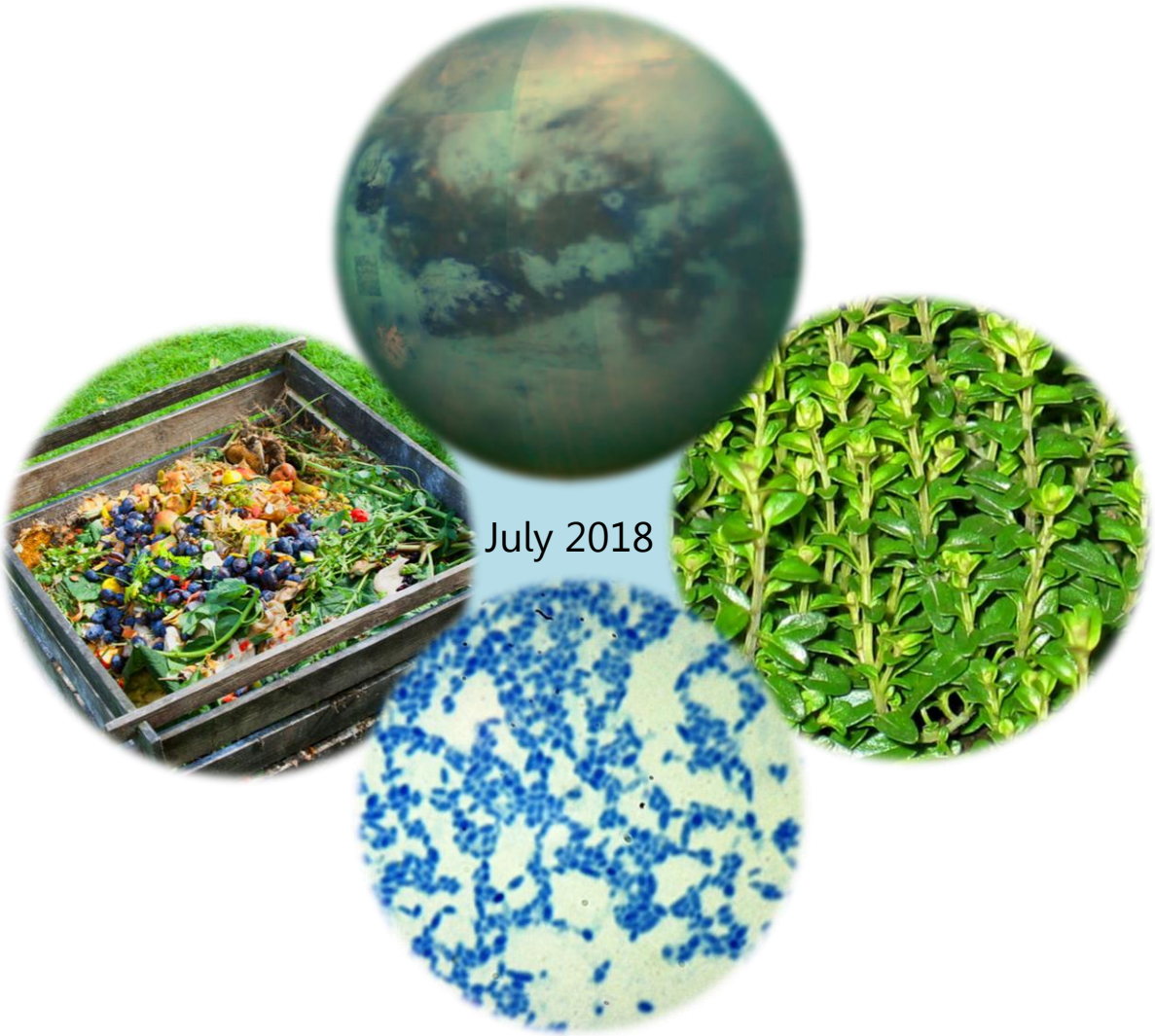
High School Students for Agricultural Science Research

Volume 7



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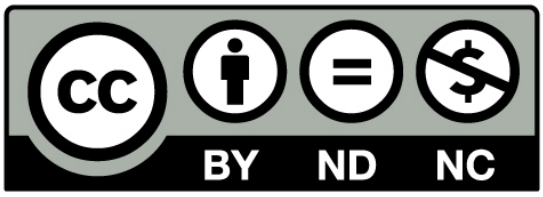

Index

Toxic effects for plants of cleaning products used at home*

Ana Cañas Rodríguez[1], Maximiliano Morteyrú Cornejo[1], Iris Luque Barrio[1], Lucía López Quesada[1], Rosa Beatriz Lens Herrera[1], Claudia Moreno Villegas[1], Javier Quirós Megías[1], Candela Burkhardt Ramírez[1], Julia Muñoz González[1], Manuel Espinosa Urgel[2], Óscar Huertas Rosales[3] and Antonio Quesada Ramos[1].

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Summary

Every day, we have more and more products for cleaning and personal care at home. We use them indiscriminately ignoring that they can have toxic effects both for living beings and the environment. In this research we have studied the effects of some products on germination and development of lentil plants. Our results show that most of them inhibit the germination of seeds and affect the growth of stems and roots and even the weight of plants. These experiments must be helpful un creating awareness about harmful action of products we use at home. As result of this research, students propose some actions to reduce the impact of these substances on the environment.

INTRODUCTION

At home, we have a lot of products for daily cleaning and personal care. In fact, there are lot of advertisements in mass media that encourage us to use them in campaigns that ignore that some of these substances may be dangerous, both for us and the environment. Detergent, fabric softener, bleach, anti-lime cleaner, grease remove, washing up liquid, nail varnish remover, alcohol or hydrogen peroxide are common products we use at home that are indiscriminately spilled out in waste water. Although this substances pass through seawage treatment plants, the water is nor completely cleaned of chemicals when is release back to the environment and can affect living beings. But firstly, we must be careful when we use some of them. A good advice is to read the labels the bottles or containes have, where we can get some information about composition and effects.

For example, detergents and soaps have chemicals with irritating effects on the skin in human beings, even they can cause dermatitis (Van Scott, E.J. Lyon, J.B., 1953); in plants, these products can lead to a loss of enzymatic activity that reduces its survival (Sadaf et al, 2014). Those that contains sodium hypochlorite, the active agent of bleach, can induce chemical burn in skin and mucoses (Piggott et al, 2007). This chemical can react with organic matters giving rise to organic chlorine compounds that are toxic for acuatic organisms and persist for a long time in ecosystems (Emmanuel *et al*, 2004). Other products, as grease remover, usually have high concentrations of sodium hydroxide; anti-lime cleaner, on the

* All the students from 1st course of ESO have collaborated in this project. Those with a greater degree of implication are mentioned in the author's list, but the work of all the group is acknowledged.

contrary, have acids in its composition. Acids and bases can damage our skin and mucoses too and both of them cause changes in water's pH levels; these variations are harmful for animals, plants, even microorganisms as they can modify the spacial structure of enzymes, even denaturate them. Toxic effects of acids on plants were studied in our laboratory in experiments with simulated acid rain (Molina *et al*, 2017).

