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Testování aeroponického systému za účelem pěstování rostlin na Marsu

Diplomová práce

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Testování aeroponického systému za účelem pěstování rostlin na Marsu

Souhrn

Předmětem této diplomové práce bylo testování specifického designu vertikálního aeroponického systému, který byl v areálu České zemědělské univerzity v Praze původně vybudován v rámci projektu Marsonaut. Cílem projektu Marsonaut bylo zkoumat možnosti pěstování rostlin v tomto systému a jeho potenciální využití na planetě Mars.

V rámci experimentu se prováděl růstový test listového salátu ve výše zmíněném aeroponickém systému. Cílem bylo ověřit, zda je tento konkrétní design vhodný k pěstování listového salátu. Sazenice salátu byly předpěstovány v inkubátoru po dobu 10 dnů, poté jsme zaznamenali váhu a počet listů každé rostliny. Následně byly rostliny přesazeny do dvou identických aeroponických jednotek s identickými podmínkami. V každé jednotce bylo 24 rostlin. Výživa rostlin byla zajištěna prostřednictvím živného roztoku na bázi dvousložkového hydroponického hnojiva s přídavkem hydroponického hnojiva s obsahem mikronutrientů. Kořeny rostlin byly tímto roztokem ostřikovány v pravidelném intervalu. Po 9 dnech experimentu jsme byli nuceni přesadit rostliny do jedné aeroponické jednotky kvůli technickým problémům s druhou jednotkou. Po 23 dnech byly rostliny sklizeny, byla zaznamenána jejich váha a počet listů a při teplotě 70 °C byly po dobu 48 hodin sušeny. Následně byla zaznamenána váha sušiny kořenů a nadzemních částí.

Růstová data byla statisticky vyhodnocena. Rozdíl v růstu mezi oběma skupinami rostlin nebyl statisticky významný. Výnos salátů z našeho experimentu byl v porovnání s ostatními studiemi velmi nízký, což bylo pravděpodobně způsobeno technickými vlastnostmi aeroponického systému. Rostliny v jednotkách byly umístěny v nepřirozené poloze a délka ostřiku živným roztokem byla v porovnání s ostatními studiemi velmi krátká. Na základě tohoto srovnání jsme došli k závěru, že tento design aeroponického systému není vhodný pro pěstování listového salátu. Možnost jeho využití na planetě Mars je velmi nepravděpodobná vzhledem k nízkému výnosu a velmi náročné údržbě.

Na základě informací a zkušeností získaných v průběhu experimentu byl aeroponický systém nedávno přestavěn na horizontální verzi.

Klíčová slova: alternativní produkční systémy, hydroponie, aeroponie, živný roztok, listový salát

Testing of an aeroponic unit with the view of growing plants on planet Mars

Summary

The aim of this diploma thesis was testing of a specific design of vertical aeroponic system that was previously constructed at the Czech University of Life Sciences in Prague as a part of the Marsonaut project. The goal of the Marsonaut project was to research the possibilities of growing plants in the system and its potential use on the planet Mars.

Within the scope of the experiment a growth test of lettuce was performed in the aeroponic unit in question. The objective was to verify whether this specific design of an aeroponic unit is suitable for growing lettuce. The seedlings of lettuce were pre-grown in an incubator for 10 days, then we noted the weight and number of leaves of each plant. Afterwards the seedlings were transplanted into two identical aeroponic units with identical conditions. There were 24 plants in each unit. The nutrition for the plants was supplied by a nutrient solution containing a two component hydroponic fertilizer combined with a micronutrient-rich hydroponic fertilizer. The roots of the plants were sprayed with this solution periodically. Technical issues with one of the aeroponic units forced us to replant all the plants into one unit after 9 days of the experiment. All the plants were harvested after 23 days, their weight and number of leaves were noted and they were dried for 48 hours at a temperature of 70 °C. After that the dry weight of roots and shoots was noted.

The growth data were statistically evaluated. The difference between the growth of the two groups of plants was not statistically significant. Compared to other studies, the yield of lettuce was very low in our case which was probably caused by the technical properties of this aeroponic system. The plants were positioned in the units in an unnatural way and the duration of nutrient solution spraying was very short compared to the other studies. Based on the comparison we came to the conclusion that this design of an aeroponic system is not suitable for growing lettuce. Given the low yield and very difficult maintenance, the prospect of using this system on planet Mars is very improbable.

Based on the information and experience gathered during our experiment, the aeroponic system has been rebuilt to a horizontal version recently.

Keywords: alternative production systems, hydroponics, aeroponics, nutrient solution, lettuce

Table of Contents

1	INTR	ODUCTION	10
2	THE A	AIM OF THE THESIS AND HYPOTHESIS	11
3	LITER	ATURE REVIEW	12
	3.1 Pc	PULATION GROWTH IN THE CONTEXT OF FOOD SECURITY AND CLIMATE CHANGE	12
	3.1.1	Population growth	12
	3.1.2	Food security	12
	3.1.3	Food production and climate change	13
	3.2	L.3.1 Drought	14
	3.2 AL	TERNATIVE FOOD PRODUCTION SYSTEMS	15
	3.2.1	Hydroponics	16
	3.2	2.1.1 Hydroponic techniques/methods	16
		3.2.1.1.1 Standing Aerated Nutrient Solution	16
		3.2.1.1.2 Nutrient Film Technique (NFT)	17
		3.2.1.1.3 Ebb-and-Flow	18
		3.2.1.1.4 Raft/Deep Water Culture	19
	3.2.2	Aeroponics	20
	3.2	2.2.1 Aeroponics in Space	21
	3.2.3	Aeroponics system possibilities	22
	3.2.4	Nutrient solution	23
	3.2	2.4.1 pH	24
	3.2	2.4.2 Electrical conductivity	24
	3.2	2.4.3 Nutrient solution temperature	26
	3.2	2.4.4 Oxygenation	26
	3.2.5	Aeroponics in detail	26
	3.4 2.4	2.5.1 Nutrient solution droplet size	26
	3.4 2 ·	2.5.2 Pressure	/ ۲
	3.4 2 '	2.5.3 Electrical SoleTiolu	27 27
	3.2	2.5.4 Flessure switch	27
	3.2	2.5.6 Disadvantages	
л	METI	HODOLOGY	29
-			25
	4.1 IE	CHNICAL PROPERTIES	30
	4.1.1	Parts used in the deroponic unit	32
	4.1.2	Additional laboratory equipment	34
	4.2 CL		35
	4.2.1	Daily care methodology	37
	4.2.2	Harvesting and measurements	37
	4.2.3	Statistics	38
5	RESU	LTS	38
	51 Pi	ANT GROWTH	38
	5.2 RC	ροτ/ςμοοτ βάτιο	44
	5.2 NC		ΔΔ
~			44
6	DISC	JSSION	45
	6.1 GF	OWTH CHARACTERISTICS RESULTS	45
	6.2 Us	ING THE AEROPONIC SYSTEM IN SPACE	48
	6.3 Di	SADVANTAGES OF THE SYSTEMS AND PROPOSED TECHNOLOGICAL UPGRADES	49
7	CON	CLUSIONS	51
8	REFE	RENCES	52

1 Introduction

For the purpose of my diploma thesis I have decided to focus on a topic involving testing of an aeroponic system located at the Czech University of Life Sciences in Prague. My main interest was in the functionality of the specific technical design of an aeroponic unit previously built at the university, its drawbacks and its potential for growing lettuce.

I was further motivated to find out more about this modern growing method because I was under the impression that innovations in agriculture are not as fast and common as in the other industries. My personal opinion is that there should be much more interest in new agricultural technologies because the successful cultivation of crops is essential for the long-term survival of humankind.

Experts are often pointing out the problematic issue of sustainibility of conventional agriculture. One of the reasons is the extensive cultivation of monocultures on fields which requires using vast amounts of fertilizers, pesticides and other agrochemicals in order to have high yields. Not only it has a long-term negative impact on the soil fertility and quality, it can also harm the environment and health of people and animals because the agrochemicals can enter soil and subsequently also the produce. Because the agricultural chemicals are often toxic for non-target organisms, reduced biodiversity comes as a consequence of using them.

The conventional agriculture's ability to produce large yields of crops is highly dependent on climate conditions in a given area and the particular growing season. There is no doubt that the climate is changing and episodes of extreme weather (drought, flash floods, torrential rain, storms etc.) occur more frequently than in the past. This may endanger the steady large yields in conventional agriculture or the quality of crops. In extremity, it can result in a total loss of produce.

All this has led to my increased interest in alternative soil-less growing methods which can be used anywhere because they do not require any particular environmental conditions. They are most often placed in interior or exterior greenhouses, which makes them less prone to pest attacks. Since there is no soil in the systems, there is no problem with growing weeds, hence there are no herbicides being used. These systems usually use very low amount of water, which makes them suitable also for use in arid areas where growing crops conventionally is difficult or impossible.

Perhaps these alternative soil-less systems do not have potential to replace conventional agriculture at this point, but they could satisfy some part of the demand for local fresh produce – especially in big cities or in areas where conventional agriculture is problematic to use. Local use of these methods of growing crops may have a positive environmental impact because there is no need for long distance produce transportation, therefore there is less usage of fossil fuels. Considering the changing climate, it would be beneficial to dedicate the research to the innovations in alternative soil-less systems. Such systems might play an important role in the future of agriculture. Furthermore, they present a quality and safe produce for the people.

2 The aim of the thesis and hypothesis

The focus of this diploma thesis was testing of an aeroponic system located on the premises of the Czech University of Life Sciences in Prague through a growth test. The aeroponic system was previously built as a part of the Marsonaut project, which aimed at testing of crop growing technologies that could be possibly used in space and on the planet Mars. Since the system was not in a working conditions before our experiment, we also wanted to optimize the methodological approach of the aeroponic growing.

