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Fertilization strategies for peas (*Pisium sativum* L.) in a growing medium simulating Mars regolith

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Plant Science

Acknowledgments

I’d like to thank Unifor for providing me with the grant.

If this thesis was a ride, and I was the pilot. Susanne would be the V8 engine. Trine the watchful copilot. Wieger the starter, Irene the wheels, Odny the axles, Valentina the brakes, Kurt the blinkers, Pia the windshield. Tore the spare tire. My parents Tino and Gloria the (diesel) fuel, my brother Francois the (winding) way. Narta would be the air conditioner, Suzanna the whippers and Khaled the radio.

As much as we like to divide things, it is in essence all one and the same (ride). Thank you all!

Ås, 2020

Marc Monarcha

Abstract:

In 2011, Andy Weir published the thought-provoking novel "The Martian", in which a fictional astronaut, Mark Whatney gets left behind on Mars where in order to survive, he must find a way to grow food. As of March 2020, the book is considered a science fiction. History shows that time and time again, ideas such as the moon landing were largely considered intangible, until certain people put in the joint effort and made them real. Now, with the hope of going to Mars permanently appearing more and more likely, it is worthwhile to test ways of growing food there. In this experiment, the Mojave Mars simulant (MMS) has been used as a Martian regolith analog to simulate Martian conditions. The analog shares similar chemical and physical features with sampled material from Mars. This study is aimed at investigating how well peas (*Pisium sativum)* grow in MMS compared to a nutrient poor sandy soil; which kind of fertilizer (inorganic, organic, biofertilizer) gives the best plant development and yield; whether repeated additions of organic matter improve growing conditions; and finally whether plants grown in MMS will have a lack of potassium (since a lack of potassium has been speculated in a different experiment). Two pot experiments were conducted under controlled conditions. In the first, peas were sown in the MMS and in a nutrient poor sand with the following fertilizer treatments: without nutrients (control), synthetic fertilizer, rhizobium without potassium, rhizobium with potassium, and digested sewage sludge. In the second experiment, the MMS pots with soil from the first experiment were used again with an addition of the same amount of sewage sludge each. Again, peas were used as a test crop. Results showed that plants in the MMS grew poorly compared to those in the nutrient poor sand. Plants grown in the MMS had symptoms of boron toxicity. Addition of sewage sludge lowered the pH of the alkaline MMS and reduced boron availability, increasing its biomass and yield. Finally, plants showed no sign of potassium deficiency in the MMS treatments, even when no additional potassium was supplied. The results of this experiment are not conclusive as to whether peas can grow on Mars. To answer this question, there is still much more research and experimentation to be done.

Outline:

Fertilization strategies for peas *(Pisium sativum L.)* in a growing medium simulating Mars regolith

I. Introduction

Colonizing mars. To the average person, let alone scientist, this may sound like a far-fetched project, but how much do we really know? Is such a thing feasible? And why would we think about doing it in the first place? The fact of the matter is, we are running low on resources, whilst the world population continues to increase exponentially. And as much as we think that sustainable ways of life are the go to solutions, if you extend the timeline for life on earth long enough, with the given patterns of say population growth (Roser et al, 2013) as our only means of predicting the future, our blue planet might not be able to keep up with the resources at stake. So if we were to keep a foothold in future times, some alternatives are worthwhile examining.

Some researchers, scientists, and entrepreneurs through their determined work and effort, are slowly but surely proving that our neighboring red planet has the potential to foster life. In fact, for us to make mars a habitable place, we need to start from scratch and work on basic human needs for survival. In other words, look for solutions outside the box. Breathable air, water, energy, and food are at the basis of what we need. Theoretically, with all the scientific data as a backup, we know that we can create breathable oxygen, drinkable water, and energy.

When it comes to food, growing plants on Martian-like soil has also been proven to be successful to a certain extent, and this is where this thesis is concerned. One possible way of investigating whether we can grow food on Mars is simulating a Martian growth experiment using a soil analog that is similar to the actual Martian soil. Having access to the Mojave Mars Simulant (MMS) (also referred to as "MMS soil" in the paper), we can build on the very little research that has been done on it and alter parameters in a plant growth experiment to try and optimize growing food in this medium.

Rockets have been sent to Mars for decades now, with vehicles roaming the Martian surface all the while, and still. Those rovers have among many other things, collected soil samples that have been analyzed *(Figure 1).*

Figure 1: Selfie from NASA's Curiosity rover taken on October 11th, 2019. (Taken from NASA's official website)

Spacex, a leading private company in the aerospace industry, is aiming to send people to mars in as soon as 2024. But for the journey to become a permanent residence, some work needs to be done.

Water is at the forefront of our survival needs if we were to colonize Mars. As a matter of fact, Mars has two polar ice caps with water in ice form. There is also some optimism in the science community about the likelihood of there being water in liquid form as well beneath the surface of the ice (Titus et al, 2003).

Having access to this water, we can produce oxygen through the process of electrolysis. Electrolysis of CO_2 or H_2O are the most evident forms in which we can produce oxygen. When CO_2, the "major ingredient of the Martian atmosphere" (James et al,1992) is exposed to very high temperature and pressure, it separates the carbon monoxide and oxygen from the molecule and thus oxygen is created through separating the two.
As a matter of fact, this electrolysis process is what is applied to support life on the International Space Station (ISS), a satellite that orbits earth. There however, water is used for the process (Samsonov et al. 2002).

As mentioned, electrolysis needs high temperature and pressure, both of which require energy. One way of generating this energy is through wind turbines. Even though the air density on Mars is much lower than that on Earth, there still is some airflow, which makes it a viable source of energy.
Another way to get it is through solar energy. In fact, the Opportunity rover that is roaming Mars at this moment is powered by solar panels (Landis, 2005).
A third way is through nuclear energy. The Curiosity rover, also still roaming Mars, is powered by nuclear energy (Witze, 2014).

With water, oxygen, and electricity available, the fourth basic need for surviving is food. This is where this experiment is concerned. We will test and see how well of a growth medium the Martian soil simulant can be. The most recently discovered Martian-like soil was that from the Mojave Desert. The Mojave Mars

Simulant (MMS) has been identified by scientists at NASA to be the best simulant of Martian soil known so far, based on sampling from the red planet. (Peters et al, 2008). Both the soil on Mars and the MMS have very similar chemical and physical properties.

At Wageningen University and Research, researchers have already been using Mars soil simulants to grow plants. Since 2013, they have been experimenting with various crops and have proved that plants can indeed grow in such soils. There, an initial study using the John Space Center (JSC) soil (a different simulant) was conducted to try and grow plants. The experiments showed that the JSC soil was able to nurture most plants with over 90% survival rate (after 50 days, the time at which germination and leaf initiation have already taken place) in *Sedum. reflexum* plants and even a 20% flowering rate in *Sinapsis. arvensis* plants (Wamelink et al., 2014), which is critical if plants were to regenerate for a long term settlement on Mars.

However, now that a better simulant in the MMS is available, researchers are using it instead for their experiments. Peas have now been tested on the MMS at Wageningen, but the results have not been published yet. When those peas grew, it was presumed that they might have had symptoms of potassium deficiency (Wamelink, 2019 pers. comm.).

<u>Objectives:</u>

As we can artificially create an environment here on earth that would in theory, putting aside some variations, depict a good representation of an actual Martian plantation, we may develop ways of potentially growing plants on Mars with the resources that will be available or possible to transport there.

Meaning sewage sludge (or similar) produced there or rhizobium which needs limited space for transport.

The main objective of this study was to determine how different fertilization strategies affect plant growth and development in the MMS. In particular, the following questions were addressed:

1-How well do peas grow in MMS compared to a nutrient poor sandy soil?

2-How well can organic fertilizers (from sewage sludge) supply peas with nutrients in a soil low in organic matter (MMS)?

