Title. Habitability Zone: The challenge of the five kingdoms.

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Abstract

In 2019 Chinese scientists explained their aspiration to grow plants, such as potatoes and cress, and breed silk worms on the dark side of the Moon.

From this starting point we wondered if these species were the best options to be the first lunar settlers, the “ambassadors” of planet Earth on another celestial body, so we set off “The Challenge of the five Kingdoms”.

Based on two physical properties, light and temperature, we compared the five kingdoms of the natural world in order to find the super-organism which could have the best chance to survive on the Moon.

Our project studies the concept the habitable zone and the characteristics of living organisms in accordance with the Primary school science curriculum. In the course of this research, students have analysed scientific papers, investigated about animals and plants development processes and performed scientific experiments with species from all the kingdoms in order to contrast their hypotheses.

This work is also a continuation of our astrobiology projects in which we work with students of different ages and their families.

Summary

The search for life on planets other than Earth is studying the habitability zone, which is defined as a region in space, at a certain distance from a star, so that the temperature of the planet allows the existence of liquid water.

In addition, other requirements must be taken allowed such as atmospheric pressure allowing water to exist in a liquid state, the greenhouse effect does not raise the planet's temperature above a limit, volcanic activity does not increase the greenhouse effect, magnetic fields protect from bombardment of highly energetic particles, albedo does not forward to space a high percentage of radiation from the star.

Chinese scientists from several universities, led by Chongqing University, published an article expressing their intention to grow certain plants and silkworms on the hidden face of the Moon.

From that article we have wondered if plants and silkworms are really the two best candidates to become the first “selenitas”.

Chinese scientists expressed concern about the temperature and light conditions that these species will need to survive on the Moon. Therefore we have focused on these two physical magnitudes to realize “The Challenge of the Five Kingdoms”.

During this 2019-20 academic year, we have investigated with primary school students the faculties of the five kingdoms to choose the perfect candidate, the "super-terraqueo" organism, with the best characteristics to represent earthly life on the Moon.

It is an annual, multidisciplinary and family integrative project, faithful to the official science curriculum and a continuation of our studies in the field of astrobiology initiated in previous courses.

Development

1. Introduction
The astrobiology project that was carried out during the 2019-20 academic year with primary school students, is based on knowing the habitability zone of our solar system, experimenting with certain physical conditions in the different species to extrapolate to other places in the Universe. The work is a continuation of the project of the past course; we continue to be based on the basic science curriculum at this educational stage. We saw it appropriated to involve families in scientific knowledge with accessible language, which motivates them to self-learning and research. The experiences at home and the substantive function were to raise awareness, motivate, persuade and invite to think about the subject developed. The College-Family-Science tandem fulfills several objectives: participation be organized for families from the academic field, revaluing the Family-School binomial and improving the school and social perception of science by citizens.

2. General objectives
   - Study the general characteristics of the five realms of terrestrial nature.
   - Understand what habitability zone means.
   - Study how living things appeared on Earth.
   - Recognize the physical conditions that affect life.
   - Continue working in the field of astrobiology from the primary curriculum based on the scientific method.
   - Encourage families to carry out home experiences in an orderly and rigorous way.
   - Revalue the family-school binomial through interinstitutional participation in the project

3. Methodology
The project has been developed with a constructivist methodology that emphasizes the open expression of what students think about the subject. On the other hand it is based on science system programs, which encourages experimentation based on the scientific method, developing research skills.

Communication, after the developed experience, has been fundamental to unifying criteria and conclusions, therefore we have made magazines that we distribute to all families so that they could know the group results.

Due to the situation experienced in the last trimester of the school year, the telematics methodology has been fundamental. The use of video conferencing, the school platform, together with digital magazines (by e-mail) facilitated the transfer of information.
4. Sequencing

First quarter
The first quarter was planned to work in the classrooms of the different courses participating in the project. Among other topics we studied in the science subjects the following:

**Natural Sciences.**
Unit 1. Living things. The five Kingdoms. The importance of light.

**Social Sciences.**
Unit 1. Our planet and the moon. Our solar system.
Unit 2. The atmosphere, the weather and the climate. Importance of climate and water in living things.