The experiment dealt with growing lettuce (*Lactuca sativa*) in a specific design of a vertical aeroponic unit in the Marsonaut laboratory. A part of this thesis is also dedicated to a final evaluation and proposition of technological upgrades that could be applied to this aeroponic system.

Hypothesis:

The specific design of a vertical aeroponic system built as a part of the Marsonaut project is a suitable cultivation method for growing lettuce.

3 Literature review

3.1 Population growth in the context of food security and climate change

3.1.1 Population growth

The predictions issued by the UN (United Nations) state that the world population will rise to approximately 8 billion by 2030 and more than 9 billion by 2050 (Gerland et al. 2014). The peak in population is projected to come slightly after the year 2100, when there should be around 10.9 billion people on Earth. From that point on, the population should stabilize or begin to decline (UN, 2019). However, these figures are predictions. Some researchers are questioning them - Keilman (2019) explains that the UN world population predictions have been erroneous in the past when the UN predicted higher population growth than it turned out to be. This error was caused by the birth rates falling stronger than was previously predicted.

The number of born babies per woman in the last 5 years has been low in western countries (1.61 in Europe, 1.75 in North America), on the other hand in sub-Saharan Africa the figure is 4.72 (Leridon 2020). Based on these fertility levels, the population increase could mainly take place in low income and developing areas of the world. The continent of Africa as a whole is expected to double its population from about 1 billion today to 2 billion by 2050 (UK Foresight 2011). This is likely going to put high pressure on the agriculture and food industries.

3.1.2 Food security

The definition of food security according to FAO (Food and Agriculture Organization) is following: "Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" (World Food Summit, 1996).

Thanks to the technological advances in the agro-industrial complex, the food production in the last century increased. However, given the high population growth and a shrinking supply of resources on one hand and complex problems like the climate change on the other hand, it is likely that it will not be enough to meet the growing demand (Prosekov & Ivanova 2016). According to FAO (2009), the food requirements will increase by 50 % by 2030 and by 80-100 % by 2050. In order to meet them, agricultural production in developing countries would need to double. Even at this moment, there are more than 820 million people experiencing hunger and another 2 million experiencing some level of food insecurity (FAO et al. 2019). In the year 2050, the shortage of food might be as high as 25 % (Prosekov & Ivanova 2016). According to McCarthy et al. (2018), simply increasing the world food production is unfortunately no longer a viable option. The reason for this is that while the world population is growing rapidly, the food production relies on natural resources, which are shrinking and finite.

Food security issues are not limited to developing countries. As of 2016, 1 in 7 Americans suffered from food insecurity. The EU report from 2013 says that 55 million

people were unable to "afford a meal with meat, chicken, fish (or vegetarian equivalent to meat) every second day.

In the past several years, the impacts of climate change have become a major threat to food security worldwide (Tripathi et al. 2016; Islam & Nursey-Bray 2017).

3.1.3 Food production and climate change

Climate change is a statistically significant difference in either the mean state of the climate or in its variability, continuing for a long period - usually decades or longer (VijayaVenkataRaman et al. 2012).

At this point, there is no doubt that the climate of planet Earth is changing. There is enough evidence to support this claim and it can be traced through the global temperature changes, rising of sea level as a consequence of melting glaciers, acidification of the oceans, high variability of precipitation and often events of extreme weather (Adger et al. 2005; Solomon 2007).

In 2017, the increase in global surface temperature has reached approximately 1 °C compared to pre-industrial levels. The pace of the increase was around 0.2 °C per decade (Allen et al. 2018). By the end of the 21st century, global temperature is projected to further increase (IPCC 2013), which would likely affect agricultural production (Singh & Singh 2017) – particularly lower the yields and increase food prices (Hatfield et al. 2014; Bandara & Cai 2014). During this period, the average growing season temperature for top ten world crops has risen by 0.5 - 1.2 °C. In western and southern Europe, yields of the dominant crops lowered by 6.3 - 21.2 % as a consequence of climate change. A similar pattern appears in other parts of Europe (Ray et al. 2019).

It is apparent that agriculture as an industry is highly sensitive to weather pattern changes which play a key role in the seasonal yields of the global crops (Howden et al. 2007). In southern Europe, where a big part of Europe's fruit supply comes from, high fluctuations of yields caused by episodes of extremely hot temperatures and drought are expected (EEA 2015). The impact of climate change might be even more severe in developing countries with a warmer climate (whose economy is often agriculture-based) because they are frequently exposed to extreme weather events. At the same time, these countries lack available finances that could be invested in adaptation methods (Parry et al. 2001; Tubiello & Fischer 2007; Morton 2007; Touch et al. 2016). Some of these developing countries have adopted a new approach to agriculture called "Green Revolution" in the 1960s, which presented new technologies in order to increase yields of local farms (Shiva 1991). Although these measures helped to boost the agricultural production, they have been widely criticized. The large volumes of used agrochemicals, fossil fuels for heavy machinery and natural resources combined with growing high-yield varieties of plants in monocropping have been blamed for pollution, loss of biodiversity, land degradation and farmers' health issues have been reported (Phungpracha et al. 2016).

It is important to realize that while climate change definitely affects agriculture, agriculture itself plays a part in climate change. This is mostly by the emissions of greenhouse gases (GHG) – particularly methane (CH₄) which comes from livestock, nitrous oxide (N₂O)

which is released by the use of both mineral and organic nitrogenous fertilizers and carbon dioxide (CO₂) (Tellez-Rio et al. 2017). In 2015, agriculture in the European Union contributed approximately 10 % to the total GHG emissions. On the other hand, thanks to a reduction in the use of nitrogenous fertilizers, GHG emissions from agriculture declined by 20 % between 1990 and 2015 (EEA 2017).



Figure 1: Correlation between agriculture and climate change (Singh & Singh, 2017)

According to Hart et al. (2017) we can expect higher biological stress in the plants caused by heat (especially in southern and western parts of Europe), damaged crops and limited fertility of the arable land.

3.1.3.1 Drought

Approximately 70 % of water consumed by humans is used for agriculture, with even a greater percentage in arid areas of the world. Water is often lost due to poor water management, which leads to salinisation and land degradation (Maggio et al. 2015).

Drought has become a serious problem for conventional agriculture in the last years and together with salinisation accounts for reductions in crop yields (Cattivelli L. et al. 2008; Pardo J.M. 2010). As Eslamian & Eslamian (2017) say, drought often goes without much public attention because of its slow effects, but the impacts may be grave - decline in groundwater supplies, depletion of water resources leading to scarcity of both irrigation and drinking water, reduction in agricultural production and soil productivity, food insecurity etc. Furthermore, there are also indirect impacts, e.g. poverty, mass migration and social changes. For example, Matiu et al. (2017) proved that consecutive years with dry and warm seasons had a negative effect on crop yields, including maize and wheat. We have witnessed the damaging drought in the last years directly in our countries in Europe. In 2018, there was a 50 % yield reduction (with respect to the previous 5 years mean) in the main crops caused by drought in central and northern Europe. Climate projections suggest that these drought conditions we experienced in 2018 could become a common occurrence as early as 2043 (Toreti et al. 2019). Therefore, a widespread consensus exists that an approach called "sustainable intensification" should be used, meaning increasing food production while at the same time minimizing the environmental impacts caused by such increase (Davis et al. 2016).

3.2 Alternative food production systems

Since the increase in the area available for cropping has been low over the last three decades and the population growth high, the available cropland per person has shrunk from around 0.4 ha in 1960 to less than 0.25 ha in 2015. Furthermore, the estimates show that the area of arable land will increase by only 4,3 % by 2050 (Maggio et al. 2015).

The cumulation of the factors mentioned above might have a negative effect on the availability of food for the world population (Gwynn-Jones et al. 2018). This prompted intensive scientific research focused on increasing the area for food production and led to an evolution of a new kind of agriculture called "closed environment agriculture", which enables growing plants in a closed system (Jensen 2002). More recently these systems have been called "controlled environment agriculture". These systems allow to set the individual parameters – such as lighting intensity and period, ambient temperature etc., so that the conditions can be optimized for ideal growth and the nutrient intake efficiency can be improved (Chen et al. 2020). All this allows for the cultivation of plants even outside of their natural season, furthermore there can be several growing cycles in one year (Sabir & Singh 2013). The main advantage of these closed systems is that they can be used in areas which are not suitable for field agriculture (e.g. arid areas like parts of Africa and Asia), as well as in cities, which usually do not have enough space for conventional agriculture (Gwynn-Jones et al. 2018). The closed environment agriculture systems are particularly hydroponics, aeroponics and aquaponics.

Conventional agriculture systems need a large volume of water and fertilizers in order to produce high yields of crops, and soil plays a crucial role. Hydroponics, aeroponics and aquaponics are systems which use a growing medium instead of soil, mostly for supporting the roots. This can be for example sand, gravel, perlite, rockwool, pine bark, coconut fibre etc. (Jones Jr. 2004). The nutrients necessary for proper growth of the plants are brought in nutrient-rich water solution (Bridgewood 2003). The fact that these systems do not use soil can be a big advantage in areas which lack fertile soil or where the soil has degraded to such an extent that it can no longer be used for cultivating plants. Furthermore, much less water is needed to nourish the crops, which is perhaps the biggest advantage considering the challenges that the world is facing like climate change, changing precipitation patterns, soil degradation, rising temperatures, food security issues etc. Because these food production technologies can be used in a practice called vertical farming where plants grow in various levels above each other, the yield per square meter can be higher than in conventional agriculture (Marginson 2010).