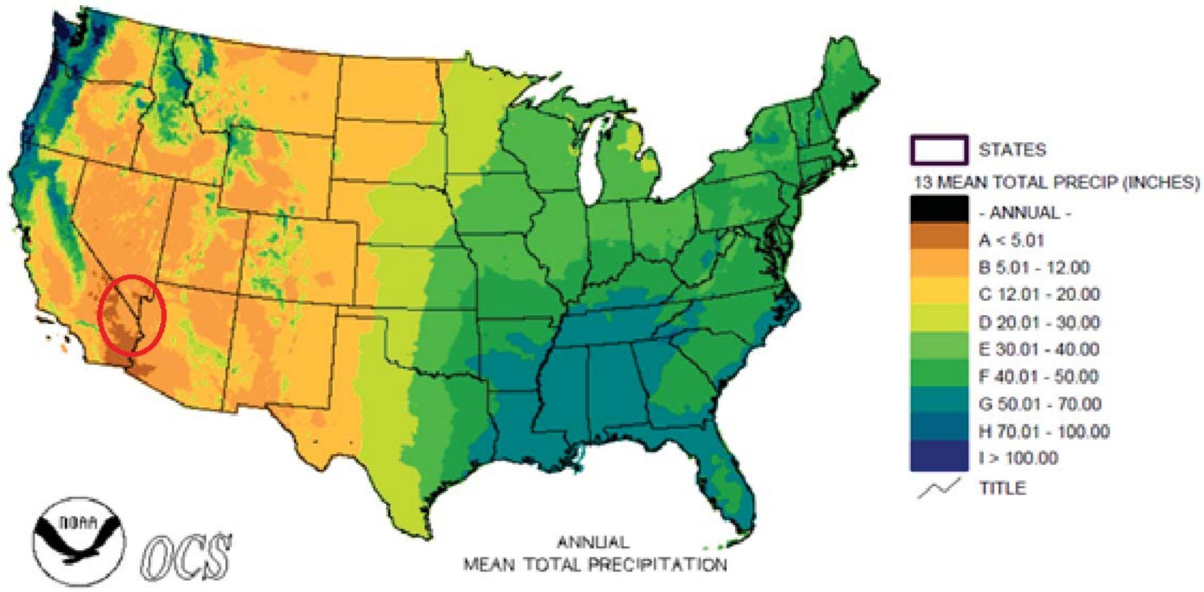
3-Will repeated additions of organic fertilizers (from sewage sludge) improve MMS growing conditions?

4-Will peas grown in the MMS have a lack of potassium?

II. Literature review

II.1. MMS Background:

The MMS soil originates from the Mojave Desert located in North America, South-West of the United States. It constitutes a large part of both Nevada and California. Located on the leeward side of the mountain ranges of California, precipitation is very scarce making it a rain-shadow area and the driest desert in North America. On average, the Mojave Desert receives 137mm/year of rainfall (Hereford et al, 2004) *(Figure 2).*



<u>Figure 2</u>: Average annual precipitation on the USA from 1961 to 1990. The Mojave Desert is around the brown South-West area of the map. Source: North Carolina Climate Office.

The desert is still home to many plant species, most notably the Joshua tree, *Yucca brevifolia (Figure3)*, an indicator species of the desert that is native to the Mojave.

Figure 3: *Picture from the Mojave Desert. At first sight the landscape and colors seem akin to Mars. The picture also suggests limited but existing plant growth along with the prevalence of the Joshua tree (picture taken from the national wildlife federation)*

For this experiment, the MMS was bought online at *www.themartiangarden.com*. The process in which the soil is made available for selling is as follows: First, rocks and boulders from the mining site in an area of Saddleback basalt deposits that came from a volcano eruption 22 million years ago (Armstrong, 1973) are transported into a different location where they are pressure-washed to get rid of contaminants from transport. Whole rocks are then crushed, a process which "closely resembles the weathering/comminution processes on Mars." (Peters et al, 2008) into coarse, fine and super fine sized particles sold either as "coarse", "fine", "super fine", or in the case of this experiment, a mixture as "unsorted". After sorting, each batch of regolith is sterilized at high temperatures before being inspected, vacuum-sealed and packed.

II.2. MMS Chemical Properties:

Preliminary analysis has been done on the MMS soil. In 2007, scientists discovered the similarities that the Mojave Desert soil bears with the Martian soil. They note "the MMS suite is similar and provides a good mineralogic analog for the igneous rocks of Mars… Plagioclase feldspar and pyroxene, along with minor olivine and magnetite, tend to be the dominant minerals in both MMS and the Martian rocks"(Peters et al., 2008).

Table 1 shows the geochemical properties of the MMS with regards to that of the sampled soil from the different landing sites collected by four different rovers and the alternative Martian simulant dubbed JSC. Nine samples from the MMS soil were collected for the calculation of the average composition and there was no significant variation in the bulk from the MMS which is curiously similar in its geochemical composition (Peters et al., 2008).

<u>Table 1</u>: Geochemical composition of the sampled soil from Martian rovers, the JSC, and the MMS. (Peters et al, 2008)

	Martian regolith								Martian simulants	
	Viking Landers		Pathfinder		MER Spirit		MER Opportunity		JSC Mars-1	MMS
	VL-1	VL-2	"Soil"	SFR	"Soil"	Rocks	"Soil"	Bounce		
Concentration in wt%										
SiO_2	43	43	42.0	57.7	45.8	45.6	43.8	50.8	43.48	49.4
TiO_2	0.66	0.56	0.8	0.50	0.81	0.55	1.08	0.78	3.62	1.09
Al_2O_3	7.3	(7)[a]	10.3	12.3	10.0	10.6	8.6	10.1	22.09	17.1
Cr_2O_3	–	–	0.3	–	0.35	0.56	0.46	0.12	0.03	0.05
Fe_2O_3	18.5	17.8	21.7						16.08	10.87
FeO			14.2	15.8	17.8	22.3	15.6			
MnO	–	–	0.3	0.5	0.31	0.38	0.36	0.43	0.26	0.17
MgO	6	(6)[a]	7.3	0.8	9.3	10.7	7.1	6.4	4.22	6.08
CaO	5.9	5.7	6.1	6.7	6.10	7.50	6.67	12.5	6.05	10.45
Na_2O	–	–	2.8	4.2	3.3	3.0	1.6	1.3	2.34	3.28
K_2O	<0.15	<0.15	0.6	1.2	0.41	0.15	0.44	0.10	0.70	0.48
P_2O_5	–	–	0.7	0.4	0.84	0.67	0.83	0.95	0.78	0.17
SO_3	6.6	8.1	6.0	0.25	5.82	1.79	5.57	0.52	0.31[b]	0.10
Cl	0.7	0.5	0.9	0.4	0.53	0.23	0.44	0.06	–	–

(Further chemical analysis that was conducted in our project will be presented in the methods part page 16)

II.3. pH levels and nutrient availability for plants

It is worthwhile noting that pH levels for the MMS have not been recorded. However, since the weather at the Mojave Desert is on the dry end of the spectrum, one can assume that the soil is either neutral or alkaline as is suggested by the United States Department of Agriculture (USDA). With that in mind, pH can have an important effect on the properties of the soil and the nutrient uptake by plants. Soil acidity or basicity has a very big influence on the solubility of certain compounds and the binding of those compounds on the exchange sites (Alam et al, 1999).

Putting this into simpler terms, plant nutrients can be either soluble in the soil or bound to a soil particle depending on the pH of the soil. For the plant to be able to absorb it, the nutrient needs to be dissolved in the soil solution. Evidently, research has been done on the effect of high pH soils on nutrient availability. For example, in one study, it was shown that nitrogen concentration in plant tops decreased with decreasing pH over the range of 5.5-3.3. Decreases in rate of phosphate absorption with increasing pH were also well documented (Alam et al, 1999).

