

Halophytes on Mars

The effect of using Mars Global Simulant (MGS-1) on the germination and development of the halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis*.



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Preface

Since the 1960s, the first space missions were executed, starting the exploration of space. By introducing long-duration space missions, we as humans will probably get an even better understanding of the solar system and beyond. To support these space missions, growing food in space will be a necessity, which can be researched with the use of artificial Martian soil.

This research report was written as part of my graduation as an Applied Biology student at HAS University of Applied Sciences Venlo and commissioned by Wageningen Environmental Research (WEnR), an Institute of Wageningen University and Research (WUR). Apart from Applied Biology, I have much interest in astronomy. Therefore, my goal was to find an internship in which I could combine both interests. After listening to the vision and ideas on how plants can be grown on Mars and support a bioregenerative life-support system with the help of agricultural ecosystems from my internship supervisor, Wieger Wamelink, I came up with a research question for my research. To answer this research question, a descriptive research strategy was used to experiment with growing plants on Martian soil simulant in a greenhouse. I researched and wrote the research report from February 2022 to June 2022.

During this research, my internship supervisor, Wieger Wamelink, and my supervisor from my college, Mark Smits, were always available for me to ask questions or ask feedback. Therefore, making it possible for me to complete my research and internship.

I would like to take this opportunity to thank my internship supervisor, Wieger Wamelink, for his excellent guidance and support during this process, as well as my supervisor from my college, Mark Smits, for his excellent support. I would also like to thank all the respondents who helped in this research. Without their assistance, I would have never been able to complete this research.

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Abstract

After the discovery of water ice and potential nutrients on Mars, there has been a surge of interest in growing plants on Mars. Experiments with plant growth on Martian soil simulants may reveal the effects of extraterrestrial soil on plant growth. As well as supporting crop production in space, leading to the realization of long-duration space missions on Mars. Long-duration space missions could lead to a better understanding of our solar system and beyond. In addition to providing food, crop production on Mars could benefit human presence by removing carbon dioxide, as well as emitting oxygen, or by being used in a water-purification system.

Due to the presence of salts in the Martian regolith, most plant species will likely not be able to grow. Halophytes are plant species tolerant to saline conditions, therefore possibly able to grow on the Martian regolith. Using a manufactured saline Martian soil, MGS-1, the germination percentage, and growth potential of three halophyte species were examined. Besides the germination percentage, the time it took for the seeds to germinate was observed. The same was carried out for the consecutive stages: hypocotyl visible, sprout leaves showing, and the vegetative stage. As for the growth potential, the shoot height, the number of branches, coloration, and biomass were observed.

The halophytes, *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* were sown on MGS-1 as well as sandy soil collected from a beach in the Netherlands. All three halophytes possess positive properties to support long-duration space missions, besides providing biomass for the space crew to eat. Both *Salsola soda* and *Cochlearia officinalis* showed not to be suitable as crops on Mars, due to the lack of germination on MGS-1. Although, they would be very applicable as crops on Mars, suggesting follow-up studies of altering the MGS-1 to support their germination and growth may be interesting. As for *Salicornia europaea*, showed no significant difference in germination percentage (67% on MGS-1 and 73% on sandy soil) ($p = 0.3615$). *S. europaea* expressed a lower growth rate on MGS-1 at the end of the experiment, especially for the shoot height ($p = 0.01105$) and fresh shoot biomass ($p = 0.002068$).

Aside from the lower growth rate of *Salicornia europaea* on MGS-1, this study showed that this crop can support long-duration space missions despite its lower growth rate. As humans have a reference daily intake of sodium to prevent health problems, *Salicornia europaea* should be analyzed for salt concentration after being grown on MGS-1.

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1 Introduction

Why are we as humans so interested in exploring our solar system and space in general? This is because comparative data is essential to completely understand an event or occurrence, the same applies to Earth's science (Glassmeier, 2020). That is where exploring our solar system and space plays an important role. According to The Planetary Society (2022) discoveries gathered from explorations of our solar system and space could assist Earth in many ways, from studying how growing food in orbit or on Mars will yield insights into growing food in extreme conditions on Earth, to new ways of understanding the human body under different gravitation, and the impacts of climate change. The creation of planetary outposts and habitats on the Moon and Mars will contribute to the advancement of solar system exploration (Schubert, 2017). Long-duration explorations of our solar system and space can be an option that is supported by using a bioregenerative life-support system (Dempster et al., 2004; Tong et al., 2011), wherein green plants take up a significant role (Salisbury, 1999). A bioregenerative life-support system is an example of an ISRU system, In-Situ Resource Utilization (Hall & Dunbar, 2020). Green plants will take on the roles including the extraction of carbon dioxide, and the addition of oxygen, and contribute to the water-purification system. Besides also providing food for space travelers and a familiar environment (Salisbury, 1999). Contributing to maintaining physical health for space travelers as well as better mental fitness. Therefore, plants could be cultivated on-site as an alternative to being flown in periodically, preferably using native water and soil (Wamelink et al., 2014, 2019).

In 2008, the search for habitability conditions on Mars was boosted. At that time the Phoenix lander performed a series of regolith and water chemistry experiments in the Martian north pole region. Water ice, potential nutrients, and compounds capable of supporting oxidation-reduction reactions were discovered (Boynton et al., 2009; Hecht et al., 2009; Sutter et al., 2012). Data from the Mars Odyssey Orbiter discovered water was present in the upper few meters of the Martian surface in middle and low latitudes (Feldman et al., 2003). In regolith samples from the Phoenix, high levels of perchlorate ions, ClO_4^- , were discovered (Cull et al., 2010). Both these discoveries indicated the presence of water on Mars. Perchlorate ions are highly water-soluble and poor at binding to soil minerals and organic carbon. Therefore, perchlorate ions are highly mobile in environments with surface water and groundwater (Brown and Gu, 2006). The presence of perchlorate ions on Mars could have resulted from their interaction with liquid water films during downward infiltration (Cull et al., 2010), indicating the presence of water on Mars. However, recent studies show that the formation of perchlorate can also be produced photochemically without aqueous conditions from the reaction between chloride and oxygen radicals obtained from SiO_2 and other metal oxides (Carrier and Kounaves, 2015). High concentrations of salt-forming elements besides perchlorate, like sulfates and chlorides, were found in the wind-blown fine particles from Mars that are easily released from rocks and mobilized by water (Clark and Van Hart, 1981; Malin, 1974). It has long been speculated that salts are present on Mars. Some justifications exist for assuming that all the following salts may be present: sulfates, chlorides, bromides, carbonates, and nitrates. Based upon measured geochemical limits and reactivity suggest a mineral assemblage dominated by MgSO_4 , Na_2SO_4 , NaCl , and $(\text{Mg,Ca})\text{CO}_3$ (Clark and Van Hart, 1981). Because liquid water is a requirement for life, an important issue is whether wetted regolith from Mars would provide a beneficial or toxic environment for Mars life (Nicholson et al., 2012).

Through the use of X-ray diffraction, the composition and physical properties of Martian regolith are significantly better understood compared to a decade ago. Therefore, synthetic planetary materials replicating one or more features from planetary regolith, also known as 'simulants', can be developed (Cannon et al., 2019). These features often include the geomechanical and compositional characteristics of rocks, regolith, or fine dust. For example, being used for plant growth experiments (Tack et al., 2021; Wamelink et al., 2014, 2019). The first Martian regolith simulant, JSC Mars-1(A), was developed using basaltic ash collected from Pu'u Nene cinder

cone. JSC Mars-1 has been used in investigations to explore the chemical and physical properties and the habitability of the Martian regolith (Chastain and Kral, 2010; Garry et al., 2006; Phebus et al., 2011). The Mojave Mars Simulant, MMS, was developed as one or more Martian regolith simulants because the material of JSC Mars-1 is highly weathering with hygroscopic properties (Peters et al., 2008; Ramkissoo et al., 2019). MMS was developed based on newly acquired physical and chemical data from Martian soils, similar to the igneous rocks on Mars (Peters et al., 2008). Recent studies indicated both JSC Mars-1A and MMS can support plant growth with nutritional supplements (Eichler et al., 2021). The Mars simulant JSC Mars-1A was used in a previous plant growth experiment on which the nutrient analysis was conducted, revealing traces of nitrates and ammonium. A small amount of reactive nitrogen was also found in the simulants (Wamelink et al., 2019), while previous studies were unsure whether reactive nitrogen is present in actual Mars regolith (Foley, 2003; Mancinelli and Banin, 2003). Martian regolith simulants are developed with high fidelity in mineral, chemical, volatile, and spectral properties compared to suitable reference material. For the Mars Global Simulant, MGS-1, the reference material used was the windblown soil Rocknest at Gale crater (Achilles et al., 2017; Archer et al., 2014; Bish et al., 2013; Blake et al., 2013; Leshin et al., 2013; Minitti et al., 2013; Sutter et al., 2017). The Mars Global Simulant, MGS-1, is saline due to the presence of salts, like (per)chlorate salts (Hecht et al., 2009; Sutter et al., 2017). The simulant also has a corresponding particle size as coarse sand with a bulk density of 1.29 g/cm³ (Cannon et al., 2019). These features could be improved by the addition of organic matter (Wamelink et al., 2014, 2019). Enriching the Martian simulant improves the water retention capacity of the soil (Lal, 2020), and increases the cation exchange capacity improving nutrient holding capacity (Meimaroglou & Mouzakis, 2019). Previous research showed MGS-1 not being able to support plant growth even with additional nutrients due to being highly alkaline with a pH above 9.0 (Eichler et al., 2021).

Soil salinity limits plant growth and development, as well as reduces crop yield (Van Zelm et al., 2020). Halophytes are plant species that can tolerate these salt concentrations and be able to survive in saline environments, like *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis*. The salt tolerance of halophytes relies on controlled uptake and compartmentalization of the ions Na⁺, K⁺ and Cl⁻ and the synthesis of organic 'compatible' solutes. However, not all halophytes show optimal growth in saline conditions (Flowers and Colmer, 2008; Riadh, et al., 2010). Some halophyte species grow better in the absence of salt or require exposure to salinity to be able to survive (Singh et al., 2014; Ramani, et al., 2006). Due to the mechanisms that halophytes are equipped with, such as osmotic adjustment, sodium compartmentalization and secretion, K⁺ retention, and reactive oxygen species (ROS) homeostasis (Hamed, 2022). Halophytes can tolerate osmotic, ion, and oxidative stresses. Germination of many halophytes occurs when the combination of the light regime (Gutterman, 1993), temperature regime (Badger and Ungar, 1989), soil water, and soil salinity (Khan et al., 2002; Ungar, 1995; Khan et al., 2002) are optimal (Naidoo and Naicker, 1992; Zia and Khan, 2004). Previous research reported the interaction between the environmental factors light, temperature, and soil moisture as controlling factors for the dormancy of seeds. While the interaction of extreme salinity and temperatures inhibits seed germination. In the absence of light, germination was even further inhibited (Baskin and Baskin, 2004; Benech-Arnold et al., 2000; Vleeshouwers et al., 1995). Various halophytes undergo a higher germination percentage in spring because of the reduction in salinity and diurnal fluctuations in temperature (Badger & Ungar, 1989). Furthermore, halophytes are adequate to restore saline and contaminated soils, which is interesting for the 21st-century farming system (Behera & Ramachandran, 2021). Halophytes also have numerous commercial applications and potential, besides being able to grow in saline conditions. Halophytes can be used as a source of oilseeds with a high nutritional value, food additives, secondary metabolites in pharmaceuticals, biofuel precursors, and nutraceuticals (Buhmann et al., 2015; Fan et al., 2013; Liu et al., 2005).

Marsh samphire (*Salicornia europaea*) is an annual succulent halophyte, with extremely reduced leaves and a spike-like inflorescence as well as a dense branching pattern up to a height of 35cm (Singh et al., 2014). The halophyte is dark green in color, turning yellowish-green and pink or red towards the end of its lifecycle (Davy et al., 2001). Later in their lifecycle, *S. europaea* plants can produce 1-3 tiny flowers for each inflorescence on the spikes (Devlin, 2015d; Singh et al., 2014). Whilst Marsh samphire can grow successfully in saline conditions, germination of their seeds is inhibited by high salt concentrations. Therefore, germination of the seeds of a Marsh samphire occurs over an extended period when soil salinity levels are the lowest. Likely during spring when high freshwater soil moisture content and relatively lower temperatures are occurring (Singh et al., 2014; Khan and Weber, 1986; Ungar et al., 1979). The optimal germination conditions of *Salicornia europaea* are at a temperature of 25°C during the day and 15°-20°C during the night with a light regime of 15-16h with light and 8-9h without light (Keiffer et al., 1994; Lv et al., 2012). Marsh samphire (*Salicornia europaea*) can be found on a wide range of the European coastline. It is also occasionally found in saline inland waters (Davy et al., 2001). They grow in intertidal habitats on a range of marine sediments, including silts, fine clays, gravels, and shelly sand. Habitats include sandy and/or muddy salt marshes, sand dunes, and the transitional area in between, as well as mudflats, sandflats, and open saline areas (National Parks and Wildlife Service, 2014; Davy et al., 2001; Jefferies et al., 1981). In the inland saline areas, the substrate may vary from fine clays to coarse sands (Davy et al., 2001).

Opposite-leaved saltwort or Monk's beard (*Salsola soda*) is a Mediterranean native annual succulent halophyte. In Italy, the plant buds are called 'agretti', and are edible and commonly consumed (Iannuzzi et al., 2020; Tundis et al., 2009). Monk's beard has fleshy green leaves growing on either green or red stems with a height up to 0.7m on average. They produce tiny flowers for each inflorescence developed out of the base of the leaves near the stem (Jepson, 1993; Tundis et al., 2009). Monk's beard is known for being difficult to germinate, with a germination potential of around 30% and a very short viability period for the seeds (Doughty, 2021; Grant, 2022; Taylor, 2020). *S. soda* is an endemic species in grasslands that are alkaline and typical for Central and Eastern Europe (Török et al., 2012). Monk's beard was traditionally used for the treatment of hypertension, constipation, and inflammation (Tundis et al., 2009). Recently, *S. soda* has been shown to possess the potential to be used as a phytostabilization in polluted areas, as it can accumulate moderate levels of trace metals (Milic et al., 2012; Lorestani et al., 2011). They can be used as an alternative crop to manage excessive solubility like Na, B, and Se in the soil (Centofanti and Banuelos, 2015). *S. soda* has been observed to increase the yields of peppers and tomatoes when cultivated together by accumulating Na⁺ and reducing sodium's impact on the peppers and tomatoes (Colla et al., 2006; Graifenberg et al., 2003).

Scurvy grass (*Cochlearia officinalis*) is a salt-tolerant short-lived perennial plant species (De Vos et al., 2013), which is mostly found in the coastal areas of Northwest Europe. *Cochlearia officinalis* grows primarily on brackish, sandy soils (Koningen and Heirman, 2014; Weeda et al., 1987; Van der Meijden, 2005). In the past, scurvy grass was used by mariners to prevent scurvy, a vitamin C deficiency disease, because of considerable levels of ascorbic acid and vitamin C present (Gustafson, 1954; Hughes, 1990; Maat, 2004). *C. officinalis* also contains glucosinolates (Griffiths, et al., 2001). *C. officinalis* has evergreen leaves with heart-shaped rosette leaves and a long stem, reaching a height up to 0.10-0.40 m while producing white four-petaled flowers (Damavandi, 2020; Van Moorsel, 2014). Previous research indicated *C. officinalis* as non-photoblastic, meaning light was not required for seed germination. While salinity was shown to negatively affect the germination of seeds, the higher the concentration of NaCl the less germination was observed (Pegtel, 1999). Furthermore, the research known on the germination and growth of Scurvy grass is still very little.

From a pilot experiment, it was concluded that the halophyte Marsh samphire (*Salicornia europaea*) can germinate and grow on a Martian soil simulant, MMS (Peters et al., 2008), in

saline conditions (Wamelink, personal communication, 2022). However, no research was conducted with halophytes on the Mars Global Simulant, MGS-1. Halophyte *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* are edible and thus can be used as a crop that is able to grow on saline soils (Guil et al., 1997; Iannuzzi et al., 2020; Tundis et al., 2009). Being able to grow halophytes on this newer Martian simulant will create a new field to research in the future to help certain aspects important for developing bioregenerative life-support systems for long-duration explorations of space. Therefore, the aim of this study was to determine whether the halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* can germinate on MGS-1 and exhibit similar growth to those grown on sandy soil. This resulted in the research question ‘What effect will the saline Martian soil simulant, MGS-1, have on the germination and development of halophytes: *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis*?’. A greenhouse experiment was conducted to answer the research question. Halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* were sown on the Mars Global Simulant (MGS-1), which contains a high concentration of salts, as well as on a saline sandy soil. The germination rate for the three halophytes, as well as the growth rate and development, were assessed. The expectations of this research were that the halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* should be able to germinate on the Mars Global Simulant (MGS-1), just as well as on the saline sandy soil. The overall growth, development, and final yield would not differ between both soils for the halophytes. The cultivated plants of halophyte *Salicornia europaea*, on the Mars Global Simulant (MGS-1), may display an abnormal appearance due to the presence of heavy metals or trace metals in the simulant. While this was not expected for the halophyte *Salsola soda* and *Cochlearia officinalis*.