3.2.1 Hydroponics

Although some might think that hydroponics is a new technology of growing food, it has been known for a long time. Some kind of hydroponic techniques can be traced back to ancient Babylon and its Hanging Gardens or to Mexico, where the Aztecs used to grow food in "floating gardens" (Jones Jr. 2004). Hydroponics became popularized in the 1930s in the USA and was even used during the World War II by the U.S. Army, which set up several hydroponic gardens on some of the islands in Pacific Ocean in order to supply fresh vegetables to the soldiers in the area (Eastwood 1947).

According to Jones Jr. (2005), hydroponics refers to a technique in which plant roots are suspended in either a static, continuously aerated nutrient solution or a continuous flow or mist of nutrient solution.

3.2.1.1 Hydroponic techniques/methods

In the following pages, some of the most commonly used methods of hydroponics are presented. While the foundation of these hydroponic methods is the same, the technological approach differs.

3.2.1.1.1 Standing Aerated Nutrient Solution

This is the oldest and a very simple hydroponic technique which was actually primarily used in the 1840s to investigate the effect of various substances on the plant growth (Russell 1950). It consists of some kind of container or vessel in which a plant is placed with its roots suspended in the nutrition solution. The solution is aerated by a pump in order to bring oxygen to the roots as well as stirring the solution. The solution is to be replaced every 5 to 10 days and water lost due to evaporation has to be replaced by either pure water or a diluted nutrient solution (Jones Jr. 2004)



Figure 2: A scheme of a standing aerated nutrient solution hydroponic system (Jones Jr. 2004)

3.2.1.1.2 Nutrient Film Technique (NFT)

Nutrient Film Technique (NFT) represents a method where the roots of the plants are kept coated in the nutrient solution without using any growing medium. NFT is considered to be one of the most popular techniques for commercial hydroponics because it offers the advantage of growing a lot of plants in a relatively small area (Baras 2018). There are usually troughs put on a slope of 0.3 - 2 % stacked vertically with drilled holes that house plastic holders with plants in them. Roots of these plants are submerged in troughs and nutrient solution is flowing through at the desired rate. Troughs can be shaped in a certain way to allow better distribution of the roots so that they can get as much nutrients from the solution as possible. The solution is pumped from the storage tank to the top of the system. From there it flows down the sloped throughs by gravity. The material used for troughs is usually polyethylene liner, polyvinyl chloride, polypropylene or coated metal. Whichever of these materials is used, it has to be opaque to keep the light out. If light entered the trough, the growth of algae in the system would become a problem. Additionally, it should be UV resistant and have good structural strength (Jones Jr. 2004). It is important to keep the flow rate fast enough so that even the plants at the bottom of the system can get enough nutrients through the roots. The speed of the flow in the system should be around 1-2 L/min (Thorarinsdottir 2015). It is crucial to take measures to ensure that the roots are never left without water and nutrients. If there is an issue and the flow of the nutrient solutions stops, the roots wither very quickly (Raviv et al. 2019).



Figure 3: A scheme of nutrient film technique hydroponics (source: Green and Vibrant website)

3.2.1.1.3 Ebb-and-Flow

This method consists of a storage tank with the nutrient solution and grow bed filled with an inert grow media such as gravel or sand. The grow bed is periodically flooded with the nutrient solution and once it reaches a certain level, it is drained through a siphon back into the storage tank, which is placed right under the grow bed (Jones Jr. 2004).



Figure 4: A scheme of ebb-and-flow hydroponics (source: Green and Vibrant website)

3.2.1.1.4 Raft/Deep Water Culture

This hydroponic method uses storage tanks which are filled with the nutrient solution. On the surface of the water are floating rafts made of some kind of lightweight material (e.g. polystyrene) to ensure they float. There are holes in the rafts which house plants in holders. Their roots are submerged in the nutrient solution at all times to ensure that plants are properly nourished. There are air pumps places in the storage tanks in order for the roots to receive enough oxygen. This method is often used commercially because of its simplicity and easy maintenance (Thorarinsdottir 2015).



Figure 5: A scheme of raft/deep water culture hydroponics (source: Superior Organics website)

3.2.2 Aeroponics

Aeroponics is a soilless food production technique based on hydroponics, where the plants are growing with their roots suspended in the air in an enclosed chamber with 100% available moisture. The nutrients are supplied to the plants by a nutrient solution, which adsorbs on the plant roots in the form of mist or a very fine spray (Lakkireddy et al. 2012; Lakhiar et al. 2018; Li et al. 2018). The growth of plants in aeroponics is very good thanks to the highly aerobic environment in the root zone, where the roots have access to 100 % of the available oxygen in the air (Buckseth et al. 2016). The very high humidity and aeration of the root zone result in an increased metabolic rate and plant growth (Chiipanthenga et al. 2012).

Researchers came up with the idea of aeroponics after observing plants growing near waterfalls. They realized that even though the plant's roots are openly hanging in the air the plants grow successfully. Therefore, growing plants in the air under the spray mist condition was a logical extension (Lakhiar et al. 2018).

The practice of aeroponics has been increasingly used, especially for crops where roots are harvested as the end product. It has also been used to cultivate various vegetable crops such as lettuce, tomato, herbs, cucumber, melon and floral crops (Li et al. 2018). It has proved to be useful in highly arid areas because it uses a very small quantity of water. It has been reported that aeroponics uses 95 % less irrigation water than conventional agriculture (Darling 2019). It has been recommended as a plant growing technique for countries lacking arable land, facing rapid environmental changes and being challenged by food security issues (Sardare et al. 2013). Similarly, it can be a good option for very small countries with a big population that do not have any space for agriculture (Lakhiar et al. 2018). Aeroponic systems have been successfully used to produce seed potatoes in India (Bag et al. 2015), Ethiopia (Tessema & Dagne 2018), China (Wang et al. 2017), Rwanda (Khadka et al. 2018), seed

yams in Ghana (Oteng-Darko et al. 2017) and elsewhere. The prospect of using a hypogravity aeroponic technology for growing food during space missions has been studied by NASA scientists (Clawson et al. 2000).

3.2.2.1 Aeroponics in Space

Experiments focused on growing plants in space have been performed for more than 40 years (Zabel et al. 2016). For example, there was an experiment done in the early 90s on board of the MIR Soviet space station (Maggi & Pallud 2010). The systems conditions usually relied on some analogue medium to the soil, such as arcillite, to secure nutrients for the plants. If these systems were to be used in space (e.g. on ISS) they would require lots of room in the spacecraft (Moffatt et al. 2019). Also, these mediums have substantial weight. As Maggi & Pallud (2010) explain, hydroponics and aeroponics have a big advantage over soil or soil analogue medium systems because they are much lighter and can be very much automated to control nutrient uptake and water usage. In 2014, NASA's Aerospace Engineering Team cooperated with the Biosystems Engineering Team in research and design of an aeroponic system that would supplement the diet for a four-man crew of the mission to planet Mars for at least 500 days (Bennett et al. 2014). NASA also developed a prototype of a lightweight inflatable aeroponic system that could be used in space. Its advantage is that when deflated, it does not take up much space, therefore might be ideal for space missions. NASA claims it is able to produce 1000 bunches of lettuce, herbs and vegetables in around 25 days (NASA).

There have been studies that dealt with the possibility of growing plants in the Martian soil simulants. Wamelink et al. (2014) grew different types of plants including field crops in a Martian soil simulant, Moon soil simulant and Rhine river soil as a control. While the latter Moon simulant not perform very well and many plants grew only very small or died, the Martian simulant performed surprisingly good and had the highest biomass production. It even outperformed the Rhine river soil, which was very nutrient poor. The authors debated that such a good performance compared to the other soils might be caused by the fact that the Martian simulant held water better than the other two. While the results looked very promising, the authors pointed out that there is a lack of reactive nitrogen and on Mars which is neccessary for the growth of plants. There is also a question of the representativeness of the used soil simulants. Leshin et al. (2013) add that low amount of organic matter in the Martian soils discovered by the Mars Curiosity rover presents another problem for a good growth of plants. Wamelink et al. (2005) think that the high pH of Martian soils ranging from 8 to 9 might be problematic for many plant species. However, the biggest issue for growing plants on Mars seems to be the limited availability of liquid water (Möhlmann 2004). Peyrusson (2021) studied the growth of mint (Mentha spicata) in Martian soil analog consisted of sand and clay-rich material collected in the vicinity of the Mars Desert Research Station in Utah. He discovered that the growth of mint in clay-rich soil analog was very limited which was likely caused by the nutrient poor and too alkaline environment. However, when he supplemented the Martian soil analog with a hydrogel polymer (which is able to hold large quantities of water) the overall growth of mint improved.

Given the uncertainties about the possibility of growing plants in the Martian soil and the availability of liquid water, it appears that some kind of a growing system for plants would have to be brought from Earth and assembled on Mars, at least in the early stages. Because water would be an essential commodity on Mars or in space, such growing system would need to use very small amounts of water and ideally be able to recycle it. Aeroponics seems to be one of the options. For example, Rahaim & Czysz (2008) presented a detailed study design of a deployable aeroponic greenhouse that could be used for a mission to Mars.

3.2.3 Aeroponics system possibilities

The design of an aeroponics system can differ based on the requirements of the grower, but normally it consists of a closed chamber made of material such as polystyrene, metal, plastic etc. The chamber might be lined with a black polysheet to ensure darkness in the chamber and optimal humidity. There are holes made in the growing table and plants are put in these holes supported by a foam ring. This helps to hold the stem of the plant and also to separate the root zone (at the bottom) from the upper part (at the top). The nutrient solution is stored in a tank and PVC or other material pipes distribute it to the nozzles. The pipes are connected to a water pump which pumps the nutrient solution at high pressure. The high pressure is used to atomize water through a small orifice to deliver a fine mist to the roots (Darling 2019). Atomization is a method of breaking up molecules of liquid into fine droplets (Avvaru, et al. 2006). The roots are sprayed in a set time interval for a set duration, which is controlled by a control unit through a timer connected to the water pump. The spacing between each nozzle, pressure in the system, the spacing between plants, the interval of spraying and the duration of spraying can all vary and depend on the scale of the aeroponic unit as well as the cultivated crops. As the nutrient solution sprays the plant roots, small droplets accumulate on the roots. All the excessive nutrient solution drips from the roots and through some kind of piping goes back into the nutrient solution tank, where it is recycled (Sharma et al. 2018). In aeroponics, plants can be grown either horizontally or vertically.