Nevertheless, living beings have protective mechanisms for some products we use. For example, catalase is an ubiquitous enzyme that protect us from the toxic effect of hydrogen peroxide, one of the products we use to treat our wounds.

In order to reduce the impact of home toxic products on living beings is important to know their effects first-hand. The main objective of this research is that young students carry out some easy experiments to demonstrate the toxic effect of these substances and, with their families, make decisions to reduce their impact in nature.

Figure 1. Products for cleaning used in the experiments with lentils.

MATERIAL AND METHODS

Toxic products and plants

The effect of home products on plants have been studied with lentil (*Lens culinaris*), both seeds and germinate plants. The tested products were: alcohol, anti-lime cleaner, bleach, concentrated washing up liquid, detergent, fabric softener, hydrogen peroxide, liquid grease remover and nail varnish remover. All of them were bought at nearby supermarkets and prepared at different concentrations.

Effect of toxic products on the germination of lentil seeds

In order to know if one product affects the germination of seeds, Petri dishes were prepared with absorbent paper; 16 lentil seeds were disposed regularly spaced in every plate. Six of them were irrigated only with water, this was the control set. Groups of two plates were watered with products solutions at 5% and 10% v/v. As seeds didn't germinate with detergent and nail varnish remover at 5%, seeds were treated with 0,5% and 1%

dilution of these products. Percentage of germinated seeds was studied in the following days.

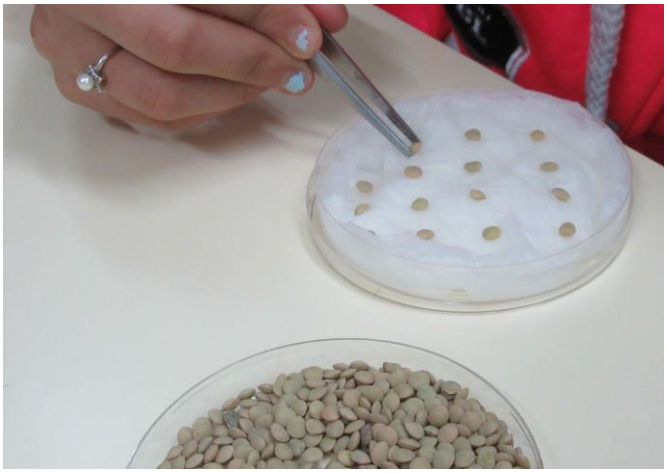


Figure 2. Preparing Petri dishes with lentil seeds.

Effect of products on the growth of plants

Different sets of pots were prepared with soil and ten lentil seeds were sown in every container. The control set, with five pots, was irrigated only with water; nine sets of three pots were irrigated with products solutions (alcohol, anti-lime cleaner, detergent, nail polish remover and liquid grease remover at 5%; bleach, hydrogen peroxide, fabric softener and washing up liquid at 10%). After ten days, plants were collected and the length of stems and roots and weight were measured by students.

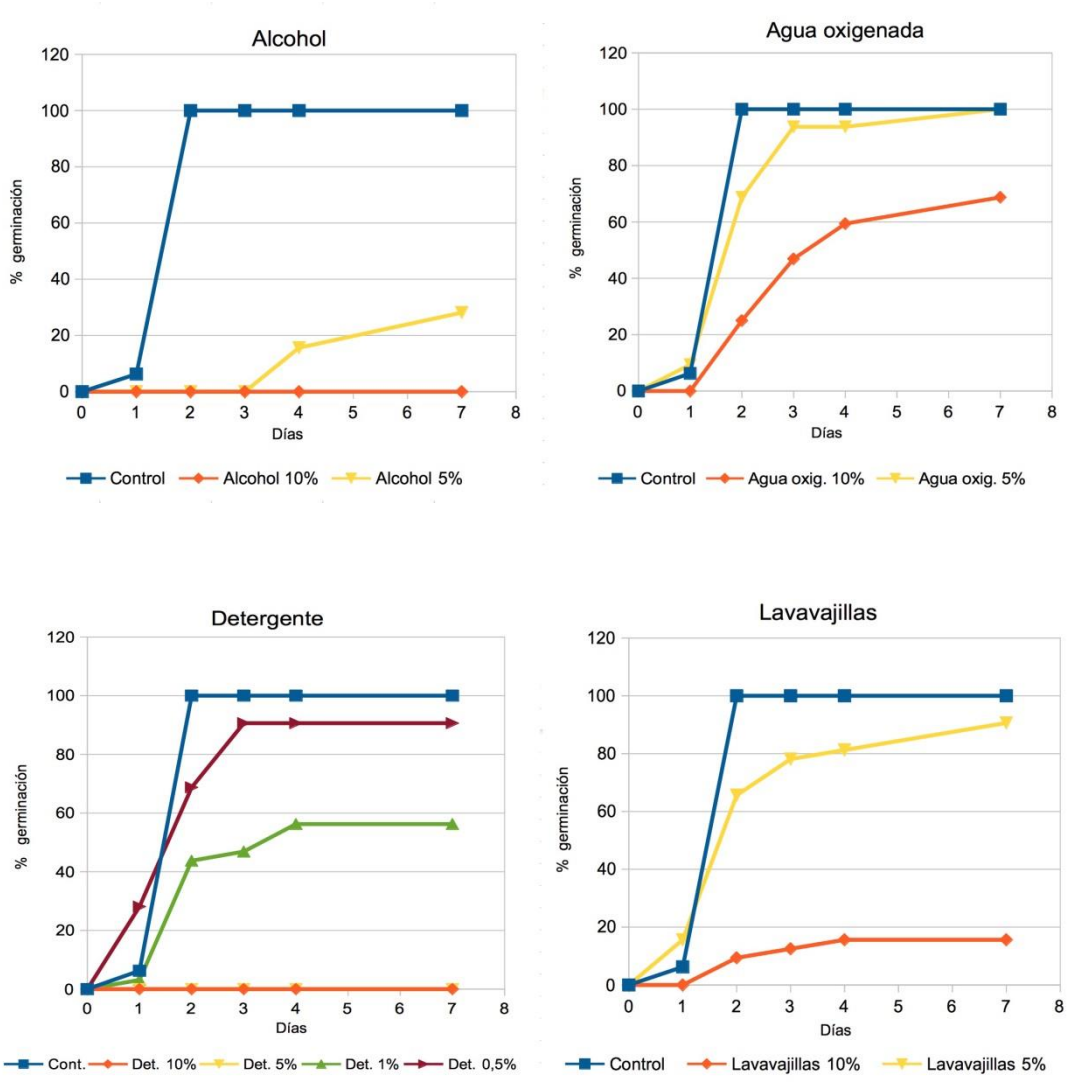
Figure 3. Sowing lentils for studying the effect of products on growth of plants.