1.1 Research report outline

Research statement: The halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* can germinate on the Mars Global Simulant (MGS-1), developed to mimic the Martian regolith, just as well as on the sandy soil collected from their natural habitat. As well as showing no significant difference in growth and development between the two soils.

In chapter 1 *Introduction*, the problem that brought about this research statement was introduced and defined as to why this is a problem that needs to be explored. The methodology used to test the research statement is outlined in chapter 2 *Methodology*. The outcome of the experiment is presented in chapter 3 *Results*, using graphs and charts for clarification. In chapter 4 *Discussion and Conclusion*, the results are compared to the existing literature, underpinning whether the results are reliable and able to answer the research statement. As well as the conclusion of this research with the answer to the research question.

2 Methodology

This chapter defines the research method used to conduct this research in determining whether halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* can germinate on the Mars Global Simulant (MGS-1). As well as the effect on the development of *Salicornia europaea* and *Cochlearia officinalis* during the first stages of their life cycle. The research was conducted starting February 7, 2022, to July 8, 2022. The greenhouse experiment extended over the period of March 7, 2022, until May 27, 2022, while lasting 82 days.

2.1 Greenhouse experiment

2.1.1 Greenhouse conditions

To determine whether the previously mentioned halophytes can germinate and grow into full-grown plants on the Mars Global Simulant (MGS-1), a greenhouse experiment has been carried out at Greenhouse Nergena, Wageningen University and Research. The greenhouse conditions were kept at the standard settings of the greenhouse, in which the day temperature is set at 20 degrees Celsius and the night temperature at 18 degrees Celsius. The light was on for 16 hours, making it dark for 8 hours. The deviations in the greenhouse conditions were determined over the period of the experiment in which the average temperature appeared to be 21.1 degrees Celsius with a standard error of ± 2.84 degrees Celsius. The air humidity appeared to be 55.5% with a standard error of $\pm 14.3\%$ (Figure 1). Appendix 1 shows the detailed graphs of the temperature and air humidity extended over the period of the experiment.

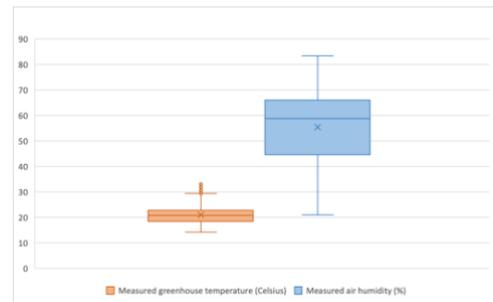


Figure 1. Boxplot illustrating the average temperature and air humidity observed extended over the period of the experiment alongside the standard error of the average. The orange box indicates the average temperature over a period of 82 days and the blue box indicates the average air humidity in the greenhouse over the same number of days.

2.1.2 Essential materials

All seeds used were purchased from a commercial retailer 'Vreeken's Zaden' (*Monniksbaard*, n.d.; *Lepelblad*, *Echt*, n.d.; *Zeekraal*, *Europese*, n.d.) in Dordrecht, The Netherlands. The Mars Global Simulant (MGS-1) purchased was developed by the University of Central Florida and sold by Exolith Lab (Exolith Lab, n.d). Sandy soil was collected from a beach on the coast of the province of Zuid-Holland (51.988700350021425, 4.104224363786213) in the Netherlands, where *Salicornia europaea* and *Cochlearia officinalis* are known to occur (NDFP, 2022). *Salsola soda* does not occur in the Netherlands but grows on sandy soils. Therefore, sandy soil was utilized as the control during this experiment.

2.1.3 Experiment set-up

The set-up of the experiment consisted of two separate sets of 20 pots for the halophytes *Salicornia europaea* and *Salsola soda*/*Cochlearia officinalis*. Both sets of 20 pots were arranged in a five-by-four block, completely randomized (Figure 2). Each set of 20 pots contained ten pots of MGS-1 (± 400 grams per pot) and ten pots of sandy soil (± 300 grams per pot), to which 10% potting soil of the total weight was added to both treatments (Table 1). To uphold the soil moisture, all pots were placed on separate plates to supply water to the soil. The saturation point of both soils with the addition of the organic soil was observed at ± 100 grams of water.

1 SE-S-7	5 SE-S-4	9 SE-M-9	13 SE-S-3	17 SE-S-5	21 SS-M-8	25 SS-S-2	29 SS-M-9	33 SS-M-4	37 SS-S-10
2 SE-M-6	6 SE-S-8	10 SE-M-7	14 SE-M-5	18 SE-M-4	22 SS-S-5	26 SS-M-6	30 SS-S-6	34 SS-S-9	38 SS-S-4
3 SE-M-2	7 SE-S-1	11 SE-S-9	15 SE-S-10	19 SE-M-10	23 SS-M-3	27 SS-S-7	31 SS-S-1	35 SS-M-10	39 SS-S-3
4 SE-S-2	8 SE-M-8	12 SE-M-3	16 SE-M-1	20 SE-S-6	24 SS-M-1	28 SS-S-8	32 SS-M-2	36 SS-M-7	40 SS-M-5

Figure 2. Set-up of the experiment in which the pots were placed in a randomized block design. The halophyte species, *Salicornia europaea* (SE) on the left and *Salsola soda* / *Cochlearia officinalis* (SS) on the right, were tested separately in a five-by-four block set-up. Both experiments implement the Martian Global Simulant (M) and saline sandy soil (S), containing ten replicas for each treatment (1-10).

Table 1. Overview of the different treatments used for the greenhouse experiment.

	Species	Number of seeds	Soil
Treatment 1 (10 replicas)	<i>Salicornia europaea</i>	100 seeds (10 replicas per pot)	Mars Global Simulant (MGS-1) (\pm 400 grams per pot + 10% potting soil)
Treatment 2 (10 replicas)	<i>Salicornia europaea</i>	100 seeds (10 replicas per pot)	Saline sandy soil (control) (\pm 300 grams per pot + 10% potting soil)
Treatment 3 (5 replicas)	<i>Salsola soda</i> / <i>Cochlearia officinalis</i>	50 seeds (5 replicas per pot)	Mars Global Simulant (MGS-1) (\pm 400 grams per pot + 10% potting soil)
Treatment 4 (5 replicas)	<i>Salsola soda</i> / <i>Cochlearia officinalis</i>	50 seeds (5 replicas per pot)	Saline sandy soil (control) (\pm 300 grams per pot + 10% potting soil)

For each pot, a total of ten or five seeds were sown on March 7th, 2022 and placed in a circle formation (Picture 1). A control tray with organic potting soil was included in the experiment containing seeds of *Salicornia europaea* and *Salsola soda*/*Cochlearia officinalis* as a viability control. During the germination stage of the halophytes, every workday the plates were monitored on the amount of water present and kept at a sufficient water level (1-2 cm depth). The pots received a spray of water from a plant watering spray in addition to the water in the plates. This mimics precipitation during the germination stage causing a slight reduction in the salt concentration, by creating a more optimal environment for the seeds to germinate. The spray of water was given from above at a height of around 10 cm from the soil. The nutrient solution was started administering to pots 1 to 20 on day 23 of the experiment and to pots 21 to 40 on day 58. The nutrient solution used was a nutrient solution for tomatoes grown in greenhouses (Appendix 2). When started administering the nutrient solution, spraying water with plant watering spray was concluded. A black cover of plastic was put onto the plates, with a cutout of the pot itself, to prevent the formation of algae or reduce the growth of possible fungi and/or bacteria in the water. Also preventing water loss through evaporation. Pots 1 to 20 received these covers on day 37 of the experiment, while pots 21 to 40 received these on day 44 of the experiment. On day 28 of the experiment, five new seeds per pot of the halophyte *Salsola soda* were sown in addition to the preliminary seeds of *Salsola soda* still present in the soil, on which a water test was performed to determine the viability of the seeds as well as undergoing cold treatment by placing them in a refrigerator on day 17 of the experiment. Five seeds of the halophyte *Cochlearia officinalis* were sown in the pots, which were prior used for *Salsola soda*, on day 43 of the experiment. All previously sown seeds were still present in the soil and served as organic material. The possibility of the sandy soil leaching out from beneath the pots into the plates with water, causing sinkholes in the soil, was resolved by filling up these holes with the sandy soil mixture saved from day 0.



Picture 1. Design of the circle formation used for sowing of the seeds (represented by colored spots) for this experiment.

2.2 Intermediate measurements

Starting March 8th, 2022 during the germination stage, data was gathered by observing the number of seeds/plants for the different stages every workday. The stages monitored were germination, the visibility of the hypocotyl, the first sprout leaves, and the vegetative stage. These observations ended on day 30 of the experiment for the halophyte *Salicornia europaea*, present in pots 1 to 20. Starting this date, all present plants per pot were identified by adding a piece of tape to the side of the pots with a number. Only a maximum of five seeds were observed per pot. From this point on, the shoot height and the number of branches were observed of the plants present in pots 1 to 20. The germination observations for pots 21 to 40 ended on day 77 of the experiment, which was 22 days after sowing the seeds of halophyte *Cochlearia officinalis*.

2.3 Harvest

On May 27th, 2022 all plants were harvested. The observations conducted at the time of harvest consisted of measuring the length of the shoot, the number of branches, and the coloration of the shoot for *Salicornia europaea*. As for *Cochlearia officinalis*, the measurements consisted of measuring the length of the shoot, leaf width, and the number of leaves. After harvesting, the above-ground biomass was collected by separating and cutting the plants above-ground. The below-ground biomass was collected after washing the roots. The fresh weight was determined for the above- and below-ground biomass. All biomass was dried in an oven at 70 °C for two days. After drying, the dry biomass weight was determined.

2.4 Statistical analysis

The data obtained during the experiment were analyzed in two stages. First, the germination data for each observed halophyte were sorted in Excel and fitted into a regression curve with a regression analysis in RStudio (RStudio Team, 2021). Furthermore, the survival data were plotted in a graph by plotting the fraction of the number of seeds/plants that survived against the time for both treatments (MGS-1 and sandy soil), conveying a clear overview. The following stages observed of the halophyte species: hypocotyl visible, sprout leaves, and vegetative stage were analyzed by collecting the timing of the first plant per pot to reach this stage. On which a t-test was performed on all three stages separately to determine if there was a significant difference between the two treatments (MGS-1 and sandy soil). Subsequently, for the obtained data during the intermediate measurements and harvest, multiple statistical analyses were conducted. This data included repeated measurements for shoot length and number of branches, as well as the coloration of the shoot, leaf width, number of leaves, and the fresh and dry weight from the above-ground biomass and below-ground biomass. A Shapiro-Wilk test and the boxplot method were performed to determine whether the sample data was normally distributed. If not, the data was transformed to fit the normal distribution. For the shoot height and the number of branches of *Salicornia europaea*, a repeated-measures ANOVA was conducted to compare the growth process between the two treatments, using RStudio. The remaining data were analyzed by performing multiple two-sample t-tests to test the links between the two treatments and the dependent variables for *Salicornia europaea*. The significance level was set at 5% ($\alpha = 0.05$). No interaction between the halophytes was tested.

3 Results

3.1 Germination of the halophytes

During the experiment, the germination potential of the halophytes *Salicornia europaea* and *Cochlearia officinalis* on the Mars Global Simulant, MGS-1 (SE-M), as well as on sandy soil (SE-S) were observed. The examined halophyte *Salsola soda* did not show any visible signs of germination for a period of 43 days, even after resowing new *Salsola soda* seeds that were subjected to colder temperatures to break dormancy. Therefore, no further results of *Salsola soda* are given.

3.1.1 Effect of the MGS-1 on the germination of *Salicornia europaea*

During 28 days of incubating, a total of 73 seeds germinated on sandy soil, and 67 seeds germinated on MGS-1 (Figure 3). After those 28 days, only 50 seeds remained viable on sandy soil and 60 seeds on MGS-1. Therefore, *Salicornia europaea* showed a germination potential of 73% on sandy soil with a survival potential of 50%. On MGS-1, the germination potential was lower than on sandy soil as it was 67% yet showing a higher survival potential of MGS-1 with 60% (Figure 4). Two days after sowing, seed germination on sandy soil was observed. On the third day, germination was observed on MGS-1. For the germination of *Salicornia europaea*, the null hypothesis was accepted with a p-value of 0.3615 ($p > 0.05$), proving there is no significant difference in the number of seeds germinated between the two treatments (MGS-1 and sandy soil). The raw data on the germination and survival rate of *Salicornia europaea* can be found in Appendix 3 and the raw statistical data of the regression analyses in Appendix 4.

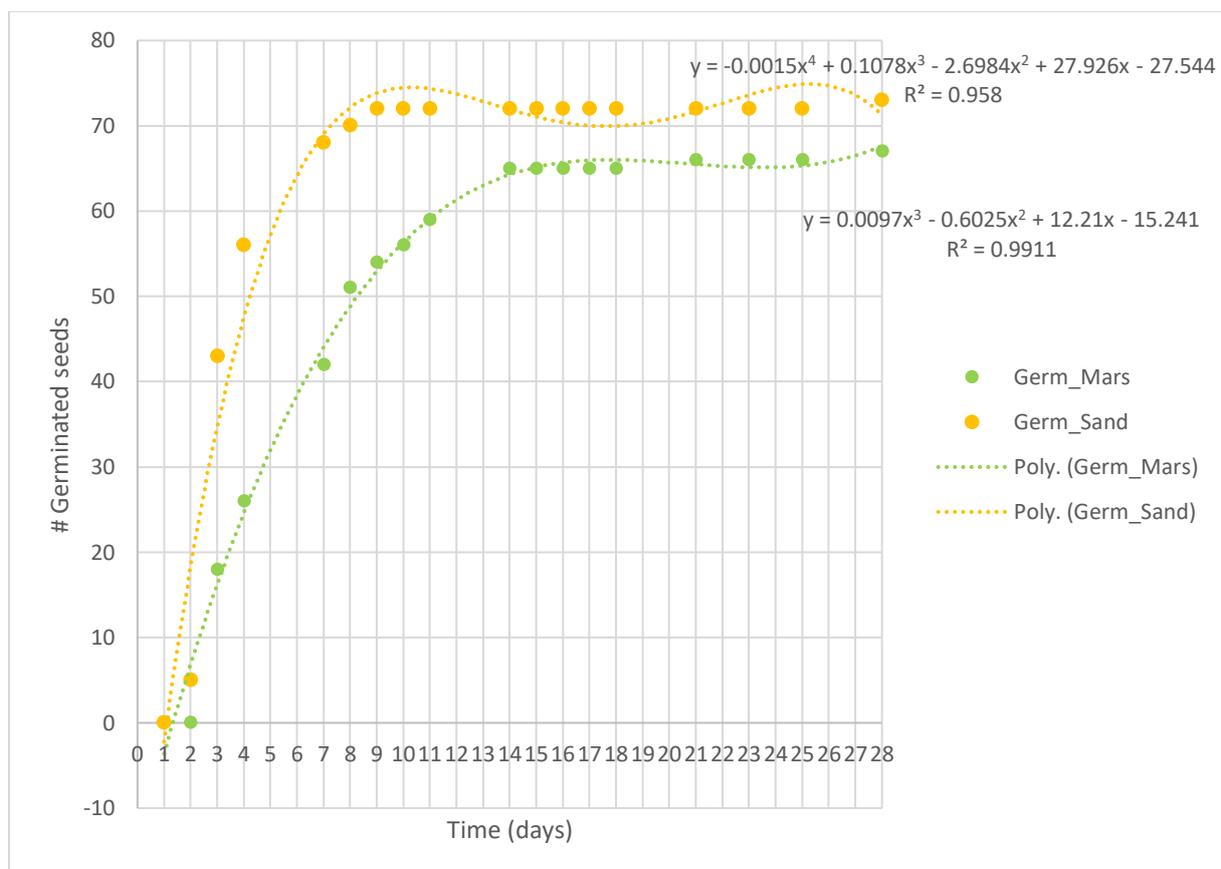


Figure 3. Scatter plot of the number of seeds germinated of *Salicornia europaea* with time as an independent variable, lasting 28 days. A logistic regression for the germination on sandy soil (Germ_Sand) indicated an R^2 -value of 0.958 (Poly.(Germ_Sand)) with a fourth-degree, and for the germination on MGS-1 (Germ_Mars) indicated an R^2 -value of 0.9911 (Poly.(Germ_Mars)) with a third degree.