Figure 6: A typical design of a horizontal aeroponic system (source: Genesis India website)

3.2.4 Nutrient solution

Hydroponic systems require relatively pure water. There are many substances present in water that can affect the growth of the plants. In some parts of the world, it can play a major role due to its content of organic and inorganic substances. Natural wells near agricultural areas may contain pesticides, herbicides or other chemicals, which can have a negative impact on the grown plants. Water sources from natural aquifers might have high concentrations of certain chemical elements and therefore using such water for the preparation of nutrient solution would impact the final content of those elements in the solution. Water samples should always be taken and laboratory analysis should be done before using any source of water in hydroponics (Jones Jr. 2014). Proper water analysis will also help to determine the content of the essential elements necessary for the plants. This should be taken into account during the preparation of the nutrient solution - e.g. if there is 10 ppm of Ca in the raw water, the formulation of the nutrient solutions has to be adjusted accordingly (Resh 2013). It is a common practice to use reverse osmosis in order to get distilled/deionized water, which is then used to prepare the nutrient solution.

In hydroponics, all the essential elements for proper growth of the plants are supplied by fertilizer salts dissolved in water, which makes the nutrient solution. Only highly soluble salts can be used in hydroponics because they must remain in the form of solution to be available to the plants (Resh 2012). The essential elements are present in the form of ions and when the plant roots are in contact with the nutrient solution, uptake of these ions into the plant takes place (Resh 2002). In general, the plants require 14 essential elements. Six macronutrients - nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, and eight micro nutrients - iron, manganese, zinc, boron, copper, molybdenum, chloride, nickel (Mattson & Peters 2014). However, not all the plants have the same requirements for the ratio and concentration of the elements in the nutrient solution. Therefore hydroponic growers have to experiment with various nutrients solutions in order to get the maximum utilization of the nutrients by the plants, while trying to achieve a yield as high as possible (Ghehsareh et al. 2011). There are numerous published nutrient solution formulations with the definition of the desired concentrations of elements based on a specific crop (Hosseinzadeh et al. 2017).

References	Ca	Mg	Na	K	N	N	Р	S	CI	Fe	Mn	Cu	Zn	в	Mo
					(NH4 ⁺)	(NO ₃ ⁻)	(PO4 ³)	(SO4 ²)							
Hoagland and Arnon (1938)	160	48	-	254	14	203	31	64	-	0.6	0.5	0.02	0.05	0.5	0.01
Hoagland and Arnon (1950)	224	49	-	232	-	242	31	113	-	-	0.5	0.02	0.48	0.45	0.0106
Hoagland and Arnon (1950)	179	49	-	230	12.6	220	24	113	-	7	0.05	0.02	0.48	0.45	0.0106
Steiner (1961)	33	20	-	514	-	184	25	39	_	2.5	2.0	0.02	0.01	0.5	0.05
Hewitt (1966)	160	36	31	156	-	170	41	48	-	5.6	0.6	0.06	0.07	0.5	0.05
Cooper (1979)	185	50	-	300	-	236	60	68	-	12	2.0	0.1	0.1	0.3	0.2
Johnson (1980)	85	25	_	138	-	105	33	33	-	2.3	0.26	0.01	0.024	0.23	0.007
Steiner (1984)	180	48	-	273	-	168	31	336	-	4.0	0.62	0.02	0.11	0.44	-
Barry (1996)	70-200	15-80	-	150-400	-	70-250	15-80	20-200	_	0.8-6.0	0.5-2.0	0.05-0.3	0.1-0.5	0.1-0.6	0.05-0.15
Jones (1997)	100-200	30-70	-	100-200	-	100-200	30-50	-	_	2-12	0.5-2.0	0.1-1.0	0.05-0.10	0.2-0.4	0.05-0.20
Yuste and Gostincar (1999)	50-500	22-484	-	65-993	14-33	47-284	4-448	32-640	-	20	0.1-1.67	0.005-0.15	0.05-0.59	0.1-1.0	0.001-2.5

Table 1: Composition of some of the commonly used nutrient solutions in hydroponics (mg/L) (Hosseinzadeh et al. 2017)

In modern hydroponics, the nutrient solution is being recirculated in the system and then collected for reuse up to the point when the plants have taken all the nutrients from the solution. The solution needs to be monitored and analyzed for the nutrient content. If needed, nutrients have to be added (Jensen 1997). Any lost water has to be replaced and at the same time, pH has to be maintained at the desired level. One of the disadvantages of a closed system is that any disease or any unwanted organism will recirculate in the system and potentially spread through the root system unless it is somehow removed or inactivated (Jones Jr. 2004).

3.2.4.1 pH

One of the important parameters of the nutrient solution used in hydroponic systems is pH. It measures the acidity or alkalinity of the solution and is determined by the concentration of acids and bases in it (Hosseinzadeh et al. 2017). The proper nutrition of the plants highly depends on the pH of the nutrient solution because it determines which nutrients are available to the plants, as they can only uptake certain ions with a specific pH range (Clark 1982). The desired pH range for most of the plants is between 5.5 and 7. Optimum pH range for some of the crops grown in hydroponics is shown in Table 2 on the next page. If the pH is not at a level it should be, the plants lose their ability to uptake the essential elements, which affects their growth (Hosseinzadeh et al. 2017). If the pH drops below 4.0, the availability of potassium (K), sulfur (S), calcium (Ca), magnesium (Mg) and phosphorous (P) to the plant can be reduced (Marschner 1995). If it rises above 7.0, the availability of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and phosphorous (P) can be reduced (Woodward et al. 1979).

The pH range suggested for most hydroponic nutrient solutions is between 5.8 and 6.5 (Hosseinzadeh et al. 2017), and most of them, when prepared, will have a pH between 5.0 and 6.0 (Argo & Fisher 2002). If pH needs to be adjusted, it can be done by adding an acid or a base (De Rijck & Schrevens 1997). pH of the nutrient solution should be monitored every day, for example using a "pen" type pH meter, which has an accuracy within 0.1 pH unit (Resh 2013). Understanding the pH of the nutrient solution might be very useful for diagnosing possible nutrient deficiencies (Baras 2018).

3.2.4.2 Electrical conductivity

Another important paremeter of the hydroponic nutrient solution is EC – electrical conductivity. It is a measure of the concentration of the nutrient solution through its ability to conduct electricity (Resh 2013). Mattson & Peters (2014) explain that it represents a sum of the total dissolved salts in water, including any unwanted contaminants (e.g. sodium) – therefore it can be viewed as the water's purity. For example, "kitchen salt" - NaCl - can be broken down to cation Na⁺ and anion Cl⁻. These ions conduct electricity, which means that the higher the content of ions in the nutrient solution, the higher the EC gets. It is important to measure the EC in hydroponic systems. If it gets too high, it may induce osmotic stress in the

plants, ion toxicity or nutrient imbalance. On the other hand, low EC suggests nutrient deficiencies, which usually cause worse growth of the plants (Singh & Bruce 2016). It has been a practice for hydroponic growers to filter the water used for preparing the nutrient solution, often by using reverse osmosis, which purifies the water (Mattson & Peters 2014). When preparing the nutrient solution, it is usually necessary to use an instrument to measure the EC called EC meter, because some fertilizer salts conduct electricity better than others. For example, ammonium sulfate conducts nearly twice as much electricity as calcium nitrate and more than three times that of magnesium sulfate, whereas urea does not conduct electricity at all (Hosseinzadeh, et al., 2017). Hence EC only gives us the general idea of the overall total dissolved salts in the nutrient solution. In order to determine the exact levels of each element, an atomic absorption analysis would have to be done (Resh 2013).

An ideal EC is crop specific and also depend on environmental conditions (Sonneveld & Voogt 2009). Most nutrient solutions have relatively low EC when prepared (< 3.0 dS/m). EC should be monitored frequently. If large quantities of water are removed from the solution due to evapotranspiration and are not replaced immediately, the EC of the nutrient solution rises (Jones Jr. 2014) and may have a damaging effect on the plants, as described above.

Crops	EC (mS/cm)	pН
Asparagus	1.4 to 1.8	6.0 to 6.8
African Violet	1.2 to 1.5	6.0 to 7.0
Basil	1.0 to 1.6	5.5 to 6.0
Bean	2.0 to 4.0	6.0
Banana	1.8 to 2.2	5.5 to 6.5
Broccoli	2.8 to 3.5	6.0 to 6.8
Cabbage	2.5 to 3.0	6.5 to 7.0
Celery	1.8 to 2.4	6.5
Carnation	2.0 to 3.5	6.0
Courgettes	1.8 to 2.4	6.0
Cucumber	1.7 to 2.0	5.0 to 5.5
Eggplant	2.5 to 3.5	6.0
Ficus	1.6 to 2.4	5.5 to 6.0
Leek	1.4 to 1.8	6.5 to 7.0
Lettuce	1.2 to 1.8	6.0 to 7.0
Marrow	1.8 to 2.4	6.0
Okra	2.0 to 2.4	6.5
Pak Choi	1.5 to 2.0	7.0
Peppers	0.8 to 1.8	5.5 to 6.0
Parsley	1.8 to 2.2	6.0 to 6.5
Rhubarb	1.6 to 2.0	5.5 to 6.0
Rose	1.5 to 2.5	5.5 to 6.0
Spinach	1.8 to 2.3	6.0 to 7.0
Strawberry	1.8 to 2.2	6.0
Sage	1.0 to 1.6	5.5 to 6.5
Tomato	2.0 to 4.0	6.0 to 6.5

Table 2: Optimal range of EC and pH values for hydroponic crops (Singh & Bruce 2016)

3.2.4.3 Nutrient solution temperature

Studies have been done to examine the effect of nutrient solution on the growth of the plants. For example Nxave et al. (2009) have studied the growth of spinach seedlings irrigated with 3 different temperatures of water. In other study, Falah et al. (2010) observed the rates of water and nutrients uptake by tomato plants. As Jones Jr. (2014) explains, the temperature of the nutrient solution should never be lower than the temperature of the ambient air because it can cause biological stress in the plants and wilting. At the same time roots placed in cold water cannot uptake enough water and essential elements to meet the demands of the upper parts of the plant. When the plant is repeatedly exposed to these conditions, it results in slower growth, poor fruition and delayed maturity. The nutrient solution can be warmed before being pumped into the system, if necessary.