RESULTS

Effect of home products solutions on the germination of lentil seeds

Figure 4 shows the percentage of germinated seeds in treatments with different products dissolutions. All seeds germinated in control plates with water (100%). No germination was observed neither at concentrations of 10% of alcohol, liquid grease remover, detergent and nail varnish remover nor 5% of detergent and nail vanish remover. Less than 20% of seeds germinated with anti-lime cleaner and concentrated washing up liquid at concentrations of 10%. Bleach and fabric softener didn´t seem to affect seeds germination at tested concentrations.

As acetone is the main component of nail polish remover, we have compared the germination in seeds treated with 10% and 5% disolutions of acetone from our laboratory; all seeds germinated at 5% and nearly 95% at 10%.



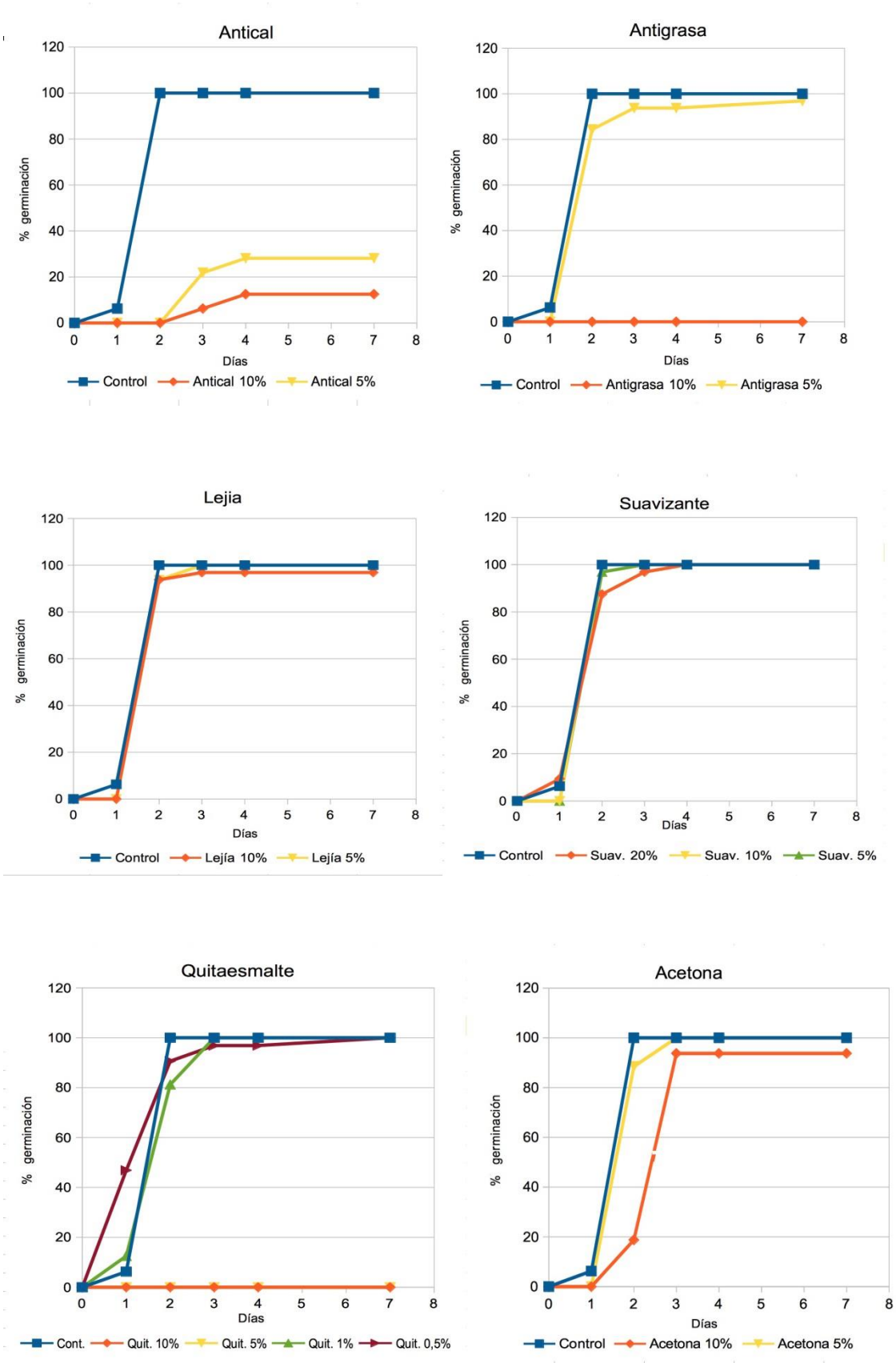


Figure 4 (previous page and this page). Proportion of seed germination with different treatments.

Figure 5. Germinated seeds treated with different concentrations of liquid grass remover compared with control irrigated with water.

Effect of products on the growth of plants

Students have observed the effect of cleaning products on the growth of plants. Lentil seeds were grown in pots. One set were irrigated with water; this was the control; the rest were watered with products dissolutions at 10% and 5%. Ten days later plants had grown and were collected, measured and weighted. Average and standard deviation for every sample were calculated with Excel. Results are shown in table 1.

Table 1. Stems and roots length and plants weight in control and treated plants.

	Stem length (cm)		Root length (cm)		Plant weight (cm)	
	Average	St. dev.	Average	St. dev.	Average	St. dev.
Control (water)	20,80	3,66	6,37	2,78	0,30	0,02
Alcohol 5%	3,76	2,19	3,52	2,74	0,19	0,05
Anti lime liquid 5%	7,48	4,22	4,43	1,62	0,18	0,04
Bleach 10%	17,49	6,56	5,26	2,71	0,25	0,04
Detergent 5%	13,52	6,30	5,14	3,44	0,20	0,04
Fabric softener 10%	14,98	7,01	5,18	3,76	0,22	0.03
Grease remover 5%	12,79	5,44	4,62	2,69	0,18	0,01
Hydrogen peroxide 10%	17,46	6,93	5,33	3,12	0,24	0,04
Nail polish remov. 5%	16,96	4,52	5,81	3,13	0,25	0,06
Washing up liquid 5%	9,51	6,37	6,49	3,73	0,22	0,04
Total	14,95	7,18	5,43	3,13	0,23	0,05

Average stem length in control plants was 20,80 cm. Stem growth in all treated plants was less than in controls. Most affected plants were those treated with alcohol (5%), anti-lime liquid (5%) and washing up liquid (5%).

