) who found that high salinity levels are lowering chlorophyll content of *Ocimum basilicum* . (El-Taher *et al*., 2021) observed a similar pattern, finding that high salinity levels are decreasing photosynthetic pigments of Cowpea (*Vigna unguiculata* L.). Moreover, high salinity levels can cause damage to the thylakoid membrane (Hamouda *et al*., 2023), resulting in reduced carbon dioxide absorption (Yusuf *et al*., 2024), rapid closure of stomatal pores, and the inhibition of enzymes needed to trigger dark-reaction (Zahra *et al*., 2022).

Pruning is a crucial practice for maintaining plant height or extending the harvesting season. It promotes new growth, flowering, fruiting, and high-quality yields in flowering and crop plants (Mawarni & Siahaan, 2022; Bora *et al*., 2024; Peng *et al*., 2024). Moreover, pruning promotes plant health by removing damaged or diseased branches and improving air and sunlight circulation (Rahman *et al*., 2024). During this study, Chlorophyll *a*, chlorophyll *b*, and total chlorophyll content under 20%SW were also significantly increased by pruning intervals on weeks 4 and 12. These findings are consistent with those of (Tripathi *et al*., 2020), who discovered a significant effect on chlorophyll *a* and chlorophyll *b* during the pruning of guava (*Psidium guajava* L.). However, a different trend was observed on week 8, where the control treatment significantly showed higher values of chlorophyll *a*, chlorophyll *b*, and total chlorophyll. This might be due to the increased availability of light in plants after pruning on week 4.

## 3.6 Conclusion and recommendations

The results demonstrated significant variations in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content under different treatments. Notably, *T. decumbens* plants subjected to 20%SW with a 15 cm and 45 cm pruning intervals exhibiting high chlorophyll levels. This suggests that low to moderate salinity levels and different pruning intervals can positively influence chlorophyll accumulation, potentially enhancing photosynthetic efficiency and stress tolerance in plants. The findings align with previous research highlighting the role of environmental factors and management practices in chlorophyll regulation. Understanding these dynamics is crucial for optimizing agricultural strategies aimed at improving crop yield and quality, particularly in saline environments where chlorophyll levels are often compromised. In conclusion, the study underscores the importance of integrating saline management practices and pruning strategies to sustainably enhance chlorophyll production in *T. decumbens*. Future research could explore the underlying physiological mechanisms and long-term effects of these interventions on plant metabolism and overall productivity.

## 3.7 Acknowledgements

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# CHAPTER FOUR

# RESPONSES AND RESILIENCY OF *TETRAGONIA DECUMBENS* MILL. TO PRUNING AND SEAWATER DILUTIONS IN HYDROPONIC CULTIVATION

**Responses and resiliency of *Tetragonia decumbens* Mill. to pruning and seawater dilutions in hydroponic cultivation**

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## 4.1 Abstract

This research aimed to investigate how diluted seawater and varying pruning levels affected *Tetragonia decumbens* (Dune spinach) vegetative growth, by exploring sustainable and environmentally friendly cultivation methods and evaluating its potential as an edible wild vegetable crop. The treatments in the study included variable amounts of seawater dilutions (100% tap water/50L, 20% SW/50L, 40% SW/50L, and 60% SW/50L) and different pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm cut). A Nutrifeed fertiliser was added to boost each treatment. During weeks 8 and 12, the number of branches (NB) and stem length (STL) were not significantly affected by diluted seawater. However, data collected at weeks 4 and 12 showed that low seawater concentrations significantly ($p < 0.05$) increased the shoot length (SHL), fresh weight (FWS), and dry weight (DWS) of *T. decumbens*. This demonstrates that *T. decumbens* may survive and thrive in seawater at low concentrations. In general, these findings contribute to a greater understanding of sustainable cultivation strategies for *T. decumbens*, emphasising its potential as a resilient and viable crop, particularly in areas with water scarcity or salinity challenges.

**Keywords:** Dune spinach, hydroponics, sustainable agriculture, *T. decumbens*, vegetative growth

## 4.2 Introduction

Climate change and unpredictable weather patterns are making it difficult for farmers in Sub-Saharan Africa to meet the global food shortage. As the global population is expected to exceed 9.7 billion by 2025, there will be a significant rise in demand for food supplies, which may not be met by cultivating conventional agricultural crops (Altaf *et al*., 2023; Barkla *et al*., 2024). Agricultural land use continues to decline due to the effects of climate change, population growth, soil salinization, freshwater shortages, and waterlogging of the soils (Munir *et al*., 2022; Sousa *et al*., 2024). Regenerative efforts have prompted farmers to source new crops and manage the complexity of plant resilience to changing systems such as extreme salinity levels (Srivarathan *et al*., 2023).

Several communal farmers have been experimenting with new edible wild plants that are high in nutrients and vitamins for human dietary consumption and that can tolerate saline conditions (Anju *et al*., 2022; Bulawa *et al*., 2022). Many wild plants are used in African countries for supplementing local diets (Leal Filho *et al*., 2022; Sultanbawa & Sivakumar, 2022). One wild edible species found in South Africa, *Tetragonia decumbens* (Aizoaceae), remains one of the most underutilised wild edible vegetables due to a lack of cultivation protocols, prospective uses, and nutritional values (Sogoni *et al*., 2021). This species thrives on offshore coastal sandy dunes from Namibia to the Eastern Cape as a halophyte with lance-shaped, succulent dark green leaves with a woody base stem and long, rigid fibrous roots (Klak *et al*., 2017). The salty-tasting leaves and tender stems of this species are cooked with a variety of vegetables or used similarly to spinach in green salads (Forrester, 2004; Mncwango *et al*., 2020). Additionally, part of the plants is suitable for pickling, fermenting, and cooking in various restaurant dishes such as stir fries, stews, and soups (Sogoni *et al*., 2021). Due to the species nutritional value, it is imperative to establish growing protocols to analyse its essential vitamins and nutrients to aid food security in Africa and globally. The most recent agricultural research on this species focused on improving crop yield and quality while reducing production expenses (Nkcukankcuka *et al*., 2021).

In agriculture, pruning is a very important technique used to maintain plant height or extend the harvesting period. It stimulates new growth, blooming, fruiting, and high-quality yields in flowering and crop plants (Mawarni & Siahaan, 2022; Bora *et al*., 2024; Peng *et al*., 2024). Furthermore, pruning enhances the health of plants by eliminating unhealthy or diseased branches and promoting better air and sunlight circulation (Rahman *et al*., 2024). Lack of pruning results in shorter, thinner emerging buds, which disrupts the overall volume of canopy growth and reduces the support growth of the plant (Mozumder *et al*., 2021). This study aims to assess the impact of pruning on vegetative growth of *T. decumbens*. It also focuses on the development of a production protocol for *T. decumbens* in a hydroponic system to evaluate its resilience to various seawater dilutions.

## 4.3 Materials and methods

### 4.3.1 Experimental location

This research was carried out at the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa (33°55'56" S and 18°38'25" E). The research project was conducted in an experimental greenhouse with temperature regulation of 21–26 ºC during the day and 12–18 ºC at night. The average relative humidity was maintained at 60%.

### 4.3.2 Plant material and experimental description

*Tetragonia decumbens* cuttings were collected on February 27, 2023, from a specific clone plant that was flourishing alongside the coastline at Granger Bay, Western Cape. A stem cutting propagation technique was used to root new plant material, as reported by (Sogoni *et al*., 2021). A total of one hundred and ten plants were propagated with rooting powder (DynarootTM No. 1 with active ingredient 0.1% IBA) in polyethylene seedling flat trays filled with a ratio of 50:50 sand/peat. The trays were placed in the propagation greenhouse on heated beds with intermitted mist irrigation. Seventy cuttings were selected out of the total number rooted and moved to a shaded area for five weeks to allow them to acclimatise. Thereafter, the cuttings were transplanted into 12.5 cm black plastic pots filled with Consul silica sand. Only healthy plants were selected and arranged in a randomised block design of three treatments and a control with nine replicates per treatment, totalling forty-eight plants.

**Figure 4.1**: Stem cuttings of *Tetragonia decumbens* placed on a heated bed in the environmentally controlled greenhouse (Picture: Ntoyaphi).

### 4.3.3 Nutrient solution

Nutrifeed$^{TM}$ fertiliser sourced from Starke Ayres in Cape Town, South Africa, was used to prepare a hydroponic aqueous solution and used as general feeding to ensure healthy plant development. Nutrifeed contains the following ingredients: 65 g/kg Nitrogen [N], 27 g/kg Phosphorus [P], 130 g/kg Potassium [K], 70 mg/kg Calcium [Ca], 20 mg/kg Copper [Cu], 1500 mg/kg Iron [Fe], 10 mg/kg Molybdenum [Mo], 22 mg/kg Magnesium [Mg], 240 mg/kg Manganese [Mn], 75 mg/kg Sulphur [S], 240 mg/kg Boron [B], and mg/kg Zinc [Zn]. For the first three weeks before the experiment started, the system operated using tap water only to ensure plants would adapt to their new environment. Thereafter, the Nutrifeed concentration was maintained at a concentration of 1:500 (1 kg/500 litres, or approximately 10 g per 5 L) in each hydroponic sump.


**Figure 4.2:** Nutrifeed, a balanced fertiliser for shrubs, trees and vegetables used as a soluble water treatment (Picture: Ntoyaphi).

### 4.3.4 Hydroponic design

The hydroponic system was constructed in accordance with (Bulawa *et al*., 2022) and built with four identical Nutrient Film Technique (NFT) systems. Each system was constructed on a wire-mesh square table (2.5 m) to provide stability and a flat surface. A 50-litre low-density polyethylene (LDPE) reservoir and a single submersible water pump capable of producing 2000 litres per hour (1 x 2000 L/h) were present in each of these Nutrient Film Technique (NFT) systems, which were referred to as Tables 1–4 (T1–T4). The low-density polyethylene (LDPE) reservoir served as a container for the nutritional solution that would be used in the experiment. The submersible water pump was designed to generate a nutrient solution and transport it from the reservoir to the gutters. White polyvinyl chloride (PVC) square gutters of 1.36 metres long were used to construct the hydroponic system and were labelled G1, G2, G3, and G4. Each white polyvinyl chloride (PVC) square gutter was covered with black plastic polyethylene sheets to prevent algae growth, and three holes per gutter were also created to accommodate 12.5 cm of plastic pots. A 50-litre low-density polyethylene (LDPE) reservoir, 1 x 2000 l/h submersible water pump, 20 mm low-density polyethylene (LDPE) irrigation pipe, 20 mm elbow irrigation fittings, 20 mm flow regulators, 20 mm T-piece irrigation fittings, and 20 mm end cap irrigation fittings were all used during the construction of each system. Each gutter on each system had three pots with a consul silica sand medium. For preventing leaks in the system, silicon glue (PVC) was used to seal the two components firmly together and when inserting irrigation fittings into the polyvinyl chloride (PVC) square gutters. A portable digital electrical conductivity (EC) metre (Hanna Instruments®TM HI 98312) that has been calibrated was used to measure the electrical conductivity (EC) in the nutritional solution on a daily basis, and a calibrated hand-held digital pH metre (Eurotech®TM pH 2 pen) was used to measure the pH in the nutritional solution on a daily basis. The pH of the nutritional solution was increased with potassium hydroxide [KOH] and reduced with phosphoric acid [$H_3PO_4$]. The pH and electrical conductivity (EC) were maintained at 4.5, 6.5, and 3.38 to 1.10 ds m-1 (S), respectively.

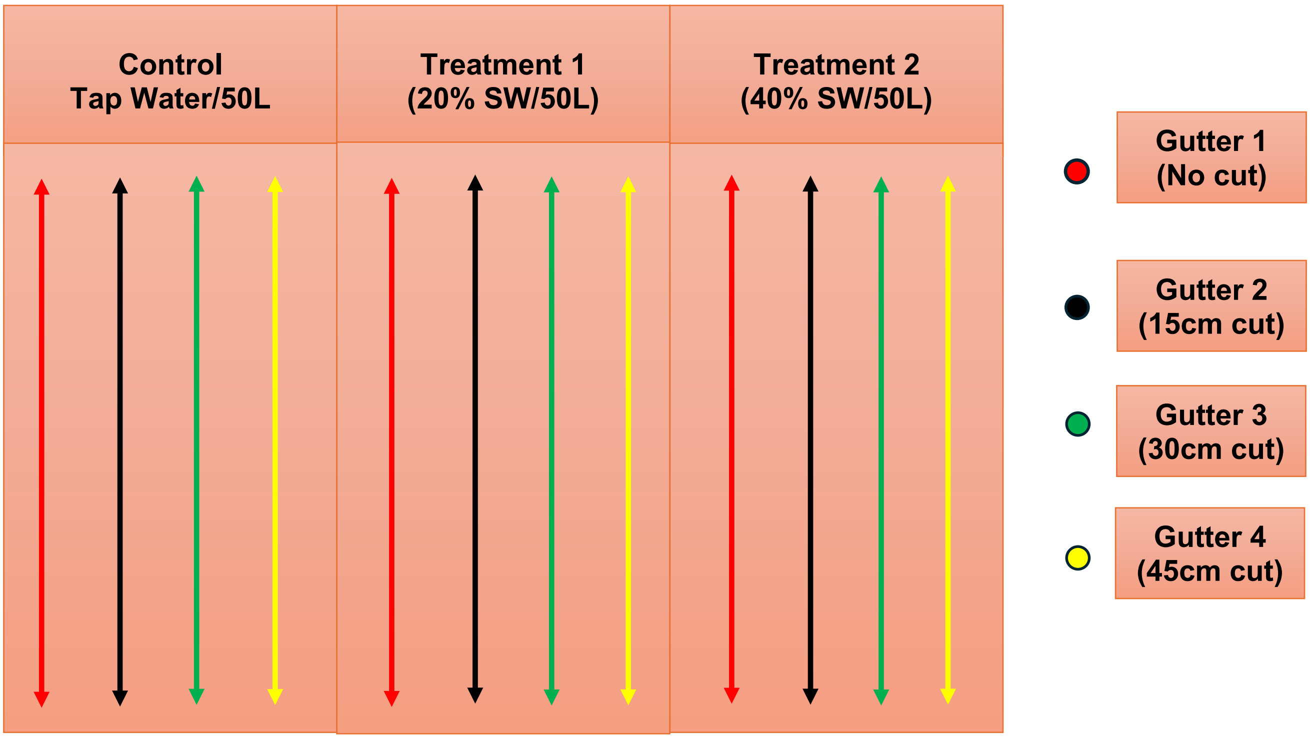


**Figure 4.3:** Layout and random design of the experiment showing the seawater dilution treatments and pruning cuts applied.

### 4.3.5 Treatments

#### 4.3.5.1 Seawater treatments

Seawater was collected from Granger Bay, Cape Town, and diluted with tap water to prepare three diluted seawater treatments and clean tap water as the control. All the sumps (S2, and S3) with seawater treatments were refilled every week (Fridays) to avoid the buildup of algae. The dilutions were as follows:

- S1 Sump 1 = clean tap water (control)
- S2 Sump 2 = 20% SW/50L (Treatment 1)
- S3 Sump 3 = 40% SW/50L (Treatment 2)

#### 4.3.5.2 Pruning treatments

At the beginning of the experiment (week one), all plants were placed in the gutters and pruned back to 15 cm. This was done to ensure uniform height in all plants. Thereafter plants were pruned again at weeks 4 and 8 to three different heights as follows:

- G1 Gutter 1 = No cut (S1G1, S2G1, and S3G1)
- G2 Gutter 2 = 15 cm (S1G2, S2G2, and S3G2)
- G3 Gutter 3 = 30 cm (S1G3, S2G3, and S3G3)
- G4 Gutter 4 = 45 cm (S1G4, S2G4, and S3G4)

At week 12, no pruning occurred, however, all plant samples were harvested and prepared for analysis. At weeks 4 and 8, all the unpruned plants were allowed to grow throughout the experiment without being pruned, hence they are represented with “NA” on the tables. “NA” means the plants were not analysed at that particular stage.

**Figure 4.4:** Stem length growth of *T. decumbens* in a NFT hydroponic system (Picture: Ntoyaphi).

#### 4.3.6 Collecting data

##### 4.3.6.1 Shoot length and stem length

The shoot length and stem length were used to determine the height and new growth of the plants. Shoot length and stem length were measured (cm) every week (Tuesdays) using a standard measuring tape and recorded on a data spreadsheet.

##### 4.3.6.2 Number of branches

The number of branches were manually counted every week (Tuesdays) and recorded on a data spreadsheet.

#### 4.3.6.3 Pruned branches

The branches were pruned every four weeks from weeks 4, 8, and 12. A standard laboratory scale (g) (RADWAG® Model PS 750.R2) was used to determine both fresh and dry weights of pruned plant materials. Thereafter, the pruned plant material was separately dried at 40 °C using a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd., 3269 Ranjit Nagar, New Dehli, 110008) oven until moisture was completely removed from the tissues. The fresh and dry weights of pruned plant material were then measured and recorded on a data spreadsheet.

#### 4.3.6.4 Plant weight

The plant weight was determined using a standard laboratory scale (g) (RADWAG® Model PS 750.R2). The shoots, stems, and roots were separated after week 12, and the fresh weights of the individual samples were recorded on a spreadsheet. The plant material was then oven-dried at 50 °C in a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd., 3269 Ranjit Nagar, New Delhi, India) to a constant weight and recorded.

### 4.3.7 Statistical analysis

The data obtained from growth parameters, harvesting intervals, and essays in this study were statistically computed using a two-way examination of variance (ANOVA). The Fisher's least significant difference will be used to compare the significant differences between treatment means at $p \leq 0.05$ using MINI-TAB statistical software.

## 4.4 Results

### 4.4.1 Effect of diluted seawater and different pruning intervals on plant growth parameters

#### 4.4.1.1 Effect of diluted seawater and different pruning intervals on shoot height

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on the shoot height of *T. decumbens* (Table 4.1). The control treatment recorded the highest mean value (85.13 cm) of shoot height, while 40%DSW recorded the least mean value of shoot height (41.77 cm). The highest mean value was significantly higher than all other treatments but did not differ much from 20%DSW (81.93 cm).

**Pruning (Week 4):** Table 4.1 showed no significant effect on the shoot height of *T. decumbens* shoots. The application of 45 cm pruning intervals in the control treatment resulted in the highest shoot height of dune spinach, while plants without pruning in 40%DSW treatment resulted in the lowest shoot height.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had no significant effect on the shoot height of dune spinach (Table 4.1).

**Diluted seawater (Week 8):** Diluted seawater showed no significant effect on the shoot height of dune spinach (Table 4.2). The plants cultivated in 20%DSW recorded the highest mean value of shoot height (96.3), while the control recorded the least shoot height value (44.17 cm). The highest mean value was significantly higher than all other treatments but was comparable to the control without pruning interval (91.1 cm).

**Pruning (Week 8):** The application of different pruning intervals had a significant effect on the shoot height of *T. decumbens*. The plants without pruning intervals in 20%DSW showed the highest mean value of shoot height in dune spinach. The lowest mean value was obtained 15 cm pruning intervals in the control treatment. The highest mean value was significantly higher than all other treatments but did not differ from the control without pruning.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals had no significant effect on the shoot height of dune spinach (Table 4.2).

**Diluted seawater (Week 12):** Diluted seawater had a significant impact ($p \leq 0.05$) on the shoot height of *T. decumbens* (Table 4.3). The plants subjected to 20%DSW recorded the highest mean value (118.6 cm) of shoot height, while the least shoot height value (34.40 cm) was recorded in the control. The highest mean value was significantly higher than all other treatments but was comparable to the control without a pruning interval (106.6 cm).

**Pruning (Week 12):** Table 4.3 showed a significant increase on the shoot height of dune spinach. The plants without pruning intervals in 20%DSW recorded the highest mean values of shoot height. The lowest mean values were obtained in 15 cm pruning intervals in the control treatment.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and different pruning intervals had no significant impact on the shoot height of dune spinach (Table 4.3).

#### 4.4.1.2 Effect of diluted seawater and different pruning intervals on shoot length

**Diluted seawater (Week 4):** Diluted seawater had a significant impact ($p \leq 0.05$) on the shoot length of *T. decumbens* (Table 4.1). The highest mean value of shoot length (11.60 cm) was recorded in 40%DSW and was significantly higher when compared to other treatments. The control recorded the least shoot length value (5.80 cm).

**Pruning (Week 4):** Different pruning intervals had no significant impact on the shoot length of *T. decumbens*. The plants subjected to 30 cm pruning intervals in 40%DSW recorded the highest shoot length, while 30 cm pruning intervals in the control recorded the lowest mean value.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had no significant impact on the shoot length of *T. decumbens* (Table 4.1).

**Diluted seawater (Week 8):** Diluted seawater different pruning intervals had no significant impact on the shoot length of *T. decumbens*. The results showed that there was no difference between the control treatment and 20%DSW treatment.

**Pruning (Week 8):** Different pruning intervals showed nonsignificant effect on the shoot length of *T. decumbens* (Table 4.2). The application of different pruning intervals showed no difference between the treatments (Table 4.2).