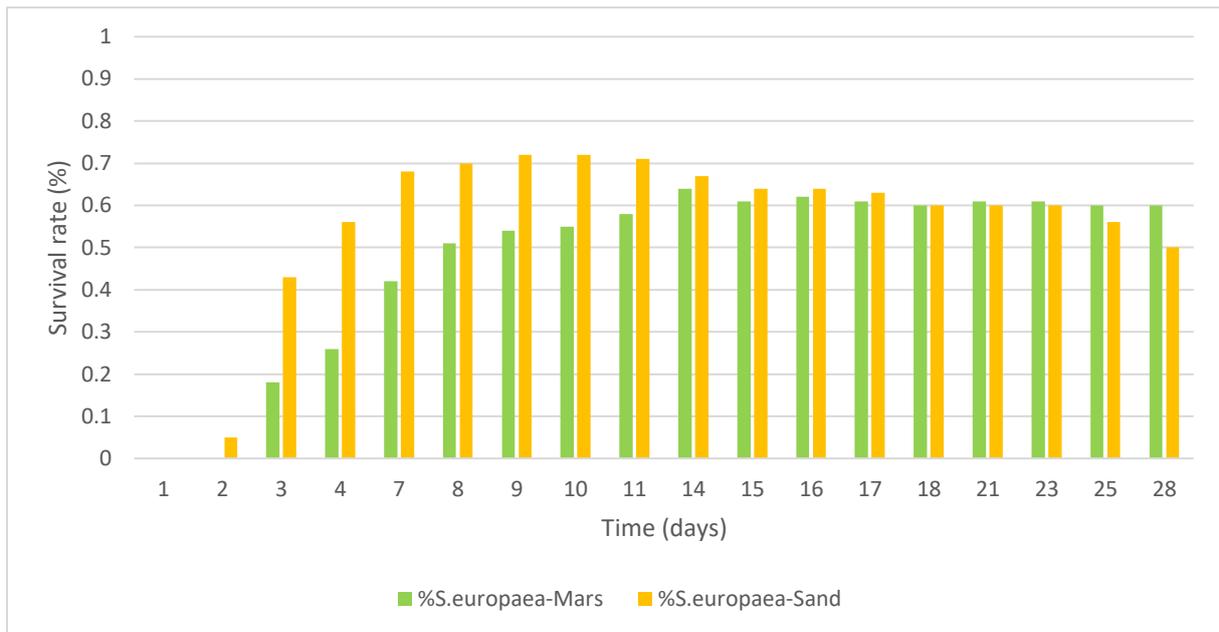


Figure 4. The survival observations over time as a fraction of the total seeds of the halophyte *Salicornia europaea*. The survival observations are the number of seeds showing viable growth signs at each observation. The y-values are the quotient of the survival observations and the total number of seeds sown at the beginning of the experiment. For the x-axis, only the observation days are included in the graph.

The three consecutive stages observed, after germination, showed no significant difference between the two treatments in time (Figure 5). The stage in which the hypocotyl is visible had a p-value of 0.08191, indicating that there is no significant difference between the plants on sandy soil and MGS-1. The same was seen for the stages in which the first sprout leaves were observed ($p = 0.5158$) and the vegetative stage was reached ($p = 0.07594$). The raw data of the consecutive stages of *Salicornia europaea* can be found in Appendix 5 as well as the raw statistical data of the multiple t-tests.

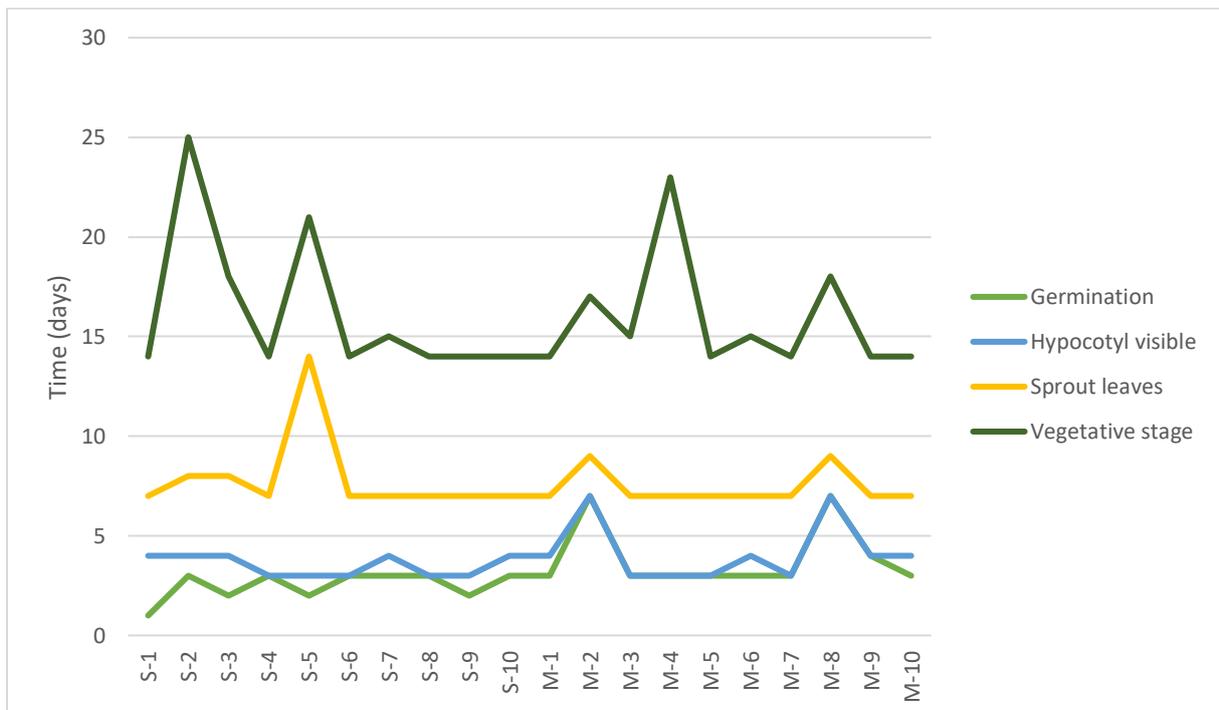


Figure 5. Graph illustrating the rate of reaching the different development stages of the plants between the two treatments for *Salicornia europaea*. The y-axis indicates the time the first seeds/plants of each pot (x-axis) were observed for the different stages of both treatments. S = sandy soil, M = MGS-1. The number 1-10 indicates the replicas for each treatment.

3.1.2 Effect of the MGS-1 on the germination of *Cochlearia officinalis*

For the duration of the experiment in which the seeds of *Cochlearia officinalis* were grown, the ones on the MGS-1 soil did not show any signs of germination except for four seeds. While nearly all seeds present in the sandy soil germinated (Figures 6 and 7). Therefore, the alternative hypothesis was accepted, rejecting the null hypothesis. This indicated there is a significant difference in germination between the two treatments with a p-value smaller than 0.05 ($p < 0.001$). The raw data on the germination and survival rate of *Cochlearia officinalis* can be found in Appendix 6 and the raw statistical data of the regression analyses in Appendix 7.

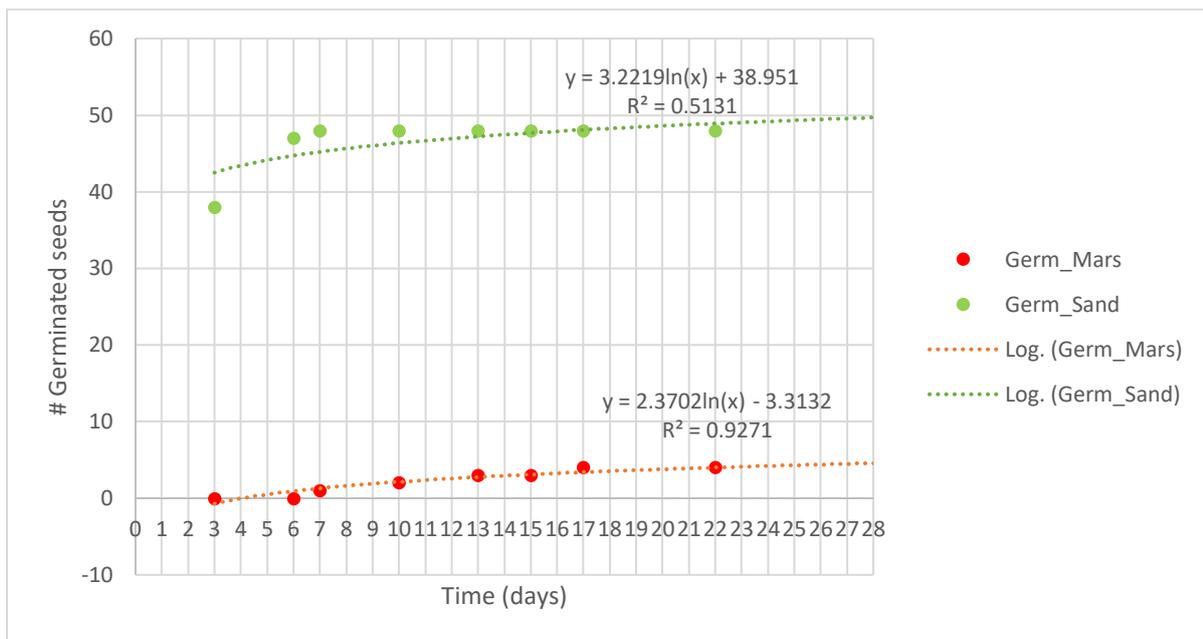


Figure 6. Scatter plot of the number of seeds germinated of *Cochlearia officinalis* against time, lasting 22 days. A logistic regression for the germination on sandy soil (Germ_Sand) indicated an R^2 -value of 0,5131 (Log.(Germ_Sand)), and for the germination on MGS-1 (Germ_Mars) indicated an R^2 -value of 0,9271 (Log.(Germ_Mars)).

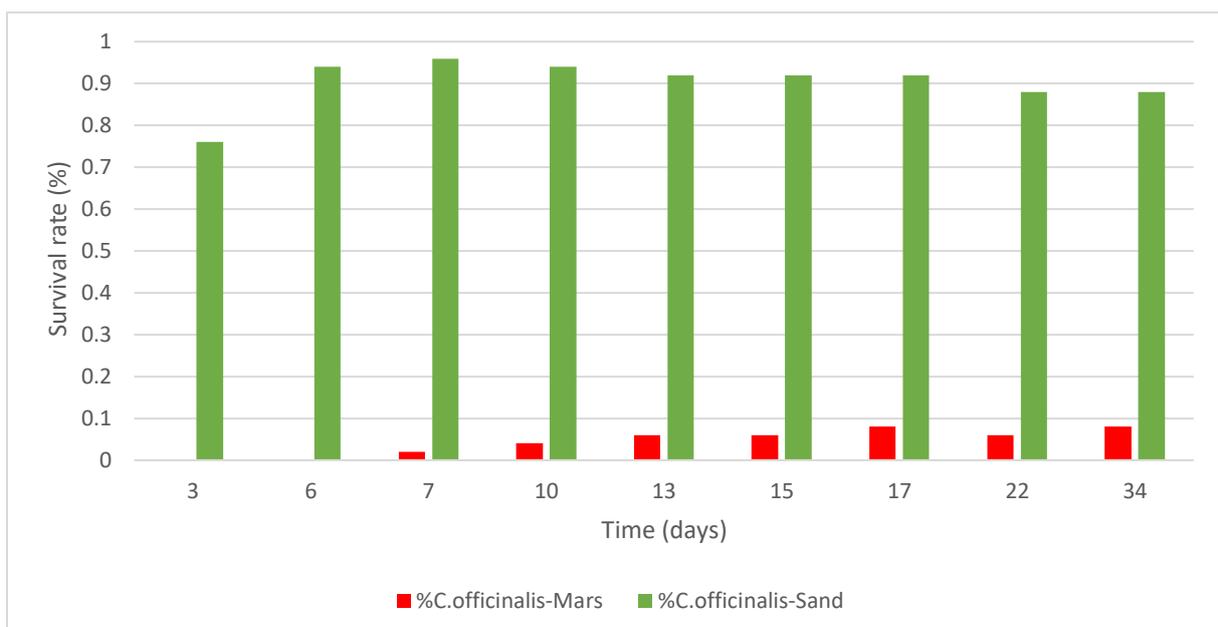


Figure 7. Graph illustrating the survival observations over time of halophyte *Cochlearia officinalis*. The survival observations are the number of seeds showing viable signs at each observation. The y-values are the quotient of the survival observations and the total number of seeds sown at the beginning of the experiment. For the x-axis, only the observed days were included in the graph.

3.2 Growth of the halophytes

3.2.1 Effect of the MGS-1 on the growth and development of *Salicornia europaea*

S. europaea was able to grow on both soils, judging by first appearances there seems to be no significant difference in growth between the two treatments. Both treatments contained plant growth corresponding with the expectations of the halophyte species, concurrent with a few outliers observed for both treatments. Almost all data showed to be not normally distributed after performing a Shapiro Wilk test and plotting the data within a 95% confidence interval. After transforming the data, normal distribution was still not reached. Therefore, parametric statistical analyses were applied knowing the data did not fit the assumption of normal distribution. The raw data of the growth observations of *Salicornia europaea* can be found in Appendix 8 and the raw statistical data for the Repeated Measures ANOVA in Appendix 9 as for the multiple performed t-tests in Appendix 10.

3.2.1.1 Shoot height

The shoot height of *Salicornia europaea* was recorded on six occasions. The plants grown on the sandy soil show faster growth in time compared to the plants grown on MGS-1, as indicated in Figure 8. The whiskers of all observations for both treatments reach around equal values, except the mean for the treatment on sandy soil (SE-S) lies higher than on MGS-1 (SE-M). According to a Repeated Measures ANOVA, the shoot height combined with the variable time indicates a significant difference between the two treatments ($p < 0.001$). In addition, the shoot height shows a significant difference between the two treatments at the time of harvest ($p = 0.01105$). Both treatments (MGS-1 and sandy soil) show outliers, in which the ratio between the two outliers is lesser for sandy soil (Pictures 2 and 3).