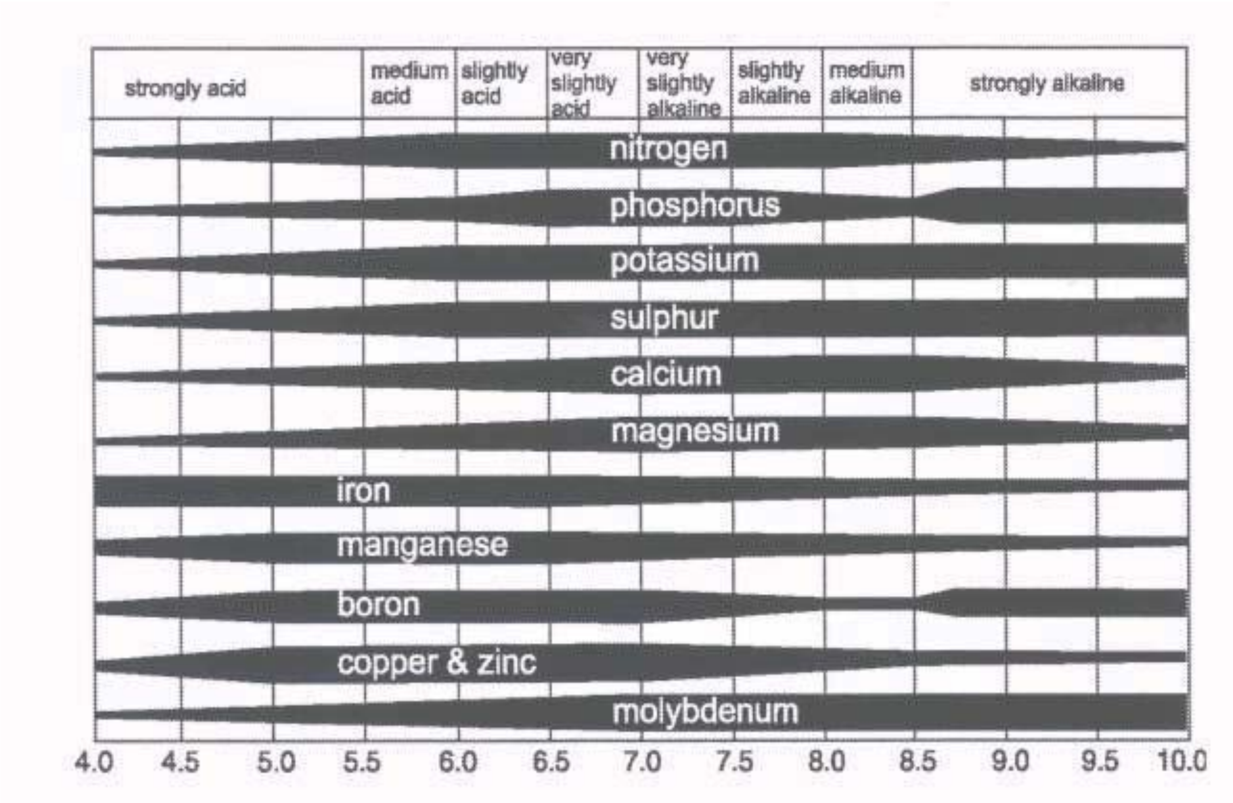


Figure 4: Effect of pH on availability of plant nutrients (Al-Omran et al, 2004)

II.4. Soil organic matter and organic amendments

Since there are no studies that show the presence or absence of soil organic matter (SOM) in the Martian simulant soil, it is important to discuss the role that SOM plays in plant growth and development and how organic amendments may affect the soil.

The presence of organic matter has an impact on the fertility of a soil. Nutrient supply, water retention, and soil structure are all affected by the presence, or absence of organic matter (Fundenberg, 2001). SOM also acts as a reservoir for plant nutrients that can be released into the soil solution as a result of mineralization by microorganisms (Fundenberg, 2001). Water holding capacity is another factor that is affected by organic matter. SOM behaves like a sponge and can retain up to 90% of its weight in water. As opposed to clay aggregates, in which some of the water retained is not available for the plants, soil organic matter will release most of the water that it absorbs to the plants (Fundenberg, 2001).

Studies show that just as organic matter plays a positive role in plant growth and development, so can the addition of organic amendments, as can be seen in the following paragraphs.

Organic fertilizers come in various forms. They can be either plant based (in the form of plant residue, compost and others), derived from animals (in the form of excreta usually), or derived from humans (also in the form of excreta). They are all labeled organic fertilizers or organic amendments because unlike mineral fertilizers, they all originate from living organisms. However, given the broad nature in the origin and production of each of these organic amendments, they may act in different ways when used to enhance plant performance.
In one experiment, the effect of adding mulch, compost, and vermicompost was studied on *Zea mays (*corn), *Phaseolus vulgaris* (common bean), and *Abelmoschus esculentus* (okra) (Roy et al, 2010). Results showed that all three organic amendments had a positive result on the aboveground biomass production (as compared to the control where no amendments were added), with the highest productivity in vermicompost treated plots. Total biomass at the end of the experiment was recorded as follows: In maize, the control produced 627 kg/ha, the mulching produced 705 kg/ha, the composting produced 974 kg/ha and the vermicomposting produced 1220 kg/ha. For both the bean and the okra, similar patterns were observed wherein on average, the vermicompost showed the most significant results followed by compost, mulching and finally the control. (Roy et al, 2010)
Another experiment involved studying the addition of organic amendments on the yield of peas. The results concluded that surprisingly, the addition of C rich

manure and compost with similar N levels did not stimulate pea productivity (Jannoura et al, 2013).

As has been said earlier, soil pH has a vital role in plant nutrient availability and consequently plant growth and development.
At high pH levels of over 9.2, for example, Aluminum (Al) phytotoxicity becomes an issue and may retard plant development (Brautigan et al, 2014). Brautigan et al (2014)tried to lower the pH of highly alkaline soil by different means of both inorganic and organic sources, in the form of gypsum (inorganic) and glucose, molasses, horse manure, green manure and humus (organic). The study concluded that all the amendments except for humus and horse manure showed positive results in getting the pH to <9.2. However, the reduction in soil pH through organic amendments was temporary, since as the experiment went on, the soil pH got back to its initial value. Gypsum showed more promising results, since there, the lowering of soil pH was most sustained throughout the experiment.

III. Materials and Methods

III.1. Soils and fertilizers

For the growth experiment, two soils were used, the MMS soil and a sandy soil from Elverum, Norway. The Elverum soil was included as a control with similar texture and very low organic matter and nutrient content.

MMS:

MMS bulk density and pH were determined before the beginning of the experiment. The MMS soil had an initial bulk density of 1.29 g/cm^3. MMS pH was recorded at 8.94. It was composed of 86% sand, 13% silt, and 1% clay, classifying it as a "sandy" according to the "*USDA Soil Texture Triangle*". *(See page 16-17 for methods of bulk density, soil pH and soil texture measurement)*

Elverum sand:

Elverum sand had an initial pH of 6. It was composed of 96% sand and 4% silt, classifying it as a "sandy" soil according to the *USDA Soil Texture Classification.* Nutrient levels for both soils are available in the following *Table 2.*

Table 2: Nutrient levels in MMS and Elverum soil. Note that methods for nutrient extraction are different for each soil type. See page 18 for methods of extraction.

	N *(g/Kg)*	**P** *(mg/Kg)*	**K** *(mg/Kg)*	**Al** *(mg/Kg)*	**Ca** *(mg/Kg)*	**Cu** *(mg/Kg)*	**Fe** *(mg/Kg)*	**Mg** *(mg/Kg)*	**Na** *(mg/Kg)*	**S** *(mg/Kg)*	**Zn** *(mg/Kg)*
MMS	*N/A*	19	6	300	2667	0.3	28	350	220	4	0.56
Elverum	0	19	6	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*

Sewage sludge

Sewage sludge was obtained from Nordre Follo Wastewater treatment plant.
It contained 117 ±*21* g N/Kg as NO_3, and 1200 ±*0* g N/Kg as NH_4.
Ammonium and nitrate (NH_4, NO_3) were measured by flow injection analysis (FIA, Tecator FIAstar 5010 Analyzer, Hillerød, Denmark) after extraction with 2M KCl, with measurements based on the fresh material (wet sample) (described on page

III.2. Experimental plan and setup

For this study, two experiments were conducted:

III.2.1Experiment A1:

In experiment A1, the MMS and Elverum soil were tested with different fertilizer strategies and pea as a test crop. Before the start of the growth experiment, pieces of cloth-like mesh were introduced to the base of every pot to avoid soil loss during the experiment. Pots used were 1L in volume. 5 different fertilizer treatments were tested for each soil. Those included a control with only the soil, an NPK treatment, a *Rhizobium* without potassium (K) treatment, a *Rhizobium* with potassium treatment, and a sewage sludge treatment. Previous pea plants grown in MMS soils had shown signs of K deficiency (Wamelink, 2019 pers. comm.), this is why K was altered.

Each treatment had 4 different replicates.

The amount of fertilizer added was as follows: The equivalent of 12 kg/daa nitrogen was used in both NPK and sewage sludge treatments (calculated based on plant-available nitrogen from the FIA results described earlier, and 4 kg/daa in the rhizobium treatments to get some plant growth started, and with the assumption that the inoculated rhizobium bacteria could fix nitrogen at a rate of around 8 kg/daa, meaning a total of 12 Kg/daa. The assumption was based on a field experiment which showed that non-inoculated peas could fix nitrogen at a rate of 6.9 Kg/daa (Mahler et al., 1979 as cited in LaRue et al., 1981). Rhizobium was obtained from the Plant Science Department at the Norwegian University of Life Science (NMBU). For the preparation of the mixture, 4 L soil were put into a container. After the right amount of fertilizers, as described in *Table 3*, and - where applicable - rhizobium (half a teaspoon), and sewage sludge were added to the soil, the container was shaken and sealed.

Two days later, the soil for each treatment was divided into four 1L pot replicates. They were placed inside of a plastic bag to avoid soil and water loss from each pot and labeled. The seeds were then sown (3 per pot, thinned down to one plant after two weeks). Plants were irrigated every 3 days to 100 % of water holding capacity for the first time, then to 65% of water holding capacity on a weight loss basis until harvest.

(See page 16 for determination of water holding capacity)

The growth room had a temperature of 22°C and a 16-hour daylength with 7000 to 8000 lux of lighting.

The experiment lasted 46 days from sowing to harvesting.