It was necessary for students to have theoretical knowledge on issues related to living beings and where we live.

**Session 1.** Project presentation to families.
In December, the project was presented to the participating families. Subsequently, a family-student commitment was signed in which they were responsible for the theoretical and experimental work that had been scheduled. 17 families participated.

Second quarter

**Session 2.** Student workbook.
On Christmas holidays, students were provided with a workbook. It was intended to remember theoretically everything learned in class. In addition, they were offered the opportunity to express, at various points in the document, their critical contribution and situation.

**Scientific Journal 1.** "The challenge of the five kingdoms. Mission: to find the super-tarráqueo".
In January, the first edition of the magazine was distributed to all families. It exposed everything that had been worked on. Individual contributions and general conclusions reached were drafted.

The main objective was that the students had information from all their peers and, after general conclusions, we all worked in the same direction. We saw appropriated to send this copy to all primary school families. Our goal was to give maximum dissemination and encourage more families to participate.

**Session 3.** Stick-Insects. Information y justification to families.
At the end of January, exemplary of stick insects were handed out to families. They were provided with information about this species: their diet, type of reproduction and behavior in nature. The ultimate purpose of this installment was for the children to become familiar with and learn by caring for them.

**First Scientific Text.** "The stick insect is able to reproduce after dead."
It was proposed the article "Potential role of bird predation in the dispersal of otherwise flightless stick insect", (2018) by the journal ECOLOGY and other articles in spanish. Documentation is part of the method and is necessary at all times to move on to experimentation.

**Scientific journal 2.** "Fásmido: Life after death".
At the end of February, the second edition of the magazine was sended to families, as we did with previous edition, for the same purpouses.

**Session 4.** Silkworms. Information, justification and distribution to families.
In early March (before confinement) families received silkworm eggs, along with information about this species: their diet, type of reproduction and behavior in nature. It was justified to have the
silkworms because the students learn more by taking care of them. At this point the experimentation phase began, subjecting each family eggs to different physical conditions, simulating the work of Chinese scientists.

Based on the article “The Chinese to grow potatoes and silkworms on the Moon” and other articles in spanish, it was prepared a workbook for the students.
We provided the documentation to justify the experimentation they were doing.

Scientific Journal 3. "We investigated astrobiology with silkworms."
Due to the confinement of early April, this edition was sent to all families by email with the conclusions from the scientific commentary and from the first results of experimentation with worm eggs.

Third quarter
Session 5. Plant Kingdom and Prototist Kingdom (Telematics).
At the end of April we held a telematics meeting (Meet) in order to give instructions for the experimentation phase with the plant and prototist kingdoms.

Session 6. Fungi Kingdom and Monera Kingdom (Telematics).
In early May we had a telematics meeting (Meet) in order to give instructions for the experimentation phase with the kingdoms of fungi and monera.

Scientific Journal 4. “Kingdoms, inferior?” Where the personal opinions of participants and the general conclusions are drafted.

5. The five kingdoms
5.1. Animal kingdom

INTRODUCTION

The Animal Kingdom brings together the most complex living things on our planet. They are pluricellular, heterotrophic (nourished by ingestion of other living beings) and most have a developed nervous system that allows them to react to the stimuli of the environment around them.

Among of all the members of the Animal Kingdom, insects are the largest and most diverse class, with more than one million species described; they have adapted to live in all the habitats of our planet thanks to their extraordinary characteristics. Could they be our champions of the "Challenge of the Five Kingdoms"?

To represent the Animal Kingdom, we have studied the behaviors of two insects, the phasmids (Phasmode order) and the silkworms (Bombyx mori L.), against variations in temperature and light.