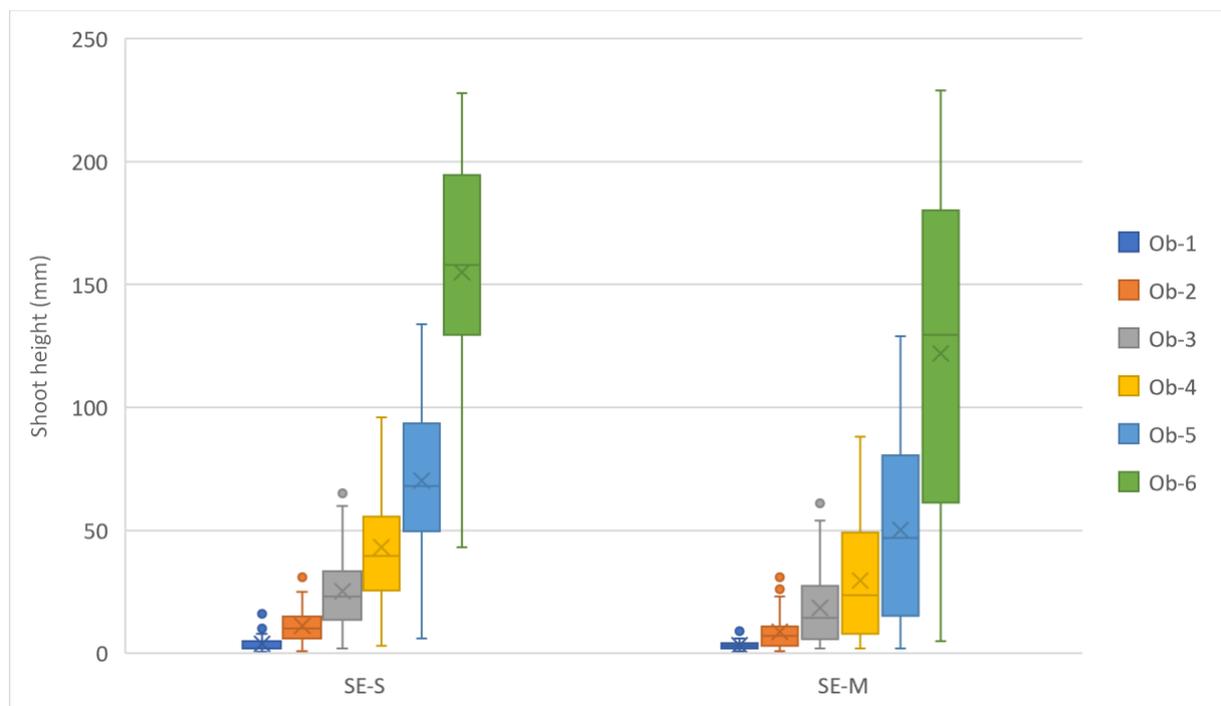
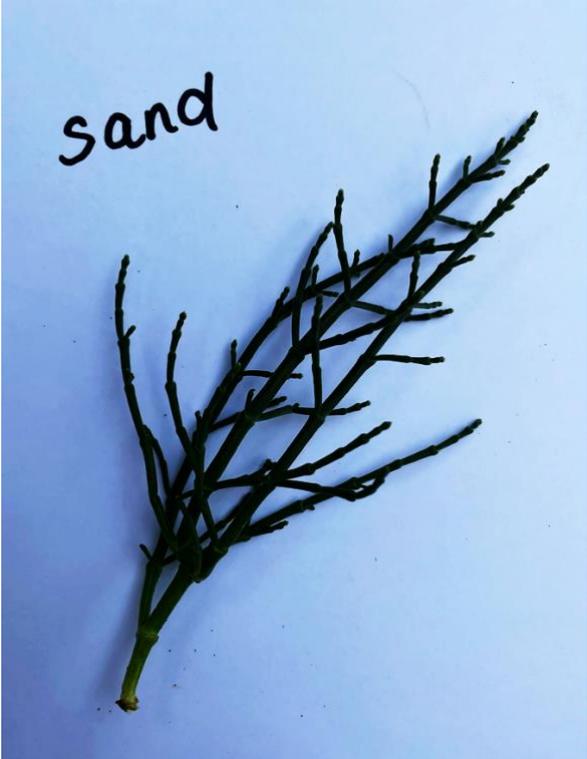
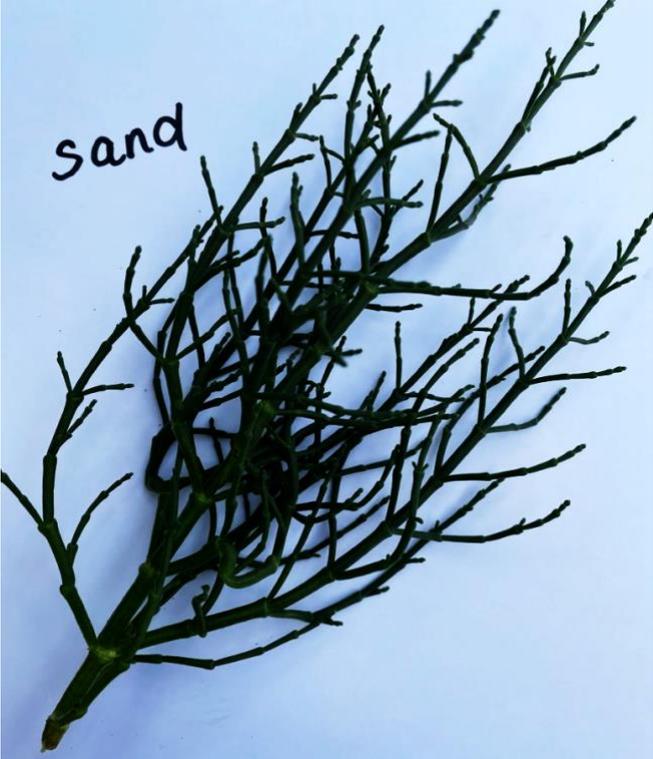


Figure 8. Boxplots illustrating the growth over a span of six observations (Ob) of the shoot between both treatments (SE-S = sandy soil and SE-M = MGS-1) for *Salicornia europaea*. The observations occurred on April 6, April 13, April 20, April 26, May 5, and May 26.

The shoot height between the two treatments shows a clear change over time (Figure 9). During the first observation, both treatments showed a similar mean in shoot height ($p = 0.1774$). From the second observation onwards, the shoot height between the two treatments showed significant differences. Although, as indicated in Figure 9, the outlier in all observations shows a relatively close corresponding shoot height. Across the observations, the box for the treatment on sandy soil (SE-S) remained relatively the same. As for the treatment on MGS-1

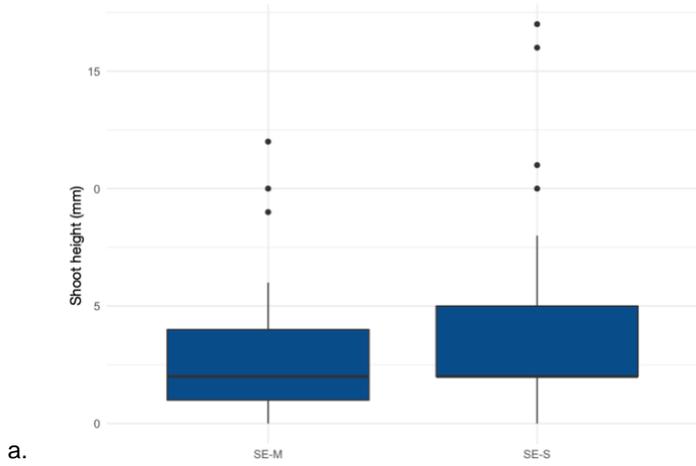
(SE-M), the box continued to expand in between the first and third quartile. For the sixth observation, the difference in the mean between the two treatments shows to have shrunken, compared to the observations before.



Pictures 2. Salicornia europaea cultivated on sandy soil at harvest from two different pots.

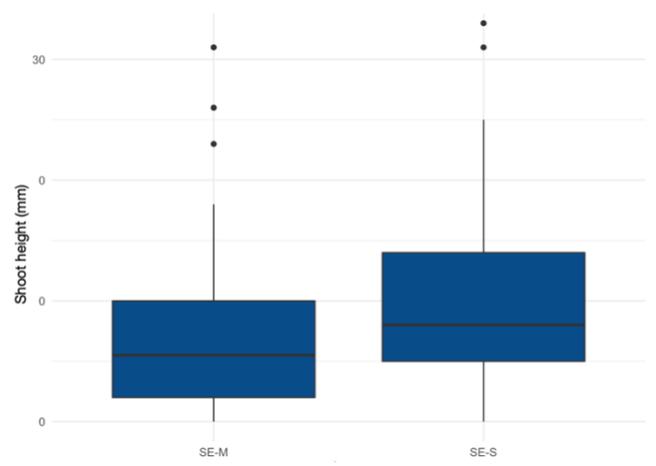


Pictures 3. Salicornia europaea cultivated on MGS-1 at harvest from two different pots.



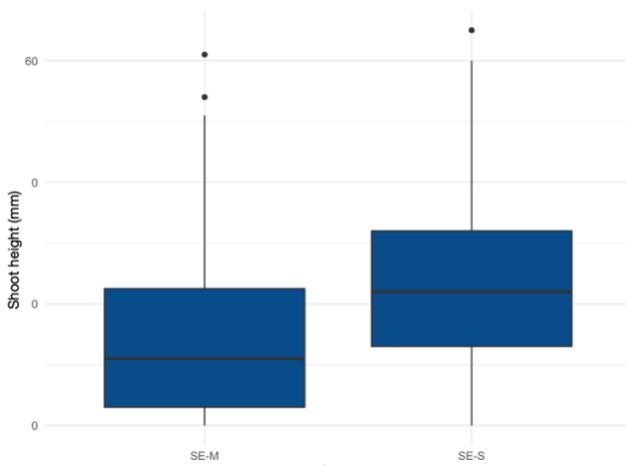
a.

$p = 0.1774$



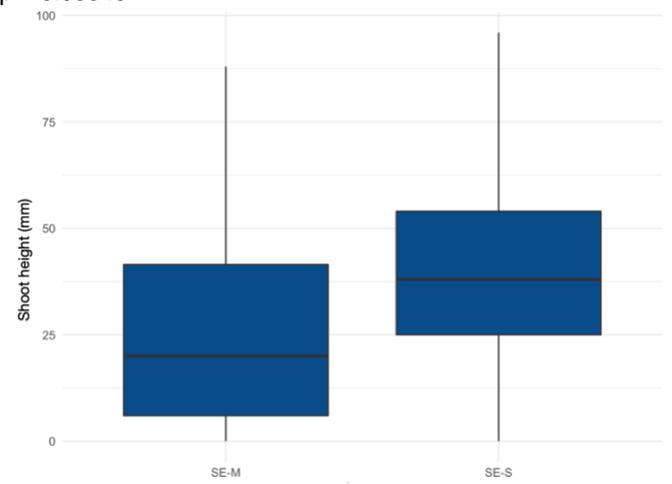
b.

$p = 0.03349$



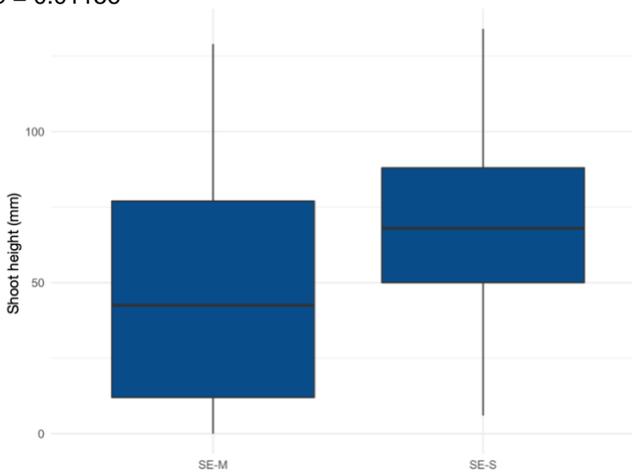
c.

$p = 0.01156$



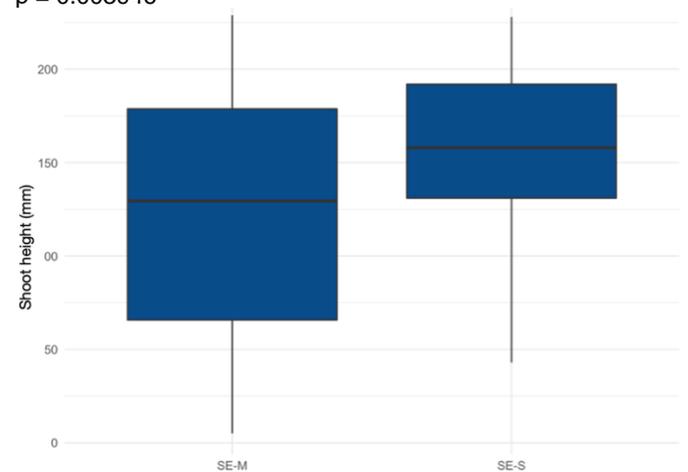
d.

$p = 0.003946$



e.

$p = 0.004001$



f.

$p = 0.01105$

Figure 9. Boxplots illustrating the shoot height between the two treatments (SE-S = sandy soil and SE-M = MGS-1) for each observation along with the p-value obtained from performing a t-test. a. Observations conducted on April 6, after growing for 4 weeks. b. Observations conducted on April 13, after growing for 5 weeks. c. Observations conducted on April 20, after growing for 6 weeks. d. Observations conducted on April 26, after growing for 7 weeks. e. Observations conducted on May 5, after growing for 8 weeks. f. Harvest observations conducted on May 25, after the plants had been growing for 11 weeks.

3.2.1.2 Number of branches

The number of branches counted on the *Salicornia europaea* has been observed on four occasions. The mean of the number of branches for the treatments corresponds reasonably to the first and second observations, as indicated in Figures 10 and 11. The third observation shows a significant difference between the two treatments for the number of branches observed ($p = 0.02898$). While during the harvest observation, no significant difference between the two treatments was observed ($p = 0.06983$), even though a higher mean for the treatment on sandy soil (SE-S) was noticeable in addition to the highest observed value belonging to the treatment on MGS-1 (SE-M). According to a Repeated Measures ANOVA, the number of branches over time indicates a significant difference between the two treatments ($p < 0.001$).

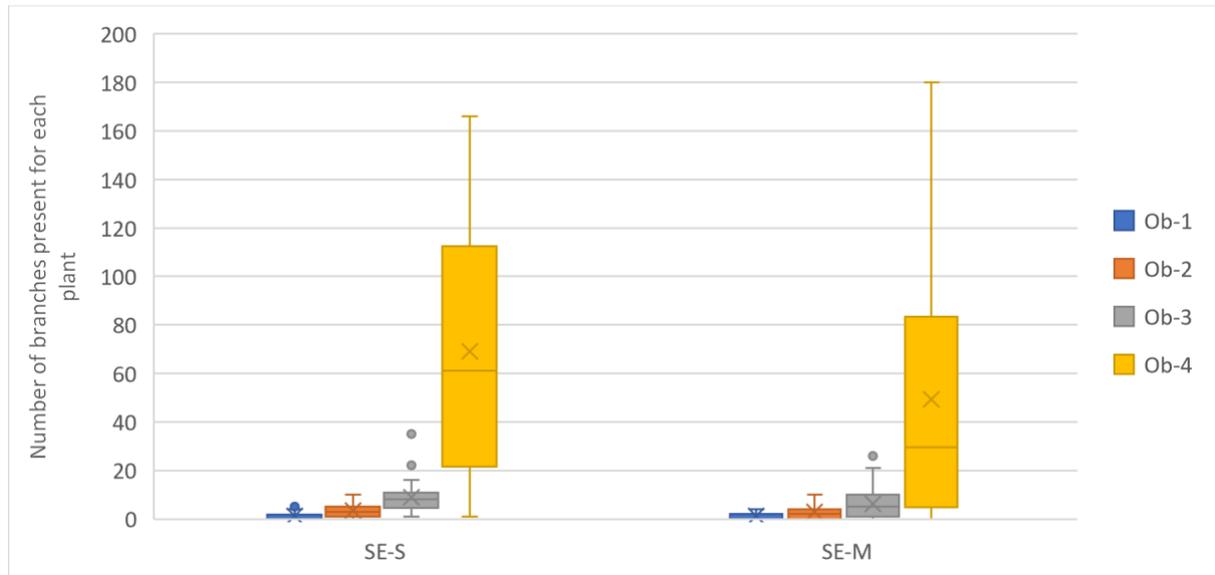
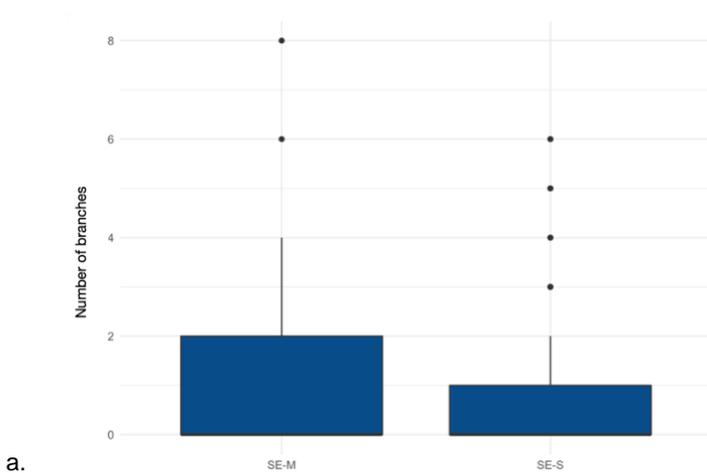
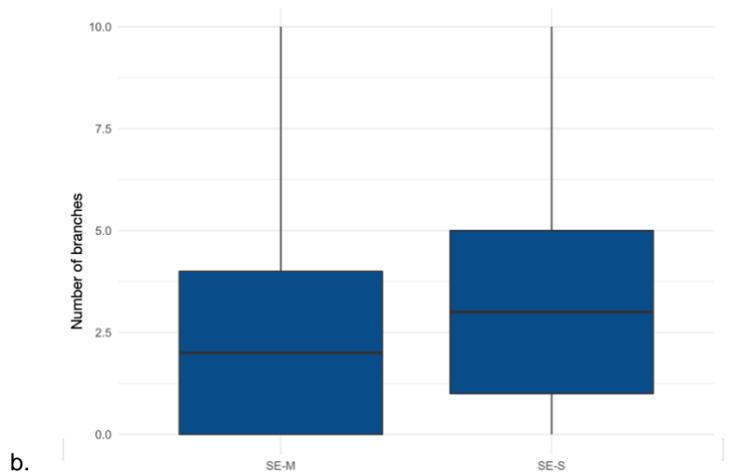


Figure 10. Boxplots illustrating the number of branches produced by the plants over a span of four observations (Ob) between both treatments (SE-S = sandy soil and SE-M = MGS-1) for *Salicornia europaea*. The observations occurred on April 20, April 26, May 5, and May 26.