Average root length was 6,37 cm. Root growth in all treated plants, but those watered with washing up liquid (5%), was less than in control. Most affected plants were those treated with alcohol (5%) and anti-lime liquid (5%).

Average weight in plants irrigated with water was 0,30 g per plant. Most affected plants were those treated with anti-lime liquid (5%), grease remover (5%) and alcohol (5%). Graphics with comparative results are shown in figures 6 and 7.

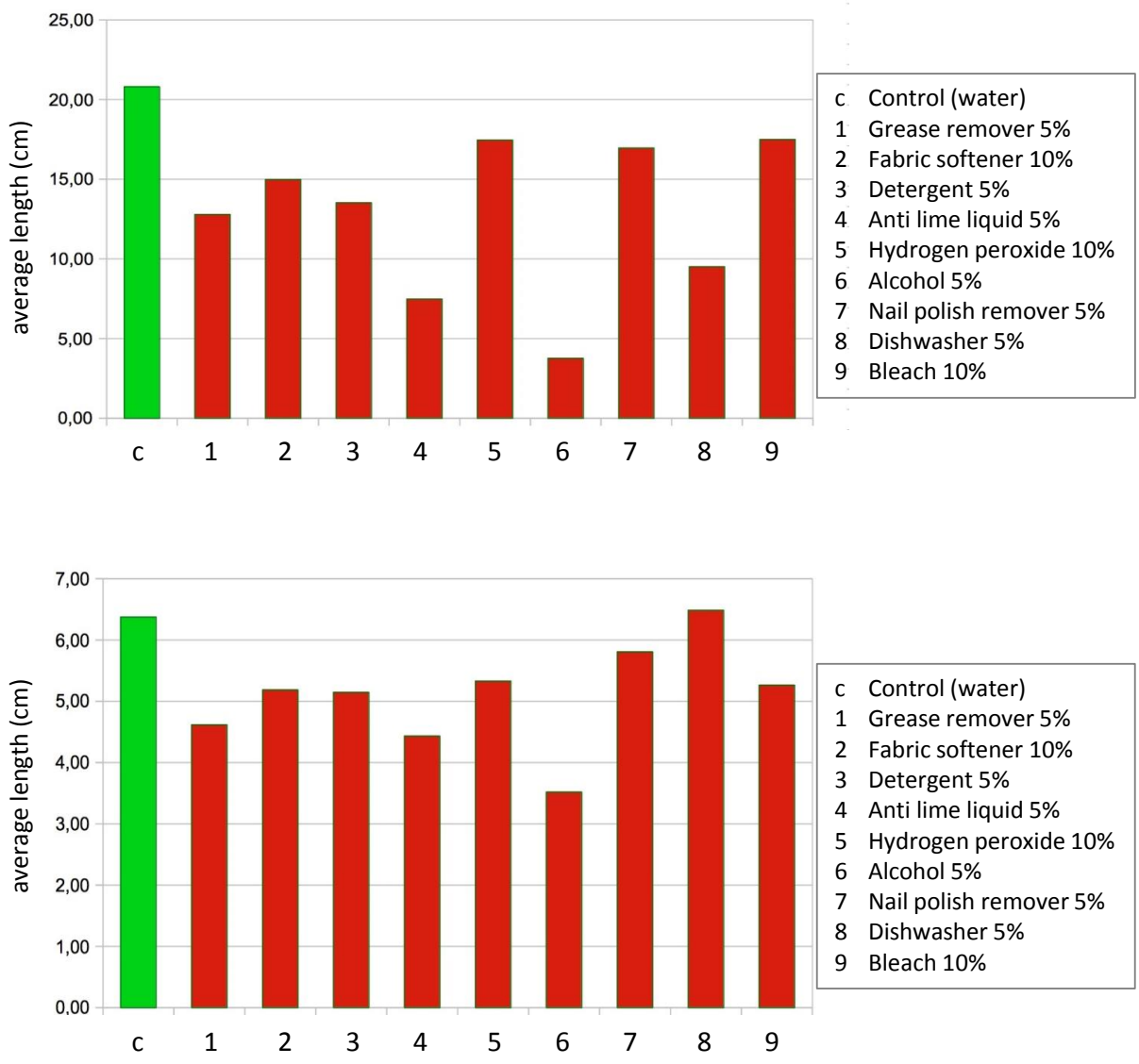


Figure 6. Average length of stems (top) and roots (bottom) in control and treated plants with cleaning products.

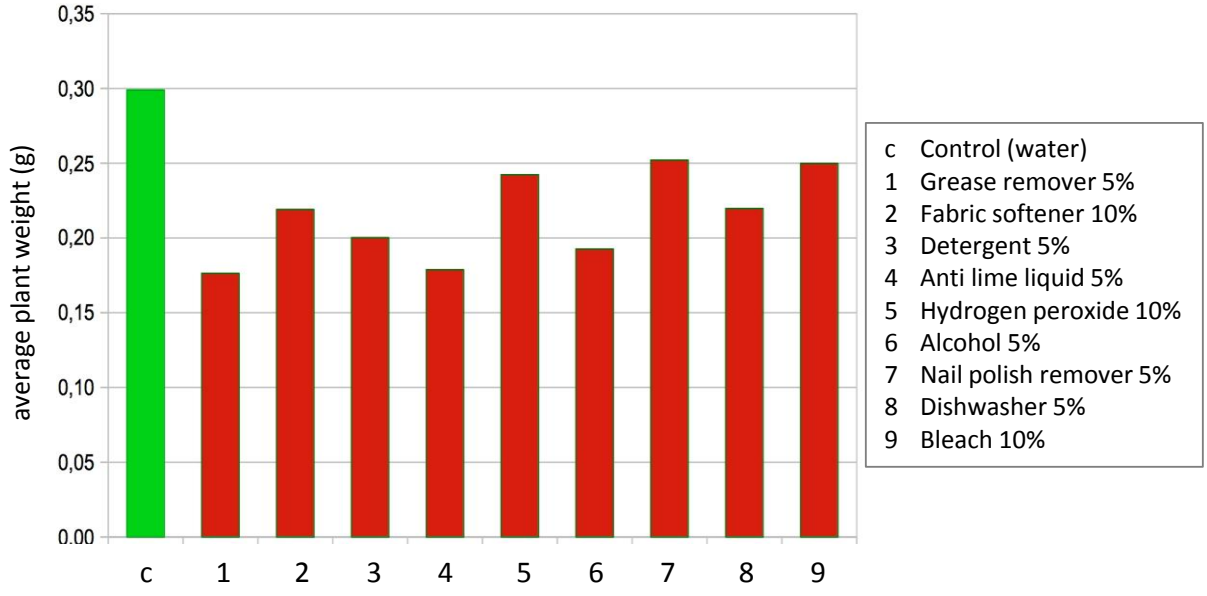


Figure 7. Average weight of plants in control and treated plants with cleaning products.