**Diluted seawater interaction with pruning (Week 8):** Table 4.2 showed nonsignificant effect on the shoot length of dune spinach. It is clear that the interaction of diluted seawater and different pruning intervals had no positive impact on *T. decumbens* shoots.

**Diluted seawater (Week 12):** Diluted seawater had nonsignificant impact on the shoot length of *T. decumbens* (Table 4.3). The results showed no significant differences between the control and 20%DSW.

**Pruning (Week 12):** Different pruning intervals showed no significant effect on the shoot length of *T. decumbens* (Table 4.3). The application of different pruning intervals showed no difference between the treatments (Table 4.3).

**Diluted seawater interaction with pruning (Week 12):** Table 4.3 showed nonsignificant effect on the shoot length of dune spinach. It is clear that the interaction of diluted seawater and different pruning intervals had no positive impact on *T. decumbens* shoots.

**Table 4.1: Effect of diluted seawater and different pruning intervals shoot height, stem length, number of branches, FWS, and DWS of hydroponically grown *T. decumbens*. (Week 4).**

| Seawater | Pruning level | SHL (cm) | STL (cm) | NB (n) | FWS (g) | DWS (g) |
|---|---|---|---|---|---|---|
| Control | No cut | 67.60 ± 9.53abc | 7.00 ± 0.15cd | 5.67 ± 0.67a | NA | NA |
| | 15 cm | 72.6 ± 12.7ab | 6.70 ± 0.78cd | 5.00 ± 0.58a | 152.4 ± 45.7abc | 22.75 ± 6.52abc |
| | 30 cm | 79.87 ± 3.72ab | 5.80 ± 0.30d | 5.33 ± 0.88a | 61.1 ± 12.9c | 8.25 ± 1.46cd |
| | 45 cm | 85.13 ± 2.78a | 6.60 ± 0.61cd | 4.67 ± 0.67a | 78.7 ± 20.4c | 10.46 ± 2.68bcd |
| 20% | No cut | 72.07 ± 3.00ab | 7.60 ± 1.50bcd | 4.33 ± 0.33ab | NA | NA |
| | 15 cm | 65.13 ± 6.52bc | 7.26 ± 0.52cd | 5.33 ± 0.67a | 269 ± 119ab | 28.1 ± 12.3ab |
| | 30 cm | 81.93 ± 2.27ab | 7.70 ± 1.16bcd | 4.00 ± 0.58ab | 290 ± 123a | 30.5 ± 12.4a |
| | 45 cm | 63.57 ± 6.71bc | 8.50 ± 0.70bc | 4.67 ± 0.88a | 65.3 ± 21.1c | 6.06 ± 2.61cd |
| 40% | No cut | 41.77 ± 1.76d | 8.50 ± 0.46bc | 4.00 ± 1.00ab | NA | NA |
| | 15 cm | 52.83 ± 7.02cd | 8.43 ± 1.93bcd | 4.00 ± 0.58ab | 123.6 ± 79.5bc | 14.78 ± 9.23abcd |
| | 30 cm | 51.93 ± 6.46cd | 11.60 ± 0.55a | 2.67 ± 0.33b | 27.5 ± 12.6c | 3.24 ± 1.55d |
| | 45 cm | 62.73 ± 7.07bc | 10.01 ± 0.64ab | 4.00 ± 0.58ab | 10.22 ± 3.33c | 1.43 ± 0.33d |
| Two-way ANOVA F-Statistics | | | | | | |
| Seawater | | 14.53* | 11.65* | 5.04* | 4.39* | 3.40* |
| Pruning | | 1.93ns | 0.76ns | 0.78ns | 5.95* | 7.23* |
| Seawater*Pruning | | 1.39ns | 1.27ns | 0.66ns | 1.38ns | 1.31ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. Ns = Not analysed. Shoot length (SHL), Stem length (STL), Number of branches (NB), Fresh Weight of Shoots (FWS), Dry Weight of Shoots (DWS), Fresh Weight of Roots (FWR), Dry Weight of Roots (DWR), Total Fresh Weight (TFW), Total Dry Weight (TDW).

**Table 4.2: Effect of diluted seawater and different pruning intervals shoot height, stem length, number of branches, FWS, and DWS of hydroponically grown *T. decumbens*. (Week 8).**

| Seawater | Pruning level | SHL (cm) | STL (cm) | NB (n) | FWS (g) | DWS (g) |
|---|---|---|---|---|---|---|
| Control | No cut | 91.1 ± 13.6ab | 9.13 ± 0.32a | 7.67 ± 1.76a | NA | NA |
| | 15 cm | 44.17 ± 2.50e | 10.17 ± 1.36a | 6.33 ± 1.86a | 14.11 ± 1.81cd | 4.22 ± 0.90cd |
| | 30 cm | 53.03 ± 0.29cde | 8.13 ± 0.68a | 5.00 ± 1.73a | 8.28 ± 2.26 d | 2.39 ± 0.42d |
| | 45 cm | 65.90 ± 7.18cd | 9.60 ± 1.23a | 5.00 ± 1.00a | 7.67 ± 1.76d | 2.70 ± 0.68d |
| 20% | No cut | 96.3 ± 10.1a | 11.50 ± 3.18a | 8.00 ± 2.52a | NA | NA |
| | 15 cm | 51.50 ± 4.15de | 8.90 ± 1.39a | 6.00 ± 1.53a | 53.1 ± 18.4ab | 8.96 ± 3.12ab |
| | 30 cm | 61.30 ± 3.56cde | 8.73 ± 1.94a | 4.00 ± 0.58a | 38.50 ± 4.73bc | 7.79 ± 0.65bc |
| | 45 cm | 72.93 ± 3.50bc | 10.30 ± 1.82a | 5.33 ± 1.20a | 69.1 ± 17.6a | 12.46 ± 2.23a |
| 40% | No cut | - | - | - | - | - |
| | 15 cm | - | - | - | - | - |
| | 30 cm | - | - | - | - | - |
| | 45 cm | - | - | - | - | - |
| Two-way ANOVA F-Statistics | | | | | | |
| Seawater | | 1.99ns | 0.25ns | 0.02ns | 24.90* | 23.88* |
| Pruning | | 16.30* | 0.46ns | 1.60ns | 6.81* | 10.95* |
| Seawater*Pruning | | 0.02ns | 0.38ns | 0.08ns | 3.78* | 3.85* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. Ns = Not analysed, (-) = Plant death. Shoot length (SHL), Stem length (STL), Number of branches (NB), Fresh Weight of Shoots (FWS), Dry Weight of Shoots (DWS), Fresh Weight of Roots (FWR), Dry Weight of Roots (DWR), Total Fresh Weight (TFW), Total Dry Weight (TDW).

**Table 4.3: Effect of diluted seawater and different pruning intervals shoot height, stem length, number of branches, FWS, and DWS of hydroponically grown *T. decumbens*. (Week 12).**

| Seawater | Pruning level | SHL (cm) | STL (cm) | NB (n) | FWS (g) | DWS (g) | FWR (g) | DWR (g) | TFW (g) | TDW (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | No cut | 106.6 ± 16.2ab | 9.13 ± 0.31a | 7.67 ± 1.76a | 305 ± 129b | 99.1 ± 34.8ab | 34.6 ± 18.1cd | 10.36 ± 5.69c | 340 ± 144bc | 109.5 ± 38.2b |
| | 15 cm | 34.40 ± 3.45e | 10.17 ± 1.36a | 6.33 ± 1.86a | 46.2 ± 11.9b | 11.84 ± 2.89c | 43.06 ± 7.44bcd | 13.84 ± 3.39bc | 89.3 ± 18.4c | 25.68 ± 5.84c |
| | 30 cm | 43.43 ± 4.24de | 8.13 ± 0.67a | 5.00 ± 1.73a | 55.3 ± 14.5b | 20.22 ± 5.68c | 31.24 ± 9.70d | 8.69 ± 2.79c | 86.4 ± 24.1c | 28.91 ± 8.09c |
| | 45 cm | 66.53 ± 3.05cd | 9.60 ± 1.23a | 5.00 ± 1.00a | 128.1 ± 17.3b | 46.78 ± 4.27bc | 35.96 ± 2.83cd | 11.33 ± 1.23c | 164.0 ± 16.2bc | 58.11 ± 5.00bc |
| 20% | No cut | 118.6 ± 17.6a | 11.50 ± 3.18a | 8.00 ± 2.52a | 444 ± 253a | 61.3 ± 15.9bc | 100.07 ± 9.26a | 31.31 ± 0.90a | 939 ± 262a | 188.4 ± 47.7a |
| | 15 cm | 56.13 ± 7.59de | 8.90 ± 1.39a | 6.00 ± 1.53a | 141.5 ± 37.3b | 26.00 ± 6.39c | 86.7 ± 38.3abc | 26.6 ± 10.9ab | 228.2 ± 75.6bc | 52.6 ± 17.2bc |
| | 30 cm | 69.33 ± 0.81cd | 8.67 ± 1.99a | 4.00 ± 0.57a | 839 ± 253a | 157.3 ± 47.5a | 110.2 ± 20.5a | 27.46 ± 4.34ab | 555 ± 273ab | 88.8 ± 20.0bc |
| | 45 cm | 85.10 ± 3.46bc | 10.30 ± 1.82a | 5.33 ± 1.20a | 380.9 ± 20.0b | 73.0 ± 10.2bc | 93.70 ± 5.43ab | 31.28 ± 1.95a | 474.6 ± 21.3bc | 104.3 ± 12.1b |
| 40% | No cut | - | - | - | - | - | - | - | - | - |
| | 15 cm | - | - | - | - | - | - | - | - | - |
| | 30 cm | - | - | - | - | - | - | - | - | - |
| | 45 cm | - | - | - | - | - | - | - | - | - |
| | | | | | Two-way ANOVA F- Statistics | | | | | |
| Seawater | | 8.96* | 0.23ns | 0.02ns | 10.98* | 4.97* | 24.29 * | 26.77* | 13.42* | 9.56* |
| Pruning | | 20.47* | 0.48ns | 1.60ns | 4.39* | 9.10* | 0.05ns | 0.17ns | 3.79* | 7.78* |
| Seawater* Pruning | | 0.20ns | 0.38ns | 0.08ns | 0.96ns | 0.37ns | 0.35ns | 0.27ns | 0.92ns | 0.41ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. Ns = Not analysed, (-) = Plant death. Shoot length (SHL), Stem length (STL), Number of branches (NB), Fresh Weight of Shoots (FWS), Dry Weight of Shoots (DWS), Fresh Weight of Roots (FWR), Dry Weight of Roots (DWR), Total Fresh Weight (TFW), Total Dry Weight (TDW).

#### 4.4.1.3 Effect of diluted seawater and different pruning intervals on number of branches

Diluted seawater (DSW) had a significant impact ($p \leq 0.05$) on the number of branches of *T. decumbens* only at week 4 (Table 4.1). Pruning and the interaction of diluted seawater with different pruning intervals had nonsignificant effect on the number of branches at week 4, week 8, and week 12. The number of branches showed no significant difference at all weeks. It is clear that different pruning intervals, the interaction of diluted seawater and different pruning intervals showed no positive influence on the number of branches of *T. decumbens*.

#### 4.4.1.4 Effect of diluted seawater and different pruning intervals on the fresh weight and dry weight of shoots

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on both the fresh and dry weights of *T. decumbens* (Table 4.1). The highest mean values of fresh and dry weights were recorded in 20%DSW treatment. These highest mean values of fresh and dry weights were significantly higher than all other treatments, including the control. The lowest fresh weight of shoots was recorded in all the treatments including the control. The lowest dry weight of shoots was recorded in 40%DSW.

**Pruning (Week 4):** Different pruning intervals showed a significant effect on fresh and dry weights of *T. decumbens* shoots. The application of 30 cm pruning intervals recorded the highest mean values of fresh and dry weights, while the lowest mean value was recorded in the following pruning intervals (30 cm and 45 cm) in all the treatments including the control.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had non-significant effect on fresh and dry weights of *T. decumbens*.

**Diluted seawater (Week 8):** Diluted seawater had a significant impact ($p \leq 0.05$) on both the fresh weight and dry weight of *T. decumbens* shoots (Table 4.2). The highest fresh and dry weights were recorded in 20%DSW treatment. These highest mean values of fresh and dry weight were significantly higher than all other treatments, including the control. The lowest fresh and dry weights were recorded in the control treatment. These were significantly lower when compared to other treatments.

**Pruning (Week 8):** Table 4.2 showed a significant effect on the fresh and dry weights of T. *decumbens* shoots. The application of 45 cm pruning intervals on the shoots of *T. decumbens* resulted in the highest fresh and dry weights, while 30 cm and 45 cm in the control resulted in the lowest fresh and dry weights.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals had a significant effect on fresh and dry weights of *T. decumbens*. This means that the interaction of diluted seawater and different pruning intervals had a positive influence of the shoots of *T. decumbens*.

**Diluted seawater (Week 12**): Diluted seawater showed a significant impact ($p \leq 0.05$) on the fresh and dry weights of *T. decumbens* shoots (Table 4.3). The highest fresh and dry weight values were recorded in 20%DSW treatment. These highest mean values of fresh and dry weight were significantly higher than all other treatments, including the control. The lowest fresh weight was recorded in the control, but it did not differ significantly when compared to other treatments. The lowest dry weight was recorded in the control and 20%DSW with 15 cm pruning interval.

**Pruning (Week 12):** Table 4.3 showed a significant effect on the fresh and dry weights of *T. decumbens* shoots. The application of 30 cm pruning intervals in 20%DSW recorded the highest mean value of fresh and dry weights, while 15 cm pruning intervals resulted in the lowest mean values.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and different pruning intervals showed non-significant effects on the fresh and dry weights of *T. decumbens* shoots.

#### 4.4.1.5 Effect of diluted seawater and different pruning intervals on the total fresh weight and total dry weight of shoots

**Diluted seawater (DSW):** Diluted seawater had a significant increase ($p \leq 0.05$) on the total fresh and dry weight of *T. decumbens* shoots (Table 4.3). The highest total fresh and dry weights were recorded in 20%DSW, while the least total fresh and dry weights were recorded in the control treatment. These highest mean values of total fresh weight and total dry weight were significantly higher than all other treatments, including the control.

**Pruning:** The application of different pruning intervals showed a significant effect on the total fresh and dry weight of *T. decumbens* shoots. The plants without pruning intervals in 20%DSW resulted in the highest mean values of fresh and dry weights of *T. decumbens* shoots. The lowest mean values were recorded in 15 cm and 30 cm pruning intervals in the control treatment.

**Diluted seawater interaction with pruning:** The interaction of diluted seawater and different pruning intervals had nonsignificant effects on the total fresh and dry weights on *T. decumbens* shoots. This is clear that the interaction of diluted seawater and different pruning intervals showed no positive influence on dune spinach shoots during week 12.

### 4.5 Discussion

Salt stress is known to be one of the primary factors that impacts enormous areas of cultivated land across the world, resulting in significant decreases in plant growth, seed dormancy, and productivity (Ahmad & Anjum, 2023b; Ahmad *et al*., 2023a). It has significant implications for plants, affecting various aspects of their physiology and growth. One of the primary impacts is on nutrient absorption and metabolic activities such as lipid and carbohydrate metabolism (Parida & Das, 2005; Zafar *et al*., 2022). This disruption can lead to reduced crop growth and yield. Additionally, salt stress affects the plant's root system and overall water balance. Elevated sodium levels create osmotic stress, leading to water shortages within plant cells and affecting water potential (Ekinci *et al*., 2022). This imbalance in water availability further exacerbates the stress conditions for plants in saline environments. Furthermore, salt stress alters nutrient availability in the soil, causing ion toxicity in plants (Ali *et al*., 2021). The accumulation of sodium and other ions can disrupt cellular processes and lead to metabolic disorders, ultimately impacting plant health and productivity.

In a nutshell, salt stress imposes multiple challenges on plants, including disruptions in nutrient absorption, metabolic imbalances, osmotic stress, and ion toxicity. These factors collectively contribute to reduced crop yields and pose significant obstacles for agriculture in saline environments. Innovative techniques for addressing salt stress in agriculture integrate advancements in genetics, physiology, agronomy, and biotechnology (Ahmad *et al*., 2023a). These approaches aim to develop resilient crops capable of sustaining productivity in saline environments while minimizing environmental impact. Continued research and implementation of these strategies are essential for ensuring global food security and agricultural sustainability in the face of increasing soil salinization and climate change challenges. Osmotic stress is primarily associated with a decrease in the development of leaves, shoots, and reproductive growth and is brought on by a deficiency of water in plant tissues (Chourasia *et al*., 2022). An increase in osmotic potential causes the plant cells to dry, allowing water to exit the root cells and reach the surrounding soil (Fu & Yang, 2023; Hualpa-Ramirez *et al*., 2024; Sharavdorj *et al*., 2024). To ensure that food is produced under such environmental stress, it is necessary to find and cultivate vegetable crops that can withstand salinity.

In this present study, the effects of diluted seawater and different pruning intervals on the growth characteristics of *T. decumbens* were assessed. Plants grown under low seawater concentrations (20% SW) with different pruning intervals (no cut, 15 cm, 30 cm, and 45 cm) showed an increase in shoot length, fresh and dry weight of shoots, and the total yield of *T. decumbens*. This supports the findings of (Azeem *et al*., 2023; Sharavdorj *et al*., 2024) and (Ngxabi *et al*., 2021), who found that low salinity concentrations increased vegetative growth whereas high salinity concentrations drastically reduced vegetative growth of halophytes, resulting in leaf yellowing and wilting. Similar results for *Cakile maritima, Arthrocnemum macrostachyum*, and *Sarcocornia fruticosa* were reported by (Pungin *et al*., 2023), who observed an increase in shoot biomass, leaf expansion, and shoot and root weight in fresh and dried plants at low to moderate salt concentrations. (Ghanem *et al*., 2021; Gill *et al*., 2024), and (Sogoni *et al*., 2021) observed comparable trends and concluded that high salinity concentrations greatly lowered the halophyte growth characteristics. However, the findings of this study revealed that *T. decumbens* is a low-salt-tolerant wild edible vegetable crop.

Pruning is considered a crucial procedure for producing optimal production and early fruit in plants (Mawarni & Siahaan, 2022). According to (Peng *et al*., 2024), pruning optimised the production, yield, and quality of *Capsicum annuum*. This further occurred in this investigation, where pruning significantly ($p \leq 0.05$) enhanced the shoot length, fresh and dry weight of shoots, and total fresh and dry weight of *T. decumbens*. (Zhong *et al*., 2024) have reported comparable results on *Ginkgo biloba*, demonstrating that pruning improved the yield and quality of crops. Pruning plays an essential aspect in developing crops that produce good quality and yields (Botanicae *et al*., 2010). Moreover, Chapagain *et al*. (2022) have made reference to this problem, stating that pruning increased productivity and fruit weight in cucumber (*Cucumis sativus*). According to some recent studies of (Peng *et al*., 2024; Rahman *et al*., 2024), pruning has been showing similar patterns and has a major influence on plant growth, quality, and productivity. Furthermore, a similar trend was observed by (Bulawa *et al*., 2022), where pruning had a significant impact on the growth of *Trachyandra divaricata*. The results above therefore indicate that in vegetable cultivation, pruning and diluted seawater concentration (20% SW) may greatly increase the yield and quality of halophytes.

### 4.6 Conclusion and recommendations

This research emphasises the adverse effects of salt stress on the growth of plants and explores viable mitigation alternatives, with an emphasis on the use of diluted seawater and pruning methods. By examining *T. decumbens* growth parameters under various conditions, the study shows that lower seawater concentrations (20%SW) combined with different pruning intervals (30 cm and 45 cm) can result in considerable increases in shoot length, biomass production, and overall yield. Furthermore, the study emphasises the importance of pruning as a management strategy to increase agricultural output and quality, as shown in diverse crops. The research study also reveals the potential of diluted seawater and pruning as strategies for enhancing plant efficiency and quality in vegetable crops, opening up novel opportunities for sustainable agricultural practices amidst increasing environmental challenges such as salinity. Additional research and implementation of such approaches can help ensure food security in salt-affected regions.

## 4.7 Acknowledgements

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# CHAPTER FIVE

# EFFECT OF DILUTED SEAWATER AND DIFFERENT PRUNING INTERVALS ON PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY OF *TETRAGONIA DECUMBENS* MILL. (DUNE SPINACH) IN HYDROPONICS

**Effect of diluted seawater and different pruning intervals on phytochemicals and antioxidant capacity of *Tetragonia decumbens* Mill. (Dune spinach) in hydroponics.**

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## 5.1 Abstract

Wild edible vegetables such as spinach are vital components of a nutritious diet, containing mineral elements, nutritional fibres, carotenoids, vitamins, antioxidants, and phytochemical components. However, the aim of the research work was to evaluate the effect of seawater dilutions and different pruning intervals in hydroponic cultivation on the antioxidants and phytochemical content of *T. decumbens* to advance its potential uses as a wild edible vegetable crop. In the present study, the phytochemical and antioxidant content of *T. decumbens* shoots cultivated at various seawater concentrations and pruning intervals was determined using total flavonol, polyphenols, ABTS, DPPH, and FRAP assays. The findings of this study showed that diluted seawater and pruning intervals significantly ($p < 0.05$) increased the phytochemical and antioxidant content of *T. decumbens*. It was further noted that low seawater concentration (20%SW) yielded significantly higher mean values for polyphenols, flavanols, FRAP, and DPPH, while the control recorded the highest values of ABTS. Low concentrations of seawater (20%SW) seem to increase antioxidant activity, polyphenol and flavonoid content, and other biochemical properties, suggesting possible methods of improving crop growth in saline environments.