p = 0.9802



p = 0.1764

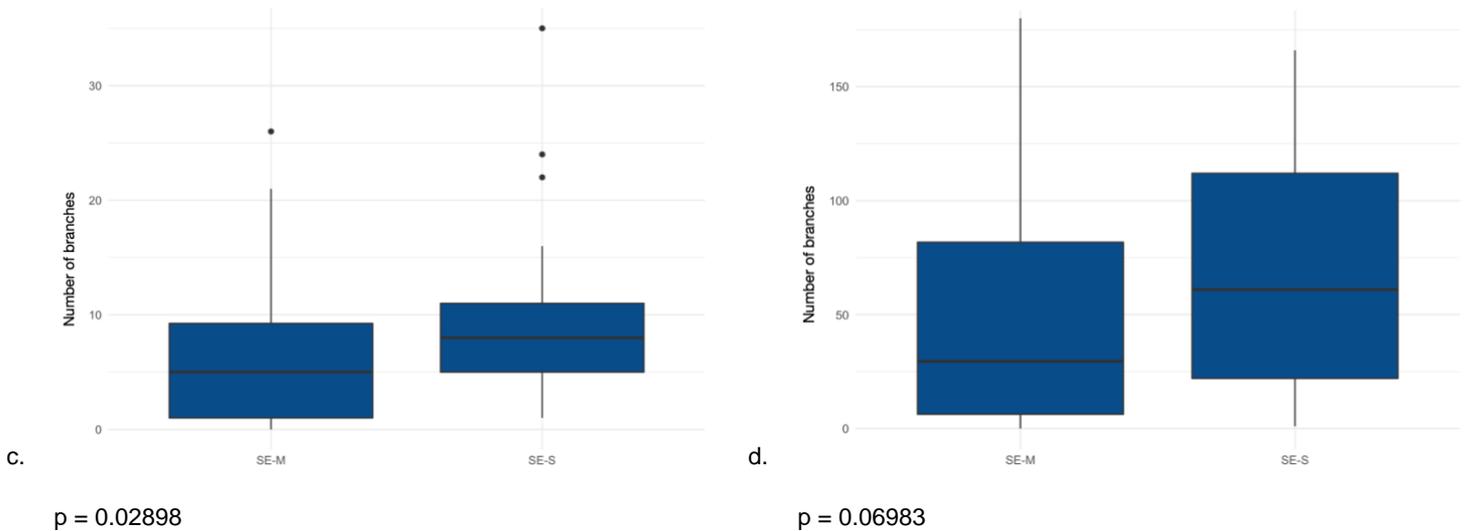


Figure 11. Boxplots illustrating the number of branches between the two treatments (SE-S = sandy soil and SE-M = MGS-1) for each observation along the p-value obtained from performing a t-test. a. Observations conducted on April 20, after growing for 6 weeks. b. Observations conducted on April 26, after growing for 7 weeks. c. Observations conducted on May 5, after growing for 8 weeks. d. Harvest observations conducted on May 25, after the plants had been growing for 11 weeks.

3.2.1.3 The coloration of the shoot

The plants grown on the sandy soil (SE-S) showed no difference in the coloration of the shoot, while the plants grown on the MGS-1 (SE-M) showed four different colorations. Therefore, a significant difference between the two treatments for coloration of the shoot was determined ($p < 0.001$). The shoot coloration on the sandy soil all possessed the color light green. The four different colorations observed on the MGS-1 (SE-M) ranged from extra light green, light green, dark green, and red (Figure 12), with the most common color being dark green.

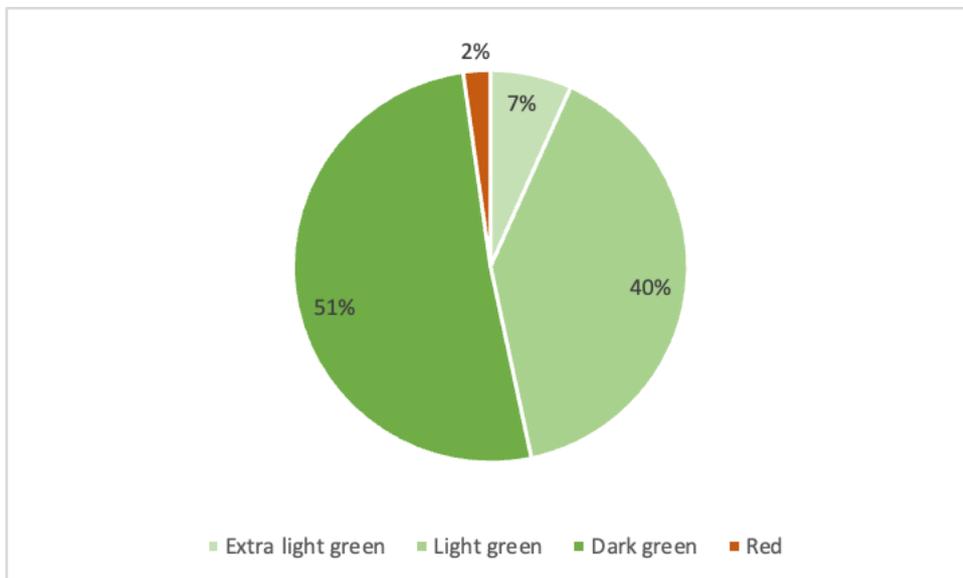


Figure 12. Pie chart indicating the proportion of the coloration of the plants grown on MGS-1 (SE-M). Including the percentage of the colors observed for the treatment.

3.2.1.4 Biomass

Salicornia europaea grown on sandy soil shows higher fresh shoot biomass than the plants grown on MGS-1 ($p = 0.008093$), which was measured for all the plants individually per pot (Figure 13). There was no significant difference found between the two treatments for the dry shoot biomass ($p = 0.09093$) as well as for water content in the shoot ($p = 0.9115$) when looking at the individual plants.

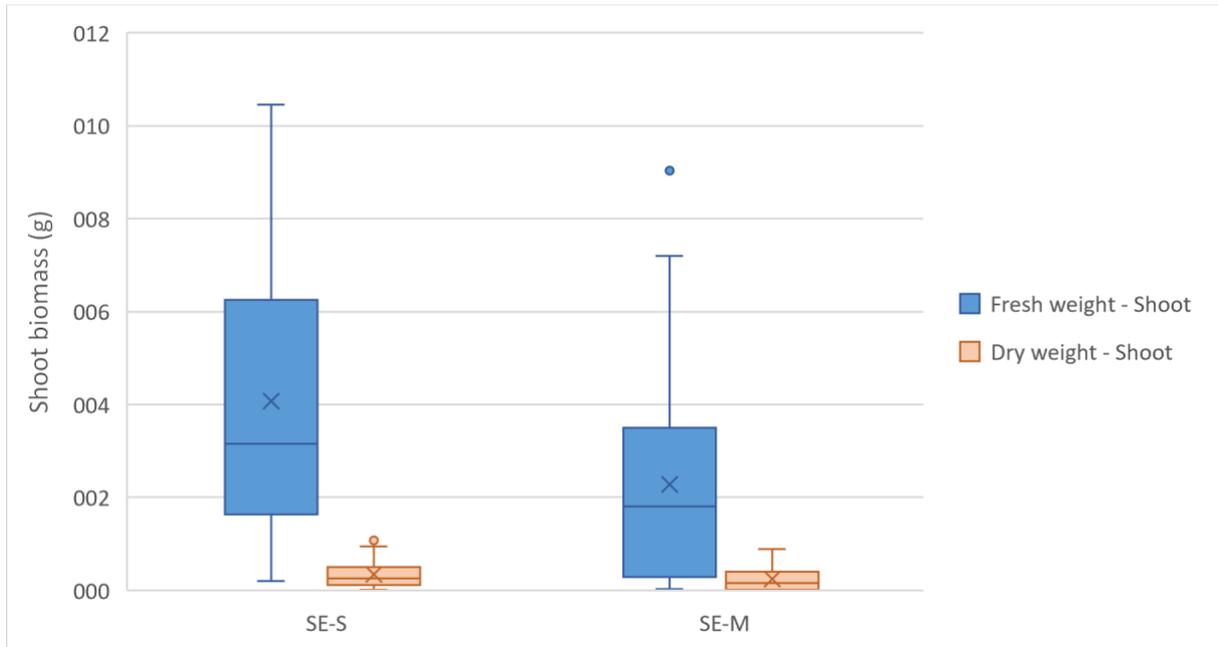


Figure 13. The fresh and dry biomass of the shoot for each individual plant grown between the two treatments (SE-S = sandy soil and SE-M = MGS-1) of *Salicornia europaea*.

The total fresh biomass of the shoot per pot shows a significant difference between the two treatments ($p = 0.002068$), in which the total fresh biomass is significantly higher for sandy soil (SE-S) in comparison to the MGS-1 soil (SE-M) for *Salicornia europaea*, as indicated in Figure 14. As opposed to the total fresh biomass, the total dry biomass shows no significant difference between the two treatments ($p = 0.1043$). The ratio between the total fresh and total dry biomass of the shoots of each pot between the two treatments also shows no significant difference ($p = 0.4196$).

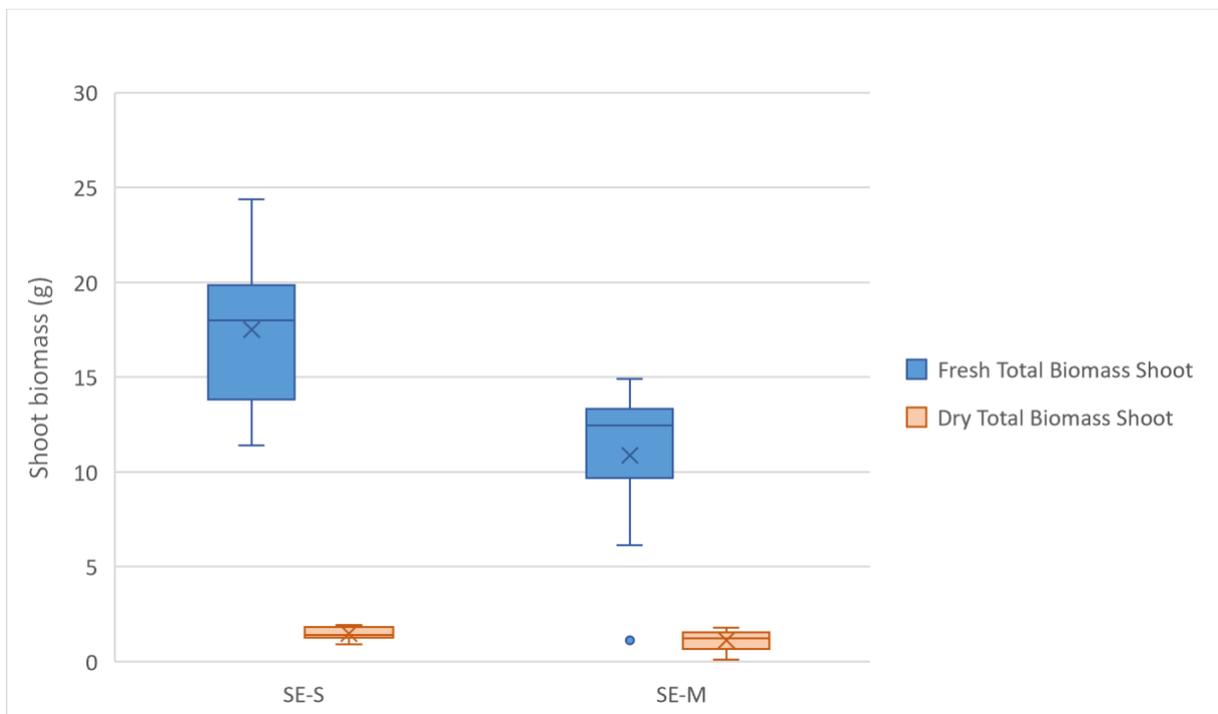


Figure 14. The fresh and dry biomass of the sum of the total shoot biomass by pot for both treatments (SE-S = sandy soil and SE-M = MGS-1) of *Salicornia europaea*.

Both the total fresh biomass ($p = 0.3782$) and the total dry biomass ($p = 0.7425$) of the roots shows no significant difference between the two treatments. The total fresh biomass of the roots grown on MGS-1 shows a wider range compared to the total fresh biomass of the roots on sandy soil (Figure 15). The ratio between the total fresh and total dry biomass of the roots of each pot between the two treatments also shows no significant difference ($p = 0.3979$).

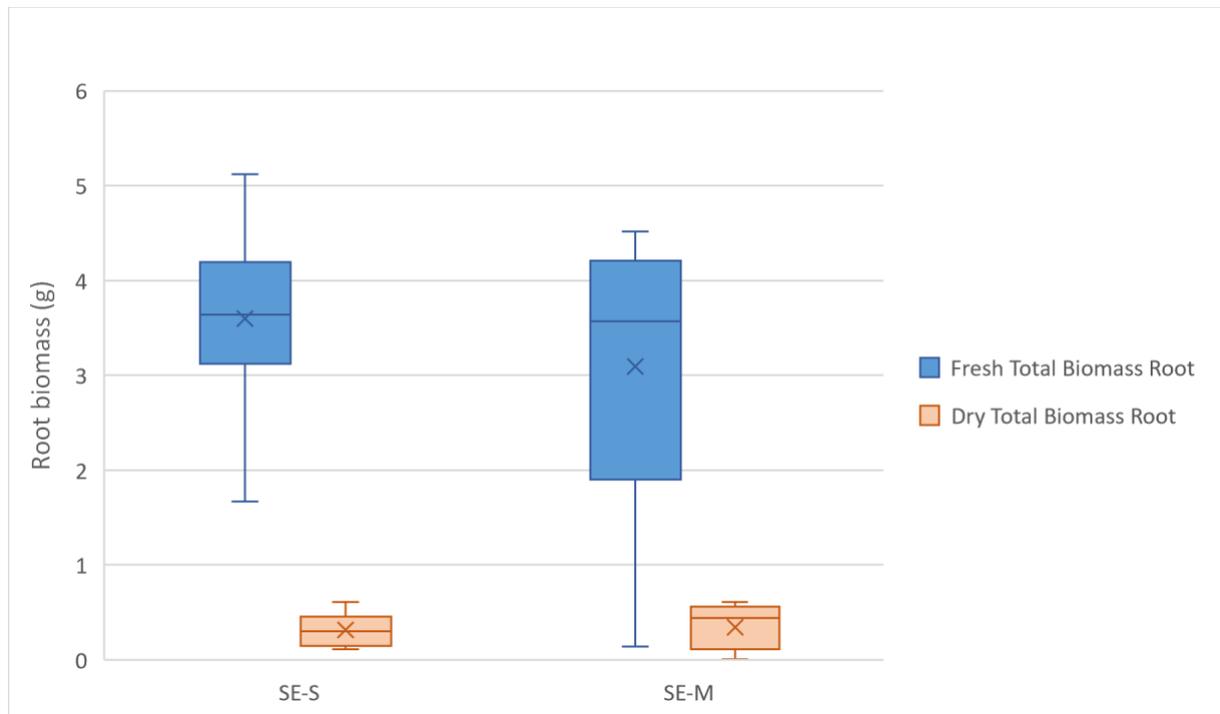


Figure 15. The fresh and dry biomass of the sum of the total root biomass by pot for both treatments (SE-S = sandy soil and SE-M = MGS-1) of *Salicornia europaea*.

3.2.1.5 Salt formation on MGS-1 and the impact on the biomass

During the harvesting of the pot, on eight of the ten pots containing the MGS-1, salt formation around the rim was observed. The salt formation does not show a significant impact ($p > 0.05$) on the fresh and dry biomass of the plant shoots and roots in the pot in which the salt is present.

3.2.2 Effect of the MGS-1 on the growth and development of *Cochlearia officinalis*

Cochlearia officinalis only showed growth on the sandy soil. Of the four seeds germinated on the MGS-1, hardly three managed to grow up to a few millimeters in height. In contrast, the *Cochlearia officinalis* on sandy soil nearly all managed to grow to a conventional height, implying there is a significant difference between the two treatments in all observed characteristics. The observations of the growth of *Cochlearia officinalis* can be found in Appendix 11.

4 Discussion & Conclusion

The aim of this study was to determine whether the halophytes *Salicornia europaea*, *Salsola soda*, and *Cochlearia officinalis* can germinate on MGS-1 and exhibit similar growth to those grown on sandy soil. Therefore, an experiment was executed in which the seeds of these halophytes had been sown on MGS-1 as well as on sandy soil to observe the germination rate and the growth afterward. It was expected that the halophytes can germinate on the MGS-1 as successfully as on sandy soil. Furthermore, showing no significant difference in the overall growth, development, and final biomass yield between the two treatments for all three halophytes. Except for *Salicornia europaea* to display an abnormal appearance due to the presence of heavy metals or trace metals. This study contributes to finding alternative crops, due to the worldwide salt accumulation problem on cultivated land (*Soil Salinization Causes & How To Prevent And Manage It*, 2022). As well as, finding crops that can thrive on Martian soil to support long-duration missions to Mars while being self-sufficient. MGS-1 has shown not to support plant growth due to salinity. Therefore, growing halophytes on this simulant gives an opportunity to grow plants on Mars and gives insight into solutions for the future when the salinization of our agricultural land becomes a problem.