DISCUSSION

Our results demonstrate that we have in our houses products we use every day for cleaning that are toxic for living beings and the environment. Some of them are more toxic than others and reduce germination or growth at lower concentrations.

Most of them affect to germination of seeds. On the contrary, other like fabric softener or bleach doesn`t have an important effect on this process. We have tested fabric softener concentrations up to 20% without significant changes in the proportion of germinated plants. Bleach concentrations up to 10% have no important effect on germination in spite of it can hurt skin and mucoses in human beings only by contact. Bleach is a toxic substance that contains sodium hypochlorite, a chemical that can produce chlorine compounds toxic for living organisms. As this product has been used for a long time, it is possible that plant have developed resistance mechanisms to it. On the other hand. we must take into account that seeds are resistant structures and in case they need special conditions to germinate, as pass through the digestive tract of animals.

On the contrary, all products affected plants growth, measured as stems and roots length and plant weight. Control plants, irrigated only with water, grew more than trated plants. Alcohol was one of the most toxic tested substances. No differences were observed in roots length between controls and plants treated with washing up liquid; this result disagree with the fact that this product was very toxic to lentils germination.

Our experiments are helpful in creating awareness about harmful action of detergents and other products used at home. As a result of this research our students have propose some actions to reduce their toxic effect:

- Reduce the consumption of detergents and toxic products at home.
- Use natural products as lemon or vinegar for cleaning instead of comercial ones.
- Use biodegradable products ir order to be broken down when they are spilled out to waste water.
- Read product labels and study composition in order to choose the most environmentally friendly.
- Maximise personal care by the use of gloves or other protector objects.
- Increase the number of waste water treatment plants to reduce the levels of pollutans in water.
- Research in new cleaning products in order to get others less toxic and more environmentally friendly.
- Make experiments about environmental toxicity, similar to those carried out by out students, to forbid the use of toxic products for environment.

Acknowledgements

This research has been carried out as part of the project "**Contaminación cotidiana**", one of the initiatives of Andalucia Mejor con Ciencia supported by Fundación Descubre.

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My own ideas

Es muy importante saber lo que estamos usando o incluso lo que echamos a nuestra ropa, casa, uñas… ¿Por qué es importante? Es importante debido a que unos experimentos nos han demostrado que muchos de los productos que utilizamos son más perjudiciales de lo que creemos. Se han regado plantas con distintos tipos de productos, los más utilizados en nuestras casas, como detergente, acetona, lejía, lavavajillas, quitaesmalte… Las conclusiones que hemos obtenido no son nada buenas. La mayoría de las plantas no han crecido lo suficiente; el alcohol no es bueno para nosotros, es muy contaminante; el lavavajillas ha afectado, también la acetona, el quitaesmalte... Lo hemos demostrado comparando con un control, solo regarla con agua.

Mi familia se ha interesado mucho; piensan que tenemos que tener mucho más cuidado al limpiar o al hacer las tareas de nuestras casas. Ellos no pensaban que podría llegar a ser algo tan importante. Desde que lo saben intentan gastar lo menos posible o ponerse guantes para no dañar sus manos.

Yo pienso que ya que hemos experimentado con la contaminación de los suelos también podríamos hacerlo con la del aire. Lo que más me ha gustado de este experimento es la facilidad de hacerlo; lo podéis hacer en casa con vasos de yogur y probar a ver que sale.

Lo que más me ha sorprendido es que la gente no sea consciente de lo que utiliza ni de lo que contaminan. Deberíamos dar esto a conocer y concienciar a la gente, que están contaminando cada vez que utilizan productos de limpieza o productos utilizados en casa para las heridas o las manos.

Ana Cañas Rodríguez 1º ESO A

Me ha gustado mucho hacer estos experimentos porque hemos aprendido a trabajar en grupo, a manejar utensilios de laboratorio y a tener más cuidado con los productos que utilizamos en nuestras casas. Este experimento tiene importancia ya que si se maneja un producto sin conciencia puede provocar graves daños. Mi propuesta es hacer experimento con semillas de otras plantas.

Tenemos que tener cuidado con los productos que tenemos en nuestras casas, no abusar de ellos y evitar echarlos al medio ambiente.

Claudia Moreno Villegas 1º ESO A

With this experiment, we saw how home products, like soap, affected plants. These products are dangerous. If someone throw out soap he can make some plants die or get infected. May be these products get into our food. If a cow eat grass with soap we will take toxic substances when we drink cow’s milk.

We can do a lot of experiments. To study how our products affect flowers, fruits and

vegetables or how they can affect us.

I love this experiment. It has been so fun and interesting. I like take care of plants and see how they grow. But the thing that surprised me was the effect of some contaminants because some of them kill plants.

We must stop this. If we use natural products for doing the same activities (vinegar as softener, for example) or put water filters in all the cities and towns for make the wáter clean. My parents think the same. They are dangerous. These experiments are awesome.

Rosa Beatriz Lens Herrera, 1º ESO A.

Mi opinión sobre el proyecto es que ha sido muy divertido; he aprendido mucho sobre la germinación de las plantas. También me ha gustado probar en mi casa con otras semillas como garbanzos o judías. La importancia es que veamos los contaminantes con los que no germinan las plantas y que nos demos cuenta de que algunos productos dañan al medio ambiente.

Me ha gustado hacer los experimentos en casa y enseñárselo a la familia. Mis padres dicen que no sabían esas cosas; que me dé cuenta de lo que podemos dañar y que tenemos que dejar de contaminar.

Lo que deberíamos hacer es reducir el uso de estos productos contaminantes y para reducir el impacto ambiental usar los que no dañen a las plantas.

Nuria Álvarez Sánchez, 1º ESO A

Mi opinión personal sobre los experimentos es que han sido muy entretenidos y nos han ayudado a desarrollar nuestra imaginación y nuestras capacidades. Su importancia es que gracias a ellos sabemos el daño que pueden hacer los productos que tenemos en nuestros hogares.

La conclusión principal que saco de los experimentos es que todos los productos, tanto los de higiene personal como los de limpieza del hogar, son dañinos para las plantas y por lo tanto para el medio ambiente.

Mi familia opina que le parece muy bien que hagamos esta clase de experimentos pues, además de ser algo dinámico, aprendemos que los productos que tenemos en nuestros hogares son dañinos para el medio ambiente.

Propongo que los productos que sean más contaminantes sean sustituidos por otros que hagan la misma función pero que sean menos peligrosos y se hagan con medios naturales.

Lucía López Quesada, 1º ESO A.

Este proyecto al principio me aburría porque no sabía nada sobre plantas ni como hacer que creciesen, pero poco a poco lo fui entendiendo y me encantó el experimento. Además aprendí sobre productos que son tóxicos y que no debemos mal usar. Una de las cosas que más me sorprendió fue que depende del producto que eches y de la semilla que pongas esta va a crecer más o menos.