*Table 3: Equivalent amounts of nutrients that were added in each treatment. *Nitrogen levels in rhizobium treatments are not exact but based on assumptions about N fixing from rhizobium.*

	Equivalent nutrient amounts per daa.									
	N *(kg/daa)*	**P** *(kg/daa)*	**K** *(kg/daa)*	**Mg** *(kg/daa)*	**Fe** *(kg/daa)*	**Mn** (kg/daa)	**Cu** (kg/daa)	**B** (g/daa)	**Mo** *(g/daa)*	**Zn** *(kg/daa)*
Control										
NPK	**12**	5	20	2.05	1.79	1.03	1.35	12	6	0.83
Rhizobium-K	**12***	5		2.05	1.79	1.03	1.35	12	6	0.83
Rhizobium+K	**12**	5	20	2.05	1.79	1.03	1.35	12	6	0.83
Sewage sludge	**12**									

III.2.2 Experiment A2

Experiment A2 was a continuation of experiment A1 in so that after the end of experiment A1, the same pots with the same soil were used with the addition of 61g sewage sludge per pot for the MMS soil. The soil from each pot of the previous experiment was mixed with 61 g of sewage sludge. Pots were placed in plastic bags to avoid soil and water loss and labelled. The seeds were then sown, and plants were thinned down to one plant per pot as in experiment A1. Plants were irrigated every 3 days to 65% water holding capacity on a weight loss basis until harvest after 46 days.

Sewage sludge was chosen here because it showed the best results in experiment A1.

III.3. Soil Analysis

III.3.1. Soil physical analysis

Particle Size Distribution

Percent of sand, silt and clay in the soil was determined using the pipette method which separates particle sizes based on sedimentation rates for the fine fractions, as well as sieving for the coarse fractions (Elonen,1971).

Bulk density

Initial bulk density of the soil was determined by filling in soil to a known volume (in a volumetric flask) and weighing it. The values were converted to g/cm^3.

Water holding capacity

A known weight of dry soil (A) of no less than 100 g was placed in a pot and filled with water until saturation. The pots had a mesh placed at their base to avoid soil loss. After some minutes, at field capacity when all the water had drained, the pots were weighed again. The weight of the soil after drainage (B) was then recorded.

Water holding capacity was then measured as **(B-A)/B x 100.**

Loss on ignition

Loss on ignition was determined after combustion (Nelson and Sommers, 1982) of a dried soil sample at 550°C using a Leco CHN-1000 instrument. It is a rough estimate of total C in a soil sample.

III.3.2. Soil chemical analysis

pH measurements

Soil pH was measured using a pH meter after mixing the soil with deionized water. Soil to solution ratio was 10 mL to 25 mL (1:2.5).

Plant available nutrients

For measuring plant available phosphorus (P), potassium, magnesium (Mg), calcium (Ca), and boron (B) in the MMS soil, the Mehlich 3 extraction method was used, described in "*Soil sampling and methods of analysis*" (Ziadi et al, 2007).

Amounts of P, K, Mg, and Ca in the extracts were measured using inductively coupled plasma optical emission spectrometry (ICP-OES). Amounts of B were measured using Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent ICP-MS 8800 TripleQ).

The Melhich 3 extraction was also used for the Elverum Sand, except for the determination of the initial P and K levels seen in *Table 2*, since those were predetermined using the ammonium lactate extractable fraction (Egnér et al, 1960).

Plant nutrient concentration

At the end of each experiment, the plants were cut at 1cm above the soil surface and placed into paper bags. The paper bags were then put inside the oven for 3 days at 55 °C for drying. After drying, the plants were crushed individually into small pieces using a plant grinder, then placed into paper bags.

Table 4: Grinded plant material inside of a cup

Since there was not enough plant material for ICP (plant nutrient) analysis on individual plants, and there were four replicates per treatment, the material of two and two replicates of each treatment were combined and mixed. The plant material was then decomposed before ICP analysis.

III.4. Statistical methods

For statistical analysis, R was used. One-way ANOVA using the Tukey HSD Comparisons was performed, with grouping information using the Tukey Method and a 95% Confidence level. Differences between treatments are considered significant when $p<0,05$.

IV. Results

IV.1. Plant growth and development

Germination/Seedling stage

When seeds were germinating and developing into seedlings, plants were growing in the Elverum sand at a slightly higher pace. Germination rates were high in all treatments (higher than 80% germination rate in all pots), and time before germination did not vary much between pots *(Figure 5).*

Figure 5: Germination and pea development in MMS soil (left) and Elverum soil (right) after four days in Experiment A1

Vegetative stage

In the early stages of vegetative growth, the only difference was the growth rate between the two soil types. Plants were still growing at a higher pace in the Elverum sand.

In the later stages of vegetative growth of experiment A1, differences in growth could be seen between treatments of the MMS soil. Plants grown in the sewage sludge treatment had better vegetative growth (bigger plants with more leaves) followed by the two treatments with *Rhizobium* and NPK which were all similar, and finally the control which had the smallest plants and fewest number of leaves (data not shown). Plants grown in the Elverum sand had better vegetative growth in general than those grown in the MMS *(Figure 6).*

Also, at the middle stages of the vegetative growth, all plants grown in the MMS soil started to display signs of chlorosis that eventually turned into necrosis that started on the outer edges before hitting the whole leaf. Those symptoms were observed in older leaves and became more severe with time. The symptoms were observed in both MMS A1 and MMS A2 experiments (possibly a little less severe in A2). *(Figure 7)*

Figure 6: Early stages of vegetative growth in peas in MMS (left) and Elverum (right) after 13 days in experiment A1.

<u>Figure 7:</u> Signs of necrosis in MMS A1 experiment at day 21 (left) and day 25 (right).

<u>Flowering/fruiting stage</u>

Time of flowering/fruiting was not different between MMS and Elverum or between treatments. Pods were bigger on average in the plants grown in Elverum sand. Plants grown in MMS sewage sludge had the biggest pods of the different treatments (within the MMS treatments).

Figure 8: Flowering-fruiting stages of peas in MMS (left) and Elverum (right) in experiment A1 at day 33.

IV.2. Plant yield

IV.2.1. Aboveground biomass

Experiment A1:

Plants grown in the Elverum sand had considerably higher aboveground biomass in all five treatments *(Figure 9)*. Aboveground biomass was determined on a dry weight basis.

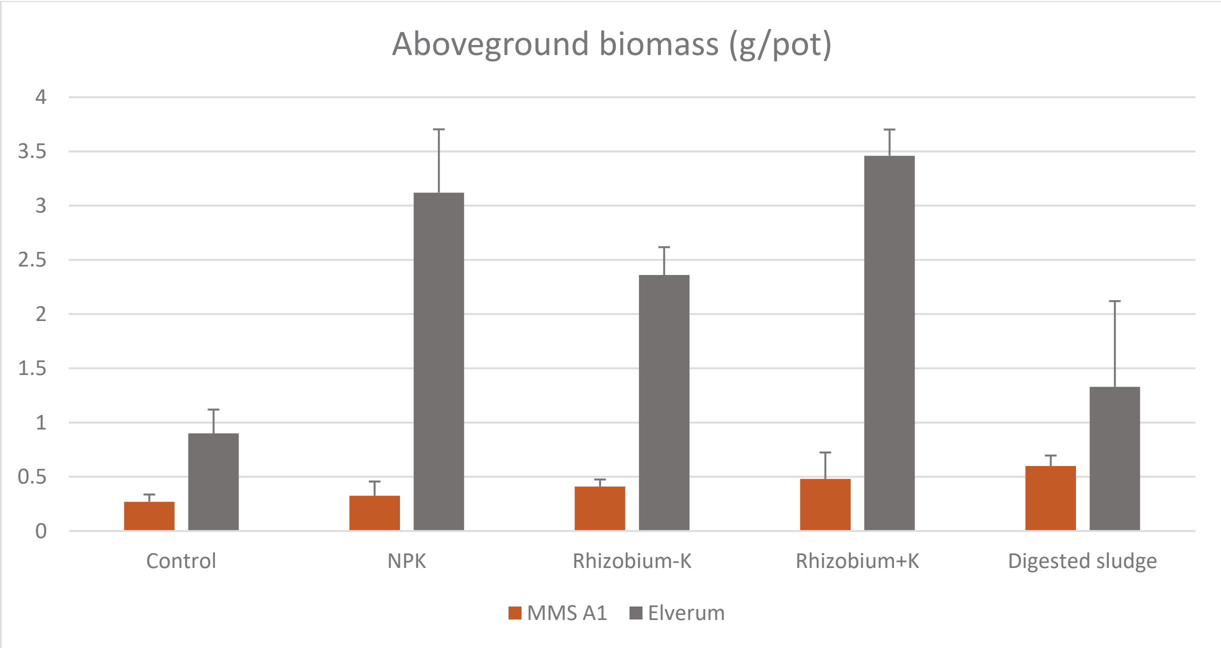


Figure 9: Aboveground biomass in the A1 experiment.