**Keywords:** crop resilience, DPPH, FRAP, polyphenols, wild edible vegetables

## 5.2 Introduction

Many environmental stressors, such as abiotic (drought, salinity, temperature, reactive oxygen species, and soil pH) and biotic (fungi, bacteria, viruses, and nematodes) factors, have detrimental effects on plant growth and development, reducing their yield (Ashapkin *et al*., 2020; Elateeq *et al*., 2023). One of the most significant elements affecting agricultural crop productivity is salinity, and it has a negative impact on crop yield, plant vigour, and germination (Atta *et al*., 2023). In saline environments, cells are dehydrated, which results in osmotic stress and the evacuation of water from the cytoplasm, which lowers the volumes of the cytosol and vacuoles. Plants under salinity stress frequently experience osmotic and ionic stress, which can cause certain secondary metabolites to accumulate or decrease (Sytar *et al*., 2018).

Nevertheless, in order for plants to survive under these critical conditions, they need to develop a number of defence mechanisms, such as primary and secondary metabolites. Plant primary metabolites are defined as compounds comprising carbohydrates, lipids, proteins, and amino acids (Elateeq *et al*., 2023). Primary metabolite production develops when necessary nutrients are present in a growth medium during the active growth phase, or trophophase (Zaynab *et al*., 2019). All plants contain primary metabolites, which are necessary for healthy plant growth and development (Erb & Kliebenstein, 2020; Elateeq *et al*., 2023). These primary metabolites have been believed to be the main components that protect plants from a variety of environmental stressors by acting as osmolytes and osmoprotectants (Ejaz *et al*., 2020).

Conversely, plant secondary metabolism describes tiny molecular compounds that are produced by metabolic pathways but are not crucial for the development or reproduction of organisms (Pang *et al*., 2021; Qaderi *et al*., 2023). In plants, secondary metabolites such as flavonoids, phenolic substances, alkaloids, and terpenes are generated close to the stationary phase of growth and do not directly contribute to the development, growth, or reproduction of plants (Rahman *et al*., 2023; Zhang *et al*., 2023). Several plant species produce secondary metabolites to protect themselves from various abiotic stresses, especially in saline environments (Pang *et al*., 2021; Rahman *et al*., 2023). Moreover, plant secondary metabolites play crucial roles in controlling microbial populations associated with hosts, warding off pests and diseases, and serving as signals for symbiosis between microbes and plants (Guerrieri *et al*., 2019).

*Tetragonia decumbens* (Aizoaceae), also known as kinkelbos, klapperbrak, and sea spinach, is an edible wild vegetable that is found along the coast from southern Namibia to the Eastern Cape (Tembo-Phiri *et al*., 2019; Nkcukankcuka *et al*., 2021; Sogoni *et al*., 2021). The plant is characterized by glossy, hairy, sessile leaves that are dark green in appearance

(Nkcukankcuka *et al*., 2021; Sogoni *et al*., 2021). Due to the salt content and crunchiness of its leaves and stems, it can be used in a variety of green foods, such as spinach, served raw in green salads, or cooked with other vegetables (Agudelo *et al*., 2021; Sogoni *et al*., 2021). The stems and leaves can be used for pickling, fermenting, and cooking in a variety of restaurant meals, including soups, stews, and stir fries (Sogoni *et al*., 2021). *T. decumbens* is underutilized due to a dearth of research on its cultivation, phytochemical, and antioxidant properties. The purpose of this study is to explore the effect of diluted seawater and different pruning intervals on the phytochemical and antioxidant content of *T. decumbens* shoots cultivated in hydroponics.

## 5.3 Materials and methods

### 5.3.1 Experimental location

This research was carried out at the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa (33°55'56" S and 18°38'25" E). The research project was conducted in an experimental greenhouse with temperature regulation of 21–26 ºC during the day and 12–18 ºC at night. The average relative humidity was maintained at 60%.

### 5.3.2 Plant material and experimental description

*Tetragonia decumbens* cuttings were collected on February 27, 2023, from a specific clone plant that was flourishing alongside the coastline at Granger Bay, Western Cape. A stem cutting propagation technique was used to root new plant material, as reported by (Sogoni *et al*., 2021). A total number of one hundred and ten plants were propagated with rooting powder (Dynaroot$^{TM}$ No. 1 with active ingredient 0.1 % IBA) in polyethylene seedling flat trays filled with a ratio of 50:50 sand/peat. The trays were placed in the propagation greenhouse on heated beds with intermitted mist irrigation. Seventy cuttings were selected out the total number rooted and moved to a shaded area for five weeks to allow them to acclimatise. Thereafter the cuttings were transplanted into 12.5 cm black plastic pots filled with Consul silica sand. Only healthy plants were selected and arranged in a randomised block design of three treatments and a control with three replicates per treatment totalling forty-eight plants.

### 5.3.3 Nutrient solution

Nutrifeed$^{TM}$ fertiliser from Starke Ayres in Cape Town, South Africa was used to prepare a hydroponic aqueous solution and used as general feeding to ensure healthy plant development. Nutrifeed contains the following ingredients: 65 g/kg Nitrogen (N), 27 g/kg Phosphorus (P), 130 g/kg Potassium (K), 70 mg/kg Calcium (Ca), 20 mg/kg Copper (Cu), 1500 mg/kg Iron (Fe), 10 mg/kg Molybdenum (Mo), 22 mg/kg Magnesium (Mg), 240 mg/kg

Manganese (Mn), 75 mg/kg Sulphur (S), 240 mg/kg Boron (B), and mg/kg Zinc (Zn). For the first three weeks before the experiment started the system operated using tap water only to ensure that plants adapt to their new environment. Thereafter the Nutrifeed concentration were maintained with a concentration of 1:500 (1 kg/500 litres, or approximately 10 g per 5 L) in each hydroponic sump.

### 5.3.4 Hydroponic design

The hydroponic system was constructed in accordance with (Bulawa *et al*., 2022) and built with four identical Nutrient Film Technique (NFT) systems. Each system was constructed on a wire mesh square table (2.5 m) to give stability and flat surface. A 50 L low-density polyethylene (LDPE) reservoir and a single submersible water pump capable of producing 2000 litres per hour (1 x 2000 L/h) were present in each of these Nutrient Film Technique (NFT) systems, which were referred to as table 1 to 4 (T1-T4). The low-density polyethylene (LDPE) reservoir served as a container for the nutritional solution that would be utilised in the experiment. The submersible water pump was designed to generate a nutrient solution and transport it from the reservoir to the gutters. White polyvinyl chloride (PVC) square gutters that were 1.36 metres long were used to construct the hydroponic system and were labelled G1, G2, G3, and G4. Each white polyvinyl chloride (PVC) square gutter was covered with black plastic polyethylene sheets to prevent algae growth and three holes per gutter were also created to accommodate 12.5 cm plastic pots. A 50 L low-density polyethylene (LDPE) reservoir, 1 x 2000 l/h submersible water pump, 20 mm low-density polyethylene (LDPE) irrigation pipe, 20 mm elbow irrigation fittings, 20 mm flow regulators, 20 mm T-piece irrigation fittings, and 20 mm end cap irrigation fittings were all used during the construction of each system. Each gutter on each system had 3 pots with a consul silica sand medium. For preventing leaks in the system, a silicon glue (PVC) was used to seal the two components firmly together and when inserting irrigation fittings to the polyvinyl chloride (PVC) square gutters. A portable digital electrical conductivity (EC) metre (Hanna Instruments®TM HI 98312) that has been calibrated was used to measure the electrical conductivity (EC) in the nutritional solution on a daily basis and a calibrated hand-held digital pH metre (Eurotech®TM pH 2 pen) was used to measure the pH in the nutritional solution on a daily basis. The pH of the nutritional solution was increased with potassium hydroxide [KOH] and reduced with phosphoric acid [$H_3PO_4$]. The pH and electrical conductivity (EC) were maintained at 4.5, to 6.5, and 3.38 to 1.10 ds m -1 (S) respectively.

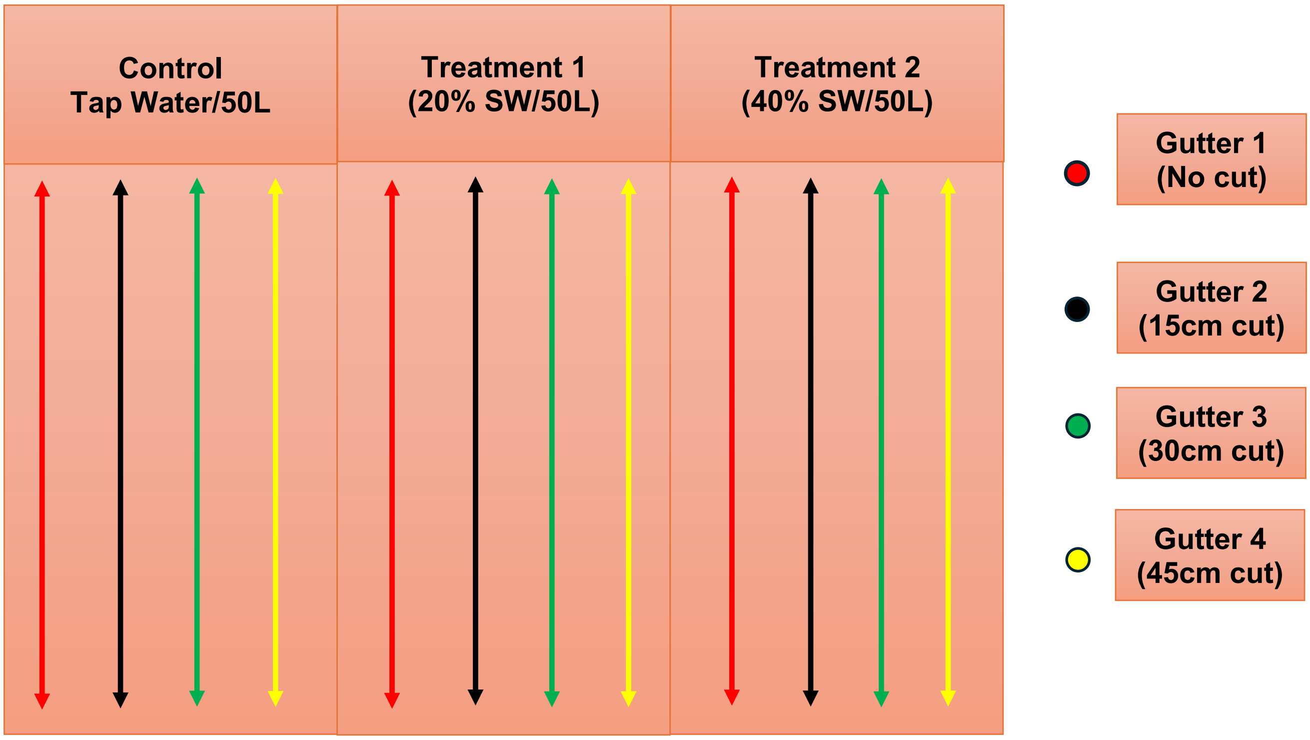


**Figure 5.1:** Layout and random design of the experiment showing the seawater dilution treatments and pruning cuts applied.

### 5.3.5 Treatments

#### 5.3.5.1 Seawater treatments

Seawater was collected from Granger Bay, Cape Town and diluted with tap water to prepare three diluted seawater treatments and clean tap water as the control. All the sumps (S2, S3, and S4) with seawater treatments were refilled every week (Friday) to avoid build-up of algae. The dilutions were as follows:

- S1 Sump 1 = clean tap water (control)
- S2 Sump 2 = 20% SW/50L (Treatment 1)
- S3 Sump 3 = 40% SW/50L (Treatment 2)

#### 5.3.5.2 Pruning treatments

At the beginning of the experiment (week one), all plants were placed in the gutters and pruned back to 15 cm. This was done to ensure uniform height in all plants. Thereafter plants were pruned again at weeks 4 and 8 to three different heights as follows:

- G1 Gutter 1 = No cut (S1G1, S2G1, and S3G1)
- G2 Gutter 2 = 15 cm (S1G2, S2G2, and S3G2)

- G3 Gutter 3 = 30 cm (S1G3, S2G3, and S3G3)
- G4 Gutter 4 = 45 cm (S1G4, S2G4, and S3G4)

At week 12, no pruning occurred, however, all plant samples were harvested and prepared for analysis. At weeks 4 and 8, all the unpruned plants were allowed to grow throughout the experiment without being pruned, hence they are represented with "NA" on the tables. "NA" means the plants were not analysed at that particular stage.

### 5.3.6 Phytochemical and Antioxidant analysis

#### 5.3.6.1 Sample preparation

*T. decumbens* shoots were pruned after weeks 4, 8, and 12 using different pruning intervals (no cut, 15, 30, and 45 cm). All materials from pruned shoots of *T. decumbens* were promptly dried for eight days at 40 °C in a fan-drying LABTECHTM model LDO 150F (Daihan Labtech India. Pty. Ltd., 3269 Ranjit Nagar, New Dehli, 110008) oven. The material that had dried out was ground to a fine powder using a Junkel & Kunkel model A 10 mill. After that, 100 mg of the dried powdered material was mixed with 25 mL of 80% (v/v) ethanol (EtOH; Merck, Modderfontein, South Africa) and left for one hour to extract the pruned shoot material. The resulting filtrates were then used for all analyses after being centrifuged for five minutes at 4000 rpm. All *T. decumbens* samples with "no cut" pruning intervals were not analysed (Na) in weeks 4 and 8. However, they were only analysed in week 12 after harvesting the entire plant.

### 5.3.7 Determination of antioxidant capacity and content

The amount of antioxidant and metabolite accumulation in the leaves was evaluated by different assays for total polyphenols, DPPH, ABTS, flavanols, and ferric reducing antioxidant power (FRAP).

#### 5.3.7.1 Polyphenol Assay

The analysis of total polyphenols in the tested plant extracts was carried out using the Folin-Ciocalteu method, as reported by (Jimoh *et al*., 2019). A solution of 7.5% sodium carbonate ($Na_2CO_3$) (Sigma, South Africa) was made by diluting Folin & Ciocalteu's phenol reagent (2N, Sigma South Africa, Sandton, South Africa) ten times with distilled water. A 96-well plate was filled with 25 µL of the crude extract, 125 µL of Folin & Ciocalteu's phenol reagent, and 100 µL of sodium carbonate ($Na_2CO_3$). The plate was then incubated at room temperature for two hours. After that, the amount of absorption was determined using a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA) at a wavelength of 765 nm. A standard curve for gallic acid (Sigma, South Africa) with concentrations ranging from 0 to 500 mg/L was used to determine the polyphenol levels of the samples. The findings were reported as mg of gallic acid equivalents (GAE) per g of dry weight (mg GAE/g DW).

#### 5.3.7.2 Estimation of Flavonol Content

The extracts of flavonol content were measured using quercetin of 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) as a standard (Tshayingwe *et al*., 2023). For every sample, 225 µL of 2% hydrochloric acid (HCl) and 12.5 µL of 0.1% hydrochloric acid (HCl) (Merck, South Africa) in 95% ethanol ($CH_3CH_2OH$) were combined with around 12.5 µL of crude sample extracts. After that, the extracts were incubated for 30 minutes at room temperature. At a temperature of 25 °C, the absorbance was measured at 360 nm. The findings were presented as mg quercetin equivalent (mg QE/g DW) per g of dry weight.

#### 5.3.7.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP content of *T. decumbens* shoots was determined using the procedure outlined by (Jimoh *et al*., 2019). The FRAP reagent was made by combining 30 mL of acetate buffer (0.3 M, pH 3.6) (Merck, South Africa) together with 3 mL of 2,4,6-tripyridyl-s-triazine (10 mM in 0.1 M hydrochloric acid), 3 mL of iron (III) chloride hexahydrate (FeCl3·6H2O) (Sigma, South Africa), and 6 mL of distilled water. 300 µL of the FRAP reagent and 10 µL of the crude sample extract were combined in a 96-well microplate, and the mixture was incubated for 30 minutes at room temperature. After that, the absorbance was determined using a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA) at 593 nm. A standard curve of L-ascorbic acid (Sigma-Aldrich, South Africa) with concentrations ranging from 0 to 1000 µM was used to determine the FRAP values of the examined plant samples. The findings were represented as µM ascorbic acid equivalents (AAE) per gramme dry weight (g DW).

#### 5.3.7.4 DPPH Free Radical Scavenging Activity

The DPPH radicals were produced using a 0.135 mM DPPH solution that was made in a dark bottle (Mndi *et al*., 2023; Cebani *et al*., 2024). A mixture of graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) solution and 25 µL of crude extract was reacted with about 300 µL of DPPH solution. The mixes were incubated for 30 minutes, and then the absorbance at 517 nm was determined. The findings were presented as µM/Trolox equivalent per g dry weight (µM TE/g DW).

#### 5.3.7.5 ABTS Free Radical Scavenging Activity

The ABTS assay was carried out using the procedure outlined by (Jimoh *et al*., 2019). A 140 mM Potassium-Peroxodisulphate ($K_2S_2O_8$) (Merck, Modderfontein, South Africa) solution and a 7 mM ABTS solution were among the stock solutions. After that, 5 mL of ABTS solution and 88 µL of $K_2S_2O_8$ were combined to prepare the working solution. After thoroughly mixing the two solutions, they were left to react for 24 hours at room temperature in the dark. A standard consisting of Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) was used with concentrations varying from 0 to 500 μM. After allowing the crude sample extracts (25 μL) to react with 300 μL of ABTS in the dark at room temperature for 30 minutes, the absorbance was measured in a plate reader at 734 nm at 25 °C. The findings were presented as μM/Trolox equivalent per g dry weight (μM TE/g DW).

#### 5.3.8 Statistical analysis

The data obtained from growth parameters, different pruning intervals, and essays in this study were statistically computed using a two-way examination of variance (ANOVA). The Fisher's least significant difference will be used to compare the significant differences between treatment means at p≤0.05 using MINI-TAB statistical software.

## 5.4 Results

### 5.4.1 Effect of diluted seawater and different pruning intervals on Phytochemicals and Antioxidant Activity of *T. decumbens* shoots

#### 5.4.1.1 Effect of diluted seawater and different pruning intervals on the accumulation of Polyphenols

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on the accumulation of polyphenols in *T. decumbens* shoots (Table 5.1). The plants cultivated under 20%DSW recorded the highest polyphenol concentrations (1.43 mg GAE/g), while 40%DSW recorded the lowest concentration of polyphenols (0.48 mg GAE/g). The highest mean value was significantly higher than other treatments but did not differ from the control treatment (1.33 mg GAE/g).

**Pruning (Week 4):** Table 5.1 shows that the application of pruning intervals had a significant effect on the shoots of *T. decumbens*. The high polyphenols were found in plants subjected to 30 cm pruning intervals. The plants subjected to 30 cm pruning intervals in 40%DSW showed the least polyphenols.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and pruning had a significant impact on the polyphenols of *T. decumbens* shoots. Table 5.1 shows that there was a significant production of polyphenols during the interaction of diluted seawater and pruning.

**Diluted seawater (Week 8):** Diluted seawater had a significant effect ($p \leq 0.05$) on the accumulation of polyphenols in *T. decumbens* shoots (Table 5.2). The highest mean value (4.21 mg GAE/g) of polyphenols was recorded under 20%DSW. The highest mean value (4.21 mg GAE/g) was significantly higher than all other treatments but did not differ from the control treatment (3.89 mg GAE/g).

**Pruning (Week 8):** Pruning intervals had a significant impact on the total polyphenols of *T. decumbens* shoots. The application of 15 cm pruning intervals in the shoots of dune spinach resulted in high total polyphenols. The lowest value of polyphenols was recorded in plants subjected to 30 cm and 45 cm pruning intervals.

**Diluted seawater interaction with pruning (Week 8):** Table 5.2 showed a significant effect on the interaction of diluted seawater and pruning in the shoots of dune spinach. These interactions showed that there was a high polyphenol production in the shoots of dune spinach.

**Diluted seawater (Week 12):** Diluted seawater showed a significant effect ($p \leq 0.05$) on the accumulation of polyphenols in *T. decumbens* shoots. The plants cultivated under 20%DSW recorded the highest mean value (2.72 mg GAE/g) of polyphenols, while the control treatment recorded the least polyphenol concentration (1.20 mg GAE/g). The highest mean value was significantly higher than all other treatments, including the control (Table 5.3).

**Pruning (Week 12):** Table 5.3 showed a significant impact on the total polyphenols of dune spinach shoots. The plants without any pruning resulted in high polyphenols in the shoots of dune spinach, while the one's subjected to 45 cm pruning intervals recorded the lowest values of polyphenols.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and different pruning intervals showed a significant impact on the polyphenols in *T. decumbens* shoots.