Lo que más me ha gustado ha sido poner las semillas con detergente en mi casa y ver como la planta crecía poco. Cuando se lo enseñé a mi familia, dijeron que era un proyecto en el podíamos aprender mucho sobre los contaminantes tóxicos que tenemos en nuestras casas.

Javier Quirós Megías, 1º ESO A.

Recycling bio-waste by small-scale composting

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HIGHLIGHTS

- Composting at small scale is a feasible methodology for the bio-waste treatment.

- Thermophilic temperatures of 50ºC were registered in the composting reactors.

- A reduction of 17 and 50% of the initial composting mixture mass and volume of the composting substrate were recorded.

SUMMARY

Bio-waste is a biodegradable organic waste composed by garden and park cuttings, and food and kitchen residues from households and catering establishments. Composting is an effective biological process for its treatment and recycling, converting this organic waste into an organic amendment. In this research, some small-scale composting reactors have been design using recycled materials. Also, the biological process has been studied by monitoring the temperature, mass and volume evolution of the composting substrate during two months. Thermophilic temperature (50ºC) and a significant reduction of mass (17%) and volume (50%) of the bio-waste mixture was found during the process. The main conclusion was that small-scale composting reactors can be effectively used for the bio-waste treatment.

INTRODUCTION AND OBJECTIVES

The generation of organic waste is directly related to human activity and as a consequence, these residues are increasing. Bio-waste is an example of such organic waste, which includes garden and park cuttings and food and kitchen wastes from households and catering establishments, among others [1]. The annual production of bio-waste in the European Union is estimated at 118-138 million tonnes [2]. Furthermore, global warning and climate change are contributing to desertification, which lead to a decrease in the organic matter of soils compromising their fertility, especially in Mediterranean countries. Composting bio-waste is a feasible strategy to both reduce the environmental impact of bio-waste generation and to produce high-quality organic amendments which can be used for increasing the organic matter within soils.

Composting is a biological process in which, a succession of microorganisms (mainly fungi and bacteria) transform the organic matter from the raw waste into a humified organic matter called compost. Composting is a low-cost technology for organic matter recycling, being nowadays the principal treatment technology for municipal organic waste in the European Union.

The aim of this project was to design small composting reactors for bio-waste composting and to study the biological process of organic matter degradation.

MATERIALS AND METHODS

Bio-waste collection.

The experiment was carried out by the students of 5º of Primaria and 1st of E.S.O. of the Colegio Internacional de Granada in their school grounds. The bio-waste collection was undertaken by the students and consisted in food wastes from their morning snaks and meals prepared in the school canteen (mainly fruit peels and vegetable scraps), as well as dry leaves from the school garden. In order to improve the composting process, the bio-waste was chopped into small size using scissors. Also, soil from the school garden was used as a microbial inoculant.

Reactors design and construction.

The composting reactors used in this experiment were adapted from those described previously [3]. The composting reactors were done using a recycled 25L water bottles of polyethylene terephthalate (PET). For each composting reactor, the water bottles were divided in two parts: the former was used as the base and to collect leachate (Part A) and the latter, to store the composting mixture (Part B). An example of a composting reactor is shown in Figure 1.

Figure 1. Construction of a small-scale composting reactor used in this experiment.

The initial bio-waste mixtures consisted in equal volumes of food scraps, dry leaves and sieved garden soil. The composting reactors were filled by adding consecutive layers of 2-3 cm of soil, dry leaves and food scraps until 80 % of the Part B volume capacity. In order to avoid insect proliferation, the last layer added to the composting reactors was soil, which covered the food scraps. Small holes were punched randomly in the part B of the reactors to ensure aeration of the composting mixture. 200-300 ml of tap water was added to composting mixtures to ensure a moisture of 30-40 %. The composting reactors were stored at classroom during the experiment (Figure 2).

Figure 2. Small-scale composting reactors used at the beginning of the process.

Monitoring the composting process.

The biological process of composting was studied during 2 months, from October 2017 to December 2017. Throughout the process, the temperature was registered at different heights and depths with a digital thermometer probe (Figure 3). Also, room temperature was recorded. The mass of the composting reactors were measured using a 5000 g electric mass balance with a precision of 100 g. The compost volume was calculated as the difference between initial volume level and the volume at each measurement.

Figure 3. Measuring temperature in a small-scale composting reactor.

RESULTS AND DISCUSSION

The temperature profile of a small-scale reactor is showed in Figure 4. The temperature rose from ambient values to 50-30ºC in the first three weeks of the process, which indicates that the thermophilic phase occurred. The biological process was also noted by a reduction in the mass of 17% (Figure 5) and a volume reduction of 50% (data not shown). These reductions can be related to CO_2 emissions from microbial development in the composting substrates [4]. The composting process lasted 30 days and the composting mixture turned into a brown compost with wet soil small by the end of the process.

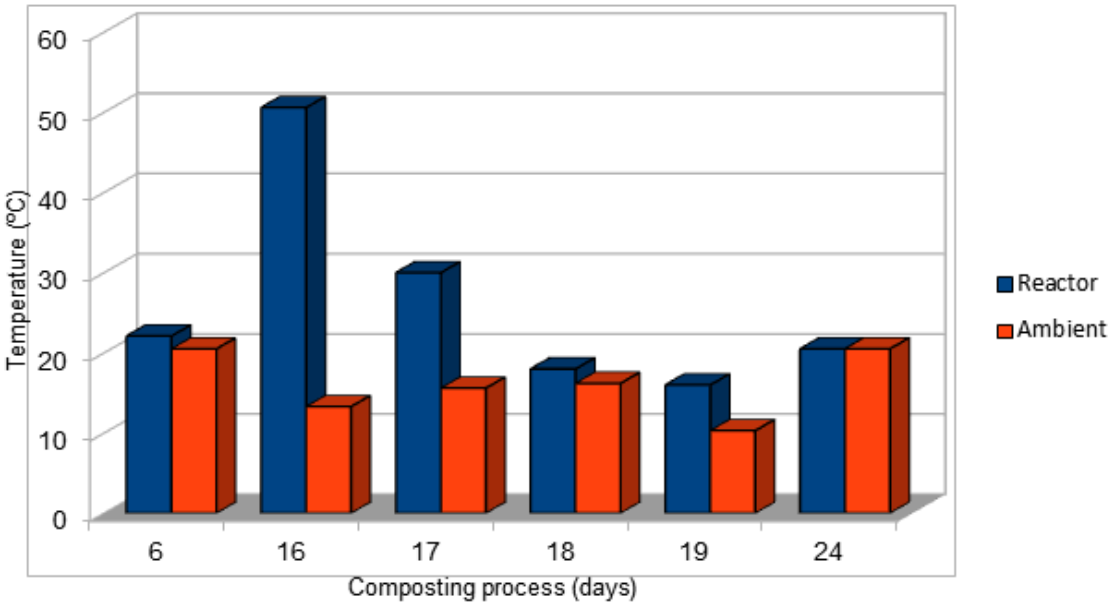

Figure 4. Temperature profile of a small-scale composting reactor.