The outcome of the experiment indicates that the halophyte *Salicornia europaea*, Marsh Samphire, can be cultivated on the saline MGS-1, with no significant difference between the number of seeds germinated. Previous research suggests *S. europaea* does not have a physiological need for salt during germination, with an optimal germination percentage of 72 percent in distilled water (Calone et al., 2020). Although the salt concentrations were unknown in the soils used during this experiment, the germination percentage for the sandy soil was 73 and 67 on MGS-1. These percentages are equivalent to the germination in distilled water as reported by previous research. The salt-forming around the rim of the pots with MGS-1 indicates the presence of salts in MGS-1. As well as previous research stating MGS-1 contains a conductivity of 38 mS/cm of soluble salts (Kuklis et al., 2021), as chloride deposits are present on Mars (Osterloo et al., 2008). The sandy soil used for the experiment was collected near the sea, which has a conductivity of approximately 50 mS/cm (Hanna Instruments, n.d.; Metresys, 2022; *Water Conductivity - Lenntech*, n.d.). Therefore, assuming this corresponds with the soluble salts in the sandy soil. Concluding *Salicornia europaea* can germinate better in saline conditions than stated in previous research.

After germination, the *Salicornia europaea* continued to grow steadily. Due to the presence of physiological and biochemical adaptations, this halophyte was able to survive in saline conditions (Smillie, 2015). *S. europaea* can accumulate a considerable amount of sodium in their shoots, up to 50% of their dry weight, without the presence of salt glands or salt bladders (Ushakova et al., 2005). The salt tolerance of *Salicornia europaea* during growth could vary from 850 to 1700 mM NaCl, depending on the geological placement (Khan and Gul, 2006). Therefore, the significant differences between the two treatments (MGS-1 and sandy soil) could possibly be due to the high pH of MGS-1 (pH > 9.0) (Cannon et al., 2019).

The plants grown on MGS-1 showed significant differences in the shoot height starting from the second observation. This is likely due to nutrient deficiency, as phosphorus is only present in small amounts in unaltered MGS-1 (Kuklis et al., 2021). On the effect of nutrient deficiency for *Salicornia europaea* has no research been conducted yet. In general, phosphorus deficiency tends to prevent or inhibit shoot growth while also turning darker in color (PennState, n.d.). Explaining the stunted shoot growth as well as the significant difference in shoot color for MGS-1. However, the turning darker in shoot color can also be indicated as a response to stress. As stress can lead the plant to end its life cycle earlier to faster reproduce and *Salicornia europaea* tends to turn darker green toward the end of its life cycle (Bray, 2000; Davy et al., 2001). Although during harvest there was no significant difference in the number of branches for each plant, due to the alpha value of 0.05, the results still indicate a difference between the

two treatments. While the second to last observation showed there was a significant difference, likely due to the same reason as for the shoot height.

Besides the accessibility of nutrients, the soil structure can affect plant growth in many ways (Passioura, 1991). MGS-1 has a bulk density of 1.29 g/cm^3 (Cannon et al., 2019), while sandy soil tends to have higher bulk densities with less pore space ($1.3\text{-}1.7 \text{ g/cm}^3$) (*Density of Soil: Bulk Density and Particle Density*, 2017). Except MGS-1 tends to aggregate right after watering, which can clear all the oxygen from the soil (Eichler et al., 2021), possibly also reducing pore space due to small particle size ($<1\text{mm}$). This can lead to a negative impact on plant growth. As for the biomass, there was a significant difference between the fresh biomass for the shoot, for the individual plants as well as the total shoot biomass per pot. The dry biomass showed no significant difference, indicating that there is a difference in the water content present in the shoot between the treatments, which was higher on sandy soil. *Salicornia europaea* has expressed dehydration, in a previous study, while being exposed to higher salinity. This, however, showed no connection to a lower growth rate (Glenn and O'Leary, 1984). This could explain the significant difference between the fresh biomass in the shoot, while there is no significant difference between the dry biomasses. This indicates the salt concentrations present in both soils were rather high, especially in MGS-1. Concluding the hypothesis stating *Salicornia europaea* should be able to germinate on the Mars Global Simulant (MGS-1) just as well as on the saline sandy soil was true. The hypothesis stating *Salicornia europaea* can grow, develop, and show no difference in the final yield between the two treatments (MGS-1 and sandy soil) was not true. Except for displaying an abnormal appearance, like the coloration of the shoot, was true. The presence of this abnormal appearance is not confirmed to be caused by the presence of heavy metals in MGS-1.

Cochlearia officinalis did not show signs of germination on MGS-1, except for a few seeds. Inhibition of seed germination can be caused by several stress factors, of which water stress is a major concern (Ahmad et al., 2009; Zhou et al., 2020). Because of the lack of previous research on the seed germination of *Cochlearia officinalis*, the abiotic factors influencing seed germination are unknown. While MGS-1 is very alkaline ($\text{pH} >9.0$), making several critical nutrients possibly inaccessible for plants (Eichler et al., 2021). The pH of beach sand ranges from 7.6 to 8.6 (Zakaria et al., 2011), indicating the germination between both treatments is likely not due to the soil pH. Furthermore, the presence of heavy metals is a possible explanation, because of the low levels present in the sandy soil derived from the beach (Pit et al., 2017). *Cochlearia officinalis* belongs to a genus descended from a relatively salt- and heavy metal-tolerant ancestral species (Nawaz et al., 2017). This genus is known for having an unusually high Zn-tolerant in comparison to other methallophytic species (Schat and Ten Bookum, 1992; Schat et al., 1996; Schat et al., 2002). Implying it to be implausible that heavy metals were the cause of the low germination percentage. High levels of vitamin C are present in *Cochlearia officinalis*, a cure against scurvy, making it a highly interesting crop to grow on Mars. Therefore, continuing research on how to germinate *Cochlearia officinalis* on MGS-1, possibly by altering certain easily manipulated features of MGS-1, can be used to support long-duration space missions on Mars.

The halophyte *Salsola soda* showed no signs of germination for both treatments. Although *S. soda* is known for its low germination rate and difficulty to germinate. Previous research showed high germination percentages ($> 86\%$), where the highest was recorded in the range between 10 and 30°C with a germination percentage between $98\text{-}100\%$ (Sara et al., 2021). Considering the fact that the seeds were not able to germinate on the MGS-1 and on sandy soil, as well as in the control tray containing just potting soil. The viability of the seeds was probably expired. Previously, several studies have been conducted with *Salsola soda* as a 'biodesalinating companion plant' for tomatoes and peppers in saline soils, which led to a higher yield for the tomatoes and peppers. These studies impute this higher yield to the accumulation of sodium and thereby reducing the impact of sodium on the tomatoes and peppers (Colla et al., 2006; Graifenberg et al., 2003). *S. soda* can also accumulate high

concentrations of sodium, boron, and selenium without showing any toxicity symptoms (Centofanti and Banuelos, 2015). Therefore, further research on the possibilities of incorporating *Salsola soda* with other crops, which are less salt or wastewater tolerant, on MGS-1 could push the field of growing food on Mars with the help of Martian soil further.

The sandy soil used for this experiment was collected from a beach close to a Marsh samphire natural habitat. During this study, no lab analyses on the sandy soil have been performed to determine the verified composition. Thereby, questioning the representation of this study due to the uncertainty of being able to recreate the experiment with the exact same results when not knowing the exact soil used. Moreover, the amount of data obtained during this experiment was dependent on the germination potential of the seeds purchased, which depends on the viability of the seeds. The predicted sample size for both treatments was not met, making the sample sizes uneven. Uneven sample sizes could lead to an unequal variance between the sample. This could affect the assumption of normal distribution for the t-test and ANOVA, as the data did not fit the assumption of normal distribution, even after data transformations.

The duration of this experiment was merely enough to let *Salicornia europaea* grow until it reached the vegetative stage, only to understand the effect of MGS-1 on the plants up until harvest. Observing the effect of the MGS-1 on the flowering of the plants as well as seed production, by counting the seeds, would be crucial to determine whether *Salicornia europaea* would be a crop useful on Mars. This can be observed by expanding the same experiment, only for a longer period to witness the reproductive stage of *Salicornia europaea*. As for *Cochlearia officinalis*, because of the delayed start, cultivating the seeds for a longer period could maybe result in more seeds germinating on MGS-1 or observe the factors influencing the low germination potential on MGS-1. A comprehensive view of the difference between the two soils used in the experiment could give more insight as to why *Salicornia europaea* was able to germinate on MGS-1 and *Cochlearia officinalis* not.

For future directions, an important follow-up study would be determining the accessibility of the heavy metals and nutrients present in the MGS-1 for the plants, while adding nutrients. Aside from the lower fresh shoot biomass for *Salicornia europaea* on MGS-1, still provides a fair amount of edible biomass, making the Marsh samphire a successful option as a crop to grow on Mars. Yet, if we would like to implement freshly grown food on Mars, the crops should be able to fit into the human diet. Therefore, we should take into consideration the reference daily intake of salt. Halophytes accumulate inorganic salts, mainly NaCl, in the vacuole and organic solutes in the cytoplasm, due to osmotic adjustments (Glenn et al., 1999). The reference daily intake is about 5 - 6 grams of salts (equal to 2 - 2.3 grams of sodium) (NHS website, 2022; *Salt Reduction*, 2020). Plants like *Salicornia europaea* or *Cochlearia officinalis* will therefore not take up a large part of a human's diet. Due to the salinity of the MGS-1, growing traditional crops would not be possible, even with modifications to the simulant (Eichler et al., 2021). Therefore, finding ways to grow traditional crops alongside halophytes to reduce the salinity in the soil would be an interesting study to continue finding ways to be self-sustainable on long space missions on Mars.

Concluding this study, *Salicornia europaea* can be cultivated on MGS-1 with the addition of nutrients and keeping the soil waterlogged, showing only reduced fresh biomass and shorter shoots on MGS-1 compared to the sandy soil. This study can be used as the foundation for further research continuing the search to find crops, most likely halophytes, convenient for long-duration space missions on Mars. As well as understanding the impacts of extreme factors on alternative crops, possibly helping the worldwide problem of salt accumulation on cultivated lands in the next following decades. Even though germination and growth of *Cochlearia officinalis* and *Salsola soda* were unsuccessful during this experiment, proving the hypotheses to be not true. These halophytes still have potential properties that will be beneficial for long-duration space missions. Therefore, it would be interesting to incorporate these halophytes in future studies.

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Appendix 1 Detailed graph of the temperature and air humidity in the greenhouse

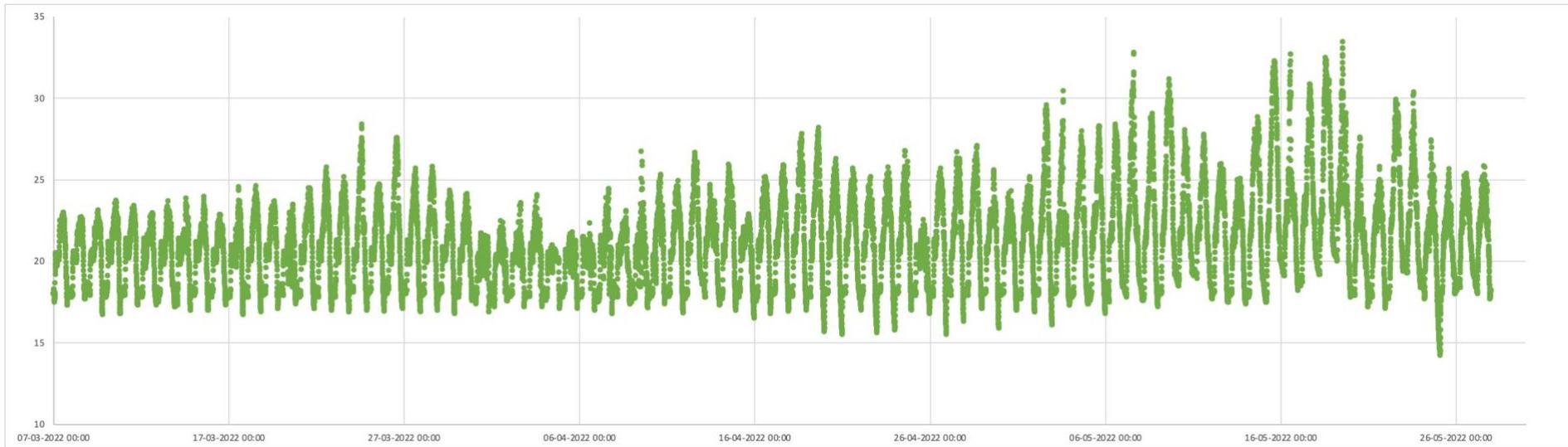


Figure 1. Graph illustrating the raw data of the fluctuations in the temperature ($^{\circ}\text{C}$) in the greenhouse over the period of the experiment, March 7, 2022 until May 27, 2022, with an interval of 5 minutes between each data point.

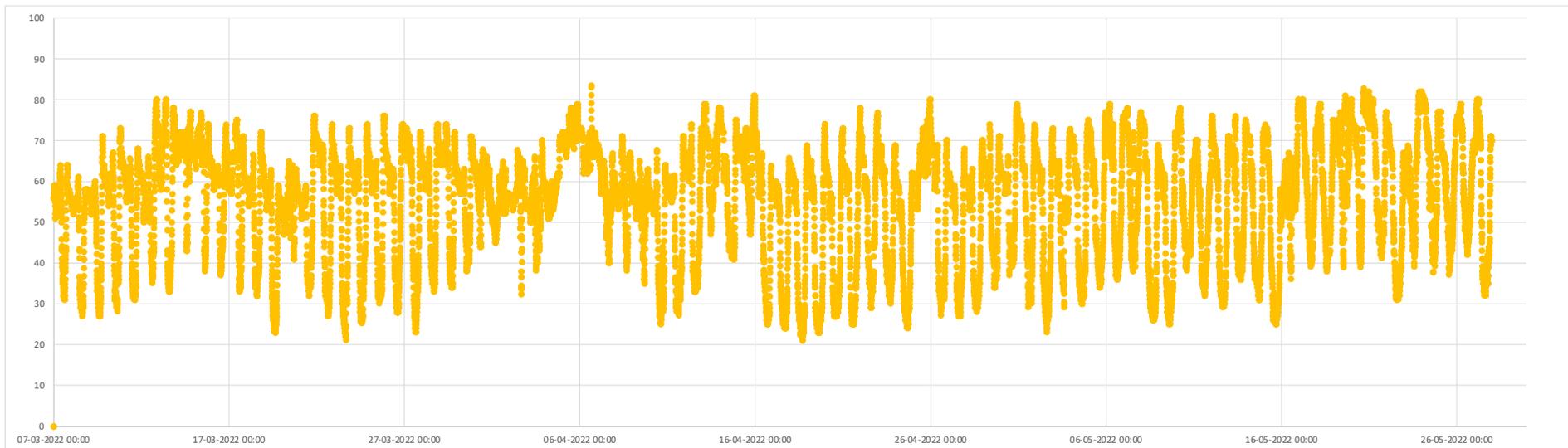


Figure 2. Graph illustrating the raw data of the fluctuations in the air humidity (%) in the greenhouse over the period of the experiment, March 7, 2022 until May 27, 2022, with an interval of 5 minutes between each data point.

Appendix 2 Nutrient solution

Table 1. The recipe to recreate the nutrient solution used during the experiment with the desired EC of 1.2.

Nutrients	mmol/m³
Copper	400
Zwakal	285
BFK	589
Baskal	504
Amnitra	100
Magnitra	162
Calsal	960
Iron chelate	300
Magnesium	500
Zinc	150
Borax	1425
Sodium	400
Nitrakal	829

Appendix 3 Raw data of the germination and survival observations of *Salicornia europaea*

Table 2. The raw data collected for the germination observations of the halophyte *Salicornia europaea* for the duration of 28 days after sowing the seeds. Exclusively the days on which observations were executed are included in the table.