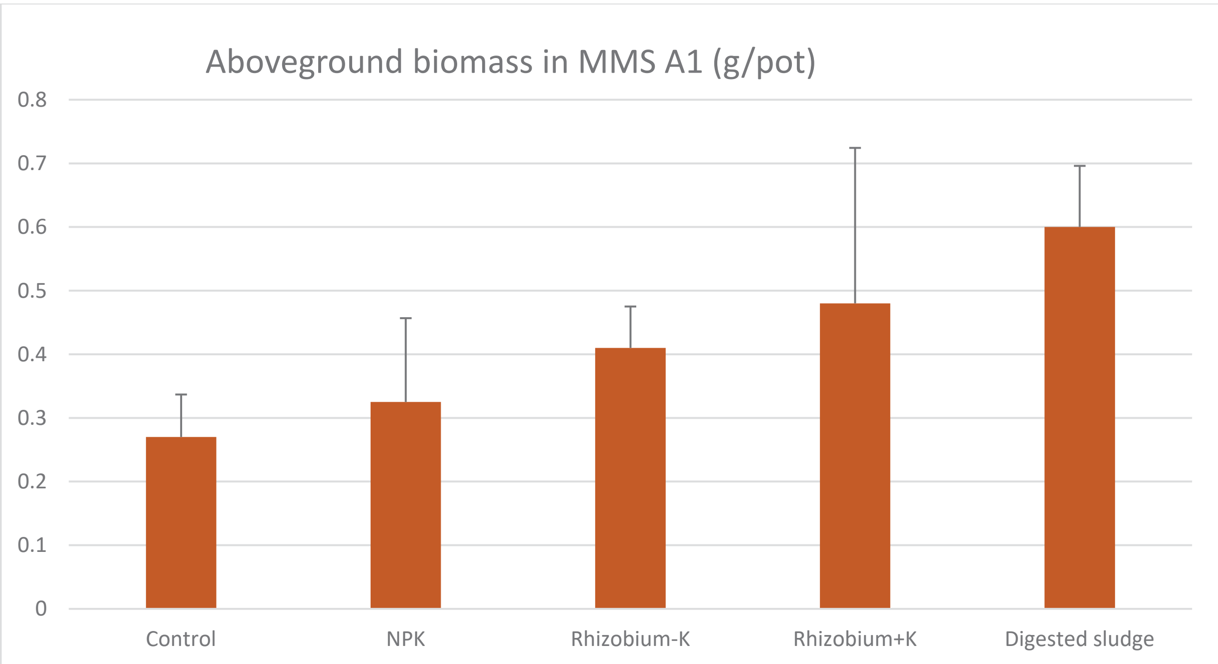


Figure 10: Aboveground biomass of the pea plants in the five treatments in the MMS A1 experiment.

In the MMS A1 experiment alone, aboveground biomass was different within each treatment. It was highest in the sewage sludge treatment (0.6g) followed by *Rhizobium*+K (0.48g), Rhizobium-K (0.41g), NPK (0.33g), and control (0.27g) *(Figure 10).*

In experiment A1, aboveground biomass was correlated with soil pH (*see Table 9 for pH values)*

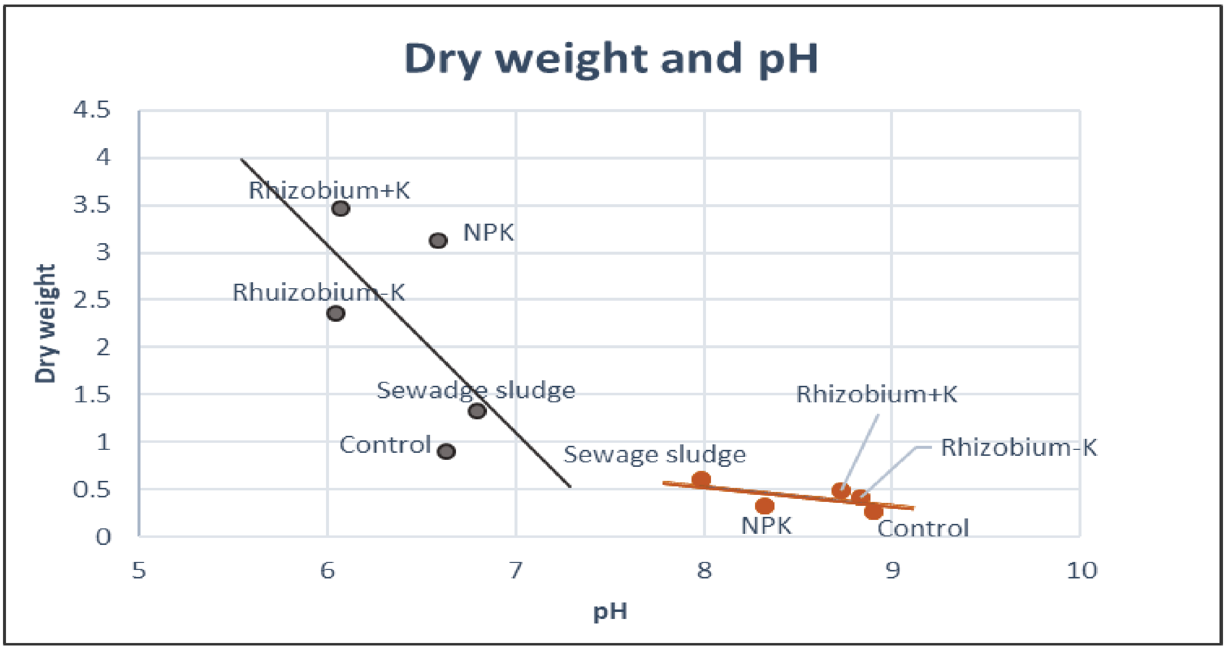


Figure 11:Plant dry weight (aboveground biomass) in relation to pH in Experiment A1. Grey represents Elverum sand. Brown represents MMS. Correlation between pH and dry weight was R=-0.61291 in Elverum (moderate negative correlation) and R=-0.61563 in MMS (moderate negative correlation).

Experiment A2:

In the follow-up experiment A2 where manure was added to all the pots using the same soil, aboveground biomass increased in all five treatments. It increased by 33% in the control, 61% in the NPK, 41% in the *Rhizobium*-K, 51% in the *Rhizobium*+K, and 12% in the digested sludge treatment *(Figure 12)*