3.2.4.4 Oxygenation

Proper root oxygenation is important in hydroponics as it affects the root function, particularly water and nutrient uptake. Many of the hydroponic systems use air pumps in the storage tanks with the nutrient solution to saturate it with oxygen. However, Jones Jr. (2014) questioned the value of such measure. According to him, although bubbling air through the nutrient solution in an open environment will add some oxygen to it, but especially in NFT systems, it is difficult to keep the O_2 level for all the plants. While plants at the top of the system will benefit from the added O_2 , in the lower troughs of the system there will be little O_2 left for the plants. The aeroponic systems are founded on the principle of an abundant supply of oxygen for the roots and in this way aeroponics presents some advantage over certain hydroponic technologies, e.g. NFT (Lakhiar et al. 2018). Nichols (2002) studied that the extent of oxygenation of the nutrient solution also depends on its temperature. In general, the warmer the nutrient solution is, the less oxygen it contains.

3.2.5 Aeroponics in detail

3.2.5.1 Nutrient solution droplet size

The size of the nutrient solution droplets plays a crucial role in aeroponics. If the droplets are too big, it brings less oxygen to the roots and may affect their growth. On the other hand, if they are too fine, the plants produce excessive root hair but do not develop lateral roots needed for sustained growth (Margaret 2012). According to Lakhiar et al. (2018) the ideal droplet size for most of the crops is between 30 and 100 microns (0.03 - 0.1 mm). Darling (2019) puts the optimal size of the droplets at 50 microns (0.05 mm).

The size of the droplets depends on the diameter of the atomization nozzles. Nozzles with an orifice diameter of 0.635 mm under the operating pressure of 80 psi (5.5 bar) produce 5 - 50 microns large droplets (Lakhiar et al. 2018) which should be optimal. The nozzles are

prone to get clogged by the mineral deposits in water (Darling 2019). In order to avoid to clogging, mesh filters are usually used (Lakhiar et al. 2018).

3.2.5.2 Pressure

Pressure in the aeroponic system is very important and affects the size of the droplets. There are low-pressure systems which rely on a simple fountain pump which pumps water through the nozzles resulting in more of a light spray than mist. They usually spray the roots on a 24/7 basis. This option is inexpensive and suitable for a home made DIY (do it yourself) kind of system. For the real mist, professional water pumps being able to generate a pressure of 60 - 90 psi (4.1 - 6.2 bar) should be used in the system (Darling 2019).

Additionally, a pre-pressurized accumulator tank is usually used in the system to help keep the pressure in the piping. This tank is placed between the water pump and the nozzles. It has a rubber bladder that can either contract or expand according to the actual water pressure in the system. It creates two chambers in the tank – one filled with liquid (in our case nutrient solution) and other filled with pressurized air. The main purpose of the accumulator tank is to maintain enough pressure in the system. Once the water pump starts pumping the nutrient solution, there is already a pre-pressurized nutrient solution in the accumulator tank which moves straight to the nozzles. If the accumulator tank was missing from the system, once the water pump turned on, there would be a brief period when the pressure would not be high enough to produce small enough droplets (Darling 2019).

3.2.5.3 Electrical solenoid

An electrical solenoid is electronically operated shut off valve connected to the timer which begins and ends the flow of the nutrient solution in the system (Darling 2019).

3.2.5.4 Pressure switch

A pressure switch controls and maintains the pressure in the water pump. It is possible to set when it should switch on and off. Once it reaches the set value for maximum pressure, it turns off the electricity to the water pump. Similarly, if the pressure reaches a set minimum value, it turns on the electricity to the water pump, which pressurizes the system.

3.2.5.5 Lighting

In indoor plant cultivation without a natural light source, light energy is a significant factor for the plants (Naoya et al. 2008) because it affects the concentrations of

phytochemicals in plants (Kopsell et al. 2008; Perez-Balibrea et al. 2008). Light delivers energy to the plants as well as it provokes some physiological and morphogenesis responses in the plants, therefore its intensity and quality is important (Rajapakse et al. 1992; Fukuda et al. 2008; Li et al. 2009). The colour of the used light has to be considered as plants need a different spectrum of light in each phase of their growth. The other parameter is luminous intensity - Darling (2019) reports that for vegetative growth, it should be anywhere between 15 000 and 20 000 lx, for flowering between 35 000 and 40 000 lx. However important light is to proper growth of plants, similarly important are periods of dark in order for the plants to respirate, which is a very important part of their growth. The optimum photo periods are different depending on the species (Darling 2019). It seems that light-emitting diodes (LED) are a good source of light in indoor production systems. Mori et al. (2002) observed high photosynthesis and growth rate in plants growing under LED lights. They have a narrow spectrum of light, produce only a little heat and their operation does not consume large amounts of electricity (Kobayashi, et al., 2013). According to Darling (2019), the blue LED with a wavelength around 400 nm are suitable for the vegetative growth, while the red LED with a wavelength around 600-640 nm are suitable for flowering and fruition.

3.2.5.6 Disadvantages

While aeroponics has many advantages over conventional agriculture, there are some disadvantages as well. The irrigation system pumping water into the chambers in the form of mist must work. If it stops working (e.g. when the electricity goes out) the roots will dry out and desiccate very fast. If the nozzles get clogged and stop producing the fine mist, the same thing can happen to the roots. Therefore it is important to periodically clean the mineral deposits in the nozzles in order for them to work correctly. In conventional agriculture, soil works as a buffer and if there is an imbalance of nutrients or lack of water, plants are still able to survive for a while. In aeroponics there is no buffer. If anything goes wrong with the irrigation, the plants die very quickly. Same as hydroponics or aquaponics, aeroponics is very dependant on electricity – not only the water pumps but also the lighting, which is used indoor where there is no natural light. However, this drawback can be very well solved by using renewable energy sources, e.g. solar power (Darling 2019).

4 Methodology

We have performed a growth experiment in the aeroponic laboratory located on the premises of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences. The laboratory was established as a part of the Marsonaut project with the aim to study how plants could be grown on planet Mars with a limited supply of water. The laboratory consisted of 6 identical aeroponic units. Other equipment in the lab was reverse osmosis water system, 2 plant growth incubators for seedlings preparation, air-conditioning unit, fans and LED panel lights.

Firstly, it was necessary to get the aeroponic units to work because only one unit was in working conditions. The other ones had issues with water pumps and the overall pressure in the system, which was too low for the units to operate correctly. At the end we managed to get two units to work.

Before starting the experiment, we ran a pre-test to check how well the systems perform. We cleaned the whole system and set up and positioned the LED panel lights. The pre-test lasted 10 days.



Figure 7: The aeroponic units in the Marsonaut laboratory (source: Ábíčko website)

4.1 Technical properties

This chapter contains a technical scheme of the aeroponic units we had at hand. The Marsonaut lab consisted of six independent identical aeroponic units. They were vertical high-pressure aeroponic systems, each had a capacity of 72 plants.



Figure 8: Technical scheme of the vertical aeroponic unit we used during the experiment (source: Michal Najman)

Figure 8 shows the technical scheme of the aeroponic units. The unit is separated into two chambers, the bottom one contains the nutrient solution tank, high-pressure water pump, compressor, pre-pressurized accumulator tank and all the piping. The main pipe leads into the top chamber where it turns into a rectangular shape circuit pipe with eight nozzles. There are ventilators in the top chamber which turn on once the nutrient solution is sprayed from the nozzles and help spread the mist through the whole chamber. All the excessive nutrient solution that drips from the roots is collected at the slightly inclined bottom of the chamber. There is a strainer mesh filter in the center that filters any solid material from the nutrient solution. The nutrient solution drips through the filter back to the nutrient solution tank located below.



Figure 9: Spraying nozzles inside the top chamber of the aeroponic unit (source: own photo)

The top chamber is covered with a plastic growing table with circular holes for the plants, as shown in Figure 10. There are LED panel lights pointed at each of the unit. The LED lights are suspended from the ceiling and secured on strings. They can be adjusted using pulleys.



Figure 10: LED panel pointed at the plant table (own photo)

4.1.1 Parts used in the aeroponic unit

Nutrient solution tank

For the nutrient solution, we used a simple plastic box with quite high walls to ensure that the nutrient solution dripping from the strainer filter would stay in the box.

Piping

Standard PVC plumbing piping was used in the units.

Nutrient solution heaters/aerators

In order to maintain a appropriate temperature of the nutrient solution, we used an aquarium heater Platinum Heater 100 by a manufacturer Aquael. It is possible to regulate the temperature between 20 and 33 °C.

Conventional aquarium stone aerators were used in order to keep the nutrient solution aerated. They also help to maintain a movement of the nutrient solution so that the nutrients are properly mixed.