With regard to stick insects, phasmids, a comment was made on a research article published by Japanese scientists, in which the resistance of the eggs of these animals was disclosed. This article described the ability to hatch outside phased eggs, having been ingested by certain birds and transited through their digestive systems. This fact makes it possible to assume that nature uses, as with the seeds of plants, this system to contribute to the expansion of these species.
The ability of eggs to survive the acidity of the digestive system and the body temperature of birds makes the phasmids firm candidates to participate in the challenge of the "Challenge of the Five Kingdoms".

Believing the convenience that students should familiarize themselves with this species to know them more, we distributed some copies to each family.

About the silkworms, we start from the Chinese researchers' scientific article referred to in paragraph 4 “Sequencing”. Housed in an aluminum container, a "minibiosphere", with water, nutrients and air are proposed to check the possibility of hatching the eggs.

After working on the document and informing families about the experience done, we handed out worm eggs to all of them.
PROCEDURE AND RESULTS

Given the importance of experimentation with silkworms, directly mentioned in the article, we subjected our worms to three different parameters (temperature, light and diet) in three different stages of their development (egg, caterpillar and chrysalis) to study the maximum possible situations:

The following table explains all the itineraries followed during the egg phase:

**Table 1. Experiments with silkworm eggs**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Light</th>
<th>Nº Exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intenso Cold (24h A -28ºc)</td>
<td>Absolute Darkness</td>
<td>1</td>
</tr>
<tr>
<td>Day/Night Alternation</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Low Temperature (North Facing Place)</td>
<td>Absolute Darkness</td>
<td>3</td>
</tr>
<tr>
<td>Day/Night Alternation</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Environmental Temperature</td>
<td>Absolute Darkness</td>
<td>5</td>
</tr>
<tr>
<td>Day/Night Alternation</td>
<td>6 Control</td>
<td></td>
</tr>
</tbody>
</table>

Not all families made all the experiences, they were divided to facilitate the work.

The results are shown in the following table:

**Table 2. Results experiments with silkworm eggs**

<table>
<thead>
<tr>
<th>Nº Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability Eggs</td>
<td>No</td>
<td>Only 1</td>
<td>Most</td>
<td>All</td>
<td>Most</td>
<td>All</td>
</tr>
<tr>
<td>Caterpillar size</td>
<td>---</td>
<td>Abnormal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Next, the individuals who remained alive had two different types of diet and we subjected them to two different light conditions.

The experiment and the results of the caterpillar phase are shown below:

**Table 3. Experiment with caterpillers of silkworms**

<table>
<thead>
<tr>
<th>Light</th>
<th>Diet</th>
<th>Grothw</th>
<th>Chrysalis formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute Darkness</td>
<td>Mulberry</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mulberry/Lettuce Alternation</td>
<td>Less</td>
<td>Littel Densa</td>
<td></td>
</tr>
<tr>
<td>Day/Night Alternation</td>
<td>Morera</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Mulberry/Lettuce Alternation</td>
<td>Less</td>
<td>Littel Densa</td>
<td></td>
</tr>
</tbody>
</table>

The worm that was born after being its egg 24 hours in the freezer was much smaller in size than the others and died with the exclusive diet of mulberry leaves.
The cocoons from an exclusive mulberry diet were then divided into three groups as shown in the following table: some were introduced, some grew to and the remaining a):

Table 4. Experiment with silkworms chrysalis

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Cold (24h a - 28°C)</th>
<th>Low Temperature</th>
<th>Environmental Temperature (Control)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatching</td>
<td>No</td>
<td>No</td>
<td>All</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS OF THE ANIMAL KINGDOM

As seen above, the cold, even moderate, greatly affects silkworms, although we got one of the eggs exposed to -28°C hatching, was viable for a few days. The chrysalis were also not able to withstand the cold and although the caterpillars were not exposed to different temperatures, we can infer that the result would have been similar.