**Table 5.1: The effect of diluted seawater and different pruning intervals on phytochemicals and the antioxidant activity of *T. decumbens* shoots (Week 4).**

| Seawater | Pruning | Polyphenols (mg GAE/g) | Flavonols (mg QE/g) | FRAP (µmol AAE/g) | DPPH (µmol TE/g) | ABTS (µmol TE/g) |
|---|---|---|---|---|---|---|
| | No cut | NA | NA | NA | NA | NA |
| | 15 cm | 1.03 ± 0.08c | 0.45 ± 0.01b | 36.52 ± 0.15b | 10.40 ± 1.75c | 47.81 ± 1.29bc |
| Control | 30 cm | 1.33 ± 0.14a | 0.59 ± 0.08a | 36.28 ± 1.01b | 12.27 ± 0.94bc | 50.22 ± 1.79b |
| | 45 cm | 1.07 ± 0.09bc | 0.32 ± 0.02c | 35.91 ± 0.35b | 12.15 ± 1.30bc | 49.15 ± 0.86b |
| | No cut | NA | NA | NA | NA | NA |
| | 15 cm | 0.67 ± 0.06de | 0.40 ± 0.02bc | 39.73 ± 1.23a | 13.14 ± 2.72bc | 43.65 ± 0.52de |
| 20% | 30 cm | 1.43 ± 0.11a | 0.65 ± 0.01a | 40.89 ± 0.64a | 11.27 ± 1.78c | 42.93 ± 1.05e |
| | 45 cm | 0.76 ± 0.05d | 0.43 ± 0.08bc | 40.21 ± 1.06a | 11.63 ± 1.12bc | 42.52 ± 0.56e |
| | No cut | NA | NA | NA | NA | NA |
| | 15 cm | 0.59 ± 0.03de | 0.37 ± 0.02bc | 34.74 ± 0.54b | 14.60 ± 1.82bc | 46.04 ± 1.74cd |
| 40% | 30 cm | 0.48 ± 0.08e | 0.41 ± 0.05bc | 35.02 ± 0.22b | 17.51 ± 1.09b | 49.59 ± 0.62b |
| | 45 cm | 1.30 ± 0.07ab | 0.62 ± 0.08ba | 32.38 ± 0.57c | 28.52 ± 5.20a | 53.10 ± 0.40a |
| Two-way ANOVA F- Statistics | | | | | | |
| Seawater | | 36.41* | 10.86* | 5.06* | 12.99* | 32.86* |
| Pruning | | 126.97* | 112.99* | 2495.27* | 42.25* | 1859.29* |
| Seawater*Pruning | | 8.16* | 3.21* | 22.09* | 4.49* | 7.17* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA= Not analysed.

**Table 5.2: The effect of diluted seawater and different pruning intervals on phytochemicals and the antioxidant activity of *T. decumbens* shoots (Week 8).**

| Seawater | Pruning | Polyphenols (mg GAE/g) | Flavonols (mg QE/g) | FRAP (µmol AAE/g) | DPPH (µmol TE/g) | ABTS (µmol TE/g) |
|---|---|---|---|---|---|---|
| | No cut | NA | NA | NA | NA | NA |
| | 15 cm | 3.89 ± 0.09a | 0.60 ± 0.03a | 33.34 ± 0.35c | 18.08 ± 1.46a | 62.50 ± 0.79b |
| Control | 30 cm | 2.76 ± 0.08b | 0.51 ± 0.01b | 43.63 ± 0.93b | 9.84 ± 1.09d | 67.51 ± 0.20a |
| | 45 cm | 1.42 ± 0.11c | 0.60 ± 0.01a | 44.40 ± 1.68b | 12.71 ± 0.93cd | 56.03 ± 0.81d |
| | No cut | NA | NA | NA | NA | NA |
| | 15 cm | 4.21 ± 0.26a | 0.32 ± 0.02c | 33.34 ± 0.35c | 15.26 ± 1.19abc | 58.97 ± 0.89c |
| 20% | 30 cm | 1.31 ± 0.13c | 0.58 ± 0.02a | 55.24 ± 0.18a | 17.02 ± 1.13ab | 54.67 ± 0.47de |
| | 45 cm | 1.45 ± 0.02c | 0.37 ± 0.03c | 34.48 ± 0.17c | 14.08 ± 1.33bc | 53.01 ± 0.83e |
| | No cut | - | - | - | - | - |
| | 15 cm | - | - | - | - | - |
| 40% | 30 cm | - | - | - | - | - |
| | 45 cm | - | - | - | - | - |
| Two-way ANOVA F-Statistics | | | | | | |
| Seawater | | 365.46* | 40.40* | 444.19* | 3.79ns | 208.65* |
| Pruning | | 221.05* | 233.67* | 1688.97* | 101.79* | 4521.36* |
| Seawater*Pruning | | 50.93* | 26.37* | 80.33* | 8.23* | 48.31* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 5.3: The effect of diluted seawater and different pruning intervals on phytochemicals and the antioxidant activity of *T. decumbens* shoots (Week 12)**

| Seawater | Pruning | Polyphenols (mg GAE/g) | Flavonols (mg QE/g) | FRAP (µmol AAE/g) | DPPH (µmol TE/g) | ABTS (µmol TE/g) |
|---|---|---|---|---|---|---|
| Control | No cut | 2.41 ± 0.04b | 0.36 ± 0.02c | 41.46 ± 0.15ab | 17.51 ± 1.61ab | 53.66 ± 0.94cd |
| | 15 cm | 1.67 ± 0.14d | 0.51 ± 0.31b | 42.34 ± 0.45ab | 14.46 ± 2.88abc | 67.51 ± 0.20a |
| | 30 cm | 2.14 ± 0.07bc | 0.45 ± 0.04bc | 42.01 ± 0.63ab | 8.77 ± 1.41d | 54.82 ± 0.32cd |
| | 45 cm | 1.20 ± 0.08e | 0.36 ± 0.02c | 45.06 ± 0.46a | 12.87 ± 1.69bcd | 59.79 ± 1.73b |
| 20% | No cut | 2.72 ± 0.06a | 0.41 ± 0.05c | 43.99 ± 1.86a | 18.88 ± 2.17a | 55.83 ± 0.84c |
| | 15 cm | 2.05 ± 0.13c | 0.43 ± 0.02bc | 38.24 ± 0.37bc | 10.39 ± 0.95cd | 52.29 ± 0.39d |
| | 30 cm | 1.55 ± 0.06d | 0.40 ± 0.02c | 34.89 ± 0.60c | 16.34 ± 1.33ab | 53.09 ± 1.22cd |
| | 45 cm | 1.54 ± 0.04d | 0.63 ± 0.01a | 42.42 ± 3.55ab | 15.16 ± 2.09abc | 54.02 ± 0.70cd |
| 40% | No cut | - | - | - | - | - |
| | 15 cm | - | - | - | - | - |
| | 30 cm | - | - | - | - | - |
| | 45 cm | - | - | - | - | - |
| Two-way ANOVA F-Statistics | | | | | | |
| Seawater | | 101.64* | 3.86ns | 7.39* | 1.87ns | 61.51* |
| Pruning | | 30.63* | 4.07* | 5.25* | 4.24* | 16.47* |
| Seawater*Pruning | | 8.13* | 10.76* | 3.73* | 3.30* | 32.39* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

#### 5.4.1.2 Effect of diluted seawater and different pruning intervals on the accumulation of Flavanol

**Diluted seawater (Week 4):** Diluted seawater significantly affected ($p \leq 0.05$) the accumulation of flavonols in *T. decumbens* shoots (Table 5.1). The 20%DSW recorded the highest mean value of flavonol (0.65 mg QE/g), while the control recorded the least flavonol value (0.32 mg QE/g). The highest mean value was significantly higher than other treatments but was also comparable the control (0.59 mg QE/g).

**Pruning (Week 4):** Pruning intervals had a significant effect on the accumulation of flavonols in the shoots of dune spinach. The application of 30 cm pruning intervals resulted in high flavonol content, while 30 cm pruning intervals in 40%DSW recorded the lowest flavonol content.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had a significant effect on the accumulation of flavonols.

**Diluted seawater (Week 8):** Diluted seawater had a significant influence ($p \leq 0.05$) on the accumulation of flavonols in *T. decumbens* shoots (Table 5.2). The control treatment recorded highest flavonol values (0.60 mg QE/g). The highest mean value recorded in the control was significantly higher than other treatments but did not differ significantly to 20%DSW (0.58 mg QE/g).

**Pruning (Week 8):** Table 5.2 showed a significant impact on the accumulation of flavonols in the shoots of *T. decumbens*. The plants pruned with 15 cm, 30 cm, and 45 cm recorded the highest mean values, while one's subjected in 20%DSW with 45 cm pruning recorded the lowest values of flavonols.

**Diluted seawater interaction with different pruning intervals (Week 8):** These interactions had a significant effect on the accumulation of flavonols in the shoots of dune spinach.

**Diluted seawater (Week 12):** Diluted seawater had no significant effect on the accumulation of flavonols in the shoots of *T. decumbens*. The plants cultivated in 20%DSW recorded the highest flavonol content (0.63 mg QE/g). The lowest mean value was found in the control and was significantly lower than all other treatments but did not differ when compared to 20%DSW without pruning and 30 cm pruning.

**Pruning (Week 12):** Table 5.3 showed a significant effect on the accumulation of flavonols in *T. decumbens* shoots. The application of 45 cm pruning intervals in 20%DSW treatment recorded the highest mean value, while plants without pruning recorded the lowest mean values of flavonols.

**Diluted seawater interaction with pruning (Week 12):** The interactions had a significant effect ($p \leq 0.05$) on the accumulation of flavonols in *T. decumbens* shoots (Table 5.3).

#### 5.4.1.3 Effect of diluted seawater and different pruning intervals on the accumulation of FRAP Antioxidant Content

**Diluted seawater (Week 4):** Diluted seawater had a significant impact ($p \leq 0.05$) on the FRAP antioxidant content of *T. decumbens* shoots (Table 5.1). The plants subjected under 20%DSW recorded the highest mean value of FRAP antioxidant content (40.89 μmol AAE/g), while 40%DSW recorded the least FRAP antioxidant content (32.38 μmol AAE/g). The highest mean value was significantly higher than all other including the control.

**Pruning (Week 4):** The application of different pruning intervals showed a significant effect on the FRAP content of dune spinach shoots. The plants subjected to the following pruning intervals (15 cm, 30 cm, and 45 cm) recorded the highest FRAP values, while 45 cm pruning intervals in 40%DSW recorded the lowest FRAP content.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and pruning intervals had a significant effect on the FRAP content of *T. decumbens* shoots.

**Diluted seawater (Week 8):** Diluted seawater had a significant effect ($p \leq 0.05$) on the FRAP antioxidant content of *T. decumbens* shoots (Table 5.2). The plants cultivated under 20%DSW recorded the highest FRAP antioxidant content (55.24 μmol AAE/g) and were significantly higher than all other treatments. The least FRAP antioxidant content (33.34 μmol AAE/g) was recorded in the control treatment.

**Pruning (Week 8):** Different pruning intervals showed a significant increase on the FRAP content in *T. decumbens* shoots. The application of 30 cm pruning intervals resulted in the highest mean value of FRAP content, while 15 cm pruning intervals resulted in the lowest mean values.

**Diluted seawater interaction with pruning (Week 8):** The interactions showed significant effect on the FRAP antioxidant content of *T. decumbens* shoots.

**Diluted seawater (Week 12):** Diluted seawater had a significant effect ($p \leq 0.05$) on the FRAP antioxidant content of *T. decumbens* shoots (Table 5.3). The plants subjected under the control recorded the highest FRAP antioxidant content (45.06 µmol AAE/g), while the least FRAP antioxidant content (34.89 µmol AAE/g) was recorded in 20%DSW treatment. The highest mean value was significantly higher than other treatments but did not differ from 20%DSW without pruning interval (43.99 µmol AAE/g).

**Pruning (Week 12):** Table 5.3 showed a significant increase on the FRAP content of *T. decumbens* shoots. The highest mean value was found in plants without pruning and 45 cm pruning intervals, while the least mean value was found in 30 cm pruning intervals

**Diluted seawater interaction with pruning (Week 12):** Diluted seawater interaction with pruning significantly affected the FRAP antioxidant content of *T. decumbens*.

#### 5.4.1.4 Effect of diluted seawater and different pruning intervals on the accumulation of DPPH Antioxidant Content

**Diluted seawater (Week 4):** Diluted seawater significantly increased ($p \leq 0.05$) the DPPH activity of *T. decumbens* shoots (Table 5.1). The plants cultivated in 40%DSW resulted in the highest DPPH antioxidant content (28.52 µmol TE/g), while the control resulted in the least DPPH antioxidant content (10.40 µmol TE/g). The highest mean value was significantly higher than other treatments, and the lowest mean value was significantly lower than all other treatments but did not differ from 20%DSW with a 30 cm pruning interval (11.27 µmol TE/g).

**Pruning (Week 4):** Different pruning intervals had a significant effect on the DPPH activity of *T. decumbens* shoots. The application of 45 cm pruning interval recorded the highest DPPH activity, while 15 cm pruning interval in the control recorded the lowest mean value.

**Diluted seawater interaction with different pruning intervals (Week 4):** The interactions of diluted seawater and different pruning intervals had a significant effect on the DPPH activity of *T. decumbens* shoots.

**Diluted seawater (Week 8):** Diluted seawater had no significant effect on the DPPH activity of *T. decumbens* shoots. The plants cultivated in the control treatment resulted in the highest and lowest DPPH activity depending on the pruning intervals made.

**Pruning (Week 8):** Different pruning intervals had a significant effect on the DPPH activity of dune spinach shoots. The plants subjected to the control with 15 cm pruning intervals recorded the highest DPPH activity, while the one's subjected to 30 cm pruning intervals recorded the lowest mean values of DPPH activity.

**Diluted seawater interaction with pruning (Week 8):** The interactions of diluted seawater and pruning had a significant effect ($p \leq 0.05$) on the DPPH activity of *T. decumbens* shoots (Table 5.2).

**Diluted seawater (Week 12):** Diluted seawater had no significant effect on the DPPH activity of *T. decumbens* shoots. The plants cultivated under 20%DSW resulted in high DPPH activity (18.88 µmol TE/g), while the least DPPH activity (8.77 µmol TE/g) was recorded in the control treatment. The highest mean value was significantly higher than all other treatments including the control.

**Pruning (Week 12):** The application of pruning intervals had a significant effect on the DPPH activity of *T. decumbens* shoots. The plants without pruning intervals recorded the highest mean values of DPPH activity, while plants with 30 cm pruning intervals in the control recorded the lowest mean values.

**Diluted seawater interaction with pruning (Week 12):** The interactions of diluted seawater and pruning had a significant effect ($p \leq 0.05$) on the DPPH activity of *T. decumbens* shoots (Table 5.3).

#### 5.4.1.5 Effect of diluted seawater and different pruning intervals on the accumulation of ABTS

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) on the ABTS values of *T. decumbens* shoots (Table 5.1). The plants subjected to 40%DSW recorded the highest ABTS value (53.10 µmol TE/g), while the least ABTS value (42.52 µmol TE/g) was recorded in 20%DSW treatment. The highest mean value was significantly higher than all other treatments, and the lowest mean value was significantly lower than all other treatments but did not differ from 20%DSW with 30 cm pruning interval (42.93 µmol TE/g).

**Pruning (Week 4):** Different pruning intervals showed a significant increase on the ABTS content in the shoots of *T. decumbens*. The application of 45 cm pruning intervals recorded the highest mean values, while 30 cm and 45 cm pruning intervals in 20%DSW treatment recorded the lowest mean values of ABTS.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals showed a significant effect on the ABTS content of *T. decumbens* shoots.

**Diluted seawater (Week 8):** Diluted seawater had a significant effect ($p \leq 0.05$) on the ABTS values of *T. decumbens* shoots (Table 5.2). The plants cultivated in the control recorded the highest ABTS values (67.51 µmol TE/g), while the least ABTS value (53.01 µmol TE/g) was recorded in 20%DSW treatment. The highest mean value was significantly higher than all other treatments.

**Pruning (Week 8):** Table 5.2 shows a significant impact on the ABTS content of *T. decumbens* shoots. The plants subjected to 30 cm pruning intervals resulted in high ABTS values, while 45 cm pruning intervals resulted into low ABTS values.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals showed a significant effect on the ABTS content of *T. decumbens* shoots.

**Diluted seawater (Week 12):** Diluted seawater significantly affected ($p \leq 0.05$) the ABTS values of *T. decumbens* shoots (Table 5.3). The plants cultivated in the control recorded the highest ABTS value (67.51 µmol TE/g), while the least ABTS value (52.29 µmol TE/g) was recorded in 20%DSW treatment. The highest mean value was significantly higher than all other treatments.

**Pruning (Week 12):** Different pruning intervals had a significant increase on the ABTS values of *T. decumbens* shoots. The application of 15 cm pruning intervals recorded the highest mean values in the control treatment. The least mean values of ABTS were obtained in 15 cm pruning intervals in 20%DSW treatment.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and different pruning intervals showed a significant effect on the ABTS content of *T. decumbens* shoots.

## 5.5 Discussion

Salinity poses significant challenges to agricultural productivity, impacting the growth of crops, their morphology, and their biochemical parameters (Jameel *et al*., 2024). It is therefore imperative to develop growing protocols for wild vegetable crops that tolerate salt to maximize the production of food around the world. Edible stalk portions of vegetables such as spinach are known to be essential constituents of a well-balanced diet enriched with phenolic compounds consisting of polyphenols, alkaloids, saponins, proteins, polysaccharides, carotenoids, minerals, dietary fiber, vitamins, and phytochemicals (Nirmala *et al*., 2022; Sarkar *et al*., 2023). Among these phytochemicals, polyphenols play an important function in suppressing proinflammatory transcription factors by linking with proteins responsible for cell signalling (Zhang & Tsao, 2016; Muscolo *et al*., 2024). These polyphenols further gained interest due to their possible beneficial effects on cardiovascular diseases (Abd El-Hack *et al*., 2023). Certain phenolic-rich plant components serve to safeguard human tissues against oxidative damage through eliminating free radicals and preventing lipid peroxidation (Akbari *et al*., 2022; Bulawa *et al*., 2022). They improve the nutritional quality of food while mitigating any possible adverse health effects associated with excessive intake of synthetic chemicals. The importance of fruits and vegetables with high antioxidant capacity goes beyond their nutritional value as they provide added-value goods that are increasingly sought after by consumers and the food industry (Pinto *et al*., 2022).

The present study emphasizes the vital significance of comprehending the effects of salinity on the growth and biochemical composition of agricultural crops, with a specific emphasis on phytochemicals and antioxidant activities. The results show a complex relationship between salinity levels and the physiological responses of *T. decumbens* shoots, highlighting both positive and negative effects on polyphenol production and antioxidant capacity. Low concentrations of diluted seawater seem to increase antioxidant activity, polyphenol and flavonoid content, and other biochemical properties, suggesting possible methods of improving crop growth in saline environments. However, elevated levels of salinity may trigger a range of alterations in the anatomy, physiology, biochemistry, and molecular biology of tissues and cells in numerous plant species (Zhao *et al*., 2019; Roșca *et al*., 2023). Furthermore, plant development is inhibited by high salinity concentrations due to a decrease in hyperosmotic stress and the buildup of salts at toxic levels for the plants (Van Zelm *et al*., 2020; Balasubramaniam *et al*., 2023). Plants adapt to these challenges by changing their physiological and metabolic processes.

According to (Pinto *et al*., 2022), fruits and vegetables containing high levels of antioxidants enhance the value of food products and are favourably acknowledged by customers and the food business. The consumption of food items with elevated phenolic content helps preserve human tissue oxidation through scavenging free radicals and reducing lipid peroxidation, enhancing nutritional quality, and avoiding potential difficulties brought about by excessive amounts of synthetic chemicals (Akbari *et al*., 2022). Therefore, it is of paramount importance to improve the phytochemical elements found in fruits and vegetables during cultivation. From the results obtained in this present study, the application of diluted seawater had a significant impact ($p \leq 0.05$) on the polyphenol content of *T. decumbens* shoots, with high-profile content in plants watered with low seawater concentration (20%DSW), while plants irrigated with high seawater concentration (40%DSW) showed significantly lower mean values of polyphenols, flavanols, and antioxidant capacity, and this further resulted in the death of plants. These findings validate those of (Ngxabi *et al*., 2021) on *T. ciliata,* where low to moderate salinity concentrations showed a positive effect on polyphenols, while high salinity concentrations demonstrated lower mean values. A similar trend was further observed by (Pungin *et al*., 2023) on *Glaux maritima*, where low salinity stress effectively enhanced the amount of phenolic compounds.