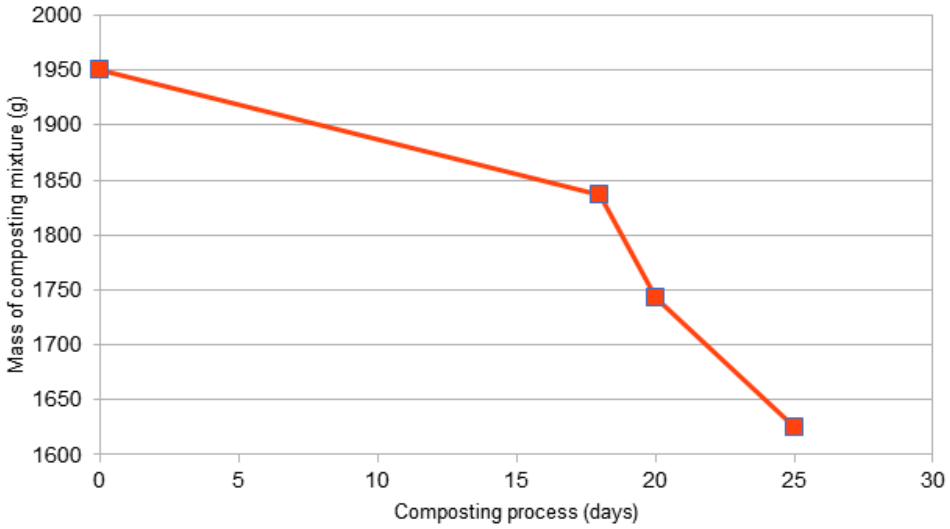

Figure 5. Mass evolution of a small-scale composting reactor during the process.

CONCLUSIONS

- The small-scale composting reactors can be used for the bio-waste treatment.

- The biological process of composting was noted with an increase of temperature (50ºC) and a significant reduction of mass (17%) and volume (50%) of the bio-waste mixture occurred.

Acknowledgements

This experiment was included in ¡Apuesto por el compost!, an educational scientific project for primary students supported by Fundación Descubre and co-financed by Fundación Española para la Ciencia y la Tecnología (FECyT) and Consejería de Economía y Conocimiento from Junta de Andalucía. More information of ¡Apuesto por el compost! project can be found in:

- Blog of the project:

https://andaluciamejorconciencia.fundaciondescubre.es/apuesto-por-el-compost-dilar-cig/es/

- Book: Buenas prácticas educativas en las iniciativas ANDALUCÍA,mejor con ciencia. 2017. Edita: Descubre, Fundación Andaluza para la Divulgación de la Innovación y el Conocimiento. ISBN: 978-84-09-01205-3.
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Do home cleaning products affect photosynthesis and oxidative metabolism in plants? Preliminary experiments

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SUMMARY

We have performed a pilot project to determine chlorophyll contents and catalase activity in plants subjected to irrigation with solutions containing common household cleaning products. The results show that certain treatments cause a reduction in chlorophyll production and catalase activity. These experiments are designed for advanced High School students, and the preliminary results obtained deserve to be explored further in the future.

INTRODUCTION

Photosyntesis is the process by which plants and some microorganisms convert water, carbon dioxide and minerals into organic compounds, basically carbohydrates, and oxygen. These transformations are possible because the photosynthetic organisms are able to transform light energy into chemical energy with the help of pigments like chlorophyll.

Photosynthesis is affected by several factors. On the one hand, there are natural elements; the most important ones are light intensity and wavelenght, carbon dioxide concentration and temperature. On the other hand, photosynthesis, as other important physiological processes in plants, may be affected by anthropogenic factors like air pollution (Darrall, 1989) and soil contamination (Baryla et al 2001; Stoeva and Bineva 2003).

At home, we use a lot of products for daily cleaning that may be somehow dangerous, both for us and the environment when used in excess or improperly disposed of. Detergent, grease remover, sanitary alcohol or hydrogen peroxide are chemicals that we indiscriminately spill out in waste water. Although these substances pass through sewage treatment plants, water is not completely cleaned of chemicals when it is released back to the environment. In this way, water with residues from our domestic cleaning products may be absorbed by plants and even affect their growth and production.

Previous experiments carried out in our laboratory have demonstrated that common cleaning products bought in nearby supermarkets affect both seed germination and plant growth (Cañas et al, in this issue, pg. 1-12). As a complementary work, the main goal of this research was to test if plant events related to photosyntesis, such as the level of chlorophyll or the protective mechanisms against reactive oxygen species (ROS) are affected as a result

of the exposure of *Vigna unciculata* plants to cleaning products. We have been able to use a simple extraction method and a spectrophotometric assay to determine the concentration of chlorophyll in control plants compared to plants treated with different household products. Besides, a qualitative method to test the catalase activity in plant extracts was also developed.

MATERIAL AND METHODS

Plant treatments

Vigna unciculata seeds were sown and, once germinated, they were watered with solutions containing different home cleaning products. The tested products were: sanitary alcohol, anti-lime cleaner, bleach, detergent, dishwasher powder, fabric softener, hydrogen peroxide, grease remover and nail varnish remover. One control set was irrigated only with water. Plants were grown for three weeks under those conditions.

Chlorophyll determination

To determine the chlorophyll content, acetone extracts (80% acetone) were prepared as follows: 0,3 g of fresh plant leaves were placed in a mortar and grinded with 1 ml of distilled water. Acetone (4 ml) were then added and vigorously mixed. Extracts were protected from light until they were centrifuged at 15,000 g for 10 min. Supernatants were collected and absorbance was measured at 650 nm in a spectrophotometer.

The amount of chlorophyll was calculated using the absorption coefficients (Arnon, 1949) and the following formula:

mg chlorophyll/ml = E652 x 5/34.5 x vol sample (microliters)

Catalase activity

The catalase activity was assayed by following the decomposition of hydrogen peroxide into water and oxygen. Leaf extracts were prepared with the same method as above, but using 50 mM K-phosphate buffer, pH 7.8, instead of acetone, in order to prevent catalase denaturation. Hydrogen peroxide solution (200 μl) was deposited onto petri dishes and then 50 μl samples were deposited onto the hydrogen peroxide drops. The course of the reaction was followed by observing bubbling of the mix due to the production of oxygen by the enzyme.