Regarding the diet, those worms with a combined lettuce-brunette diet grew smaller than those on an exclusive mulberry diet and formed lower-density silk chrysalis. Another characteristic of these animals is their great voracity, a negative aspect if we want to breed them in such a remote area. The only parameter that didn’t seem to affect them was the light conditions.

That is why it seems to us that silkworms would have very little chance of overcoming the "Challenge of the Five Kingdoms", they need very specific conditions to survive.

However, if we consider stick insects we may have a strong candidate. According to the observations of our little scientist Ariadna Castaño, phasers do not need to feed as much as silkworms. She has observed that they may be 15 days without eating and should not be given to drink. They are also reproduced by partogenesis (females produce eggs without fertilization) and their eggs are very resistant.

Therefore we consider that the survival chances of stick insects on the moon are very high, but could they win over other Kingdoms?
5.2. **Plantae Kingdom**

**INTRODUCTION**

The members of the Plantae Kingdom are living beings that are distinguished by performing photosynthesis, that is, they are autotrophic, so they are extraordinarily independent since they do not need to be supplied with food to survive, the presence of CO\(_2\), mineral salts and light is sufficient for them to develop.

From that point of view, Chinese researchers’ project to achieve plant growth on the Moon is not far-fetched. Through photosynthesis and cellular respiration plants regulate and balance the gases necessary for their survival, CO\(_2\) and O\(_2\); through perspiration they renew the water and if they are supplied with a substrate and light they can live without problem. However, on the Moon they would encounter a great inconvenience, fluctuating temperature due to the absence of a significant atmosphere.

Remember that the lunar temperature can fluctuate from 123ºC in the sunny part to -153ºC in the dark part, and at the poles it reaches below 200ºC. Maintaining a stable temperature and within habitability limits is going to be a challenge for scientists, because how do plants react to the cold?

In this Kingdom we will experiment with two different groups of individuals: briophytes (without conductive vessels) and vascular plants (with conductive vessels).

**Briophytes: Mosses**

**PROCEDURE AND RESULTS**

Moss samples were collected in the drain gutters.

The mosses were divided into five vessels and exposed to five different environments:
- Vessel 1: Direct exposure to the Sun
- Vessel 2: Ambient temperature but in the shade
- Vessel 3: Ambient temperature but in the dark
- Vessel 4: Moderate cold, 4 ºC for 24h
- Vessel 5: Intense cold, -22 ºC for 24h

Table 5 explains the distribution of mosses and the results of experiments. Moss activity was measured indirectly, observing color and volume.

**Table 5. Results of moss exposure to different temperatures and light.**

<table>
<thead>
<tr>
<th>Vessel 1</th>
<th>Vessel 2</th>
<th>Vessel 3</th>
<th>Vessel 4</th>
<th>Vessel 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct exposure to the sun</td>
<td>Ambient temperature but in the shade</td>
<td>Ambient temperature but in the dark</td>
<td>Moderate cold (4 ºC) for 24h</td>
<td>Intense cold (-22 ºC) for 24h</td>
</tr>
<tr>
<td>1º Week</td>
<td>Green colour</td>
<td>Green colour</td>
<td>Green colour</td>
<td>Green colour</td>
</tr>
<tr>
<td>2º Week</td>
<td>Green colour</td>
<td>Dark Green colour</td>
<td>Dark Green colour</td>
<td>Green colour</td>
</tr>
<tr>
<td>3º Week</td>
<td>Green colour</td>
<td>Dark Green colour</td>
<td>Dark Green colour</td>
<td>Green colour</td>
</tr>
</tbody>
</table>

We see no significant differences even those specimens that were in total darkness and at low temperatures.
Vascular plants: Legumes

PROCEDURE AND RESULTS

We work with legume seeds because it is easy to get and germinate. 4 groups of seeds were made:

- Group 1: In a bowl we put 30 chickpea, bean and lentil seeds (10 of each) and put them in the freezer. After 24 hours we take the seeds out of the freezer and put them in water for another 24 hours.