Plants also frequently suffer from ion imbalance and osmotic stress when exposed to high salt concentrations, which reduces their uptake of vital minerals like potassium and phosphorus (Shabala & Pottosin, 2014; Ngxabi *et al*., 2021). This limits the amount of potassium and phosphorus that are available for metabolic activities, such as the production of polyphenols. A similar trend was observed regarding flavonol content, where low seawater concentrations (20%DSW) significantly enhanced the flavonol content of *T. decumbens* shoots, while high seawater concentrations (40%DSW) showed significantly lower mean values that resulted in the death of plants. However, these findings were in correspondence with those of *(*Ngxabi *et al*., 2021) and (Ksouri *et al*., 2007) on *T. ciliata* and *Cakile maritima*, where low salt concentrations recorded significantly higher mean values of flavonols.

The antioxidant activity (FRAP and DPPH) on *T. decumbens* shoots irrigated with low seawater concentrations revealed greater mean values than the control. These findings contradict those of (Pungin *et al*., 2023) on *Glaux maritima* plants, where high salt concentrations increased the amount of FRAP, DPPH, and ABTS. Furthermore, these findings corroborate those of (Chrysargyris *et al*., 2018) on edible flowers, where salinity increased the amount of antioxidants. A similar trend was observed by (Ngxabi *et al*., 2021) on *T. ciliata*, where low salinity concentrations also recorded slightly higher antioxidants. The correlation between elevated production of secondary metabolites and oxidative stress caused by salinity could provide validity to these findings. Moreover, excessive salinity may be the cause of this since it can be toxic and inhibit photosynthesis, which in turn reduces the synthesis of secondary metabolites. It was not the case for ABTS, where the control recorded the highest mean values when compared to other treatments. Low seawater concentrations (20%DSW) recorded the lowest mean values of ABTS. These results corroborate the findings of (Sogoni *et al*., 2021) on *T. decumbens*, which demonstrated significantly lower antioxidant capacity in the leaves of plants subjected to different salinity concentrations than the control. The findings of this research demonstrate that *T. decumbens* can thrive in environments with low to moderate salinity levels and suggest that it might be used as a substitute food source in regions with low to moderate salinity soils.

## 5.6 Conclusion and recommendations

Nonetheless, elevated quantities of seawater lead to a reduction in polyphenol levels, a compromise in antioxidant activity, and even the mortality of plants, indicating that agricultural techniques must carefully regulate salinity stress. This study highlights the significance of taking into account both the nutritional value of crops and their resilience to environmental difficulties, and it provides insightful information on the mechanisms underlying plant responses to salinity stress. The research provides opportunities for the development of sustainable farming practices and the improvement of food security in countries affected by soil salinity by clarifying the intricate relationships between oxidative stress, salinity, and the synthesis of secondary metabolites. In the end, the results indicate that *T. decumbens* is a resilient crop species with possibilities to enhance dietary health and agricultural production in areas with low to moderate salinity. Further research into the molecular mechanisms governing plant responses to salinity stress will be crucial for harnessing the full potential of salt-tolerant crops and addressing global challenges in food production.

## 5.7 Acknowledgements

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# CHAPTER SIX

## SEAWATER DILUTIONS AND DIFFERENT PRUNING LEVELS MODULATE FULL FEED OF HYDROPONICALLY CULTIVATED *TETRAGONIA DECUMBENS* MILL.

**Seawater dilutions and different pruning levels modulate full feed contents of hydroponically cultivated *Tetragonia decumbens* Mill.**

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## 6.1 Abstract

Vegetables are crucial for a balanced diet and the major source of nutritional stability. Although underutilised vegetables use have increased to combat poverty, hunger, and economic growth, essential nutritional content for many of these species remained underutilised. In this study, the effect of diluted seawater (100% tap water/50L, 20% SW/50L, 40% SW/50L, and 60% SW/50L) and different pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm cut) on proximate, micronutrient, and macronutrients of hydroponically cultivated *T. decumbens* was investigated. Results showed greater ash content and higher amounts of important minerals, such as magnesium (Mg), potassium (K), and zinc (Zn), at 20% seawater dilution, and 30cm and 45cm pruning levels. The study emphasises high content of minerals such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) in *T. decumbens* which are required for human health. These findings suggest that *T. decumbens* has the potential to be a nutrient-dense and robust crop that can increase food security and nutrition, particularly in areas with environmental stresses under optimal growing conditions. Further study to optimise cultivation procedures, field trials for practical application, and consideration of incorporating *T. decumbens* into regional food systems are encouraged to meet nutritional demands and improve food security.

**Keywords:** ash content, Dune spinach, food security, micronutrients, macronutrients, neglected vegetables

## Introduction

Global agricultural production needs to increase by 70–100% by 2050 in order to meet the food demands of approximately seven billion people worldwide (Loconsole *et al*., 2019). According to (Timmer, 2010) and (Jagtap *et al*., 2022), more than one billion people lacked consistent access to affordable and nutritious food. Commercial agricultural land worldwide of 1.5 billion hectares, is too small to generate enough food to feed the increasing world population (Herrero *et al*., 2012). Additionally, it has been projected that over the next 50 years, urbanisation will reduce agricultural land by 13%, and by 2030, the global productivity of agriculture will decline by 1.5 percent annually (Berchoux *et al*., 2023).

The yields of several important crops, such as rice, wheat, and maize, are negatively impacted by unfavourable environmental changes that occur all over the world, such as high temperatures, climate change, salinity of the soil, soil degradation, and a lack of clean irrigation water (Haj-Amor *et al*., 2022; Çakmakçı *et al*., 2023). Due to this, the world food supply has decreased in many developing nations, making it more difficult for many people to have access to sufficient nutritional food for daily dietary needs to support healthy and active lifestyles (Malhi *et al*., 2021). Nevertheless, this suggests that in the coming years, food production will need to increase in order to meet the growing demand for agricultural products (Sahu *et al*., 2013). To meet this demand, exploring underutilized plants become necessary to increase leafy options and meet daily demand for vital nutrients.

Vital nutrients in plants include macronutrients and micronutrients. Macronutrients are required in larger quantities for the growth of plants, and micronutrients are required in smaller quantities but are still essential for the growth of plants (Vejan *et al*., 2021). The macronutrients are nitrogen (N), potassium (K), phosphorous (P), magnesium (Mg), calcium (Ca), and sulphur (S) (Daramola & Hatzell, 2023). Macronutrients are essential for plant and human life as they execute vital metabolic and physiological functions like respiration, photosynthesis, and growth (Tariq *et al*., 2023; Monib *et al*., 2023). A balanced availability of these vital nutrients in soil is crucial for the healthy growth of plants (Tariq *et al*., 2023). Micronutrients are characterised as important small-molecule nutrients that plants require in small amounts (Sherefu *et al*., 2021; Faizan *et al*., 2024). The micronutrients include manganese (Mn), molybdenum (Mo), iron (Fe), chloride (Cl), zinc (Zn), aluminium (Al), boron (B), and copper (Cu) (Thapa *et al*., 2021). These small-molecule nutrients serve as the building blocks for both coenzymes and enzymes. They are crucial for the resilience of plants and productivity, particularly during times of stress. However, the absence of any of the micronutrients has a significant detrimental effect on the crop's development, yield, and overall nutritional value (Zaib *et al*., 2023). Furthermore, these micronutrients help produce antioxidants that safeguard cells in plants from oxidative damage brought on by a variety of stressors like salt content, pollution from heavy metals, and nutritional imbalances (Nandi *et al*., 2024).

Due to their high nutrients, vitamins, and mineral composition, green vegetables are essential to a healthy diet and are the primary contributors to world nutritional stability (Ebert, 2020). However, vegetables that are underutilised are being used more frequently and successfully to combat poverty, hunger, and economic growth (Jena *et al*., 2018; Shree *et al*., 2022). These neglected vegetable crops are vital biological resources for the impoverished in rural areas and have the potential to improve the health of millions of people living in tribes. Furthermore, neglected vegetable crops have high levels of antioxidant activity and are abundant in minerals, vitamins, and other elements that promote health (Yasin *et al*., 2018; Bulawa *et al*., 2022). Neglected vegetable crops may further feed the underprivileged by satisfying the dietary needs of vulnerable populations and are resistant to many biotic and abiotic stresses (Hossain *et al*., 2021; Barooah *et al*., 2023).

*Tetragonia decumbens*, also referred to as dune spinach, is one of the underutilised vegetables that may be eaten raw or cooked with other vegetables. It may also be used as an alternative for traditional spinach (Sogoni *et al*., 2021). This species can thrive in a wide range of environments, such as salinity and soils deficient in nutrients with minimal water needs (Nkcukankcuka *et al*., 2021). The importance of cultivating underutilised edible halophytes has become problematic in crop production (Bueno & Cordovilla, 2020). Additionally, it has been noted that edible halophytes such as *T. decumbens* are a good source of nutrients and bioactive elements (El-Amier *et al*., 2021; Sogoni *et al*., 2021), which are thought to have significant influences on many health outcomes (Shah & Smith, 2020; Barreca *et al*., 2021). The aim of this study was to determine the effects of graded salinity derived from diluted seawater and different pruning levels on nutritional composition of *T. decumbens* to guide future production of green vegetables in food security.

## 6.3 Materials and methods

### 6.3.1 Experimental location

This research was carried out at the research greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology, Bellville campus, Cape Town, South Africa(33°55'56" S and 18°38'25" E). The research project was conducted in an experimental greenhouse with temperature regulation of 21–26 ºC during the day and 12–18 ºC at night. The average relative humidity was maintained at 60%.

### 6.3.2 Plant material and experimental description

*Tetragonia decumbens* cuttings were collected on February 27, 2023, from a specific clone plant that was flourishing alongside the coastline at Granger Bay, Western Cape. A stem cutting propagation technique was used to root new plant material, as reported by (Sogoni *et al.*, 2021). A total number of one hundred and ten plants were propagated with rooting powder (Dynaroot™ No. 1 with active ingredient 0.1 % IBA) in polyethylene seedling flat trays filled with a ratio of 50:50 sand/peat. The trays were placed in the propagation greenhouse on heated beds with intermitted mist irrigation. Seventy cuttings were selected out the total number rooted and moved to a shaded area for five weeks to allow them to acclimatise. Thereafter the cuttings were transplanted into 12.5 cm black plastic pots filled with Consul silica sand. Only healthy plants were selected and arranged in a randomised block design of three treatments and a control with three replicates per treatment totalling forty-eight plants.

### 6.3.3 Nutrient solution

Nutrifeed™ fertiliser from Starke Ayres in Cape Town, South Africa was used to prepare a hydroponic aqueous solution and used as general feeding to ensure healthy plant development. Nutrifeed contains the following ingredients: 65 g/kg Nitrogen (N), 27 g/kg Phosphorus (P), 130 g/kg Potassium (K), 70 mg/kg Calcium (Ca), 20 mg/kg Copper (Cu), 1500 mg/kg Iron (Fe), 10 mg/kg Molybdenum (Mo), 22 mg/kg Magnesium (Mg), 240 mg/kg Manganese (Mn), 75 mg/kg Sulphur (S), 240 mg/kg Boron (B), and mg/kg Zinc (Zn). For the first three weeks before the experiment started the system operated using tap water only to ensure that plants adapt to their new environment. Thereafter the Nutrifeed concentration were maintained with a concentration of 1:500 (1 kg/500 litres, or approximately 10 g per 5 L) in each hydroponic sump.

### 6.3.4 Hydroponic design

The hydroponic system was constructed in accordance with (Bulawa *et al*., 2022) and built with four identical Nutrient Film Technique (NFT) systems. Each system was constructed on a wire mesh square table (2.5 m) to give stability and flat surface. A 50 L low-density polyethylene (LDPE) reservoir and a single submersible water pump capable of producing 2000 litres per hour (1 x 2000 L/h) were present in each of these Nutrient Film Technique (NFT) systems, which were referred to as table 1 to 4 (T1-T4). The low-density polyethylene (LDPE) reservoir served as a container for the nutritional solution that would be utilised in the experiment. The submersible water pump was designed to generate a nutrient solution and transport it from the reservoir to the gutters. White polyvinyl chloride (PVC) square gutters that were 1.36 metres long were used to construct the hydroponic system and were labelled G1, G2, G3, and G4. Each white polyvinyl chloride (PVC) square gutter was covered with black plastic polyethylene sheets to prevent algae growth and three holes per gutter were also created to accommodate 12.5 cm plastic pots. A 50 L low-density polyethylene (LDPE) reservoir, 1 x 2000 l/h submersible water pump, 20 mm low-density polyethylene (LDPE) irrigation pipe, 20 mm elbow irrigation fittings, 20 mm flow regulators, 20 mm T-piece irrigation fittings, and 20 mm end cap irrigation fittings were all used during the construction of each system. Each gutter on each system had 3 pots with a consul silica sand medium. For preventing leaks in the system, a silicon glue (PVC) was used to seal the two components firmly together and when inserting irrigation fittings to the polyvinyl chloride (PVC) square gutters. A portable digital electrical conductivity (EC) metre (Hanna Instruments®TM HI 98312) that has been calibrated was used to measure the electrical conductivity (EC) in the nutritional solution on a daily basis and a calibrated hand-held digital pH metre (Eurotech®TM pH 2 pen) was used to measure the pH in the nutritional solution on a daily basis. The pH of the nutritional solution was increased with potassium hydroxide [KOH] and reduced with phosphoric acid [$H_3PO_4$]. The pH and electrical conductivity (EC) were maintained at 4.5, to 6.5, and 3.38 to 1.10 ds m -1 (S) respectively.

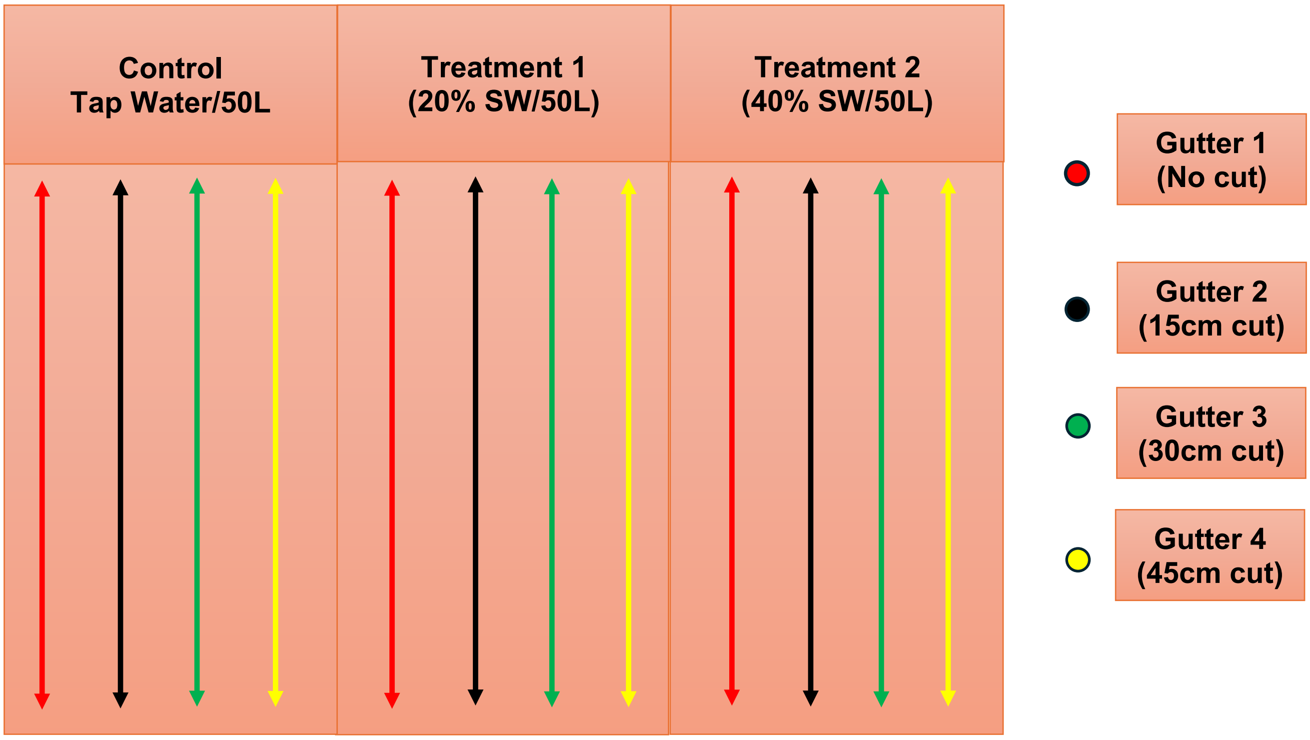


**Figure 6.1:** Layout and random design of the experiment showing the seawater dilution treatments and pruning cuts applied.

### 6.3.5 Treatments

#### 6.3.5.1 Seawater treatments

Seawater was collected from Granger Bay, Cape Town and diluted with tap water to prepare three diluted seawater treatments and clean tap water as the control. All the sumps (S2, S3, and S4) with seawater treatments were refilled every week (Friday) to avoid build-up of algae. The dilutions were as follows:

- S1 Sump 1 = clean tap water (control)
- S2 Sump 2 = 20% SW/50L (Treatment 1)
- S3 Sump 3 = 40% SW/50L (Treatment 2)

#### 6.3.5.2 Pruning treatments

At the beginning of the experiment (week one), all plants were placed in the gutters and pruned back to 15 cm. This was done to ensure uniform height in all plants. Thereafter plants were pruned again at weeks 4 and 8 to three different heights as follows:

- G1 Gutter 1 = No cut (S1G1, S2G1, and S3G1)
- G2 Gutter 2 = 15 cm (S1G2, S2G2, and S3G2)

- G3 Gutter 3 = 30 cm (S1G3, S2G3, and S3G3)
- G4 Gutter 4 = 45 cm (S1G4, S2G4, and S3G4)

At week 12, no pruning occurred, however, all plant samples were harvested and prepared for analysis. At weeks 4 and 8, all the unpruned plants were allowed to grow throughout the experiment without being pruned, hence they are represented with "NA" on the tables. "NA" means the plants were not analysed at that particular stage.

### 6.3.6 Nutritional analysis

#### 6.3.6.1 Sample preparation

The nutrient uptake was analysed on the harvested shoots of *T. decumbens*. Only materials from pruned shoots of *T. decumbens* were dried for eight days at 40 °C in a fan-drying LABTECHTM model LDO 150F (Daihan Labtech Pty. Ltd. New Dehli, India) oven. The materials that had dried out was ground to a fine powder using a Junkel & Kunkel model A 10 mill. After the experiment, all the samples were packed, labelled, and sent out to the Department of Agriculture and Rural Development analytical laboratory, of KwaZulu Natal in powdery form for minerals and proximate analysis.

### 6.3.7 Proximate Analysis

#### 6.3.7.1 Moisture content

The moisture content was determined through modified techniques that was used by (Mndi *et al*., 2023). A temperature of 105 °C was used to dry empty porcelain containers for an hour. After cooling, the containers were weighed (W1). About one gram of the pulverized samples of *T. decumbens* were placed in a receptacle and oven-dried to a constant weight at 105 °C. The glass container was allowed to cool in a desiccated environment before being weighed again (W3). To find the moisture content percentage, the following equation was used.

$$\%\ \text{Moisture content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

#### 6.3.7.2 Crude fat content

The crude fat was calculated using a minor modification that corresponded with the description provided by (Tshayingwe *et al*., 2023). One gram of the powdered material was extracted in 100 millilitres of diethyl ether using an orbital shaker for a duration of twenty-four hours. The mixture was filtered, and the filtrate was collected in clean beakers that had been weighed previously (W1). To equilibrate the ether extract, 100 millilitres of diethyl ether was added. After mixing the mixture for an additional twenty-four hours on an orbital shaker, the filtrate was collected into a beaker (W1). The ether filter was weighed in the beaker (W2) after being dried out in a steam bath and oven-dried at 55 °C. As a result, the following was used to determine the crude fat content.

$$\text{\% Crude fat content} = \frac{\text{W2} - \text{W1}}{\text{Original weight of the pulverised sample}} \times 100$$

#### 6.3.7.3 Ash content

A procedure described by Bulawa *et al*., (2022) was used to ascertain the percentage ash content of *T. decumbens* shoots that were examined. A 105 °C oven was used to heat porcelain dishes that had been labelled with samples for one hour. A desiccator was used to cool the crucibles before they were weighed. (W1). Then, a crucible containing one gram of ground shoots was reweighed (W2). The crucible containing the plant sample was put in a muffle oven and heated to a temperature of 250 °C for 1 hour. Thereafter, the temperature of the muffle oven was increased to 550 °C for five hours in order to thoroughly ash the samples. Samples were weighed and the porcelain bowls were kept cool in a desiccator (W3). The ash content of the samples was calculated using the following equation.

$$\text{\% Ash content} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100$$

#### 6.3.7.4 Crude protein

This was discovered by heating 2 grams of pulverized materials and 20 millilitres of concentrated sulfuric acid ($H_2SO_4$) to a clear mixture while using as a catalyst (Cebani *et al*., 2024). Following filtration and distillation, the digested extracts were dissolved in 250 millilitres. Thereafter a second distillation in 500 millilitres round-bottom flask using the aliquot containing 50 millilitres of 45% sodium hydroxide (NaOH), 150 millilitres of the distillate was placed into a flask containing 100 millilitres of 0.1 M hydrochloric acid (HCl). A yellow colour signalled the endpoint of the titration process, and equation below was used to compute the nitrogen content percentage.

$$\text{Crude protein} = \frac{[(\text{ml std acid} \times \text{N of acid}) - (\text{ml bank} \times \text{N of base})] - (\text{ml std base} \times \text{N of base}) \times 1.4007}{\text{Original weight of the pulverised sample}}$$

Where N= stands for the crude percentage and normality were acquired by multiplying the nitrogen value by a constant factor of 6.25 (Idris *et al*., 2019).