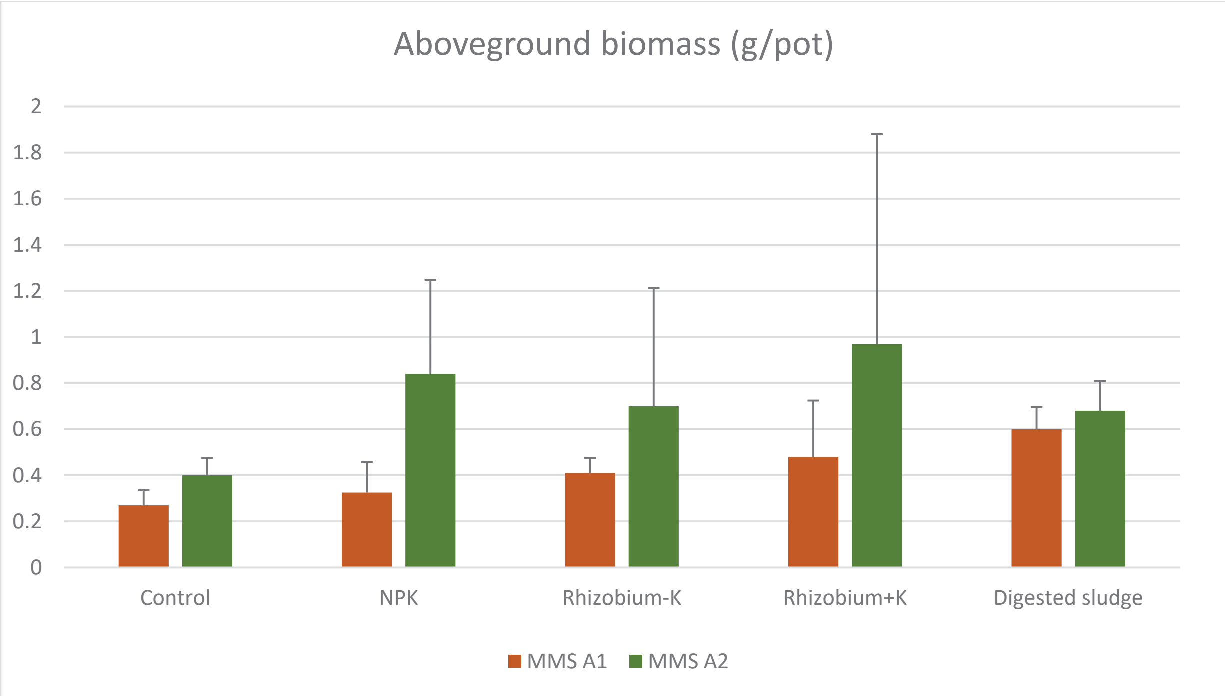
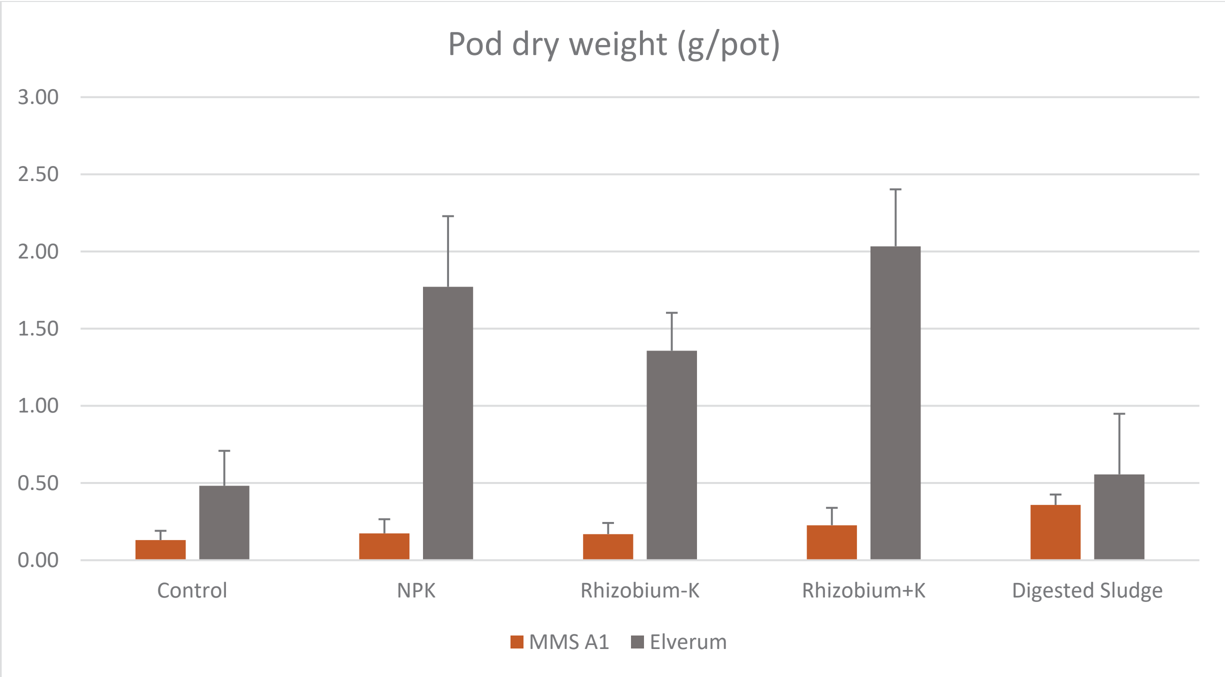


Figure 12: Aboveground biomass of plants grown in MMS soil in Experiments A1 and A2.

IV.2.2. Pod yield

Experiment A1:

The Elverum sand showed considerably higher pod dry weights in all five treatments as seen in the following *Figure 13.* Pod dry weight was calculated as: pod fresh weight * (1-plant moisture content).



<u>Figure 13:</u> Average pod dry weight of the five treatment in the A1 experiment.

<u>Experiment A2:</u>

In the follow-up experiment A2, pod yield increased in all five treatments compared to results from the A1 experiment. Pod weight increased by 21% in the former control, 66% in the former NPK, 57% in the former *Rhizobium*-K, 57% in the former *Rhizobium*+K, and 15% in the A1 digested sludge treatment *(Figure 14).*

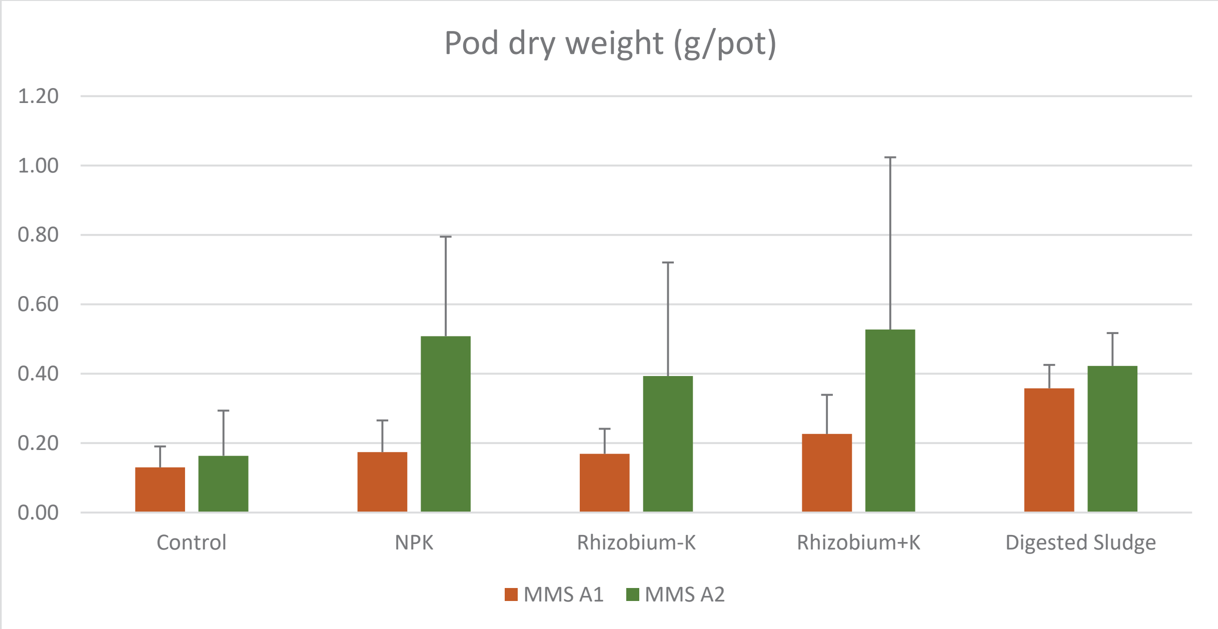


Figure 14: Average pod dry weight of the four treatments in MMS A1 and the follow-up MMS A2 experimen

The number of seeds that measured over 0.7 cm in diameter was also recorded *(Table 6).*

Table 5: Average number of seeds with at least 0.7 cm in diameter.

	Average number of seeds per plant		
	MMS A1	Elverum	MMS A2
Control	0.5	1	0.75
NPK	1	7.5	2.75
Rhizobium-K	0	8	1
Rhizobium+K	0.25	7.75	1.25
Sewage sludge	2.5	1	2

IV.3. Plant Analysis

Table 6: Plant nutrient content for experiments A1 and A2.

Plant nutrient content	Ca g/Kg			Mg g/Kg			P g/Kg			K g/Kg			B mg/Kg		
	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2
Control	14.5	7.5	20.5	2.8	1.6	3.3	2.4	1.4	2.4	21.5	14.5	22.5	1150	15	1050
NPK	18.0	12.5	20.5	3.2	2.7	3.5	2.7	2.1	1.9	21.5	20.5	18	1400	53	635
Rhizobium-K	19.0	9.4	19.5	2.9	2.3	3.2	2.7	2.2	2.5	22	19	20.5	1550	51	910
Rhizobium+K	17.5	10.4	21.0	3.2	2.5	3.6	2.5	2.2	2.5	24	11	20	1250	54	835
Sewage Sludge	21.0	9.4	21.5	3.2	4.7	3.5	2.0	1.0	2.0	16.5	14.5	16	795	22	515
Standard deviation															
Control	±2.2	±0.8	±1.1	±0.1	±0.1	±0.3	±0.4	±0.3	±0.1	±3.6	±2.2	±2.2	±70.8	±2.9	±70.8
NPK	±0	±0.8	±0.8	±0.1	±0.1	±0.2	±0	±0.1	±0.2	±0.8	±2.2	±0	±1767.8	±23	±1467.3
Rhizobium-K	±2.8	±2.4	±2.2	±0.5	±0.5	±0.5	±0.4	±0.5	±0.1	±1.5	±4.3	±2.2	±5957.4	±66.5	±5137.2
Rhizobium+K	±2.1	±0.8	±0	±0.4	±0.1	±0.1	±0.1	±0	±0.4	±0	±1.4	±0	±70.7	±4.2	±374.8
Sewage Sludge	±0	±3.5	±0.7	±0.1	±0.1	±0.2	±0	±0.1	±0.1	±0.7	±0.7	±0	±21.2	±0.7	±63.6

Nutrient concentrations in plants are expressed on a kg dry basis.