- Group 2: In the same way we take another 30 seeds and put them in water. After 24 hours we put the seeds in the freezer for another 24 hours.

- Group 3: We put in water 30 seeds for 24h. Once the seeds had germinated and the plants reached a certain size, the pot with all the plants was put in the freezer for 24 hours.

- Group 4: We put 30 seeds in water, this group will act as control.

![Image 7. Procedure with legume seeds.](image)

All seeds were planted in four different pots respecting the four groups as indicated in the table along with the results obtained.

Table 6. Results of exposure of the vascular plants to the cold

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germination</td>
<td>25</td>
<td>15</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Growth</td>
<td>Good</td>
<td>Good</td>
<td>Slow</td>
<td>------</td>
</tr>
<tr>
<td>Growth after the cold</td>
<td>Doesn’t apply</td>
<td>None</td>
<td>Doesn’t apply</td>
<td>Doesn’t apply</td>
</tr>
<tr>
<td>Growth after 2 weeks</td>
<td>Good</td>
<td>None</td>
<td>Slow</td>
<td>Doesn’t apply</td>
</tr>
</tbody>
</table>

KINGDOM PLANTAE CONCLUSIONS

The results suggest that the problem that plants must overcome in order to survive at low temperatures is the freezing of water. Low temperatures slow its growth and paralyze it, but if the water freezes:

- It can destroy plant cells by forming crystals that break down cell membranes or by increasing the volume of water when frozen.

- Prevents the absorption of water by the roots, water is needed to transport substances inside the plant, sap, and to make photosynthesis.

To resist low temperatures, the Plantae Kingdom has adopted several strategies:
- Secretion of chemical compounds of antifreeze action.
- Reduced the size of your leaves to reduce the surface area of exposure.
- Darker colored leaves to absorb more heat from the Sun.
- Hairs in the aerial areas that protect the organs of the plant.
- Loss of leaves in winter to avoid freezing.
- Low water content in the leaves.

Tundra is the biome where we find the members of the Plantae Kingdom with the most capacity to withstand the cold. Its vegetation consists of pastures, mosses and lichens that do not pass 10cm high.

In this part of the planet the soil remains frozen most of the year as the average temperature is -34ºC, reaching even -50ºC occasionally during the colder months. In addition, daylight hours are scarce during those months.

Due the mosses don’t have vascular system, they absorb the water directly by "hojitas"( little sleves), that gives them an advantage, a superpower, when it comes to combating the cold. Its small size also plays in its favor because it reduces the surface area of exposure to low temperatures.

On the other hand, legume seeds are true survival capsules, but when liquid water is needed to restart and germinate, they suffer cell ruptures caused by ice. The same happens in its aerial parts.

So what if Chinese scientists were able to insulate their plants from extreme temperatures, could the Kingdom Plantae the Five Kingdoms Challenge?

5.3 Fungi Kingdom

**INTRODUCTION**

The members of the Fungi Kingdom were for a long time included in the Plantae Kingdom because they had, some of them, a plant-like appearance. However, they are heterotrophic, pluricellular, and single-celled living things that need wet places to live. They feed on decaying matter and play an essential role in nature by breaking down organic matter and making it inorganic.

To investigate the "superpowers" of fungi we decided to use yeast, a single-celled fungus that is used in the manufacture of bread and is therefore easy to obtain.

The yeast ferments the starch of the flour and transforms it into glucose that it subsequently uses as food. Two waste substances are produced in this process:
- ethyl alcohol that evaporates during baking
- CO₂ that converts mass from compact to fluffy

Fermentation also generates heat.

Will yeast be a good candidate to survive on the moon?