#### 6.3.7.5 Neutral detergent fibre (NDF)

The NDF composition of the samples was determined using the equation below, as described by Jimoh *et al*., (2024).

$$\% \text{ NDF } = \frac{(\text{W1} + \text{W2}) - \text{W1}}{\text{Weight of the sample}} \times 100$$

#### 6.3.7.6 Acid detergent fibre (ADF)

The NDF composition of the samples was determined using the equation below, as described by (Obregón-Cano *et al*., 2019).

$$\% \text{ ADF } = \frac{\text{W3} - \text{W1}}{\text{W2} - \text{W1}} \times 100$$

## 6.4 Results

### 6.4.1 Macronutrients

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) in all macronutrients of *T. decumbens* shoots (Table 6.1). The plants subjected to 20%DSW produced the highest amount of magnesium (880 mg/100g), phosphorus (810 mg/100g), nitrogen (4695 mg/100g), and sodium (5135 mg/100g). The least mean values of magnesium (630 mg/100g) and sodium (955 mg/100g) were obtained in the control treatment, while for phosphorus and nitrogen were obtained in 40%DSW treatment. The highest amount of calcium (710 mg/100g), potassium (5470 mg/100g), and K/Ca+Mg (1595 mg/100g) were recorded in the control treatment and their lowest amounts were recorded in 40%DSW treatment.

**Pruning (Week 4):** Different pruning intervals showed a significant effect in all the macronutrients in the shoots of *T. decumbens*. The application of 30 cm pruning intervals in the shoots of dune spinach resulted in the highest amount of magnesium and sodium, while their lowest amounts were recorded in 15 cm pruning intervals in the control. The highest amount of phosphorus and nitrogen were recorded in 15 cm pruning intervals, while their lowest amounts were also recorded in 15 cm pruning intervals but in 40%DSW treatment. For calcium, potassium, and K/Ca+Mg 15 cm pruning intervals in the control treatment recorded the highest mean values, while 15 cm pruning in 40%DSW recorded the lowest mean values.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had a significant effect in all macronutrients in the shoots of *T. decumbens* except magnesium

**Diluted seawater (Week 8):** All macronutrients were significantly affected ($p \leq 0.05$) by diluted seawater (DSW) in *T. decumbens* shoots except for nitrogen and potassium (Table 6.2). The plants subjected to 20%DSW produced high amounts of magnesium (770 mg/100g), sodium (5105 mg/100g), phosphorus (960 mg/100g), and nitrogen (3345 mg/100g). The least mean values were recorded in the control for both magnesium (330 mg/100g) and sodium (605 mg/100g). Phosphorus, nitrogen, and potassium no significant difference. This means that the differences were so small that they are not noticeable.

**Pruning (Week 8):** Different pruning intervals had a significant impact in all macronutrient in *T. decumbens* shoots. For magnesium and sodium, plants subjected to 15 cm and 30 cm pruning intervals recorded the highest mean values. However, phosphorus, nitrogen, and potassium showed no significant difference in this stage.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals had a significant effect on calcium and K/Ca+Mg. However, for other macronutrients no significant effect was observed in the shoots of dune spinach. It is clear that the interaction of diluted seawater and different pruning intervals had no positive effect on most macronutrients.

**Diluted seawater (Week 12):** Diluted seawater significantly affected ($p \leq 0.05$) all macronutrients except for nitrogen (Table 6.3). The plants cultivated in 20%DSW produced high amounts of magnesium (955 mg/100g), nitrogen (2960 mg/100g), and sodium (6110 mg/100g), while least mean values were recorded in the control treatment. For calcium (430 mg/100g), phosphorus (565 mg/100g), potassium (5235 mg/100g), and K/Ca+Mg (2340 mg/100g), their highest mean values were recorded in plants cultivated under the control treatment. Their lowest mean values were all recorded in 20%DSW treatment.

**Pruning (Week 12):** Different pruning intervals in the shoots of *T. decumbens* showed a significant effect in magnesium, sodium, and potassium. However, the was not significant effect in calcium, phosphorus, nitrogen, and K/Ca+Mg. It is clear that the application of different pruning intervals in some macronutrients had no positive effect.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater with different pruning intervals showed a significant effect in the shoots of *T. decumbens* in calcium, magnesium, sodium, and phosphorus. However, for nitrogen, potassium and K/Ca+Mg no significant effect was observed.

**Table 6.1: The effect of diluted seawater and different pruning intervals on macronutrients of *T. decumbens* shoots (Week 4).**

| Seawater | Pruning level | Ca (mg/100g DW) | Mg (mg/100g DW) | Na (mg/100g DW) | P (mg/100g DW) | N (mg/100g DW) | K (mg/100g DW) | K/Ca+Mg (mg/100g DW) |
|---|---|---|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA | NA | NA | NA |
| | 15 cm | 710 ± 10.0a | 630 ± 0.00d | 955 ± 15.0d | 705 ± 5.00c | 3165 ± 5.00c | 5470 ± 30.0a | 1555 ± 35.0a |
| | 30 cm | 510 ± 90.0b | 715 ± 115cd | 2815 ± 1685bc | 710 ± 0.00c | 3715 ± 165b | 5215 ± 435a | 1595 ± 225a |
| | 45 cm | 700 ± 0.00a | 735 ± 5.00bcd | 1195 ± 5.00cd | 775 ± 5.00ab | 4075 ± 45.0b | 5310 ± 80.0a | 1465 ± 5.00ab |
| 20% | No cut | NA | NA | NA | NA | NA | NA | NA |
| | 15 cm | 375 ± 5.00c | 815 ± 5.00abc | 5015 ± 95.0a | 810 ± 10.0a | 4695 ± 15.0a | 4405 ± 15.0bc | 1315 ± 5.00b |
| | 30 cm | 280 ± 50.0d | 880 ± 40.0a | 5135 ± 365a | 690 ± 30.0c | 3950 ± 530b | 4270 ± 20.0c | 1270 ± 80.0b |
| | 45 cm | 265 ± 5.00d | 840 ± 20.0ab | 4580 ± 360a | 760 ± 20.0b | 3215 ± 5.00c | 4720 ± 0.00b | 1465 ± 35.0ab |
| 40% | No cut | NA | NA | NA | NA | NA | NA | NA |
| | 15 cm | 270 ± 0.00d | 725 ± 5.00cd | 4405 ± 565ab | 455 ± 5.00d | 1835 ± 5.00d | 2840 ± 40.0d | 990 ± 10.0c |
| | 30 cm | 275 ± 5.00d | 735 ± 5.00bcd | 4225 ± 275ab | 465 ± 5.00d | 1850 ± 10.0d | 2895 ± 45.0d | 995 ± 25.0c |
| | 45 cm | 275 ± 15.0d | 745 ± 25.0bc | 3985 ± 75.0ab | 480 ± 10.0d | 1860 ± 0.00d | 2985 ± 25.0d | 1020 ± 30.0c |
| Two-way ANOVA F-Statistics | | | | | | | | |
| Seawater | | 100.91* | 10.39* | 22.55* | 425.39* | 112.65* | 202.44* | 32.73* |
| Pruning | | 140.48* | 325.36* | 34.23* | 2391.10* | 300.31* | 803.58* | 247.58* |
| Seawater*Pruning | | 14.54* | 1.56ns | 3.22* | 54.31* | 16.00* | 22.83* | 4.57* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 6.2: The effect of diluted seawater and different pruning intervals on macronutrients of *T. decumbens* shoots (Week 8).**

| Seawater | Pruning level | Ca (mg/100g DW) | Mg (mg/100g DW) | Na (mg/100g DW) | P (mg/100g DW) | N (mg/100g DW) | K (mg/100g DW) | K/Ca+Mg (mg/100g DW) |
|---|---|---|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA | NA | NA | NA |
| | 15 cm | 410 ± 40.0a | 430 ± 130b | 855 ± 305b | 730 ± 0.00a | 2575 ± 775a | 3940 ± 940a | 1800 ± 20.0a |
| | 30 cm | 385 ± 15.0a | 435 ± 115b | 890 ± 310b | 849 ± 160a | 2940 ± 1150a | 4125 ± 1005a | 1890 ± 170a |
| | 45 cm | 410 ± 20.0a | 330 ± 10.0b | 605 ± 15.0b | 715 ± 15.0a | 1825 ± 15.0a | 3240 ± 50.0a | 1740 ± 40.0a |
| 20% | No cut | NA | NA | NA | NA | NA | NA | NA |
| | 15 cm | 240 ± 0.00b | 760 ± 0.00a | 4865 ± 65.0a | 960 ± 300a | 2785 ± 5.00a | 3320 ± 10.0a | 1140 ± 0.00c |
| | 30 cm | 230 ± 10.0bc | 770 ± 50.0a | 3250 ± 2950ab | 605 ± 35.0ab | 3055 ± 55.0a | 3350 ± 240a | 1145 ± 15.0bc |
| | 45 cm | 180 ± 0.00c | 685 ± 5.00a | 5105 ± 195a | 290 ± 0.00bc | 3345 ± 65.0a | 3445 ± 15.0a | 1345 ± 15.0b |
| 40% | No cut | - | - | - | - | - | - | - |
| | 15 cm | - | - | - | - | - | - | - |
| | 30 cm | - | - | - | - | - | - | - |
| | 45 cm | - | - | - | - | - | - | - |
| | | | | Two-way ANOVA F-Statistics | | | | |
| Seawater | | 132.48* | 31.77* | 13.23* | 6.77* | 1.76ns | 0.72ns | 103.35* |
| Pruning | | 165. 49* | 40.36* | 6.26* | 19.01* | 15.97* | 26.32* | 291.38* |
| Seawater*Pruning | | 16.53* | 3.54ns | 1.84ns | 1.03ns | 1.05ns | 0.46ns | 14.32* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 6.3: The effect of diluted seawater and different pruning intervals on macronutrients of *T. decumbens* shoots (Week 12).**

| Seawater | Pruning level | Ca (mg/100g DW) | Mg (mg/100g DW) | Na (mg/100g DW) | P (mg/100g DW) | N (mg/100g DW) | K (mg/100g DW) | K/Ca+Mg (mg/100g DW) |
|---|---|---|---|---|---|---|---|---|
| Control | No cut | 430 ± 0.00a | 465 ± 5.00e | 970 ± 20.0c | 565 ± 5.00a | 2790 ± 50.0ab | 4530 ± 60.0abc | 1940 ± 10.0b |
| | 15 cm | 430 ± 60.0a | 580 ± 20.0cd | 1020 ± 140c | 490 ± 10.0ab | 2800 ± 490ab | 5235 ± 555a | 1945 ± 235b |
| | 30 cm | 375 ± 45.0ab | 515 ± 25.0de | 980 ± 120c | 370 ± 10.0bc | 2630 ± 360ab | 4740 ± 370ab | 1985 ± 165b |
| | 45 cm | 305 ± 5.00bc | 450 ± 40.0e | 955 ± 20.0c | 360 ± 20.0c | 2650 ± 180ab | 4655 ± 205abc | 2340 ± 0.00a |
| 20% | No cut | 195 ± 5.00d | 635 ± 5.00c | 4545 ± 255b | 215 ± 15.0d | 2120 ± 10.0b | 2690 ± 70.0e | 1110 ± 20.0c |
| | 15 cm | 205 ± 5.00cd | 850 ± 20.0b | 6110 ± 50.0a | 325 ± 15.0cd | 2565 ± 25.0ab | 4030 ± 70.0bcd | 1285 ± 5.00c |
| | 30 cm | 235 ± 5.00cd | 955 ± 15.0a | 6070 ± 10.0a | 230 ± 0.00d | 2960 ± 40.0a | 3815 ± 15.0cd | 1080 ± 10.0c |
| | 45 cm | 285 ± 45.0bcd | 785 ± 45.0b | 5865 ± 25.0a | 330 ± 100cd | 2735 ± 45.0ab | 3605 ± 335d | 1170 ± 90.0c |
| 40% | No cut | - | - | - | - | - | - | - |
| | 15 cm | - | - | - | - | - | - | - |
| | 30 cm | - | - | - | - | - | - | - |
| | 45 cm | - | - | - | - | - | - | - |
| | | | | Two-way ANOVA F-Statistics | | | | |
| Seawater | | 49.60* | 277.23* | 3217.57* | 42.37* | 0.59ns | 41.12* | 139.39* |
| Pruning | | 0.20ns | 22.46* | 21.00* | 3.36ns | 0.81ns | 4.70* | 2.00ns |
| Seawater*Pruning | | 5.12* | 9.66* | 19.82* | 6.37* | 1.83ns | 1.08ns | 1.98ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

### 6.4.2 Micronutrients

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) in all micronutrients of *T. decumbens* shoots (Table 6.4). For manganese (Mn) and iron (Fe), the highest mean values were obtained in the control treatment, while the lowest mean values were obtained in 40%DSW treatment. For copper (Cu) and zinc (Zn), the highest mean values were recorded in plants subjected to 20%DSW treatment, while the control treatment recorded the lowest values of these two nutrients.

**Pruning (Week 4):** Different pruning intervals showed a significant effect on the micronutrients of *T. decumbens* shoots. The application of 30 cm pruning intervals in both manganese and iron resulted in the highest mean values, while high diluted seawater (40%DSW) recorded the lowest mean values of these nutrients.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had a significant effect on the micronutrients of *T. decumbens* shoots except for copper (Table 6.4).

**Diluted seawater (Week 8):** Diluted seawater had no significant effect in the micronutrients except for copper (Table 6.5). Manganese recorded the highest mean values in the control treatment, while the least mean value was recorded in 20%DSW treatment. For copper the highest mean values were obtained in 20%DSW treatment and the lowest mean values were obtained in the control treatment. For iron and zinc, both the highest and lowest mean values were obtained in 20%DSW treatment.

**Pruning (Week 8):** Different pruning intervals showed a significant effect in the micronutrients of *T. decumbens* shoots. The application of 45 cm pruning intervals in the control resulted in high mean values of manganese. The 45 cm pruning intervals in 20%DSW treatment recorded the least mean values of manganese. For copper and zinc, the application of 30 cm pruning intervals recorded the highest mean values, while 45 cm pruning intervals recorded the least mean values. A different trend was observed for iron, where the application of 15 cm pruning intervals recorded the highest mean values and the least mean values were recorded in 45 cm pruning intervals.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and different pruning intervals had a significant impact on copper and zinc. However, for manganese and iron the interaction were not significant.

**Diluted seawater (Week 12):** Diluted seawater had no significance on the micronutrients of dune spinach expect zinc (Table 6.6). The plants subjected to 20%DSW produced high amounts of iron (3.85 mg/100g) and zinc (8.55 mg/100g). The least mean values were recorded in the control treatment for both iron (1.50 mg/100g) and zinc (2.95 mg/100g). For manganese and copper, plants showed no significant difference between the control treatment and 20%DSW.

**Pruning (Week 12):** Table 6.6 showed non-significant effect on all micronutrients in the shoots of *T. decumbens* except for iron. The application of 15 cm pruning intervals in 20%DSW recorded the highest mean value of iron, while 30 cm pruning intervals in the control treatment resulted in the lowest mean value. Manganese and copper showed no significant different between the control and 20%DSW treatment. For zinc, the application of the following different pruning intervals (15 cm, 30 cm, and 45 cm) in 20%DSW recorded the highest amount, while the following pruning intervals (15 cm, 30 cm, and 45 cm) in the control resulted in lowest mean values of zinc.

**Diluted seawater interaction with pruning:** The interaction of diluted seawater and different pruning intervals showed a significant effect on manganese and zinc. However, on copper and iron non-significant effects were observed during week 12.

### 6.4.3 Proximate composition

**Diluted seawater (Week 4):** Diluted seawater had a significant effect ($p \leq 0.05$) in all proximate content of *T. decumbens* shoots (Table 6.7). The plants cultivated under 20%DSW resulted in high ash content and crude protein, while the control treatment resulted in low ash content. Crude protein resulted in the lowest mean value in plants subjected to 40%DSW treatment. For crude fat, the highest mean value was recorded in the control. The lowest mean value was recorded in 20%DSW and was similar to the control with 45 cm pruning intervals. For ADF and NDF, the highest mean values were obtained in the control and 40%DSW, while their lowest mean values were obtained in 20%DSW. Moisture content was found highest in 20%DSW and 40%DSW, while the lowest moisture was found in the control treatment.

**Pruning (Week 4):** The application of different pruning intervals showed a significant effect in all proximate content in the shoots of *T. decumbens*. The plants subjected to 45 cm pruning intervals recorded in 20%DSW recorded the highest ash content and crude protein. The lowest ash was recorded in 15 cm pruning intervals in the control, while the following pruning intervals (15 cm, 30 cm, and 45 cm) in 40%DSW recorded the lowest crude protein. The application of 15 cm pruning intervals in the control resulted in the highest crude fat, ADF, and NDF contents.

The lowest ADF and NDF contents were recorded in 45 cm pruning intervals in 20%DSW treatment. For crude fat, the lowest mean values were recorded in 30 cm pruning intervals in 20%DSW and 45 cm pruning intervals in the control. The highest moisture content was recorded in the following pruning intervals (15 cm and 30 cm) in both 20%DSW and 40%DSW, while the lowest mean values were recorded in 30 cm pruning intervals in the control.

**Diluted seawater interaction with pruning (Week 4):** The interaction of diluted seawater and different pruning intervals had a significant effect in all proximate composition of *T. decumbens* shoots except for crude fat and moisture.

**Diluted seawater (Week 8):** Diluted seawater showed a significant increase on the ash content, ADF, and NDF of *T. decumbens* shoots (6.8). However, non-significant effect was obtained in crude fat, crude protein, and moisture. The plants subjected to 20%DSW recorded the highest mean values of ash (27.64%). The lowest mean values of ash were recorded in the control treatment (12.61%). Crude fat, crude protein, and moisture showed no significant difference between the control and 20%DSW treatment. The plants cultivated under the control resulted in the highest ADF (37.19%) and NDF (55.82%) contents, while their lowest mean values were found in 20%DSW.

**Pruning (Week 8):** Table 6.8 showed a significant effect in all proximate composition of *T. decumbens* shoots except for crude fat. The application of 15 cm pruning intervals in 20%DSW resulted in the highest ash content, while 45 cm pruning intervals in the control resulted in the lowest ash content. No significant difference was obtained for crude fat, crude protein, and moisture in week 8. The plants subjected to 45 cm pruning intervals in the control recorded the highest mean value of ADF and NDF.

**Diluted seawater interaction with pruning (Week 8):** The interaction of diluted seawater and pruning had a significant effect on the ash content of *T. decumbens* shoots. However, there was no significant effect for other proximate compositions.

**Diluted seawater (Week 12):** Diluted seawater had a significant increase on the ash content, ADF, and NDF of *T. decumbens* shoots (Table 6.9). However, no significant effect was observed for crude fat, crude protein, and moisture. The plants cultivated under 20%DSW recorded the highest mean values of ash content (30.10%), while the control treatment recorded the lowest mean values (15.19%). Crude protein and moisture showed no significant difference between the control and 20%DSW treatment. The plants subjected to the control treatment resulted in the highest mean value of crude fat, ADF, and NDF, while their lowest mean values were found in 20%DSW treatment.

**Pruning (Week 12):** Table 6.9 shows that different pruning intervals had no significant impact on crude fat, crude protein, ADF, and moisture of *T. decumbens* shoots. However, ash and NDF showed a significant effect on the shoots of dune spinach. The plants subjected to the following pruning intervals (15 cm, 30 cm, and 45 cm) resulted in high ash content in 20%DSW treatment. The lowest mean values were found in plants subjected to 30 cm and 45 cm pruning intervals in the control treatment. The plants without pruning intervals in the control recorded the highest ADF and NDF mean values, while their lowest mean values were found in 45 cm pruning intervals in 20%DSW. For crude fat, 30 cm pruning intervals in the control resulted in the highest mean value, while plants without pruning in 20%DSW resulted in the lowest mean values.

**Diluted seawater interaction with pruning (Week 12):** The interaction of diluted seawater and pruning had no significant effect in the proximate composition of *T. decumbens* shoots. Only ash was significantly affected by the interaction of diluted seawater and different pruning intervals.