Boron

Boron levels in the plants were very high in all the treatments in the MMS soil (Table 7). On average, MMS A1 plant concentrations were 1229 mg B /g/kg for all five treatments; MMS A2 plant concentrations were lower at 789 mg B /kg. B concentration in plants grown in the Elverum sand were by far the lowest at 39 mg/kg.

Plants grown in the sewage sludge treatment had the lowest B concentrations in both the MMS A1 and MMS A2 experiments (795 and 515 mg/kg, respectively) out of all the treatments, including the control.

Potassium

Plant K content did not vary much between the plants grown in the Elverum sand and the ones grown in the MMS.

However, in the plants grown in MMS soil, plant K content was the lowest in the sewage sludge treatments (16.5 g K/kg in plants from MMS A1 and 16 g K/kg in plants from MMS A2)

Calcium

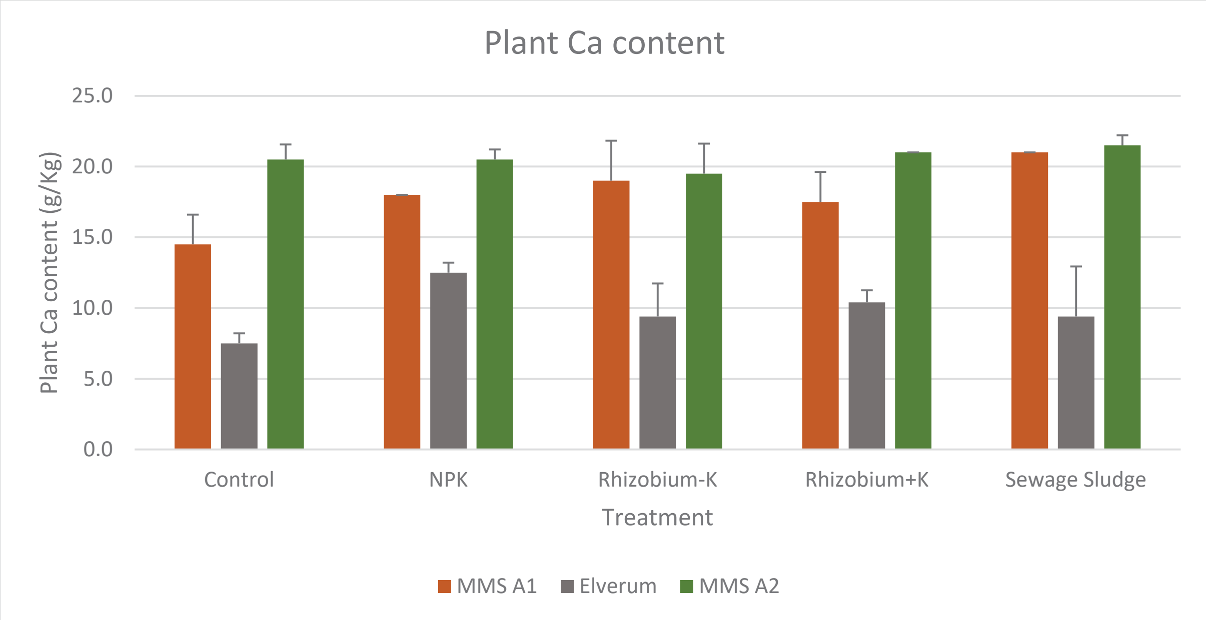


Figure 15: *Plant Ca content for all treatments in MMS A1 MMS A2 and Elverum sand.*

Calcium exhibited similar patterns in most treatments. Ca levels were generally highest in the MMS A2 plants, followed by the MMS A1 plants and finally the Elverum plants *(Figure 15)*.

Nitrogen and Carbon

Table 7: *Nitrogen and Carbon content in the plants.*

	% Plant N content			**% Plant C content**		
	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2
Control	3.4	3.1	4.1	42.0	44.0	42.6
NPK	4.1	2.8	3.5	43.1	43.1	42.9
Rhizobium-K	4.0	3.2	3.8	43.2	43.3	42.8
Rhizobium+K	3.6	3.0	3.6	42.8	43.7	42.5
Human manure	3.6	2.5	3.8	42.6	41.9	42.8
	Standard deviation					
Control	*±0.5*	*±0.3*	*±0.1*	*±0.3*	*±0.3*	*±0.4*
NPK	*±0.2*	*±0.2*	*±0.2*	*±0.1*	*±0.1*	*±0.1*
Rhizobium-K	*±0.4*	*±0.1*	*±0.3*	*±0.2*	*±0.1*	*±0.3*
Rhizobium+K	*±0.3*	*±0.1*	*±0.7*	*±0.6*	*±0.1*	*±0.4*
Human manure	*±0.1*	*±0.1*	*±0.2*	*±0.4*	*±0.8*	*±0.1*

Plant N content was lower in the Elverum sand (2.91%) than in both MMS experiments where it was similar (3.74% in MMS A1 and 3.76% in MMS A2*) (Table 8)*

Plant C did not vary much between the treatments or the soils.

IV.4. Soil Analysis

Table 8: Soil pH and %loss on ignition.

	soil pH			%Loss on ignition		
	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2
Control	8.90	6.63	8.07	1.05	0.27	1.41
NPK	8.32	6.59	7.89	1.11	0.29	1.41
Rhizobium-K	8.83	6.04	7.80	1.21	0.35	1.40
Rhizobium+K	8.73	6.07	7.86	1.11	0.37	1.50
Sewage Sludge	7.99	6.79	7.59	1.42	0.79	1.83

Soil pH

The Elverum sand had the lowest soil pH range (6.04-6.79) compared to (7.59-8.07) in MMS A2 and (7.99-8.90) in MMS A1.

Also, the average MMS pH decreased by 8.2% in the MMS A2 experiment compared to the MMS A1 (from 8.55 to 7.84).

The soil pH of the sewage sludge treatment (7.99) was 8.1% lower than the average pH of the rest of the treatments in MMS A1 (8.7). The soil pH of the sewage sludge treatment (7.59) was 4% lower than the average pH of the rest of the treatments in MMS A2 (7.9).

Loss on ignition (LOI)

LOI in MMS A1 was statistically significantly different from LOI in MMS A2.

LOI was also the highest in the treatment with sewage sludge in all three experiments (MMS A1, MMS A2 and Elverum). LOI in the sewage sludge treatments of both MMS A1 and the Elverum was also found to be statistically significantly different from all the other treatments in the respective soils.

Table 9: *Soil nutrient content for experiments A1 and A2.*

	Soil Nutrient Content														
	Ca g/Kg			**Mg** g/Kg			**P** g/Kg			**K** g/Kg			**B** mg/Kg		
	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2	MMS A1	Elverum	MMS A2
Control	3175	53	3200	418	8	423	17	10	64	383	16	380	16750	320	9275
NPK	3325	79	3450	405	9	408	26	17	63	445	33	443	11500	320	9800
Rhizobium-K	3400	69	3450	410	11	403	25	17	78	400	12	393	14000	288	11875
Rhizobium+K	3600	67	3700	408	12	385	25	17	73	420	33	390	12675	353	7175
Sewage Sludge	3600	81	3550	405	9	378	80	64	136	343	17	325	10025	155	8175
	Standard deviation														
Control	±171	±7	±82	±27	±2	±5	±1	±1	±12	±13	±9	±5	±6397	±36	±2545
NPK	±96	±10	±58	±32	±1	±19	±3	±1	±26	±32	±5	±5	±1291	±63	±876
Rhizobium-K	±142	±13	±58	±30	±2	±15	±1	±2	±7	±29	±2	±5	±2161	±39	±2323
Rhizobium+K	±231	±10	±82	±38	±1	±6	±1	±2	±23	±34	±8	±15	±2343	±120	±640
Sewage Sludge	±245	±54	±130	±20	±5	±13	±15	±48	±30	±21	±9	±18	±2018	±55	±1250

Boron

Boron concentrations were clearly higher in the MMS soil compared to the Elverum soil.

Average B concentrations of all treatments were 12990 mg/kg in MMS A1, 9260 mg/kg in MMS A2, and 287 mg/kg in Elverum sand.

Potassium

Potassium concentrations were clearly higher in the MMS soil compared to the Elverum.

Average K concentrations in all treatments were 398 g/kg in MMS A1, 386 in MMS A2, and 22 g/kg in Elverum sand.

Also, K concentrations decreased in every treatment going from MMS A1 to MMS A2. K concentrations were lowest in the sewage sludge treatment in both MMS A1 and MMS A2.