**PROCEDURE AND RESULTS**

To study the behavior of yeasts to light and cold we dissolved 7gr of granulated yeast in 50ml of warm water, and then added 300 gr of flour with a pinch of salt. The resulting mass was divided into 5 groups as shown in the table below along with their results:

**Table 7. Results of exposure to different temperatures and light of the Fungi Kingdom**

<table>
<thead>
<tr>
<th>Mass 1 Direct exposure to the sun</th>
<th>Mass 2 Ambient temperature but in the shade</th>
<th>Mass 3 Ambient temperature but in the dark</th>
<th>Mass 4 Moderate cold (4 °C) for 24h</th>
<th>Mass 5 Intense cold (-22 °C) for 24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1º day Very Active</td>
<td>Active</td>
<td>Active</td>
<td>No Active Ambient Temperature</td>
<td>No Active Ambient Temperature</td>
</tr>
<tr>
<td>2º day ****</td>
<td>****</td>
<td>****</td>
<td>Active</td>
<td>Active</td>
</tr>
</tbody>
</table>
The day the experiment was launched, the ambient temperature was 28ºC.

Yeast activity was measured indirectly, observing the sponginess of the mass. If it would press with a finger we see the mass sinks but instantly come back to its shape that means that the yeasts are active and are fermenting the flour to feed themself, if the mass remains compact it would mean that the yeasts are not active or inert.

**CONCLUSIONS OF THE FUNGI KINGDOM**

As can be seen in this experiment at higher temperature more yeast activity, so that those that were 24h subjected to –22ºC initially had no activity. However, the temperature has not affected the viability of the yeasts as at the end of the experiment they all had fermented the flour. Regarding the exposure to light, no differences were found between the mass exposed to light and the mass not exposed to light. This ability to survive in hostile environments makes yeasts a strong candidate to take over the title of super-globe.

### 5.4 PROTOCTIST KINGDOM

**INTRODUCTION**

The Protist Kingdom is a bit the tailor’s drawer of the natural world because it includes single-celled and pluricellular beings; autotrophs and heterotrophs. The individuals of the Protist Kingdom are divided into two large groups: protozoa and algae. One thing they have in common is that they all live linked to liquid water. This could be a problem for their survival off Earth. Will these incredible beings be able to resist the lack of light and low temperatures? To investigate this we will focus on pluricellular algae, autotrophic living beings that are different to plants that they do not have true tissues.

**PROCEDURE AND RESULTS**

To study the behavior of algae, we have collected samples from a pond and the Palmones River (Cadiz). After filtering the samples to remove debris, we filled five glass cups. Once labeled we have subjected them to different conditions. The results are reflected in the following table.
Table 8. Results of the experiment with algae

<table>
<thead>
<tr>
<th></th>
<th>Vessel 1</th>
<th>Vessel 2</th>
<th>Vessel 3</th>
<th>Vessel 4</th>
<th>Vessel 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1º week</td>
<td>Direct exposure to the sun</td>
<td>Ambient temperature but in the shade</td>
<td>Ambient temperature but in the dark</td>
<td>Moderate cold (4°C) for 24h</td>
<td>Intense cold (-22°C) for 24h</td>
</tr>
<tr>
<td></td>
<td>Green tone</td>
<td>Brown tone</td>
<td>Brown tone</td>
<td>Brown tone</td>
<td>Brown tone</td>
</tr>
<tr>
<td>2º week</td>
<td>More volume. They float</td>
<td>Brown tone. They don't grow</td>
<td>Precipitation</td>
<td>Green tone. Best look</td>
<td>Green/Brown tone Best look</td>
</tr>
<tr>
<td></td>
<td>Brown/Green tone. Too much volume. They float</td>
<td>Brown tone. They don't grow</td>
<td>They don't survive</td>
<td>Best look</td>
<td>Best look</td>
</tr>
<tr>
<td>Photos</td>
<td>![Photos](image9 Procedure with algae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The ambient temperature at the time was 28°C. Algae activity was measured indirectly, observing coloration, buoyancy, volume growth and precipitation of substances at the bottom.