**Table 6.4: The effect of diluted seawater and different pruning intervals on micronutrients of *T. decumbens* shoots (Week 4).**

| Seawater | Pruning level | Mn (mg/100g DW) | Cu (mg/100g DW) | Fe (mg/100g DW) | Zn (mg/100g DW) |
|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA |
| | 15 cm | 13.00 ± 0.00a | 0.20 ± 0.00d | 11.75 ± 0.05abc | 8.05 ± 0.05d |
| | 30 cm | 13.15 ± 0.05a | 0.35 ± 0.15cd | 14.80 ± 4.40a | 11.75 ± 3.05bc |
| | 45 cm | 13.45 ± 0.05a | 0.35 ± 0.05cd | 8.80 ± 0.60bcd | 11.30 ± 0.00c |
| 20% | No cut | NA | NA | NA | NA |
| | 15 cm | 9.10 ± 0.10b | 0.40 ± 0.00bc | 9.60 ± 0.00bcd | 14.25 ± 0.45b |
| | 30 cm | 8.65 ± 0.15b | 0.60 ± 0.10a | 7.90 ± 0.00cd | 17.10 ± 0.00a |
| | 45 cm | 8.20 ± 0.20b | 0.55 ± 0.05ab | 11.95 ± 0.65ab | 14.10 ± 0.30b |
| 40% | No cut | NA | NA | NA | NA |
| | 15 cm | 6.30 ± 0.20c | 0.25 ± 0.05cd | 6.20 ± 0.00d | 12.50 ± 0.10bc |
| | 30 cm | 5.75 ± 1.75c | 0.30 ± 0.00cd | 6.85 ± 0.15d | 12.75 ± 0.50bc |
| | 45 cm | 5.85 ± 0.05c | 0.30 ± 0.00cd | 6.90 ± 0.70d | 13.15 ± 0.35bc |
| Two-way ANOVA F-Statistics | | | | | |
| Seawater | | 113.44* | 11.44* | 8.77* | 15.87* |
| Pruning | | 243.35* | 33.42* | 38.80* | 154.03* |
| Seawater*Pruning | | 12.93* | 1.60ns | 3.47* | 3.07* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 6.5: The effect of diluted seawater and different pruning intervals on micronutrients of *T. decumbens* shoots (Week 8).**

| Seawater | Pruning level | Mn (mg/100g DW) | Cu (mg/100g DW) | Fe (mg/100g DW) | Zn (mg/100g DW) |
|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA |
| | 15 cm | 7.50 ± 2.70ab | 0.15 ± 0.05cd | 17.8 ± 12.1ab | 11.00 ± 2.50ab |
| | 30 cm | 6.60 ± 3.60ab | 0.15 ± 0.05cd | 11.20 ± 4.40abc | 10.30 ± 2.00b |
| | 45 cm | 10.45 ± 0.05a | 0.10 ± 0.00d | 6.85 ± 0.05bc | 8.95 ± 0.05bc |
| 20% | No cut | NA | NA | NA | NA |
| | 15 cm | 6.80 ± 0.10ab | 0.35 ± 0.50b | 23.55 ± 0.25a | 12.45 ± 0.35ab |
| | 30 cm | 5.35 ± 0.35ab | 0.50 ± 0.00a | 10.90 ± 1.50abc | 14.40 ± 0.80a |
| | 45 cm | 2.60 ± 0.00bc | 0.20 ± 0.00c | 2.60 ± 0.50c | 5.20 ± 0.10c |
| 40% | No cut | - | - | - | - |
| | 15 cm | - | - | - | - |
| | 30 cm | - | - | - | - |
| | 45 cm | - | - | - | - |
| | | Two-way ANOVA F-Statistics | | | |
| Seawater | | 4.71ns | 56.33* | 0.01ns | 0.29ns |
| Pruning | | 8.60* | 42.11* | 7.63* | 47.15* |
| Seawater*Pruning | | 2.59ns | 11.89* | 0.41ns | 3.89* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 6.6: The effect of diluted seawater and different pruning intervals on micronutrients of *T. decumbens* shoots (Week 12).**

| Seawater | Pruning level | Mn (mg/100g DW) | Cu (mg/100g DW) | Fe (mg/100g DW) | Zn (mg/100g DW) |
|---|---|---|---|---|---|
| Control | No cut | 5.20 ± 0.00a | 0.10 ± 0.00a | 2.20 ± 0.00bc | 7.10 ± 0.20ab |
| | 15 cm | 3.80 ± 1.70ab | 0.10 ± 0.00a | 3.75 ± 0.85ab | 2.95 ± 0.45c |
| | 30 cm | 2.35 ± 1.05ab | 0.10 ± 0.00a | 1.50 ± 0.20c | 3.80 ± 0.00c |
| | 45 cm | 1.10 ± 0.20b | 0.10 ± 0.00a | 3.20 ± 0.20ab | 3.60 ± 0.20c |
| 20% | No cut | 2.80 ± 0.20ab | 0.15 ± 0.05a | 3.45 ± 0.85ab | 4.30 ± 0.00bc |
| | 15 cm | 4.05 ± 0.25a | 0.35 ± 0.05a | 3.85 ± 0.05a | 7.30 ± 0.50a |
| | 30 cm | 3.85 ± 0.05ab | 0.25 ± 0.05a | 2.60 ± 0.40abc | 7.50 ± 0.10a |
| | 45 cm | 4.95 ± 1.45a | 1.65 ± 1.35a | 3.60 ± 0.60ab | 8.55 ± 2.45a |
| 40% | No cut | - | - | - | - |
| | 15 cm | - | - | - | - |
| | 30 cm | - | - | - | - |
| | 45 cm | - | - | - | - |
| Two-way ANOVA F-Statistics | | | | | |
| Seawater | | 1.64ns | 2.19ns | 3.97ns | 15.90* |
| Pruning | | 0.70ns | 1.09ns | 4.51* | 0.37ns |
| Seawater*Pruning | | 4.35* | 1.09ns | 0.59ns | 7.93* |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA =  Not analysed, (-) = Plant death.

**Table 6.7: The effect of diluted seawater and different pruning intervals on proximate content of *T. decumbens* shoots (Week 4).**

| Seawater | Pruning level | % Ash | % Crude Fat | % Crude Protein | % ADF | % NDF | % Moisture |
|---|---|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA | NA | NA |
| | 15 cm | 18.99 ± 0.33c | 2.31 ± 0.03a | 19.81 ± 0.03e | 32.56 ± 0.07a | 51.22 ± 0.14a | 4.61 ± 0.65bc |
| | 30 cm | 22.73 ± 2.94b | 2.07 ± 0.13bc | 23.23 ± 1.04c | 27.78 ± 1.73b | 44.70 ± 2.99b | 4.00 ± 0.02c |
| | 45 cm | 19.66 ± 0.14c | 1.95 ± 0.03c | 25.45 ± 0.28b | 26.80 ± 0.00b | 44.18 ± 0.42b | 5.31 ± 0.92abc |
| 20% | No cut | NA | NA | NA | NA | NA | NA |
| | 15 cm | 26.81 ± 0.18a | 2.10 ± 0.16abc | 20.10 ± 0.03e | 24.18 ± 0.02c | 39.00 ± 0.05c | 6.22 ± 0.13a |
| | 30 cm | 27.19 ± 0.56a | 1.95 ± 0.08c | 21.57 ± 0.18d | 24.02 ± 0.46c | 38.47 ± 0.66c | 6.33 ± 0.17a |
| | 45 cm | 28.82 ± 0.39a | 2.07 ± 0.04bc | 29.36 ± 0.10a | 18.73 ± 0.52d | 32.47 ± 0.17d | 5.66 ± 1.04ab |
| 40% | No cut | NA | NA | NA | NA | NA | NA |
| | 15 cm | 22.59 ± 0.27b | 2.07 ± 0.05bc | 11.47 ± 0.05f | 31.60 ± 0.00a | 49.20 ± 0.35a | 6.30 ± 0.05a |
| | 30 cm | 23.93 ± 0.18b | 2.11 ± 0.02abc | 11.55 ± 0.07f | 30.83 ± 0.42a | 48.86 ± 0.50a | 6.51 ± 0.55a |
| | 45 cm | 23.95 ± 0.30b | 2.18 ± 0.04ab | 11.62 ± 0.02f | 32.16 ± 1.76a | 48.94 ± 0.58a | 5.72 ± 0.30ab |
| | | | | Two-way ANOVA F-Statistics | | | |
| Seawater | | 36.70* | 0.93ns | 1023.44* | 91.48* | 113.10* | 7.15* |
| Pruning | | 542.10* | 704.09* | 2914.36* | 1033.18* | 1712.58* | 103.03* |
| Seawater*Pruning | | 5.37* | 2.35ns | 157.56* | 16.17* | 17.46* | 1.81ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death

**Table 6.8: The effect of diluted seawater and different pruning intervals on proximate content of *T. decumbens* shoots (Week 8).**

| Seawater | Pruning level | % Ash | % Crude Fat | % Crude Protein | % ADF | % NDF | % Moisture |
|---|---|---|---|---|---|---|---|
| Control | No cut | NA | NA | NA | NA | NA | NA |
| | 15 cm | 15.31 ± 3.36b | 2.33 ± 0.41a | 16.09 ± 4.85a | 30.98 ± 7.24ab | 49.57 ± 7.39ab | 4.90 ± 0.87a |
| | 30 cm | 15.10 ± 2.85b | 2.45 ± 0.38a | 18.38 ± 7.19a | 29.17 ± 8.36ab | 46.45 ± 8.05abc | 5.80 ± 0.44a |
| | 45 cm | 12.61 ± 0.07b | 2.19 ± 0.07a | 11.46 ± 0.12a | 37.19 ± 0.13a | 55.82 ± 0.45a | 5.27 ± 0.50a |
| 20% | No cut | NA | NA | NA | NA | NA | NA |
| | 15 cm | 27.64 ± 0.19a | 2.42 ± 0.01a | 17.40 ± 0.01a | 19.21 ± 0.18b | 34.81 ± 1.02c | 6.04 ± 1.46a |
| | 30 cm | 25.41 ± 0.56a | 1.91 ± 0.63a | 19.10 ± 0.33a | 18.99 ± 0.85b | 34.19 ± 1.37c | 7.03 ± 1.86a |
| | 45 cm | 25.48 ± 0.16a | 2.25 ± 0.08a | 20.91 ± 0.38a | 19.49 ± 0.00b | 37.96 ± 0.19bc | 7.44 ± 1.45a |
| 40% | No cut | - | - | - | - | - | - |
| | 15 cm | - | - | - | - | - | - |
| | 30 cm | - | - | - | - | - | - |
| | 45 cm | - | - | - | - | - | - |
| | | | | Two-way ANOVA F-Statistics | | | |
| Seawater | | 63.70* | 1.00ns | 1.75ns | 12.77* | 16.44* | 2.33ns |
| Pruning | | 83.74* | 1.00ns | 16.00* | 22.11* | 61.70* | 16.93* |
| Seawater*Pruning | | 7.32* | 1.00ns | 1.04ns | 1.76ns | 2.00ns | 0.36ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

**Table 6.9: The effect of diluted seawater and different pruning intervals on proximate content of *T. decumbens* shoots (Week 12).**

| Seawater | Pruning level | % Ash | % Crude Fat | % Crude Protein | % ADF | % NDF | % Moisture |
|---|---|---|---|---|---|---|---|
| Control | No cut | 16.75 ± 0.24cd | 1.70 ± 0.11bc | 17.47 ± 0.33ab | 34.30 ± 0.12a | 53.45 ± 1.00a | 7.24 ± 0.00a |
| | 15 cm | 18.63 ± 1.33c | 2.46 ± 0.12abc | 17.51 ± 3.08ab | 32.53 ± 4.42ab | 50.40 ± 4.30ab | 4.69 ± 0.36b |
| | 30 cm | 16.09 ± 0.59d | 3.12 ± 0.36a | 16.44 ± 2.24ab | 28.74± 4.16abc | 48.77 ± 2.85ab | 6.02 ± 0.31ab |
| | 45 cm | 15.19 ± 0.54d | 2.82 ± 0.07ab | 16.54 ± 1.13ab | 27.25 ± 1.32abcd | 48.38 ± 0.35ab | 6.33 ± 0.90ab |
| 20% | No cut | 22.64 ± 0.03b | 1.46 ± 0.99c | 13.27 ± 0.07b | 25.79 ± 1.22bcd | 44.76 ± 1.22b | 7.66 ± 0.10a |
| | 15 cm | 30.10 ± 0.55a | 2.32 ± 0.09abc | 16.03 ± 0.18ab | 22.34 ± 1.09cd | 36.06 ± 1.22c | 7.04 ± 0.82ab |
| | 30 cm | 28.76 ± 0.15a | 2.27 ± 0.02abc | 18.49 ± 0.27a | 21.06 ± 0.14cd | 34.28 ± 0.09c | 6.71 ± 1.09ab |
| | 45 cm | 29.87 ± 0.06a | 2.30 ± 0.30abc | 17.10 ± 0.28ab | 23.41 ± 0.80d | 37.78 ± 0.77c | 6.71 ± 1.14ab |
| 40% | No cut | - | - | - | - | - | - |
| | 15 cm | - | - | - | - | - | - |
| | 30 cm | - | - | - | - | - | - |
| | 45 cm | - | - | - | - | - | - |
| Two-way ANOVA F-Statistics | | | | | | | |
| Seawater | | 715.00* | 2.52ns | 0.59ns | 21.76* | 73.94* | 3.53ns |
| Pruning | | 21.22* | 3.35ns | 0.78ns | 2.12ns | 5.70* | 1.66ns |
| Seawater*Pruning | | 20.28* | 0.33ns | 1.83ns | 0.69ns | 1.05ns | 0.83ns |

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference. NA = Not analysed, (-) = Plant death.

## 6.5 Discussion

Drought and saltwater conditions had a significant impact on the nutritional values of commercial plants across the globe, with a noticeable impact in arid locations (Ali *et al*., 2022). This has caused an expanding global quest for the examination of the dietary and nutraceutical values of halophytes to combat malnutrition and improve food security in countries impacted by drought and salinity (Alexopoulos *et al*., 2023). Halophytes have been demonstrated to have essential beneficial minerals with numerous phytochemical compounds crucial for human consumption (Duarte *et al*., 2022). These halophytes perform an imperative function in worldwide nourishment and food security, and the consumption of beneficial wild edible vegetables can be explored to support the nutritional needs around the globe (Shahid *et al*., 2023; Beato *et al*., 2024). According to (Bulawa *et al*., 2022), these wild edible vegetables offer a variety of macronutrients, micronutrients, and proximate compounds.

An abundance of ash content indicates that the crop is high in dietary fibre, which shields digestive enzymes in the alimentary tract (Shahid *et al*., 2023). Ash contains many vital nutritive ingredients, particularly minerals, micronutrients, and macronutrients that are essential for the regular physiological processes of the body (Bulawa *et al*., 2022; Shahid *et al*., 2023). According to (Shahid *et al*., 2023), ash is made up of inorganic material from plants containing oxides and salts, such as anions like phosphates, sulphates, and chlorides, and other cations and halides like calcium, sodium, potassium, magnesium, manganese, and iron. This study found that the maximum levels of ash content on hydroponically grown *T. decumbens* shoots were found in week 4 under 20%SW + 15 cm pruning interval (Table 6.7). The ash composition of *T. decumben*s shoots varied between 12.61% and 30.10% across all treatments, which is higher than the 5% recorded in different wild edible vegetables and is consistent with the composition found in processed foods (Muñoz-Arrieta *et al*., 2021; Liu *et al*., 2022). These findings are consistent with the study of (Bulawa *et al*., 2022) on *Trachyandra divaricata* flower buds where potassium concentrations without pruning and with 15 cm pruning levels had the highest ash content. A similar trend was observed by (Ranjbar *et al*., 2021) on the ash content of *Salicornia bigelovii* (pickleweed) where increasing salinity had ascending and significant values. Similar findings were further observed by (Mndi *et al*., 2023) on *Mesembryanthemum crystallinum* where ash content increased with increasing salinity and drought conditions.

Moisture content refers to the quantity of water present in a material (Ievinsh, 2023). An elevated moisture content indicates that water-soluble enzymes may be more active in the plant (Stadlmayr *et al*., 2011). The amount of moisture is primarily determined by the plants temperature, humidity, and harvest time. The increased amount of moisture in plants from less humid and arid environments may be related to their stronger water retention capacity since they are xeric, and xerophytes retain water and have sunken stomata to minimise water transpiration (Shahid *et al*., 2023). In the current research, the highest amount of moisture on hydroponically cultivated *T. decumbens* shoots was discovered in week 4 under 40%SW + 30 cm pruning intervals. The moisture contents of *T. decumbens* shoots varied between 4.00% and 7.66% across all treatments, indicating that leaves of this plant may have lesser microbial contamination and chemical degradation, which are often linked with high levels of moisture (Mndi *et al*., 2023). Such decreased values indicate that *T. decumbens* leaves might possess a longer storage life, which benefits the growers and sellers.

The highest protein composition was obtained in (20%SW) with 45 cm pruning intervals. Availability of large amount of protein found in wild edible vegetables means that they are more nutritious than other conventionally consumed vegetables (Shahid *et al*., 2023). The protein content found in hydroponically grown shoots of dune spinach varied between 11.47% and 29.36 % in this study. Consuming food that contain around 12% of their caloric intake from proteins represent excellent sources of protein. This implies that dune spinach can play an essential function in helping people in rural regions get affordable and easily accessible proteins. These findings corroborate with similar studies to the studies on *Tetragonia decumbens*, *Mesembryanthemum crystallinum* L. and *Trachyandra divaricata* (Bulawa *et al*., 2022; Mndi *et al*., 2023; Tshayingwe *et al*., 2023)

According to (Jimoh *et al*., 2020), wild vegetable crops like *Amaranthus caudatus* L., *Solanum nigrum*, *Ipomoea plebeia*, *Ipomoea wightii*, *Limeum sulcatum*, and *Pyrenacantha kaurabassana* have been discovered to have small amounts of unsaturated oils, which vary from 1.9 to 4.8%. The fat composition of *T. decumbens* shoots ranged between 1.46% to 3.12%, and these results were consistent with those of (Bulawa *et al*., 2022; Mndi *et al*., 2023; Tshayingwe *et al*., 2023). Vegetable fats supply energy, fatty acids, and vitamins that enhance their edible qualities by retaining taste (Sarker & Oba, 2020; Kumar *et al*., 2022).

Iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential minerals for the diet of humans (Punchay *et al*., 2020; Mndi *et al*., 2023). Small amounts of trace elements are needed in not more than 20 mg per day and constitute less than 0.01% of the human weight (Tshayingwe *et al*., 2023; Islam *et al*., 2023). The small amounts of elements assessed in *T. decumbens* shoots meet the daily nutritional require as reported by (Bulawa *et al*., 2022). At weeks 4 and 12, dune spinach recorded the highest iron (Fe) content in 20%SW + 15 cm and 45 cm pruning intervals, and ranged from 1.50 to 17.8 mg/100 g. This was significantly less than the amounts recorded for other wild edible crops that are consumed in South Africa, such as *Lecaniodiscus cupaniodes* (27.8 mg/100 g), *Sterculia tragacantha* (803.7 mg/100 g), and *Ipomoea plebeian* (Ntuli, 2019; Mbatha *et al*., 2021). Fe is an important micromineral present in diets such as meat, lentils, and green vegetables (Farag *et al*., 2021; Bulawa *et al*., 2022). Iron intake also plays an essential function in the prevention of illnesses during pregnancy (Farag *et al*., 2021).

The amount of manganese (Mn) found in *T. decumbens* shoots varied from 1.10 to 13.45 mg/100 g, which was less than the amount found in *Moring oleifera* (252 mg/100 g). (Ghanbarzadeh *et al*., 2022). Manganese serves as an antioxidant and has a role in reproduction of cells, functioning of the immune system, blood sugar management, digestion, and the development of bones (Punchay *et al*., 2020; Ghanbarzadeh *et al*., 2022; Islam *et al*., 2023). Zinc (Zn) were measured in large quantities in *T. decumbens* shoots when compared with earlier investigations of wild edible vegetables (Sritalahareuthai *et al*., 2020; Alam *et al*., 2020; Punchay *et al*., 2020). Plants cultivated in 20%SW + 15 cm and 45 cm pruning intervals produced high amounts of zinc (8.55 mg/100g). The maximum amount of zinc in the shoots of dune spinach ranged from 2.95 mg/100 g to 7.10 mg/100 g. A high proportion of zinc consumed in accordance with the recommended daily allowance (RDA) may reduce the chance of developing chronic illnesses such as impotence, delayed sexual maturation, growth retardation, and skin and eye disorders (Farag *et al*., 2021; Islam *et al*., 2023). Also, zinc regulates the synthesis of cytokines and antibodies and impacts the functional ability of innate and adaptive immune system cells, all of which are important for immunological homeostasis (Weyh *et a*l., 2022; Gasmi *et al*., 2023).

At weeks 4 and 8, dune spinach growth with 20%SW + 30 cm pruning intervals produced the highest amount of copper. Copper (Cu) as an essential nutrient that is necessary for the survival of humans (Farag et al., 2021). *T. decumbens* shoots were also discovered to have significant levels of copper (Cu), and varied between 0.10 mg/100 g and 1.65 mg/100 g. Copper further plays a vital role in several biochemical processes that sustain life, including but not limited to iron homeostasis, peptide hormone processing, mitochondrial respiration, connective tissue formation, antioxidant defence, and melanin production (Farag *et al*., 2021; Islam *et al*., 2023).