Pot number	Pot ID	Treatment	Days																
			2	3	4	7	8	9	10	11	14	15	16	17	18	21	23	25	28
1	SE-S-7	S. europaea-Sand		2	3	3	4	4	4	4	4	4	4	4	4	4	4	4	3
2	SE-M-6	S. europaea-Mars		4	6	6	9	9	8	9	9	10	10	10	10	10	10	10	10
3	SE-M-2	S. europaea-Mars			3	3	4	4	4	6	6	6	6	6	6	6	6	6	7
4	SE-S-2	S. europaea-Sand		5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	6
5	SE-S-4	S. europaea-Sand		4	4	5	6	6	6	6	6	5	5	5	5	4	4	4	4
6	SE-S-8	S. europaea-Sand		3	5	7	7	7	7	7	7	7	7	6	6	6	6	5	5
7	SE-S-1	S. europaea-Sand	1	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	SE-M-8	S. europaea-Mars				3	3	3	3	4	4	4	4	4	4	4	4	4	4
9	SE-M-9	S. europaea-Mars			1	4	5	7	7	7	8	7	7	7	6	6	6	6	6
10	SE-M-7	S. europaea-Mars		3	3	4	6	6	7	7	7	7	7	7	6	6	6	6	6
11	SE-S-9	S. europaea-Sand	1	6	7	10	10	10	10	10	7	7	7	7	7	7	7	7	5
12	SE-M-3	S. europaea-Mars		1	2	5	5	5	6	7	8	8	8	8	8	8	8	8	8
13	SE-S-3	S. europaea-Sand	2	6	6	8	8	8	8	7	7	7	7	4	5	5	5	4	4
14	SE-M-5	S. europaea-Mars		4	4	4	6	6	6	6	6	6	6	6	6	6	6	6	6
15	SE-S-10	S. europaea-Sand		5	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10
16	SE-M-1	S. europaea-Mars		2	2	4	4	4	4	4	5	5	5	5	5	5	5	5	4
17	SE-S-5	S. europaea-Sand	1	4	5	8	8	9	9	9	9	9	9	9	9	9	9	9	5
18	SE-M-4	S. europaea-Mars		2	3	3	4	4	4	4	4	3	3	3	3	3	3	2	2
19	SE-M-10	S. europaea-Mars		2	5	6	6	6	6	6	7	6	6	6	6	7	7	7	7
20	SE-S-6	S. europaea-Sand		4	5	5	5	6	6	6	5	3	3	3	3	3	3	3	3

Table 3. The raw data collected for the survival observations of the halophyte *Salicornia europaea* for the duration of 28 days after sowing the seeds. Exclusively the days on which observations were executed are included in the table.

Pot number	Pot ID	Treatment	Days																
			1	2	3	4	7	8	9	10	11	14	15	16	17	18	21	23	25
1	SE-S-7	S. europaea-Sand			2	3	3	4	4	4	4	4	4	4	4	4	4	4	3
2	SE-M-6	S. europaea-Mars			4	6	6	9	9	9	9	9	10	10	10	10	10	10	10
3	SE-M-2	S. europaea-Mars					3	3	4	4	6	6	6	6	6	6	6	6	7
4	SE-S-2	S. europaea-Sand			5	7	7	7	7	7	7	7	7	7	7	7	7	7	6
5	SE-S-4	S. europaea-Sand			4	4	5	6	6	6	6	5	5	5	5	4	4	4	4
6	SE-S-8	S. europaea-Sand			3	5	7	7	7	7	7	7	7	6	6	6	6	5	5
7	SE-S-1	S. europaea-Sand		1	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
8	SE-M-8	S. europaea-Mars					3	3	3	3	4	4	4	4	4	4	4	4	4
9	SE-M-9	S. europaea-Mars				1	4	5	7	7	8	7	7	7	6	6	6	6	6
10	SE-M-7	S. europaea-Mars			3	3	4	6	6	7	7	7	7	6	6	6	6	6	6
11	SE-S-9	S. europaea-Sand		1	6	7	10	10	10	10	10	7	7	7	7	7	7	7	5
12	SE-M-3	S. europaea-Mars			1	2	5	5	5	6	7	8	8	8	8	8	8	8	8
13	SE-S-3	S. europaea-Sand		2	6	6	8	8	8	8	7	7	7	7	4	5	5	5	4
14	SE-M-5	S. europaea-Mars			4	4	4	6	6	6	6	6	6	6	6	6	6	6	6
15	SE-S-10	S. europaea-Sand			5	9	10	10	10	10	10	10	10	10	10	10	10	10	10
16	SE-M-1	S. europaea-Mars			2	2	4	4	4	4	5	5	5	5	5	5	5	5	4
17	SE-S-5	S. europaea-Sand		1	4	5	8	8	9	9	9	9	9	9	9	9	9	9	5
18	SE-M-4	S. europaea-Mars			2	3	3	4	4	4	4	3	3	3	3	3	3	2	2
19	SE-M-10	S. europaea-Mars			2	5	6	6	6	6	6	7	6	6	6	7	7	7	7
20	SE-S-6	S. europaea-Sand			4	5	5	5	6	6	6	5	3	3	3	3	3	3	3

Appendix 4 Raw statistical data of the regression analyses on the germination observations of *Salicornia europaea* in RStudio

```
glm(formula = Germ ~ Treatment, family = "binomial", data = data)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.5956	-1.4689	0.8106	0.9116	0.9116

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	0.6633	0.2111	3.142	0.00168 **
TreatmentSand	0.2812	0.3069	0.916	0.35953

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 247.64 on 199 degrees of freedom
Residual deviance: 246.80 on 198 degrees of freedom
AIC: 250.8

Number of Fisher Scoring iterations: 4

Analysis of Variance Table

Response: Germ

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	0.18	0.18000	0.8366	0.3615
Residuals	198	42.60	0.21515		

Appendix 5 Raw data of the development stages observations of *Salicornia europaea*

Table 4. The raw data collected for the development stages observations of the halophyte *Salicornia europaea* for the duration of 28 days after sowing the seeds, illustrating the first seeds for each pot to reach a certain stage in a certain number of days.

Pot ID	Germination	Hypocotyl visible	Sprout leaves	Vegetative stage
S-1	1	4	7	14
S-2	3	4	8	25
S-3	2	4	8	18
S-4	3	3	7	14
S-5	2	3	14	21
S-6	3	3	7	14
S-7	3	4	7	15
S-8	3	3	7	14
S-9	2	3	7	14
S-10	3	4	7	14
M-1	3	4	7	14
M-2	7	7	9	17
M-3	3	3	7	15
M-4	3	3	7	23
M-5	3	3	7	14
M-6	3	4	7	15
M-7	3	3	7	14
M-8	7	7	9	18
M-9	4	4	7	14
M-10	3	4	7	14

Table 5. The raw statistical data of the t-test performed to determine whether there was a significant difference between the time in which the seeds reached a certain development stage of the halophyte *Salicornia europaea*

T-test	Output
t.test(Hypocotyl ~ Treatment)	data: SED\$Hypocotyl by SED\$Treatment t = 1.7619, df = 80.067, p-value = 0.08191 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.09866392 1.62247344 sample estimates: mean in group M mean in group S 6.000000 5.238095
t.test(Germ leaves ~ Treatment)	data: SED\$Germ_leaves by SED\$Treatment t = 0.65263, df = 80.911, p-value = 0.5158 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.027963 2.031447 sample estimates: mean in group M mean in group S 10.073171 9.571429
t.test(Vegetative stage ~ Treatment)	data: SED\$Vegetative by SED\$Treatment t = -1.7983, df = 78.996, p-value = 0.07594 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -4.0497024 0.2053354 sample estimates: mean in group M mean in group S 19.26829 21.19048

Appendix 6 Raw data of the germination and survival observations of *Cochlearia officinalis*

Table 6. The raw data collected for the germination observations of the halophyte *Cochlearia officinalis* for the duration of 34 days after sowing the seeds. Exclusively the days on which observations were executed are included in the table.

Pot number	Pot ID	Treatment	Days									
			3	6	7	10	13	15	17	22	34	
21	SS-M-8	C. officinalis-Mars										
22	SS-S-5	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
23	SS-M-3	C. officinalis-Mars										
24	SS-M-1	C. officinalis-Mars										
25	SS-S-2	C. officinalis-Sand	4	5	5	5	5	5	5	5	5	5
26	SS-M-6	C. officinalis-Mars										
27	SS-S-7	C. officinalis-Sand	5	5	5	5	5	5	5	5	5	5
28	SS-S-8	C. officinalis-Sand	4	5	5	5	5	5	5	5	5	5
29	SS-M-9	C. officinalis-Mars										
30	SS-S-6	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
31	SS-S-1	C. officinalis-Sand	3	4	4	4	3	3	3	4	5	5
32	SS-M-2	C. officinalis-Mars			1	2	2	2	2	2	2	2
33	SS-M-4	C. officinalis-Mars										
34	SS-S-9	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
35	SS-M-10	C. officinalis-Mars					1	1	1	1	1	2
36	SS-M-7	C. officinalis-Mars										
37	SS-S-10	C. officinalis-Sand	4	4	4	4	4	4	4	4	4	3
38	SS-S-4	C. officinalis-Sand	4	4	5	4	4	4	4	4	5	5
39	SS-S-3	C. officinalis-Sand	5	5	5	5	5	5	5	5	1	1
40	SS-M-5	C. officinalis-Mars								1	0	0

Table 7. The raw data collected for the survival observations of the halophyte *Cochlearia officinalis* for the duration of 34 days after sowing the seeds. Exclusively the days on which observations were executed are included in the table.

Pot number	Pot ID	Treatment	Days									
			3	6	7	10	13	15	17	22	34	
21	SS-M-8	C. officinalis-Mars										
22	SS-S-5	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
23	SS-M-3	C. officinalis-Mars										
24	SS-M-1	C. officinalis-Mars										
25	SS-S-2	C. officinalis-Sand	4	5	5	5	5	5	5	5	5	5
26	SS-M-6	C. officinalis-Mars										
27	SS-S-7	C. officinalis-Sand	5	5	5	5	5	5	5	5	5	5
28	SS-S-8	C. officinalis-Sand	4	5	5	5	5	5	5	5	5	5
29	SS-M-9	C. officinalis-Mars										
30	SS-S-6	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
31	SS-S-1	C. officinalis-Sand	3	4	4	4	3	3	3	4	5	5
32	SS-M-2	C. officinalis-Mars			1	2	2	2	2	2	2	2
33	SS-M-4	C. officinalis-Mars										
34	SS-S-9	C. officinalis-Sand	3	5	5	5	5	5	5	5	5	5
35	SS-M-10	C. officinalis-Mars					1	1	1	1	1	2
36	SS-M-7	C. officinalis-Mars										
37	SS-S-10	C. officinalis-Sand	4	4	4	4	4	4	4	4	4	3
38	SS-S-4	C. officinalis-Sand	4	4	5	4	4	4	4	4	5	5
39	SS-S-3	C. officinalis-Sand	5	5	5	5	5	5	5	5	1	1
40	SS-M-5	C. officinalis-Mars								1	0	0

Appendix 7 Raw statistical data of the regression analyses on the germination observations of *Cochlearia officinalis* in RStudio

```
glm(formula = Germ ~ Treatment, family = "binomial", data = data)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.797	-0.459	0.201	0.201	2.146

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-2.1972	0.4714	-4.661	3.15e-06 ***
TreatmentSand	6.0890	1.1147	5.463	4.69e-08 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 137.989 on 99 degrees of freedom
Residual deviance: 42.312 on 98 degrees of freedom
AIC: 46.312

Number of Fisher Scoring iterations: 6

Analysis of Variance Table

Response: Germ

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	19.36	19.3600	346.22	< 2.2e-16 ***
Residuals	98	5.48	0.0559		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 10. The raw data of the coloration of the shoot of *Salicornia europaea* observed during the harvest of the plants.

			Date of measurement: 25-05-2022				
			Coloration HARVEST				
			1	2	3	4	5
Pot number	Pot ID	Treatment					
1	SE-S-7	S. europaea-Sand	LG	LG	LG		
2	SE-M-6	S. europaea-Mars	DG	DG	DG	DG	DG
3	SE-M-2	S. europaea-Mars	LG	LG	LG	LG	LG
4	SE-S-2	S. europaea-Sand	LG	LG	LG	LG	LG
5	SE-S-4	S. europaea-Sand	LG	LG	LG	LG	
6	SE-S-8	S. europaea-Sand	LG	LG	LG	LG	
7	SE-S-1	S. europaea-Sand	LG	LG	LG		
8	SE-M-8	S. europaea-Mars	DG	DG	DG	DG	
9	SE-M-9	S. europaea-Mars	DG	R	DG	DG	DG
10	SE-M-7	S. europaea-Mars	LG	LG	LG	LG	LG
11	SE-S-9	S. europaea-Sand	LG	LG	LG	LG	LG
12	SE-M-3	S. europaea-Mars	DG	DG	DG	DG	DG
13	SE-S-3	S. europaea-Sand	LG	LG	LG	LG	
14	SE-M-5	S. europaea-Mars	DG	DG	DG	DG	DG
15	SE-S-10	S. europaea-Sand	LG	LG	LG	LG	LG
16	SE-M-1	S. europaea-Mars	LG	LG	LG		
17	SE-S-5	S. europaea-Sand	LG	LG	LG	LG	LG
18	SE-M-4	S. europaea-Mars	LLG	LLG	LLG		
19	SE-M-10	S. europaea-Mars	LG	LG	LG	LG	LG
20	SE-S-6	S. europaea-Sand	LG	LG	LG		

Table 11. The raw data of the fresh weight for both the shoot, weighted for each individual plant, and the root, collective of all the roots present in each pot, of *Salicornia europaea* weighted after the harvest of the above-ground and below-ground biomass.

			Date of measurement: 27-05-2022						Date of measurement: 27-05-2022	
			Fresh weight - Above ground biomass (gram)						Fresh weight - Below ground biomass (gram)	
			1	2	3	4	5 Extra	Total	Total	
Pot number	Pot ID	Treatment								
1	SE-S-7	S. europaea-Sand	6,33	1,65	6,34			14,32	4,03	
2	SE-M-6	S. europaea-Mars	2,28	1,83	4,08	2,29	0,38	2,32	13,18	3,84
3	SE-M-2	S. europaea-Mars	0,43	3,39	1,8	5,22	0,21		11,05	2,19
4	SE-S-2	S. europaea-Sand	3,95	3,24	0,54	2,91	1,61	0,11	12,36	1,67
5	SE-S-4	S. europaea-Sand	0,75	6,18	2,2	2,26			11,39	4,68
6	SE-S-8	S. europaea-Sand	0,3	2,42	7,95	7,04			17,71	3,31
7	SE-S-1	S. europaea-Sand	10,02	8,96	5,33			0,07	24,38	2,56
8	SE-M-8	S. europaea-Mars	5,32	0,31	0,23	0,28			6,14	1,04
9	SE-M-9	S. europaea-Mars	5,24	0,04	1,87	1,91	2,8	1,07	12,93	3,48
10	SE-M-7	S. europaea-Mars	5,45	7,04	0,19	0,18	0,03	0,001	12,891	4,42
11	SE-S-9	S. europaea-Sand	10,45	4,86	1,04	1,7	0,2		18,25	3,99
12	SE-M-3	S. europaea-Mars	0,46	0,12	7,02	2,05	2,55	2,7	14,9	3,6
13	SE-S-3	S. europaea-Sand	10,39	4,7	1,33	0,34			16,76	3,7
14	SE-M-5	S. europaea-Mars	0,93	0,12	3,61	5,99	3,12		13,77	4,52
15	SE-S-10	S. europaea-Sand	1,94	5,07	3,16	2,43	1,42	8,25	22,27	3,58
16	SE-M-1	S. europaea-Mars	2,83	9,03	0,16				12,02	4,14
17	SE-S-5	S. europaea-Sand	2,88	6,83	2,47	1,47	5,03		18,68	3,38
18	SE-M-4	S. europaea-Mars	0,57	0,49	0,07				1,13	0,14
19	SE-M-10	S. europaea-Mars	0,29	1,79	7,2	1,05	0,39	0,15	10,87	3,54
20	SE-S-6	S. europaea-Sand	10,11	4,79	4,14				19,04	5,12

Table 12. The raw data of the dry weight for both the shoot, weighted for each individual plant, and the root, collective of all the roots present in each pot, of *Salicornia europaea* weighted after drying the above-ground and below-ground biomass.