CONCLUSION OF THE PROTOCTIST KINGDOM

As can be seen in this experiment at higher temperature there is more activity, in such a way that those that were 24h subjected to −22°C initially had no activity. Due to the exposure to light, large differences were found between the vessels exposed to light and those not exposed to light, the green color of the chlorophyll was more intense in the first one named. Despite their dependence on light, researchers at Aarhus University, Denmark, have published the existence of a seaweed that is able to live under Arctic ice, where the level of light is only 0.02% of the light in the surface area of the ice. Single-celled diatomea, a seaweed called cylindrus fragilariopsis, produces a particularly interesting antifreeze protein. This was the discovery of scientists from the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven. Protein not only varies the size, but also the internal structure and porosity of ice crystals. “Ice varies in such a way that the alkaline solution remains in the gutters. This prevents that cavities and grooves freeze in the ice,” says researcher Maddalena Bayer-Giraldi.

Thanks to the protein the ice is not formed in a stable block, but in a light network of ice crystals, full of voids and gutters. Scientists found that this type of algae produces large amounts of this protein, especially when the cold and salinity increase around it.
Many of the ice dwellers use another trick. They separate viscous substances, such as large polymers from sugar molecules, with which they form a protective layer around them. It cushions the body against its surroundings, similar to a neoprene suit that prevents water contacting the skin.

Could this convert the Protostist Kingdom into a winner? We'll know soon.

### 5.3. Monera Kingdom

**INTRODUCTION**

The Monera Kingdom is incredibly diverse. It is made up of single-celled individuals, bacteria, who have a simpler cell structure than cells in other kingdoms.

When we consider experimenting with the response of the members of the Monera Kingdom to light and temperature, we were hesitant to let the children, the real researchers of this project, manipulate potentially pathogenic cells. The source from which bacteria samples were taken had to be "safe," everything was not valid.

At first we thought that a possible source of samples could be the bacterial flora that grows on the teeth and could be grown in Petri plates in the lab. However, the situation generated by the pandemic forced us to change our strategy, lacking stoves and other means for the cultivation of our samples.

Investigating the Monera Kingdom seemed impossible until we came up with the idea of using yogurt with Bifids.

Yogurt is a food produced by the lactic fermentation of milk. This fermentation is performed by bacteria that oxidize lactose in the absence of oxygen and produce lactic acid. The effect of lactic acid on milk proteins, mainly on casein, gives yogurt its peculiar characteristics.

A symbiotic cultivation of two bacteria is used to make yogurt: Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus salivarius ssp. Thermophilus.

Yogurts with "Bifids" contain the bacteria responsible for the lactic fermentation of milk and a third additional bacterium of the genus Bifidobacterium. Bifidus in Latin *bifidus*, means "cleft in two parts", and refers to its form.

*Image 10. Cells of Bifids.* (nootriment.com)

For a yogurt to be considered probiotic, these bacteria must be alive in the final product. In each pack of yogurt with "Bifids" there is an amount of up to 12.5 billion Bifidobacterium. If we also join all the bacteria responsible for fermentation we find that yoghurts are a great source of individuals from the Monera Kingdom.

**PROCEDURE AND RESULTS**

To create a medium in which to study bacteria’ reaction to temperature and light we dissolved 300gr of yogurt with "Bifids" in one liter of whole milk. The mixture underwent different conditions as shown in the table below:
Table 9. Conditions of the experiment with the morera kingdom