Calcium

Calcium levels were clearly higher in the MMS soil compared to the Elverum soil.

Average Ca concentrations in all treatments were 3420 g/kg in MMS A1, 3470 g/kg in MMS A2, and 70 g/kg in the Elverum sand.

Phosphorus

Phosphorus levels were significantly higher in the sewage sludge treatments in all three soils. In the MMS A1, sewage sludge soil contained 80 g P/kg, whereas the average of the other treatments was 23 g P/kg. In the Elverum sand, sewage sludge soil had 64 g/kg where the average of the other treatments was 15. The MMS A2 soils, where sewage sludge was added to every treatment, had at least twice as much phosphorus in every treatment

V. Discussion

IV.1. Overview of the experiment

As the results have shown, plants grown in the MMS soil had a slower growth rate, less aboveground biomass, and lower yields compared to the ones grown in the Elverum sand. MMS plants also exhibited strong necrotic symptoms in the older leaves. In the following paragraphs, possible explanations for the results will be discussed.

Boron toxicity

Boron concentrations were extremely high in both plants, and soil of the MMS experiments. In chickpeas, another legume belonging to the same (Fabaceae) family as peas, any plant tissue concentration of Boron that falls above 190 mg/kg can be considered toxic (Singh et al, 2010). Plants grown in the MMS had a B concentration ranging from 515 mg/kg to 1550 mg/kg which clearly exceeds the toxicity threshold limit for chickpeas suggested by Singh et al.

B toxicity is typically seen in mature leaves as marginal or tip chlorosis and necrosis (Marschner, 2012). Symptoms also include decreased plant dry weight (Princi et al, 2016). All those symptoms were observed and recorded in the experiment as seen in *Figure 16.* Boron toxicity in peas was also observed in another experiment (Muhammad et al, 2015), as seen in *Figure 17,* where symptoms were identical. This all suggests that peas grown in the MMS soil had toxic B concentrations. The Elverum sand, on the other hand, did not show any signs of B toxicity.

Interestingly, the MMS originates from a volcanic rock formation near a town called Boron (Mungas et al, 2007), named after the element, where borax $Na_2B_4O_7{\cdot}10H_2O$ is mined (Krister and Helvaci, 1994). When borax reacts with a mineral acid, it turns into boric acid $B(OH)_3$, the form of B that can be taken up by the plants (Hu and Brown, 1997). This gives an indication as to why high levels of Boron were found in the MMS soil and plants in our experiment given its origins. It also worthwhile noting that boron was detected in situ on Mars for the first time in 2017 at levels below 0.05 wt % (500 mg/kg) (Gasda et al, 2017), a concentration that is much lower than that found in the MMS.

<u>Figure 16:</u> Signs of B toxicity in peas: Marginal and tip chlorosis in leaves. From experiment A2 in the MMS soil


Figure 17: B toxicity signs in peas from different experiment (Muhammad et al, 2015)

For human consumption, acute lethal dose of boric acid is in the range of 3 000-6 000 mg in infants and 15 000-20 000 mg in adults (Bakirdere et al, 2010). With the highest concentration of B in plants from our experiment at 1550 mg/kg (given in plant dry weight and not fresh weight), the minimum amount to be ingested for an infant lethal dose would be around 2kg of dry weight, or around 10 kg of fresh weight. This is not likely to occur.
However, it is important to keep in mind that the numbers do not represent the whole picture, and plant B concentrations are from whole plants, of which pod B concentrations may vary. It is also important to note that clinical symptoms of Boron toxicity have also been reported at a dose as low as 100 mg (Bakirdere et al, 2010).

Interestingly, B concentrations in both plants and soil were lower when sewage sludge was added to the MMS soil. This was the case for every treatment (Both in plants and Soil). The average decrease of B concentration (Experiment A1 to

Experiment A2) after incorporating the sewage sludge in plants was 36%. The average decrease in estimated plant available concentrations was 29%.This is most likely because B becomes less available when pH goes from around 8.70 (average MMS A1 without sewage sludge) to 7.84 (average MMS A2) as clearly seen in *Figure 4, page 11.* Another important element that might play a role in B availability is the presence of organic matter (Goldberg, 1997). In one of many studies that came to the same conclusion (Goldberg, 1997), Yermiyahu et al (1995) showed that Boron sorption by soil-composted organic matter mixtures increased as the organic matter content increased.

In the MMS experiment, loss on ignition was statistically significantly different in the treatments where sewage sludge was added compared to the rest of the treatments. Therefore evidently, the addition of sewage sludge contributed to a higher loss on ignition, which can be translated into more organic matter.

The incorporation of organic material in the form of sewage sludge, and the decrease of pH can therefore explain why B available concentrations in soil and subsequent B concentrations in plants were lower in the sewage sludge treatments and why severity of B toxicity was reduced.

Biomass and yield in relation to pH

According to the *USDA*, 5.5-7.0 is an ideal pH range for peas (Pavek, 2012). In this experiment, soil pH went from 8.70 in the MMS treatments without sewage sludge to 7.84 in the MMS treatments with sewage sludge. The average pH of the Elverum sand was 6.42.

As *Figure 4 page 11* suggests, nutrient availability depends on soil pH. The pH values from the experiment could therefore be a reason why plants grown in the Elverum sand (with an ideal pH) had the highest aboveground biomass and yields followed by the plants grown in the MMS A2 (second closest to the ideal pH range from the three experiments) and finally the MMS A1 (least close to the ideal pH range) *(Figure 9,12,13,14, pages 24-28).*

Effect of soil pH on aboveground biomass can be seen in one experiment, where Blanchard et al (2020) showed that cucumbers grown in an aquaponic system with four different pH levels (5, 5.8,6.5, and 7) had different yields, with the highest yield obtained at pH 5.

Phosphorus

In the MMS A1 experiment, plant-available P concentrations in the soil were highest in the sewage sludge treatments (80 g P/kg) whereas the average of the rest of the treatments was 23 g P/kg. In the MMS A2 experiment, soil P concentrations were at least double those of the respective MMS A1 treatment. However, this rise in P concentration in the soil did not translate into a rise of P concentrations in the plant. In fact, P concentrations in the plants were lowest in the MMS A1 sewage sludge treatments and second lowest in the MMS A2 sewage sludge treatments. This could be due to the decrease of pH with the addition of sewage sludge. *Figure 4, page 10* shows how P becomes less available when pH decreases from 8.7 (MMS A1) to 7.84 (MMS A2). In high pH soils, calcium reacts with phosphorus to form calcium-phosphate bonds which are least soluble at pH 8 (Hopkins and Ellsworth, 2005) and thus less available. The MMS A2 had the closest pH to that at 7.84. This could explain why P levels in the plants grown in the MMS A2 were lower.

Dysko et al. (2008) studied the impact of different nutrient solution pH(s) (ranging from 4.5 to 6.5) on P concentrations in tomatoes grown in straw, peat, and rockwool. Their results, in accordance with this experiment and *Figure 4*, showed that average P concentrations in plants can vary with changes in pH. In their experiment, P concentrations in leaves from different growth media at pH 4.5 were statistically significantly different from the mean concentration at pH 6.5.

Potassium

Symptoms of potassium deficiency in peas can be similar to boron toxicity symptoms that were observed. In fact, K deficiency in peas results in necrotic spots in older leaves, which can be more apparent in leaf margins (Government of Western Australia, 2015). Results from the ICP analysis showed that K concentrations in plants of MMS was not an issue. Even though the available concentrations were very high in the MMS soil itself, *(Table 10, page 33),* the concentrations in the plants did not vary from the ones grown in the Elverum sand *(Table 7, page 29),* in which the plants did not display any symptom of K deficiency, or any other deficiency/toxicity symptom.

VI. Conclusion

This experiment has shown that it is possible to grow peas on a Martian soil simulant. However, those plants had very low aboveground biomass and yields compared to plants grown in an infertile sand. In addition, they showed severe symptoms of boron toxicity, which seems to be the most evident problem in the soil.

For better plant growth, the high soil pH levels seem to be another problem that needs to be tackled. The addition of sewage sludge has proven to reduce the severity of the toxicity (and lower the pH), but is not sufficient to avoid B toxicity altogether.

This study could also be relevant to agricultural soils with low organic matter and are contaminated with boron in general, since in accordance with previous studies, the experiment has shown that the addition of sewage sludge could help to a small extent in that respect.

Contrary to suggestions from growth experiments in the MMS soil conducted at Wageningen, plant nutrient concentration of this experiment has shown that potassium was not deficient in the plants.

It is reasonable to think about all that can be misrepresented when it comes to an actual growth experiment on Mars: MMS being not an exact simulant, Mars not having the same gravity as Earth etc. However, it is also reasonable to see all the similarities. The results of this experiment are not conclusive in this respect. So, for the time being, more research needs to be done.

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