High-pressure water pump

A 12V Pentair Shurflo heavy duty high-pressure water pump was used in the aeroponic units. It generates pressure up to 100 psi and it is able to pump up to 6.8 L of water per minute.

There was a flow-through mesh filter placed after the water pump to filter any solid material from the nutrient solution.

Pre-pressurized accumulator tank

A pre-pressurized accumulator by a manufacturer Shurflo was used in the aeroponic units. It has a 0.7 L volume and operates under the constant pressure of 1.4 bar. It is used in the unit to reduce unwanted cycling and pulsation of the nutrient solution and to extend the water pump life.

Manometer

A standard manometer was placed behind the pre-pressurized accumulator tank in order to monitor current pressure generated by the water pump. The range of the manometer was 0 - 10 bar.

Electrical solenoid

An electrical solenoid was used to operate the frequency of the nutrient solution flowing through the piping. It was connected to the operating hardware unit and automatically opened and closed based on the set values.

Nozzles

There were eight misting nozzles used in each unit. Mesh filters with a screen size 100 mesh (0.149 mm) by a manufacturer Arag were used in the nozzles to filter any solid matter.

Interior circuit ventilators (fans)

Four interior 12V Sunon GF80251B1-000U-AE9 fans by a manufacturer Sunon Fans were used inside the growth chambers to ensure that the nutrient spray is distributed on all the plant roots. Two were placed at the top of the chamber and two at the bottom.

4.1.2 Additional laboratory equipment

Air-conditioning unit

A standard AC unit was used inside the room with the aeroponic units. Since the LED lights and other electronic equipment generate some heat, it is important to keep a steady temperature in the room for the plants.

Fans

There were in total six fans in the room with the aeroponic units. Their purpose was to distribute the air coming from the AC unit to ensure there is no build-up of warm air between the LED lights and the plants.

Reverse osmosis

A Maxquarium 000 PPM reverse osmosis filter by a manufacturer GROWMAX WATER was used in the laboratory. It has a capacity to produce up to 20 L/h of pure deionized water with EC 0.0. It also eliminates all salts and heavy metals from the water. We used it to get pure water for preparing the nutrient solution.

Plant growth incubators

There were two identical plant growth incubators manufactured by Carel with integrated LED lights in the Marsonaut laboratory which we used to grow lettuce seedlings from seeds. We were able to set a precise temperature in the incubators, as well as light period.

LED panel lights

We used two SPYDR 2X LED panel lights by a manufacturer Fluence By OSRAM for the cultivation of the plants in the aeroponic units. They are 345W LED lights with light output 860 μ mol/s (according to the manufacturer the wattage of this light is comparable to 600W HPS (high-pressure sodium vapor) light). They have a 380 – 780 nm full light spectrum meaning they are suitable for all phases of growth. The dimensions of each LED panel are 108.7 x 119.4 x 10.9 cm.

4.2 Cultivation properties

For the experiment we used seeds of Dubagold lettuce variety (*Lactuca sativa*) (SEMO, Czech Republic) which were planted in plastic trays filled with pearlite and placed in a growth incubator. They were watered with deionized water for the first 3 days. After that we started watering them with a FloraMicro (GHE) hydroponic nutrient solution dosed according to the label. After 10 days in the incubator the plants were carefully extracted from the pearlite and their roots washed with fresh water to get rid of any pearlite stuck on the roots. Roots were dried with paper tissues to get rid of any excessive humidity that would add weight to the plants.



Figure 11: Seedlings of lettuce growing in an incubator (source: own photo)

The plants were weighed on a scale, photographed in a Petri dish and the number of leaves of each plant was noted before placing them in the aeroponic units. There were 24 plants in each aeroponic unit, each plant placed in a plastic holder with foam ring for support and their roots suspended in the air.

There was one LED panel light above each of the two aeroponic units. It was placed approx. 30 cm above the growing table. We used a phone app Lux Light Meter Free (Doggo Apps) to measure the light intensity at the canopy level. Since there was a difference between the luminous intensity in the upper and lower part of the growing table, we have statistically evaluated the growth in both parts (upper and lower) in order to determine if luminous intensity had any influence on the growth of the plants.

We used an electrical socket timer and set the light period to be 18 hours of light followed by 6 hours of dark.

We used a two-component hydroponic fertilizer Hydro A + Hydro B (Plagron) combined with a supplementary hydroponic fertilizer MicroFlora (GHE). The reason for using such combination was that MicroFlora contains some secondary elements (B, Cu, Fe, Mn, Mo) which are absent in Hydro A+B. We prepared the solution based on the label on Hydro A+B fertilizers. As for MicroFlora, we used the concentration for the growing phase described on the label. Each aeroponic unit had a 10 L tank stored in the bottom chamber. We placed an aquarium aerator in each tank to ensure good aeration of the nutrient solution. Each aeroponic unit was pre-set to turn on and spray the roots for approx. 1 - 2 seconds with 30 seconds intervals.



Figure 129: A lettuce seedling placed in foam ring and a plastic holder (source: own photo)

4.2.1 Daily care methodology

We established a methodology of daily monitoring of the systems. In aeroponics, the conditions in the system should be closely monitored and any imbalances should be fixed as soon as possible. The average time spent monitoring the systems every day was about 20 minutes. Every day we would perform checks of the following:

- functionality of the water pumps
- nutrient solution pH, EC, levels in the tanks and temperature
- nutrient solution filters
- room temperature
- whether the nozzles are not clogged
- appearance of the plants

We measured pH and EC every day using a pH meter and EC meter (both by Milwaukee). The pH meter was calibrated every 3 days using a buffer solution 7.01 (Milwaukee). Every time we prepared a new nutrient solution, we measured pH and EC. We aimed for the pH to be between 6.0 and 7.0. We kept the EC between 1.3 and 1.6, which is said to be within the acceptable range for lettuce in most hydroponic studies (e.g. Brechner & Both 2013; Singh & Bruce 2016). If the prepared nutrient solution had a pH lower than 6.0, we would use a "pH plus" additive (Plagron) containing potassium hydroxide. If pH was too high, we would use a "pH down" additive (Advanced Hydroponics of Holland) containing phosphoric acid. If EC was too low, we would add 1 extra liter of the nutrient solution (prepared with the same ratio) at a time until it reached 1.3 - 1.6. If it was too high, we would dilute the nutrient solution with deionized water until EC was in the desired range.

The nutrient solution levels in the tanks declined regularly. We would refill the tanks with deionized water and adjust the pH and EC to the desired levels.

The nutrient solution was completely exchanged for a fresh one once a week.

The temperature in the laboratory was always kept in the 19 - 21 °C range using the AC unit. That range is within the recommended ambient temperature for growing lettuce hydroponically (Singh & Bruce 2016).

The temperature of the nutrient solution was always kept in the 18 - 20 °C range.

We inspected the mesh filters in the piping as well as the filter between the top and the bottom chamber three time a week and cleaned them if needed.

4.2.2 Harvesting and measurements

After 23 days, all the plants were harvested, their roots and leaves were separately weighed, photographed and number of leaves of each plant was noted. After that the plants were dried in an oven (Memmert, Germany) for 48 hours at 70 °C. The dried leaves and roots were weighed again in order to calculate the root/shoot ratio.

4.2.3 Statistics

We used a data analysis software STATISTICA 13 (Statsoft Inc. (2013), version 13. www.statsoft.com) to analyze the data we gathered and perform F-tests, one sample t-tests and two sample t-tests.

Lettuce plants growing in the aeroponic unit are considered dependent variables, therefore a two sample t-test was used to determine whether there is statistically significant difference between the fresh biomass weight of the plants in each aeroponic unit at the beginning and the end of the experiment. The same statistical method was also used to determine whether there is statistically significant difference for number of leaves. In order to compare the data from the beginning and the end of the experiment for each aeroponic unit, we used descriptive statistics.

To compare the data between the two aeroponic units from the beginning and the end of the experiment, we first performed a F-test to determine whether variances for each sample group are or are not statistically different. Later, a two sample t-test was used to determine whether or not there is a statistically significant difference between the fresh biomass weight and number of leaves of plants in the two aeroponic units.

Additionally, we performed a correlation analysis to find out whether there is a dependence between the fresh weight of the roots and the number of leaves and how strong it is. In order to quantify this dependence, a regression analysis was done to find out how the number of leaves increased with an increase of the roots weight. The correlation and regression analyses were done separately for each group of plants.

We also calculated the average root/shoot ratio (dry weight of roots/dry weight of shoots) to be able to compare our results with another research that had been done before.

Since there was a difference in the luminous intensity in different spots of the growing table of the aeroponic unit, we compared the weight and number of leaves of 3 groups (clusters) of plants from each unit. The goal was to check whether the difference in the positioning on the growing table affected the growth.

Lastly, we used correlation analysis to study whether there was any dependence between daily changes in pH and EC of the nutrient solution.

5 Results

5.1 Plant growth

After 9 days of the experiment we were forced to move all the plants from Unit 2 into Unit 1 because of a large nutrient solution leak in Unit 2. The conditions in Unit 1 were the same as in Unit 2. We noted which of the plants were replanted so we could determine if and how replanting affected their growth. In order to avoid confusion, we established labels as Group 1 and Group 2 for what was previously Unit 1 and Unit 2 because all the plants were put in one aeroponic unit.

It should be noted that there was no write-off of any plants, 100 % of the plants survived the experiment.

A two sample t-test determined that there was a statistically significant difference between the plant's weight at the beginning and the end of the experiment for both groups of plants (p < 0.05).