<table>
<thead>
<tr>
<th>Vessel 1</th>
<th>Vessel 2</th>
<th>Vessel 3</th>
<th>Vessel 4</th>
<th>Vessel 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct exposure to the sun</td>
<td>Ambient temperatura but in the shade</td>
<td>Ambient temperatura but in the dark</td>
<td>Moderate cold (4°C) for 24h</td>
<td>Intense cold (-22°C) for 24h</td>
</tr>
<tr>
<td>Half curdled</td>
<td>A curdled milk ring is observed</td>
<td>A curdled milk ring is observed</td>
<td>Liquid</td>
<td>Solid</td>
</tr>
<tr>
<td>Day 2</td>
<td>Curdled</td>
<td>Half curdled</td>
<td>Half curdled</td>
<td>A curdled milk ring is observed</td>
</tr>
<tr>
<td>Day 3</td>
<td>Curdled</td>
<td>Curdled</td>
<td>Curdled</td>
<td>Half curdled</td>
</tr>
<tr>
<td>Day 4</td>
<td>Curdled</td>
<td>Curdled</td>
<td>Curdled</td>
<td>Curdled</td>
</tr>
</tbody>
</table>

The day the experiment was launched, the ambient temperature was 28°C.

The activity of bacteria was measured indirectly, observing the consistency of the milk. If the milk is curdled it means that the bacteria are active and fermenting the milk to feed, if the milk remains liquid it means that bacterial activity is low or non-existent.

CONCLUSIONS OF MONERA KINGDOM

As can be seen in this experiment at higher temperature there are more bacterial activity, in such a way that bacteria that were 24h to -22°C initially had no activity. However, the temperature has not affected the viability of bacteria as at the end of the experiment they had all curdled the milk.

Due to the exposure to light, no differences were found between the vessel exposed to light and the one no exposed.

In view of our results we can infer that the bacteria used in our experiment are sensitive to low temperatures, such as those in a domestic freezer. However, once the temperature increases its activity recovers because they have remained "paralyzed" but alive.

There are some other type of bacteria with amazing behaviours: the extremophilic bacteria.

The "superpowers" of the most impressive extremophilic bacteria are:

1. Heat Resistance: *Thermus aquaticus* lives in temperatures between 50 and 80 °C.
2. Cold Resistance: *Polaromonas vacuolata* live in teh waters od Antarctica, at 0 °C, and have an optimal temperatura of 4 °C.
3. Gamma Resistance: *Thermococcus gammatolerans* is capable of withstanding up to a gamma-ray irradiation of up to 30 KG.
4. Resistance to salt concentration: *Haloferax volcanii* lives in the Dead Sea, where the salt concentration is 10 times more concentrated than that of the Mediterranean Sea. She is considered one of the first inhabitants of the Earth.
5. Acidity resistance: *Lactobacillus acidophilus* is a bacterium responsible for the formation of yogurt. It is able to live in pH conditions too low for other life forms.
For all this the Monera Kingdom is a very strong candidate to win the Challenge of the Five Kingdoms.

6. General Conclusions

Once we have studied all the kingdoms of nature, it is difficult for us to come to a conclusion. The variety of life on Earth makes potential candidates multiply. The table lists the overall results of our experiences.

Table 10. General results

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Bacteria</th>
<th>Algaes</th>
<th>Yeast</th>
<th>Moss</th>
<th>Legumes</th>
<th>Worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
</tr>
<tr>
<td>heat</td>
<td>Favors</td>
<td>Favors</td>
<td>Favors</td>
<td>Favors</td>
<td>Favors</td>
<td>Favors</td>
</tr>
<tr>
<td>Temp.</td>
<td>soft</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
</tr>
<tr>
<td>Cold</td>
<td>Survive</td>
<td>Survive</td>
<td>Survive</td>
<td>Survive</td>
<td>Survive</td>
<td>Survive</td>
</tr>
<tr>
<td>Food</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Doesn’t affect</td>
<td>Affect</td>
</tr>
</tbody>
</table>

On the one hand, we would have to choose the least complex kingdom, the bacteria. But on the other hand, it would be more appropriate to lead a small ecosystem made up of representatives of each of these kingdoms, hence they could complement and help each other not only survive, but grow and reproduce. A colonizing ecosystem like those organisms that appeared on Earth and were evolving, photosynthetic and decomposing first and then animals and organism consumers.

These general conclusions taken by all students together with the opinions of each of them are set out in Scientific Journal Nº.4.

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General

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