			Date of measurement: 30-05-2022						Date of measurement: 30-05-2022			
			Dry weight - Above ground biomass (gram)						Dry weight - Below ground biomass (gram)			
			1	2	3	4	5	Extra	Total	Total		
Pot number	Pot ID	Treatment										
1	SE-S-7	S. europaea-Sand	0,49	0,14	0,55				1,18	0,45		
2	SE-M-6	S. europaea-Mars	0,22	0,16	0,44	0,22	0,04	0,24	1,32	0,55		
3	SE-M-2	S. europaea-Mars	0,001	0,27	0,06	0,4	0,001		0,732	0,17		
4	SE-S-2	S. europaea-Sand	0,28	0,25	0,01	0,2	0,11	0,07	0,92	0,18		
5	SE-S-4	S. europaea-Sand	0,11	1,08	0,33	0,39			1,91	0,45		
6	SE-S-8	S. europaea-Sand	0,01	0,16	0,68	0,52			1,37	0,11		
7	SE-S-1	S. europaea-Sand	0,84	0,03	0,41				1,28	0,15		
8	SE-M-8	S. europaea-Mars	0,41	0,01	0,01	0,01			0,44	0,02		
9	SE-M-9	S. europaea-Mars	0,61	0,001	0,21	0,23	0,29	0,11	1,451	0,44		
10	SE-M-7	S. europaea-Mars	0,47	0,61	0,01	0,01	0,001	0,001	1,102	0,14		
11	SE-S-9	S. europaea-Sand	1,25	0,46	0,06	0,16	0,01		1,94	0,47		
12	SE-M-3	S. europaea-Mars	0,04	0,01	0,88	0,22	0,33	0,32	1,8	0,61		
13	SE-S-3	S. europaea-Sand	0,95	0,36	0,09	0,02			1,42	0,14		
14	SE-M-5	S. europaea-Mars	0,05	0,01	0,51	0,8	0,39		1,76	0,49		
15	SE-S-10	S. europaea-Sand	0,15	0,43	0,25	0,19	0,11	0,66	1,79	0,35		
16	SE-M-1	S. europaea-Mars	0,23	0,83	0,001				1,061	0,45		
17	SE-S-5	S. europaea-Sand	0,24	0,66	0,14	0,11	0,5		1,65	0,25		
18	SE-M-4	S. europaea-Mars	0,07	0,01	0,01				0,09	0,01		
19	SE-M-10	S. europaea-Mars	0,01	0,17	1,15	0,1	0,03	0,01	1,47	0,59		
20	SE-S-6	S. europaea-Sand	0,74	0,14	0,45				1,33	0,61		

Appendix 9 Raw statistical data of the performed Repeated Measures ANOVA for the observations shoot height and number of branches of the halophyte *Salicornia europaea* in RStudio

Repeated Measures ANOVA – Shoot height

```
model <- aov(SESHA$Shootheight ~ factor(SESHA$Time) + Error(factor(SESHA$ID)))  
summary(model)
```

Error: factor(SESHA\$ID)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	84	291998	3476		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(SESHA\$Time)	5	1067313	213463	421	<2e-16 ***
Residuals	420	212964	507		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Repeated Measures ANOVA – Number of branches

```
model <- aov(SEBA$Branches ~ factor(SEBA$Time) + Error(factor(SEBA$ID)))  
summary(model)
```

Error: factor(SEBA\$ID)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	84	73658	876.9		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(SEBA\$Time)	3	199318	66439	117.5	<2e-16 ***
Residuals	252	142514	566		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 10 Raw statistical data of the multiple t-tests on the growth observations of *Salicornia europaea* in RStudio

Table 13. The raw statistical data of the t-test performed to determine whether there was a significant difference between the treatment in which the plants were grown and the growth observations of the halophyte *Salicornia europaea*

T-test	Output
t.test(Shoot height Ob-1 ~ Treatment)	data: SESH\$`Ob-1` by SESH\$Treatment t = -1.3625, df = 70.549, p-value = 0.1774 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -2.4226753 0.4559347 sample estimates: mean in group SE-M mean in group SE-S 2.772727 3.756098
t.test(Shoot height Ob-2 ~ Treatment)	data: SESH\$`Ob-2` by SESH\$Treatment t = -2.1626, df = 81.836, p-value = 0.03349 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -6.7281201 -0.2807491 sample estimates: mean in group SE-M mean in group SE-S 7.227273 10.731707
t.test(Shoot height Ob-3 ~ Treatment)	data: SESH\$`Ob-3` by SESH\$Treatment t = -2.5829, df = 82.805, p-value = 0.01156 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -15.542190 -2.018786 sample estimates: mean in group SE-M mean in group SE-S 16.00000 24.78049
t.test(Shoot height Ob-4 ~ Treatment)	data: SESH\$`Ob-4` by SESH\$Treatment t = -2.9656, df = 82.813, p-value = 0.003946 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -25.258797 -4.978453 sample estimates: mean in group SE-M mean in group SE-S 26.95455 42.07317
t.test(Shoot height Ob-5 ~ Treatment)	data: SESH\$`Ob-5` by SESH\$Treatment t = -2.9618, df = 81.94, p-value = 0.004001 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -37.406832 -7.347048 sample estimates: mean in group SE-M mean in group SE-S 47.81818 70.19512
t.test(Shoot height Ob-6 ~ Treatment)	data: SESH\$`Ob-6` by SESH\$Treatment t = -2.6027, df = 78.692, p-value = 0.01105 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -58.34423 -7.77550 sample estimates: mean in group SE-M mean in group SE-S 121.8182 154.8780

t.test(Number of branches Ob-1 ~ Treatment)	<p>data: SEB\$`Ob-1` by SEB\$Treatment t = -0.024836, df = 82.569, p-value = 0.9802 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.8091030 0.7891473 sample estimates: mean in group SE-M mean in group SE-S 1.136364 1.146341</p>
t.test(Number of branches Ob-2 ~ Treatment)	<p>data: SEB\$`Ob-2` by SEB\$Treatment t = -1.3635, df = 82.807, p-value = 0.1764 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -2.0130764 0.3756042 sample estimates: mean in group SE-M mean in group SE-S 2.522727 3.341463</p>
t.test(Number of branches Ob-3 ~ Treatment)	<p>data: SEB\$`Ob-3` by SEB\$Treatment t = -2.2241, df = 79.451, p-value = 0.02898 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -5.8599997 -0.3251444 sample estimates: mean in group SE-M mean in group SE-S 5.931818 9.024390</p>
t.test(Number of branches Ob-4 ~ Treatment)	<p>data: SEB\$`Ob-4` by SEB\$Treatment t = -1.8367, df = 82.972, p-value = 0.06983 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -41.346953 1.645179 sample estimates: mean in group SE-M mean in group SE-S 49.29545 69.14634</p>
t.test(Fresh Shoot Biomass ~ Treatment)	<p>data: SE_Biomass_Shoot\$`Fresh weight - Shoot` by SE_Biomass_Shoot\$Treatment t = -2.7185, df = 77.284, p-value = 0.008093 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -2.7555732 -0.4255758 sample estimates: mean in group SE-M mean in group SE-S 2.280889 3.871463</p>
t.test(Dry Shoot Biomass ~ Treatment)	<p>data: SE_Biomass_Shoot\$`Dry weight - Shoot` by SE_Biomass_Shoot\$Treatment t = -1.7107, df = 81.598, p-value = 0.09093 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.23487970 0.01769271 sample estimates: mean in group SE-M mean in group SE-S 0.2343333 0.3429268</p>
t.test(Ratio Fresh-Dry Shoot Biomass ~ Treatment)	<p>data: SE_Biomass_Shoot\$Ratio by SE_Biomass_Shoot\$Treatment t = -0.11155, df = 83.318, p-value = 0.9115 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.667653 1.490523 sample estimates: mean in group SE-M mean in group SE-S</p>

	8.147937 8.236502
t.test(Total Fresh Shoot Biomass ~ Treatment)	data: SE_Biomass\$`Fresh Total Biomass Shoot` by SE_Biomass\$Treatment t = -3.5958, df = 17.988, p-value = 0.002068 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -10.500571 -2.755229 sample estimates: mean in group SE-M mean in group SE-S 10.8881 17.5160
t.test(Total Dry Shoot Biomass ~ Treatment)	data: SE_Biomass\$`Dry Total Biomass Shoot` by SE_Biomass\$Treatment t = -1.7315, df = 14.68, p-value = 0.1043 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.79730533 0.08330533 sample estimates: mean in group SE-M mean in group SE-S 1.122 1.479
t.test(Ratio Total Fresh-Dry Shoot Biomass ~ Treatment)	data: SE_Biomass\$`Ratio Fresh-Dry Shoot` by SE_Biomass\$Treatment t = 0.82713, df = 17.011, p-value = 0.4196 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.598143 3.659399 sample estimates: mean in group SE-M mean in group SE-S 9.870359 8.839731
t.test(Total Fresh Root Biomass ~ Treatment)	data: SE_Biomass\$`Fresh Total Biomass Root` by SE_Biomass\$Treatment t = -0.90693, df = 15.66, p-value = 0.3782 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.7075489 0.6855489 sample estimates: mean in group SE-M mean in group SE-S 3.091 3.602
t.test(Total Dry Root Biomass ~ Treatment)	data: SE_Biomass\$`Dry Total Biomass Root` by SE_Biomass\$Treatment t = 0.3341, df = 16.51, p-value = 0.7425 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.1652071 0.2272071 sample estimates: mean in group SE-M mean in group SE-S 0.347 0.316
t.test(Ratio Total Fresh-Dry Root Biomass ~ Treatment)	data: SE_Biomass\$`Ratio Fresh-Dry Root` by SE_Biomass\$Treatment t = 0.87008, df = 15.069, p-value = 0.3979 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -2.447041 5.825220 sample estimates: mean in group SE-M mean in group SE-S 10.22839 8.53930
t.test(Coloration ~ Treatment)	data: Col\$Coloration2 by Col\$Treatment t = 4.9895, df = 43, p-value = 1.048e-05 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval:

	0.2979049 0.7020951 sample estimates: mean in group SE-M mean in group SE-S 2.5 2.0
t.test(Total Fresh Shoot Biomass ~ Salt formation)	data: Biomass_Salt_formation\$`Fresh Total Biomass Shoot` by Biomass_Salt_formation\$Salt_formation t = 1.5145, df = 3.9465, p-value = 0.2054 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -2.710513 9.140263 sample estimates: mean in group 0 mean in group 1 13.46000 10.24512
t.test(Total Dry Shoot Biomass ~ Salt formation)	data: Biomass_Salt_formation\$`Dry Total Biomass Shoot` by Biomass_Salt_formation\$Salt_formation t = 0.91207, df = 1.6723, p-value = 0.4737 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -1.81877 2.58877 sample estimates: mean in group 0 mean in group 1 1.430 1.045
t.test(Total Fresh Root Biomass ~ Salt formation)	data: Biomass_Salt_formation\$`Fresh Total Biomass Root` by Biomass_Salt_formation\$Salt_formation t = 1.5456, df = 7.756, p-value = 0.162 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.4870791 2.4345791 sample estimates: mean in group 0 mean in group 1 3.87000 2.89625
t.test(Total Dry Root Biomass ~ Salt formation)	data: Biomass_Salt_formation\$`Dry Total Biomass Root` by Biomass_Salt_formation\$Salt_formation t = 1.9563, df = 3.8533, p-value = 0.1248 alternative hypothesis: true difference in means is not equal to 0 95 percent confidence interval: -0.1008361 0.5583361 sample estimates: mean in group 0 mean in group 1 0.53000 0.30125

Appendix 11 Raw data of the growth observations of *Cochlearia officinalis*

Table 14. The raw data of the shoot height, number of leaves, and leaf width of *Cochlearia officinalis* collected during the harvest of the plants.

Pot number	Pot ID	Treatment	Date of measurement: 25-05-2022					Date of measurement: 25-05-2022					Date of measurement: 25-05-2022						
			Shoot height (mm)					Number of leaves					Leaf width (biggest leaf) (mm)						
			1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
21	SS-M-8	S. soda-Mars																	
22	SS-S-5	S. soda-Sand	70	74	95	53	41	6	7	8	5	4	34	21	30	15	10		
23	SS-M-3	S. soda-Mars																	
24	SS-M-1	S. soda-Mars																	
25	SS-S-2	S. soda-Sand	46	120	34	51	62	4	8	5	5	6	9	34	13	16	15		
26	SS-M-6	S. soda-Mars																	
27	SS-S-7	S. soda-Sand	65	31	31	63	15	6	4	5	4	4	17	9	10	18	6		
28	SS-S-8	S. soda-Sand	68	63	56	34	50	5	5	5	5	5	17	15	15	10	13		
29	SS-M-9	S. soda-Mars																	
30	SS-S-6	S. soda-Sand	61	22	46	20	26	6	4	4	4	4	22	6	11	6	8		
31	SS-S-1	S. soda-Sand	31	51	41	18		4	7	4	3		8	24	12	4			
32	SS-M-2	S. soda-Mars	2																
33	SS-M-4	S. soda-Mars																	
34	SS-S-9	S. soda-Sand	28	41	5	39	32	5	6	2	5	5	10	10	3	10	9		
35	SS-M-10	S. soda-Mars	2	2															
36	SS-M-7	S. soda-Mars																	
37	SS-S-10	S. soda-Sand		40	54	63			5	4	7		12	15	26				
38	SS-S-4	S. soda-Sand	25	76	61	38		4	7	6	4		7	22	18	10			
39	SS-S-3	S. soda-Sand	32	78	65		38	3	7	8		4	7	27	29				
40	SS-M-5	S. soda-Mars																	

Table 15. The raw data of the fresh weight for both the shoot, weighted for each individual plant, and the root, collective of all the roots present in each pot, of *Cochlearia officinalis* weighted after the harvest of the above-ground and below-ground biomass.

Pot number	Pot ID	Treatment	Date of measurement: 27-05-2022						Date of measurement: 27-05-2022		
			Fresh weight - Above ground biomass						Fresh weight - Below ground biomass		
			1	2	3	4	5 Extra	Total	Total	Total	
21	SS-M-8	S. soda-Mars									
22	SS-S-5	S. soda-Sand	0,79	0,47	1,18	0,07	0,05	2,56	0,71		
23	SS-M-3	S. soda-Mars									
24	SS-M-1	S. soda-Mars									
25	SS-S-2	S. soda-Sand	0,05	1,59	0,04	0,15	0,17	2	0,81		
26	SS-M-6	S. soda-Mars									
27	SS-S-7	S. soda-Sand	0,28	0,11	0,07	0,2	0,01	0,67	0,16		
28	SS-S-8	S. soda-Sand	0,18	0,13	0,2	0,13	0,16	0,8	0,22		
29	SS-M-9	S. soda-Mars									
30	SS-S-6	S. soda-Sand	0,5	0,07	0,14	0,01	0,01	0,73	0,17		
31	SS-S-1	S. soda-Sand	0,1	0,68	0,19	0,07		1,04	0,31		
32	SS-M-2	S. soda-Mars	0,01					0,01	0,01		
33	SS-M-4	S. soda-Mars									
34	SS-S-9	S. soda-Sand	0,17	0,03	0,11	0,07	0,03	0,01	0,42	0,24	
35	SS-M-10	S. soda-Mars	0,01	0,01				0,02	0,01		
36	SS-M-7	S. soda-Mars									
37	SS-S-10	S. soda-Sand		0,14	0,23	0,71		1,08	0,27		
38	SS-S-4	S. soda-Sand	0,1	0,15	0,85	0,48		1,58	0,38		
39	SS-S-3	S. soda-Sand	0,06	0,96	1,2	0,15		2,37	0,51		
40	SS-M-5	S. soda-Mars									

Table 16. The raw data of the dry weight for both the shoot, weighted for each individual plant, and the root, collective of all the roots present in each pot, of *Cochlearia officinalis* weighted after drying the above-ground and below-ground biomass.

			Date of measurement: 30-05-2022					Date of measurement: 30-05-2022			
			Dry weight - Above ground biomass					Dry weight - Below ground biomass			
			1	2	3	4	5 Extra	Total	Total		
Pot number	Pot ID	Treatment									
21	SS-M-8	S. soda-Mars									
22	SS-S-5	S. soda-Sand	0,094	0,063	0,12	0,018	0,004	0,299	0,056		
23	SS-M-3	S. soda-Mars									
24	SS-M-1	S. soda-Mars									
25	SS-S-2	S. soda-Sand	0,009	0,176	0,02	0,027	0,025	0,257	0,12		
26	SS-M-6	S. soda-Mars									
27	SS-S-7	S. soda-Sand	0,043	0,001	0,006	0,028	0,001	0,079	0,021		
28	SS-S-8	S. soda-Sand	0,038	0,032	0,033	0,022	0,015	0,14	0,029		
29	SS-M-9	S. soda-Mars									
30	SS-S-6	S. soda-Sand	0,075	0,007	0,015	0,001	0,001	0,099	0,011		
31	SS-S-1	S. soda-Sand	0,009	0,078	0,013	0,001		0,101	0,02		
32	SS-M-2	S. soda-Mars	0,001					0,001	0,001		
33	SS-M-4	S. soda-Mars									
34	SS-S-9	S. soda-Sand	0,01	0,018	0,001	0,017	0,001	0,002	0,049	0,034	
35	SS-M-10	S. soda-Mars	0,001	0,001					0,002	0,001	
36	SS-M-7	S. soda-Mars									
37	SS-S-10	S. soda-Sand		0,024	0,023	0,094			0,141	0,032	
38	SS-S-4	S. soda-Sand	0,001	0,011	0,099	0,066			0,177	0,038	
39	SS-S-3	S. soda-Sand	0,001	0,111	0,132		0,013		0,257	0,01	
40	SS-M-5	S. soda-Mars									