Table 3 below shows the weight results of lettuce plants for both groups of plants. The number of plants in each group was the same (24). There was a significant difference between the minimum and maximum weight of plants at the beginning of the experiment in each group individually – 1.3 g and 1.48 g for Group 1 and Group 2 respectively. This difference became even more significant at the end of the experiment after harvesting and weighing the plants, where in Group 1 and Group 2 the difference between the minimum and maximum weight of plants was 19.53 g and 13.68 g respectively. These differences were also apparent from looking at the weight variation coefficients (v_x) for both Group 1 and Group 2.

Table 3: The weight analysis (fresh biomass) of lettuce plants for Unit 1 (Group 1) and Unit 2 (Group 2) at the beginning and the end of the experiment (N = number of plants; m = weight; $\sigma =$ standard deviation; vx = variation coefficient)

	N	Ø m ₀ (g)	Ø m1(g)	min m ₀ (g)	max m ₀ (g)	min m ₁ (g)	max m1(g)	σm_0	σm_1	v _x m ₀ (%)	v _x m ₁ (%)
Unit 1 (Group 1)	24	0.92	7.17	0.41	1.71	1.67	21.2	0.34	3.96	37.4	55.3
Unit 2 (Group 2)	24	0.88	7.79	0.47	1.95	2.92	16.6	0.35	3.2	39.9	41.1

For the number of leaves, a two sample t-test determined that there is a statistically significant difference between the number of leaves at the beginning and the end of the experiment for both groups of plants (p < 0.05).

Table 4 below includes the number of leaves, minimum and maximum number of leaves, standard deviation and variation coefficient at the beginning and the end of the experiment. The variations in the values are lower than those for the weight of the plants (Table 3) and it is apparent that the number of leaves in average more than doubled over the course of the experiment.

Table 4: The number of leaves analysis for Unit 1 (Group 1) and Unit 2 (Group 2) at the beginning and the end of the experiment (N = number of plants; L = number of leaves; σ = standard deviation; vx = variation coefficient)

	N	Ø L ₀ (pcs)	ØL ₁ (pcs)	min L ₀ (pcs)	max L ₀ (pcs)	min L ₁ (pcs)	max L ₁ (pcs)	σL_0	σL_1	v_x L ₀ (%)	v_x L ₁ (%)
Unit 1 (Group 1)	24	4.17	9.83	3	5	7	13	0.48	1.63	11.6	16.6
Unit 2 (Group 2)	24	4.29	9.83	2	6	7	15	0.81	1.95	18.8	19.8

We statistically compared the data for weight and number of leaves between the two groups of plants both at the beginning and the end of the experiment. This was done mainly because of the replanting of all the plants from Unit 2 to Unit 1 when we had the issue with nutrient solution leak in Unit 2. Figure 13 shows two boxplot graphs comparing the weight (in grams) of the plants for Unit 1 (Group 1) and Unit 2 (Group 2) at the beginning (left) and at the end (right) of the experiment.



Figure 13: Boxplot graphs comparing the weight (in grams) the two groups of plants at the beginning (left) and the end (right) of the experiment

Table 5 on the next page describes a comparison of the weight of the two groups of plants at the beginning and the end of the experiment. An F-test was previously done to check whether variances are or are not the same for the two groups. The result showed that p > 0.05,

meaning that variances were the same. Therefore a two sample t-test was used to compare the data. The result showed that p > 0.05 for both the beginning and the end of the experiment, meaning there was no statistically significant difference between the weight of plants neither at the beginning nor at the end of the experiment between the two groups of plants.

	Ν	Ø (g)	σ	DF	p value	
$U_1 m_0$	24	0.92	0.34	16	0.7	
$U_2 m_0$	24	0.88	0.35	40	0.7	
$U_1 m_1$	24	7.17	3.96	16	0.55	
$U_2 m_1$	24	7.79	3.2	40	0.55	

Table 5: A two sample t-test used to compare the weight of plants in each unit (group) at the beginning and the end of the experiment (N = number of plants; \emptyset = mean value; σ = standard deviation; DF = degrees of freedom)



Figure 14: Boxplot graphs comparing the number of leaves of the two groups of plants at the beginning (left) and the end (right) of the experiment

The same comparison was done for the number of leaves for the two groups of plants at the beginning and the end of the experiment.

An F-test was performed for variances of the data for number of leaves from the beginning of the experiment. The results showed that p < 0.05, meaning that variances of values for number of leaves were not the same for the two groups of plants at the beginning of the experiment. Therefore a Welch's test was used to compare the data.

Another F-test was performed for variances of the data of number of leaves at the end of the experiement. The results showed that with p > 0.05, therefore variances were the same and a two sample t-test was used. The results are shown in Table 6 below. P values for both Welch's test and two sample t-test were higher than 0,05, meaning there was no statistically significant difference between the number of leaves of plants neither at the beginning nor at the end of the experiment between the two groups of plants.

Figure 14 shows two boxplot graphs comparing the number of leaves of plants for Unit 1 (Group 1) and Unit 2 (Group 2) at the beginning (left) and at the end (right) of the experiment.

Table 6: A Welch's test and a two sample t-test used to compare the number of leaves of plants in each unit (group) at the beginning and the end of the experiment (N = number of plants; \emptyset = mean value; σ = standard deviation; DF = degrees of freedom)

		Ν	Ø L (pcs)	σ	DF	p value	
Welch's	$U_1 L_0$	24	4.17	0.48	16	0.52	
test	$U_2 L_0$	24	4.29	0.81	40	0.32	
two	$U_1 L_1$	24	9.83	1.63	16	1	
t-test	$U_2 L_1$	24	9.83	1.95	70	1	

A correlation analysis was performed to find out whether there is a statistically significant linear dependance between the fresh weight of roots and the number of leaves for plants in each group. In this analysis the independent variable was the fresh weight of roots, the dependant variable was the number of leaves.

For Group 1, the correlation coefficient (R) was calculated to be 0.54. The coefficient of determination (\mathbb{R}^2) was 0.3, meaning there was a 30% variability in the values. The p value was greater than 0.05, meaning that the coefficients were valid and there was a medium linear dependency and an upward trend. A regression analysis was done to quantify the dependency. It showed that with every 1 gram increase in the fresh weight of roots, there was a 0.59 increase in the number of leaves.

For Group 2, the correlation coefficient (R) was calculated to be 0.55. The coefficient of determination (R^2) was 0.3 so the variability in the values was 30% (same as in Group 1). The p value was greater than 0.05, meaning there was also a medium linear dependency and a upward trend. Again, a regression analysis was done with the results that for every 1 gram increase in the fresh weight of roots there was a 1,06 increase in the number of leaves.



Figure 10: Roots of lettuce plants in the aeroponic unit (source: own photo)

For the first 9 days (before replanting all the plants into one unit) the luminous intensity between the two aeroponic units was different. For Unit 1 the average was 17 570 lx. For Unit 2 it was only 8 170 lx. Unit 1 had more than twice more light than Unit 2. Therefore

we were interested in how this affected the growth of the plants and whether there would be a big difference in the weight and number of leaves at the end of the experiment.

Table 7 shows an average leaf number increase and weight increase between the beginning and the end of the experiment for each of the 3 groups of plants mentioned in chapter Statistics.

	Gro	up 1	Gro	up 2	Group 3		
	Ø L _{incr}	Ø m _{incr} (g)	Ø L _{incr}	Ø m _{incr} (g)	Ø L _{incr}	Ø m _{incr} (g)	
Unit 1 (17 570 lx)	6.7	8.3	5.7	5.4	4.7	5.8	
Unit 2 (8170 lx)	5.7	7.7	5.5	5.9	5.5	8.2	

Table 7: A comparison of increases in number of leaves and weight for 3 separate groups in each aeroponic unit (\emptyset L_{incr} = average increase in number of leaves; \emptyset m_{incr} = average increase in weight)

5.2 Root/shoot ratio

A root/shoot ratio is calculated by dividing the dry or fresh weight of roots of a plant by the dry or fresh weight of shoots (stem + leaves) of the same plant.

In our case we calculated our fresh root/shoot ratio to be in average 0.396 and our dry root/shoot ratio to be in average 0.299 for all the plants combined (48 plants).

5.3 pH/EC analysis

As mentioned in the daily care methodology chapter, pH and EC was measured each day during the course of the experiment. A correlation and regression analyses were done to find out whether pH value affected EC value in any way. We found out that there was no linear dependency between the values.

6 Discussion

6.1 Growth characteristics results

The results of the experiment we performed in two vertical aeroponic units showed some variation in weight of the two groups of plants both at the beginning and at the end of the experiment (Table 3). However, the average weight values were still similar. The weight variation at the beginning of the experiment can be easily explained by the fact that we did choose the plants randomly and it was not our goal to have uniform groups of plants.

The variation in weight at the end of the experiment was even bigger than at the beginning. With that being said, the difference between the average weight in the two groups of plants at the end of the experiment was only 0,62 g. The variation between the weight of the plants within the group is to be expected. Plants are living organisms so there will always be some variation in the values. In the aeroponic unit we used, the nozzles spraying the roots with the nutrient solution are positioned in a particular way (see Fig. 9) and it is possible that some of the roots are placed in a more favorable position towards the nozzles resulting in being sprayed more than the other ones. It seems impossible to be able ensure that all the roots of every plant would be sprayed evenly.

The aeroponic units we used had control units with pre-set settings for the interval of spraying and the duration of spraying. Our interval was set for 30 seconds and the spraying lasted for 1 - 2 seconds, which is much lower than in a research done by Li et al. (2018) where the interval was also 30 seconds but the spraying lasted for 20 seconds. In another paper by Tunio et al. (2021) lettuce yields were best when the spraying interval was 30 minutes and spraying lasted for 5 minutes. It could be said that the spraying settings differs very much in different researches and it would be interesting to study more into depth how the duration of spraying affects the growth of the plants. Unfortunately our experiment did not have a capacity to do that.