Minerals such as nitrogen (N), sodium (Na), phosphorus (P), magnesium (Mg), calcium (Ca), potassium (K), and K/Ca+Mg were examined in this chapter. Magnesium (Mg) is widely recognised for preventing a variety of ailments, such as heart disease, and its insufficiency has also been connected to the aetiology of diabetes mellitus (Arshad *et al*., 2020; Mathew &

Panonnummal, 2021; Gasmi *et al*., 2023). Furthermore, it is required in the human system as an intracellular electrolyte and a co-factor for the production of various enzymes, proteins, and nucleic acids (Farag *et al*., 2021; Bulawa *et al*., 2022). At weeks 4, 8, and 12, magnesium contents were found to be greater in 20%SW with three different pruning intervals (15 cm, 30 cm, and 45 cm) when compared with other treatments, including the control. The maximum composition of magnesium in *T. decumbens* shoots ranged between 330 mg/100 g to 955 mg/100 g, which is more than the USDA's recommendation of 55 mg/100 g of cooked meals. These findings are consistent with the outcomes of (Bulawa *et al*., 2022), who discovered that this component was above the RDA value in *T. divaricata* tested samples. As a result, using dune spinach will aid in the treatment of a variety of ailments.

The use of diluted seawater and different pruning intervals considerably increased the potassium (K) content in *T. decumbens* shoots. At weeks 4, 8, and 12, The control with the following pruning intervals (unpruned, 15 cm, 30 cm, and 45 cm) produced the maximum potassium content and ranged from 2690 mg/100 g to 5470 mg/100 g. Potassium is a vital element for a balanced diet. It is particularly essential physiologically as it triggers intracellular and extracellular cations necessary for blood pressure regulation, muscle contractility, and nerve impulse transmission (Farag *et al*., 2023). The WHO recommends a daily intake of 3510 mg of potassium to support a nutritional diet (Drewnowski *et al*., 2015). A study by Jimoh *et al*. (2020) suggested that adults should consume at least 2000 mg of potassium, which is in line with the amount reported in *T. decumbens* shoots in this study. Sodium (Na) is the most abundant cation in extracellular fluid. It is essential for maintaining acid-base balance and acts as a precursor for nerve impulse transmission (Jobin *et al*., 2021). Sodium is a mineral essential for the body of humans, with an RDA of 3371 mg for adults (Drewnowski *et al*., 2015). At weeks 4, 8, and 12, dune spinach cultivated in 20%SW with the following pruning intervals (15 cm, 30 cm, and 45 cm) produced the maximum composition of sodium and varied between 605 mg/100 g and 6110 mg/100 g.

Phosphorus is an essential element of nucleic acids and cell membranes (Serna & Bergwitz, 2020; Bird & Eskin, 2021). It plays an imperative role in the movement and generation of energy in the form of ATP, in regulating the pH level, as well as increasing bone minerals and triggering various pathways of metabolism such as glycolysis and gluconeogenesis (Ballestín *et al*., 2021). Approximately 750 mg of this element is needed by adults, and more during the rapid growth of children (Serna & Bergwitz, 2020; Bird & Eskin, 2021). In the present study, the maximum phosphorus content surpassed 760 mg/100 g. At weeks 4 and 8, dune spinach cultivated in 20%SW + 15 cm and 30 cm pruning intervals ranged between 215 760 mg/100 g and 960 760 mg/100 g. Nevertheless, this study quantified *T. decumbens* to be regarded as a phosphorus supplement since diluted seawater and different pruning intervals had a significant effect on the plant responses. A comparable analysis was conducted by (Bulawa *et al*., 2022), who found a greater concentration of phosphorus (P) in *Trachyandra divaricata*.

Nitrogen (N) components were greater in 20%SW + 30 cm and 45 cm pruning intervals when compared to all treatments, including the control (Tables 6.1 and 6.3). Crops use nitrogen (N) to promote leaf development and green colour, as well as to sustain their metabolic processes at low tissue water potential, thereby helping to ease drought stress in cereal crops (Nawaz *et al*., 2020). Nitrogen is also important in meals because it helps the body synthesise amino acids (Shahid *et al*., 2023). The highest amount of nitrogen varied from 1825 mg/100 g and 4695 mg/100 g). The calcium composition shows to be significantly influenced by diluted seawater and different pruning intervals (Tables 6.1, 6.2, and 6.3). At weeks 4, 8, and 12, the control with 15 cm, 30 cm, and 45 cm pruning intervals produced the maximum amount of calcium. The highest calcium content in this study ranged between 180 mg/100 g and 710 mg/100 g. These results agrees with the findings of (Tshayingwe *et al*., 2023) on *Trachyandra divaricata*. Calcium (Ca) is reported to protect membrane structure and function under salt stress (Farzana *et al*., 2023). It inhibits osteopenia and osteoporosis brought on by certain chemotherapy medications and is essential for the development and maintenance of both muscles and bones (Cope, 2022; Afuape *et al*., 2022; Costa *et al*., 2024).

## 6.6 Conclusion and recommendations

The study revealed that 20%SW with or without pruning nearly optimized all minerals, micronutrients macronutrients of dune spinach than other seawater dilution treatments. Proximate compositions such as ash, crude protein, and moisture are found to be high in dune spinach. This implies that dune spinach can play an essential function in helping people in rural regions get affordable and easily accessible food that is rich in proteins. The high content of essential minerals and nutrients, coupled with their adaptability to salinity, makes them a promising resource for addressing global nutritional needs. Therefore, cultivation of *T. decumbens* with 20% seawater dilution could provide significant nutritional benefits needed for a balanced diet and may combat malnutrition and enhance food security.

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## 6.8 Reference

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# CHAPTER SEVEN

# GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 7.1 General discussion

Global agricultural production needs to increase by 70–100% by 2050 in order to meet the food demands of approximately seven billion people worldwide (Loconsole *et al*., 2019). However, climate change, increasing soil salinity, and emerging freshwater scarcity have severe impacts on agricultural production in many different countries, notably South Africa (Sogoni *et al*., 2021). This reduction has encouraged more study on underutilised wild edible vegetables to enhance daily meals (Ngxabi *et al*., 2021). The present research demonstrated that growing wild edible vegetables such as *T. decumbens* can be effective in improving the availability of food as these wild vegetables tolerate adverse conditions like as salinity while providing nutritional contents, needed secondary metabolites, and antioxidant properties.

**In chapter 2** it was concluded that *Tetragonia decumbens* Mill., commonly called dune spinach, is a promising but underrated halophyte with enormous potential for culinary and commercial uses. This species has been reported to be grown in dry and coastal areas due to its exceptional resistance to salt and drought. Harnessing the full potential of the species requires a well-designed cultivation technique that takes into account its distinct requirements for managing nutrients, water, and soil. Because of its unique characteristics, Dune spinach can survive in harsh conditions because to its physiological and biochemical processes, which include osmotic adjustment, ROS-detoxification, and succulence. It is an important resource for food security because of these processes, which also increase its nutritional content and resistance. Beyond its usage in food preparation, *T. decumbens* has a variety of other uses, including as possible involvement in soil bioremediation and animal feed. However, in order to create efficient culture techniques and determine the market potential, further study is necessary as commercial cultivation is still mainly unknown.

**In chapter 3** the effect of diluted seawater (DSW) and different pruning intervals on the chlorophyll content in *T. decumbens* shoots was conducted. The results indicate that low levels of diluted seawater (20%SW) and different pruning intervals (15 cm and 45 cm) significantly increased the chlorophyll values on the shoots of *T. decumbens* when compared with the control on week 4 and 12. These findings suggest that 20%SW with 15 cm or 45 cm pruning could be the optimal protocol for chlorophyl yield of *T. decumbens*. These findings are consistent with those of (Parvin *et al*., 2015) and (Sogoni *et al*., 2021), who reported a significant reduction in chlorophyll content at high levels of salinity and a rise at low to moderate salinity levels. Likewise, (Ngxabi *et al*., 2021) reported a similar trend, indicating a significant increase in chlorophyll content at low salinity levels in *Trachyandra ciliata*. (Bulawa *et al*., 2022), also reported similar findings on *Trachyandra divaricata* exposed to low salinity levels with 15 cm pruning interval, which resulted in enhanced chlorophyll content.

**In Chapter 4** the effect of diluted seawater (DSW) and different pruning intervals on the vegetative growth of *T. decumbens* shoots cultivated through hydroponics was investigated. The growth parameters such as shoot length, fresh weight and dry weight of shoots, and total dry and wet weight of shoots and roots were significantly affected by diluted seawater concentrations and different pruning intervals The results indicated that low levels of diluted seawater (20%SW) and different pruning intervals (no cut, 30 cm, and 45 cm) enhanced the production of *T. decumbens* and high levels of diluted seawater (40%SW) reduced the production and resulted leaf wilting and plant death. Similar results have been reported by (Azeem *et al*., 2023; Sharavdorj *et al*., 2024) and (Ngxabi *et al*., 2021), where low salinity levels increased vegetative growth of halophytes, while high salinity levels reduced growth and caused leaf wilting and resulted in plant death. Likewise, (Pungin *et al*., 2023) reported similar findings on *Cakile maritima*, *Arthrocnemum macrostachyum*, and *Sarcocornia fruticosa*, where low to moderate salinity levels increased shoot biomass, leaf expansion, and shoot and root weight in both fresh and dried. A similar trend was observed by (Ghanem *et al*., 2021; Gill *et al*., 2024), and (Sogoni *et al*., 2021) where high salinity levels significantly reduced halophyte development. In addition, pruning stimulates new growth, blooming, fruiting, and high-quality yields in flowering and crop plants (Mawarni & Siahaan, 2022; Bora *et al*., 2024; Peng *et al*., 2024). However, in this current study, no cut, 30 cm, and 45 cm pruning intervals had the highest values in all growth parameters. A similar trend was observed by (Zhong *et al*., 2024) on *Ginkgo biloba*, where pruning showed increased yield and quality of crops.

**In chapter 5**, the effect of diluted seawater (DSW) and different pruning intervals on the phytochemical and antioxidant activity of *T. decumbens* shoots grown hydroponically was conducted. The phytochemical and antioxidant capability of T. decumbens shoots was shown to be enhanced by low concentrations of diluted saltwater (20%SW) and varying pruning intervals (15 cm and 30 cm), according to the study's findings. *T. decumbens* gathered the highest concentrations of polyphenols, flavonols, FRAP, and DPPH in this study. These outcomes are supported by the findings of (Bulawa *et al*., 2022), who applied low potassium concentrations to *Trachyandra divaricata* to promote phytochemical accumulation. When it came to polyphenols, low to moderate salinity concentrations had a favourable impact, whereas high salinity concentrations exhibited lower mean values. This trend was also noted by (Ngxabi *et al*., 2021) in their study on *Trachyandra ciliata*. The number of phenolic compounds was efficiently increased by low salinity stress, as further found by (Pungin *et al*., 2023) on *Glaux maritima* in a similar pattern. In contrast, the control and high diluted seawater (40%SW) had high mean values for DPPH and ABTS.

**In chapter 6**, the investigation on the effect of diluted seawater (DSW) and different pruning intervals on nutritional content of *T. decumbens* shoots grown hydroponically was evaluated. The results indicated that low levels of diluted seawater (20%SW) and different pruning intervals (15 cm and 30 cm) significantly enhanced the nutritional content in the shoots of *T. decumbens*. The results of this study revealed that the ash composition of *T. decumben*s shoots have shown positive results of 12.61% and 30.10% across all treatments, which is higher than the 5% recorded in different wild vegetables and is consistent with the composition found in processed foods (Muñoz-Arrieta *et al*., 2021; Liu *et al*., 2022). These results are consistent with findings of (Bulawa *et al*., 2022) on *Trachyandra divaricata*, where the ash content reported was between 13.3% to 23.6% in all treatments. High ash value indicates that the plant is high in dietary fibres, which provide shelter for digestive organisms in the gastrointestinal tract (Bulawa *et al*., 2022; Shahid *et al*., 2023).

The moisture content on hydroponically cultivated *T. decumbens* shoots was discovered in week 12 under 20%SW without pruning. The moisture content of *T. decumbens* shoots varied between 4.00% and 7.66% across all treatments, indicating that leaves of this plant may have lesser microbial contamination and chemical degradation, which are often linked with high levels of moisture (Mndi *et al*., 2023). Such decreased values indicate that *T. decumbens* leaves might possess a longer storage life, which benefits the growers and sellers. These results are similar with that of (Bulawa *et al*., 2022) on flower buds of *Trachyandra divaricata*, where the moisture content ranged between 9.9% to 12.1% in all treatments

The highest fat content in dune spinach shoots was found in week 4 under 20%SW + 45 cm pruning intervals. The fat composition of *T. decumbens* shoots ranged between 1.46% to 3.12%, and these results were consistent with those of (Bulawa *et al*., 2022; Mndi *et al*., 2023; Tshayingwe *et al*., 2023). Similar findings were also observed by (Jimoh *et al*., 2020) on wild vegetable crops like *Amaranthus caudatus* L., *Solanum nigrum*, *Ipomoea plebeia*, *Ipomoea wightii*, *Limeum sulcatum*, and *Pyrenacantha kaurabassana*, where they have small amounts of unsaturated oils, which vary from 1.9 to 4.8%. Vegetable fats supply energy, fatty acids, and vitamins that enhance their edible qualities by retaining taste (Sarker & Oba, 2020; Kumar *et al*., 2022). Diluted seawater (20%SW) + 30 cm and 45 cm pruning intervals had the highest protein composition in this study and varied between 11.47% and 29.36 %. These were similar to those of (Bulawa *et al*., 2022; Mndi *et al*., 2023; Tshayingwe *et al*., 2023) on *Tetragonia decumbens*, *Mesembryanthemum crystallinum* L. and *Trachyandra divaricata*. The protein content of this plant was comparable with that of *Moringa oleifera* leaves (Sultana, 2020).

Iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential minerals for the diet of humans (Punchay *et al*., 2020; Bulawa *et al*., 2022; Mndi *et al*., 2023). These small amounts of trace elements are needed in not more than 20 mg per day and constitute less than 0.01% of the human weight (Tshayingwe *et al*., 2023; Islam *et al*., 2023). Diluted seawater (20%SW) + 15 cm pruning interval significantly affected the iron content in dune spinach shoots. The iron content ranged between 1.50 to 17.8 mg/100 g. This is significantly less than the amounts recorded for other wild edible crops that are consumed in South Africa, such as *Lecaniodiscus cupaniodes* (27.8 mg/100 g), *Sterculia tragacantha* (803.7 mg/100 g), and *Ipomoea plebeian* (Ntuli, 2019; Mbatha *et al*., 2021). The results were also similar to the ones of (Bulawa *et al*., 2022) on flower buds of *Trachyandra divaricata*. The highest manganese content in dune spinach shoots was found in week 12 under 20%SW + 15 cm pruning intervals. The amount of manganese (Mn) found in *T. decumbens* shoots varied from 1.10 to 13.45 mg/100 g, which was less than the amount found in *Moring oleifera* (252 mg/100 g). (Ghanbarzadeh *et al*., 2022). The highest zinc and copper content in dune spinach shoots were found in weeks 4 and 8 under 20%SW + 30 cm pruning intervals. Zinc and copper were further found in large quantities in *T. decumbens* shoots when compared with earlier investigations of wild edible vegetables (Sritalahareuthai *et al*., 2020; Alam *et al*., 2020; Punchay *et al*., 2020). The maximum amount of zinc in the shoots of dune spinach ranged from 2.95 mg/100 g to 7.10 mg/100 g, and copper varied between 0.10 mg/100 g and 1.65 mg/100 g. Copper further plays a vital role in several biochemical processes that sustain life, including but not limited to iron homeostasis, peptide hormone processing, mitochondrial respiration, connective tissue formation, antioxidant defence, and melanin production (Farag *et al*., 2021; Islam *et al*., 2023).

Magnesium (Mg) is widely recognised for preventing a variety of ailments, such as heart disease, and its insufficiency has also been connected to the aetiology of diabetes mellitus (Arshad *et al*., 2020; Mathew & Panonnummal, 2021; Gasmi *et al*., 2023). Furthermore, it is required in the human system as an intracellular electrolyte and a co-factor for the production of various enzymes, proteins, and nucleic acids (Farag *et al*., 2021; Bulawa *et al*., 2022). Magnesium contents were found to be greater in 20%SW with three different pruning intervals (15 cm, 30 cm, and 45 cm) when compared with other treatments, including the control. The maximum composition of magnesium in *T. decumbens* shoots ranged between 330 mg/100 g to 955 mg/100 g, which is more than the USDA's recommendation of 55 mg/100 g of cooked meals. These findings are consistent with the outcomes of (Bulawa *et al*., 2022), who discovered that this component was above the RDA value in *T. divaricata* tested samples. As a result, using dune spinach will aid in the treatment of a variety of ailments.

The maximum potassium content ranged from 2690 mg/100 g to 5470 mg/100 g. Potassium is a vital element for a balanced diet. It is particularly essential physiologically as it triggers intracellular and extracellular cations necessary for blood pressure regulation, muscle contractility, and nerve impulse transmission (Farag *et al*., 2023). The WHO recommends a daily intake of 3510 mg of potassium to support a nutritional diet (Drewnowski *et al*., 2015). A study by Jimoh *et al*. (2020) suggested that adults should consume at least 2000 mg of potassium, which is in line with the amount reported in *T. decumbens* shoots in this study. Dune spinach growth with 20%SW with the following different pruning intervals (15 cm, 30 cm, and 45 cm) produced the highest amount of sodium in weeks 4,8, and 12. Sodium (Na) is the most abundant cation in extracellular fluid. It is essential for maintaining acid-base balance and acts as a precursor for nerve impulse transmission (Jobin *et al*., 2021). Sodium is a mineral essential for the body of humans, with an RDA of 3371 mg for adults (Drewnowski *et al*., 2015). In this study, the maximum composition of sodium varied between 605 mg/100 g and 6110 mg/100 g.

Phosphorus is an essential element of nucleic acids and cell membranes (Serna & Bergwitz, 2020; Bird & Eskin, 2021). It plays an imperative role in the movement and generation of energy in the form of ATP, in regulating the pH level, as well as increasing bone minerals and triggering various pathways of metabolism such as glycolysis and gluconeogenesis (Ballestín *et al*., 2021). Approximately 750 mg of this element is needed by adults, and more during the rapid growth of children (Serna & Bergwitz, 2020; Bird & Eskin, 2021). In the present study, the maximum phosphorus content surpassed 760 mg/100 g at the 20%SW + 15 cm pruning intervals and ranged between 215 760 mg/100 g and 960 760 mg/100 g. Nevertheless, this study quantified *T. decumbens* to be regarded as a phosphorus supplement since diluted seawater and different pruning intervals had a significant effect on the plant responses. A comparable analysis was conducted by (Bulawa *et al*., 2022), who found a greater concentration of phosphorus (P) in *Trachyandra divaricata*.

Nitrogen (N) components were greater in 20%SW + 30 cm and 45 cm pruning intervals when compared to all treatments, including the control. Crops use nitrogen (N) to promote leaf development and green colour, as well as to sustain their metabolic processes at low tissue water potential, thereby helping to ease drought stress in cereal crops (Nawaz *et al*., 2020). Nitrogen is also important in meals because it helps the body synthesise amino acids (Shahid *et al*., 2023). The highest amount of nitrogen varied from 1825 mg/100 g and 4695 mg/100 g). Calcium (Ca) is reported to protect membrane structure and function under salt stress (Farzana *et al*., 2023). It inhibits osteopenia and osteoporosis brought on by certain chemotherapy medications and is essential for the development and maintenance of both muscles and bones (Cope, 2022; Afuape *et al*., 2022; Costa *et al*., 2024). The highest calcium content in this study ranged between 180 mg/100 g and 710 mg/100 g. These results agrees with the findings of (Tshayingwe *et al*., 2023) on *Trachyandra divaricata*.

## 7.2 Conclusion

The results of this study indicate that *T. decumbens* plant growth, chlorophyll content, nutritional content, phytochemical content, and antioxidant capacity have all been positively impacted by diluted seawater (DSW) and varying pruning intervals. Among all treatments, including the control, low diluted seawater (20%SW) and pruned or unpruned intervals proved to be the most effective. On the other hand, dune spinach development was shown to be strongly influenced by the impacts of diluted seawater and different pruning intervals on plant growth, chlorophyll content, nutritional content, phytochemical content, and antioxidant capacity. These findings suggest that *T. decumbens* may be grown as a viable leafy green vegetable by using diluted seawater and different pruning intervals. When grown under salinity, edible wild vegetable plants like *T. decumbens* have the ability to be commercialised as an edible leafy green crop.

## 7.3 Recommendations

The study suggested that hydroponic cultivation of dune spinach using low levels of diluted seawater (20%SW) and different pruning intervals optimizes productivity, resulting in improved plant growth, chlorophyll content, nutritional content, phytochemical and antioxidant properties. This approach offers a sustainable solution for addressing salinization challenges and obtaining a valuable leafy vegetable. Overall, the dissertation aimed to contribute to sustainable agriculture and food security by exploring the potential of dune spinach as a valuable crop and providing insights into the cultivation practices that can optimize its growth and nutritional value. However, further research is needed on the nutritional content of *T. decumbens* to support its commercial viability as a wild edible vegetable.

# CHAPTER EIGHT

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## 8.2 APPENDICES