The average number of leaves of plants in the two groups was very similar at the beginning of the experiment with only 0.12 difference between the mean values. Such a small difference is understandable considering that all the seedlings were of the same age. It is interesting that at the end of the experiment both groups of plants had exactly the same number of leaves in average.

The correlation and regression analyses for both groups of plants showed that there is a linear dependency between the fresh weight of the roots and the number of leaves. However, the difference between the two groups was substantial. There was an increase of 0.59 and 1.06 with every 1 gram increase in the fresh weight of roots for Group 1 and Group 2 respectively. This means that the increase in Group 2 was almost double the increase in Group 1. It is difficult to assess why there is such a big difference because all the values we observed were very similar between the two groups. The fresh weight of roots between the two groups at the end of the experiment differed only by 0.02 g in average. The difference in number of leaves between the two groups at the beginning of the experiment was only 0.12 and the values were identical at the end of the experiment. We first thought this could have been caused caused by the fact that plants in Group 2 had a quite big variation in the number of leaves at the

beginning of the experiment (18.8 %) comparing to Group 1 (11.6 %). That means that there could have been more plants with a higher number of leaves in Group 2 than in Group 1. Therefore we counted how many plants had a particular number of leaves for both the beginning and the end of the experiment for both groups of plants. However, there were no significant differences that would explain the difference in the increase in number of leaves with every 1 gram increase in the fresh weigh of roots between the two groups. In order to accurately assess this difference, a more thorough analysis would have to be done or there would have to be much closer observation of the weight of the roots and number of leaves during the course of the experiment.

As for the the replanting of plants from Unit 2 into Unit 1 after 9 days of the experiment, it was interesting to observe whether this would affect the growth of plants from Unit 2 in any way. From looking at Tables 3, 4, 5, and 6 it seems that replanting did not affect the plants from Unit 2 in any way – the average values for weight at the end of the experiment are quite similar, the average values for number of leaves at the end of the experiment are exactly the same for both aeroponic units. The fact that the statistical tests did not find any statistically significant differences between the values confirms that the conditions really were very similar for the first 9 days when the plants were in two separate aeroponic units and neither the difference in luminous intensity nor replanting the plants from Unit 2 into Unit 1 caused any stress to the plants. It is important to say that the experiment aimed at having the conditions in the two aeroponic units as same as possible.

Comparing the root/shoot (RS) ratio for our plants, we found out that our values are were accordance with a research done by Li et al. (2018). In their research they studied two different varieties of lettuce – Dasusheng and Nenglv Naiyou. For Dasusheng their dry weight root/shoot ratio was $0.32 \pm 0,04$ and for Nenglv Naiyou it was $0.3 \pm 0,03$. Our dry weight root/shoot ratio was 0.299 which is very close to their results. We performed a one sample t-test to compare our dry weight RS ratio with theirs. P value was 0.96 meaning there was no statistically significant difference between the values. A boxplot graph for the test can be seen in Figure 16. Some differences existed between our design of experiment and the one of Li et al. Their aeroponic system was based on an A-frame structure. They transplanted their lettuce plants into the aeroponic unit 28 days after sowing while we did that after 10 days. Even their experiment in the aeroponic system lasted longer. They kept the plants in the system for 45 days while we harvested them after 23 days. However, the root/shoot ratio was almost the same which tells us that our plants were proportionally the same.



Figure 16: A boxplot graph of a one sample t-test used to compare our root/shoot value with the reference value from Li et al. (2018)

In our experiment, the lettuce plants were 33 days old (from seed to harvest) and their average final weight was 7.48 g. That means that there was a daily weight increase of about 0.23 g. When growing lettuce organically on a field, it takes about 5-6 weeks for the plant to mature (from seed to harvest) and the final average weight is about 400 g. That means the daily increase is roughly 10.4 g (Konvalina et al. 2007), more than 45 times higher than in our experiment. In an study done by El-Helaly & Darwish (2019) the average weight of lettuce after 42 days in an aeroponic system was 106.36 g. Their daily increase was about 2.53 g, which is exactly 11 times higher than in our experiment. However, El-Helaly & Darwish used a different nutrient solution than we did. The main difference was in the design of their aeroponic unit – their was horizontal with polystyrene tables at the top and chambers with spraying nozzles under them. Their research does not mention the interval between spraying the roots with the nutrient solution or the duration of spraying, however the design of the aeroponic system they used seems to be much more favorable for the growth of the plants because they are positioned in a natural way (as they would be positioned if they grew in soil) with their roots suspended in the air right below the canopy. In the aeroponic system we used the plants were placed in a growing table which had an incline so the plants were in an unnatural position (Fig. 17). It is apparent that the daily increase in weight in our experiment was significantly lower than in the other studies.

Vertical aeroponic systems are regularly used to produce lettuce but those are different from the one we used because the growing table with the holes for the plants usually has kind of a "pocket" where the plant is placed. That provides the plants with a more natural position (Fig. 17).





Figure 17: A comparison of the aeroponic system we used in our experiment and an aeroponic farm producing lettuce in Minnesota, USA (source: left picture: own photo; right picture: Minnpost website)

To be able to better imagine the amount of lettuce we harvested, we calculated that our system's growing floor area was roughly 0.8 m^2 . On this area we harvested in total 316 g of lettuce, which means that our yield per square meter would be 395 g. That it much less than in a research done by Touliatos et al. (2016), where they studied a difference in yield between a horizontal and a vertical hydroponic system. Aeroponics is a subcategory of hydroponics therefore we could roughly compare the results. Their growing floor area was 0.4 m^2 and 0.02 m^2 and their yield per square meter was 6.9 kg and 95 kg for horizontal and vertical hydroponic system respectively. Compared to our results it is more than 17 times and 240 times more for horizontal and vertical hydroponic system respectively.

This comparison of results from our experiment with the experiments done by others shows clearly, even without a statistical evaluation, that the aeroponic system we used performed much worse than any of the systems in other researches.

6.2 Using the aeroponic system in space

The prospect of a successful use of the indentical system that we tested in our experiment in space or on the planet Mars is very improbable. The system as it was and in the conditions we had would not be able to produce enough lettuce to support a diet of astronauts. Furthermore, this system was far from being flawless and the technical maintenance was very difficult. Space or the planet Mars are places where any technology must be absolutely reliable. Any mistake in such an inhospitable environment can be fatal. If an aeroponic system would ever be used there, it would need to be a system that works perfectly and produces enough food to support the diet of the astronauts. The system would also have to be very easy to maintain and any malfunctions would need to be easily fixed.

6.3 Disadvantages of the systems and proposed technological upgrades

It would not be very complicated to improve the system and to assemble it differently. The main issue as it was when we performed the experiment was the positioning of plants. If the growing table was positioned horizontally and the spraying nozzles would be placed underneath, the yield might be better. It would also require changing the settings of the control unit to make the spraying duration longer than it was in our case. Another disadvantage of the system was that all the piping, water pump, accumulator tank, filters and even the control unit were placed under the grow chamber and even under the floor level, therefore any maintenance was very difficult and uncomfortable to do. If the system was horizontal, it would be much more convenient to place all those components mentioned above on the floor next to the units.

The nutrient solution in the tanks would regularly decline in both units. In one of them the decline was small and could have been caused by the roots holding water and evaporation. The decline in the other unit was much larger and was caused by a leak in the piping.



Figure 18: The newly built aeroponic system in the Marsonaut laboratory (source: own photo)

The information and experience gathered during our experiment have led to a modified, new horizontal design of the aeroponic system in the Marsonaut laboratory. The newly built aeroponic system uses the control unit and the components such as piping, water pump etc. from the aeroponic system we used, however the new setup is horizontal and lettuce plants can be grown positioned in a natural way with their roots hanging freely underneath the canopy. Roots are sprayed from underneath the growing tables and the spraying interval and duration has changed. At the moment of writing this, one experiment has been done there by my colleagues aimed at growing lettuce aeroponically and also aquaponically using water from aquaculture. The results of this experiment are not finalized yet, however the experiment ran for 35 days and the heaviest lettuce plant grown aeroponically had 349 g. This figure alone demonstrates that the applied changes have led to a significant increase in the growth of lettuce. Figure 18 on the previous page shows the design of the new aeroponic system. The growing tables have handles so the piping and spraying nozzles located underneath are easily accessible.

7 Conclusions

- The aeroponic system that we tested by growing lettuce in it did produce lettuce, however the yield was very poor compared to other studies focused on growing lettuce aeroponically, hydroponically and organically in soil. The main reason for the low yield of lettuce was probably the design of the aeroponic system, especially the unnatural positioning of the plants and the distribution of the spraying nozzles inside the aeroponic chamber, as well as the interval and duration of spraying of the nutrient solution.
- The design of the aeroponic system that was used in the Marsonaut laboratory presented a new, experimental approach to the design of aeroponic system. When we consulted it with colleagues specialized in plants and they growth, they were unable to assess whether the system would successfully produce lettuce. During the experiment and at the end when we analyzed the data we realized that this design is not suitable for producing large amounts of lettuce.
- It is unlikely that the aeroponic system as it was designed and assembled when we performed the experiment would be able to produce sufficient yields of lettuce in space or on the planet Mars. The system was too prone to technical malfunctions (e.g. nutrient solution leak) and maintenance of this system would be very difficult. Any method of producing food in space or on the planet Mars would need to be extremely reliable because the lives of astronauts would be dependent on the ability to produce food. In general, the aeroponic system was not easily maintained.
- It is possible that if the system was built horizontally, the yield of lettuce in the experiment could be higher.
- From the data we collected and statistically analyzed and the comparison of our results with other studies, our hypothesis:

The specific design of a vertical aeroponic system built as a part of the Marsonaut project is a suitable cultivation method for growing lettuce

must be rejected.

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