RESULTS AND DISCUSSION

Growth of plants was affected by some cleaning products. Figure 1 shows obvious growth differences between untreated plants and plants treated with some of the products tested. The most toxic substances were alcohol, detergent and dishwasher, which cause alterations either in plant growth or in the aspect of leaves. No effect was observed in plants treated with hydrogen peroxide, fabric softener or grease remover (not shown).

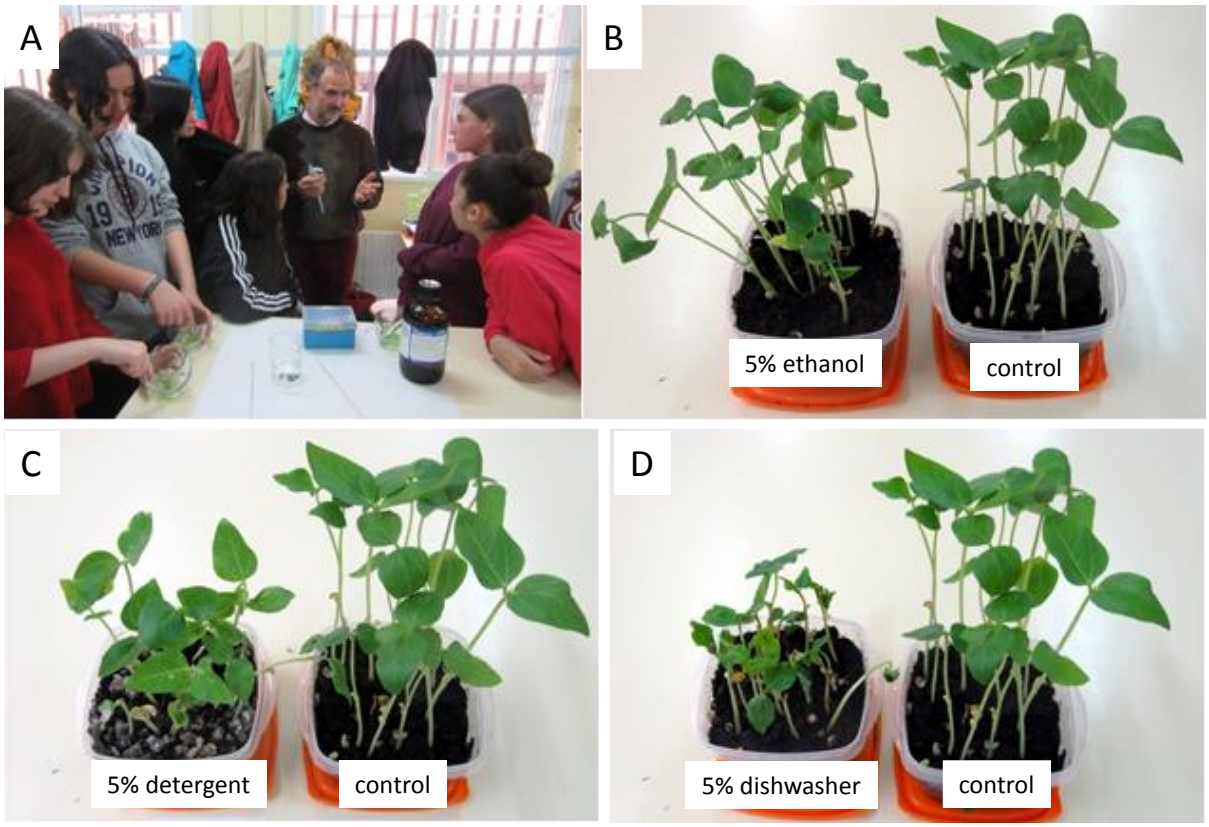

Figure 1. A) The team working in the lab. B), C) and D) Images of plants after growth for 3 weeks in the absence (control) and the presence of 5% (v/v) ethanol, 5% (w/v) washing machine detergent, and 5% (w/v) dishwashing powder, respectively.

Figure 2 summarizes the results of chlorophyll content determination in plants after various treatments, based on the spectrophotometric analysis of leaf extracts. Plants treated with dishwater or ethanol (alcohol) solutions showed lower concentrations of chlorophyll than controls. On the contrary, no differences were observed in plants treated with hydrogen peroxide. Plant catalase probably prevented the effect of this reagent.

Some cleaning products also affected catalase activity in plant leaves. Visual inspection showed differences in oxygen bubbles production (Figure 3). The most toxic treatment was caused by dishwater and detergent solutions.

Our preliminary results confirm that home cleaning products not only affect germination and growth of plants but they can alter photosynthesis and metabolism of reactive oxygen species. Dishwasher powder or detergent have a negative influence on chlorophyll production and affect catalase activity in leaves. Synthesis of protecting organic compounds in plants may therefore be reduced as a consequence of those treatments and plants can suffer increased exposure to ROS.

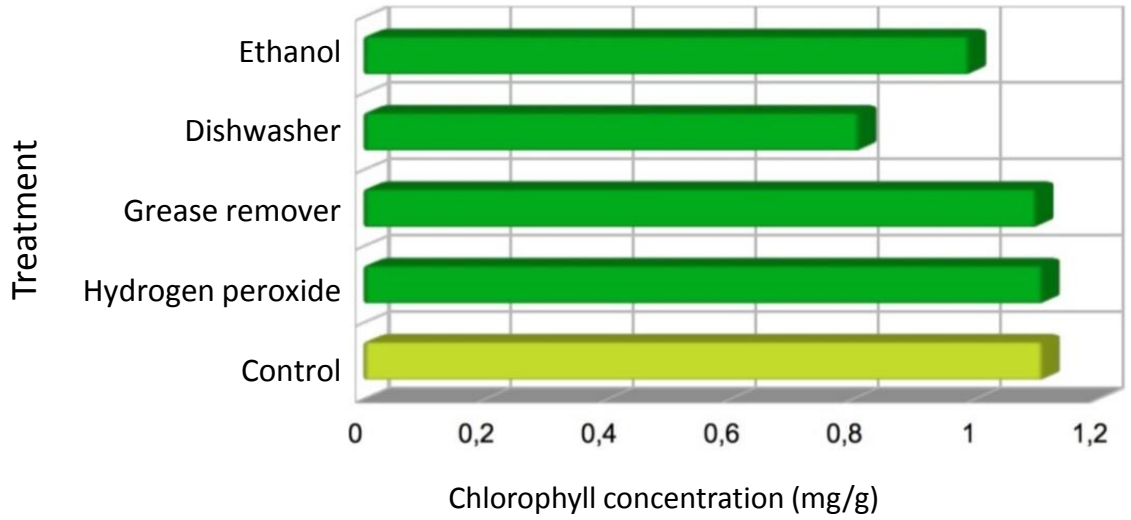

Figure 2. Effect of different treatments on chlorophyll contents of *Vigna unciculata* leaves. All the products were used at 5% (w/v or v/v) concentration.

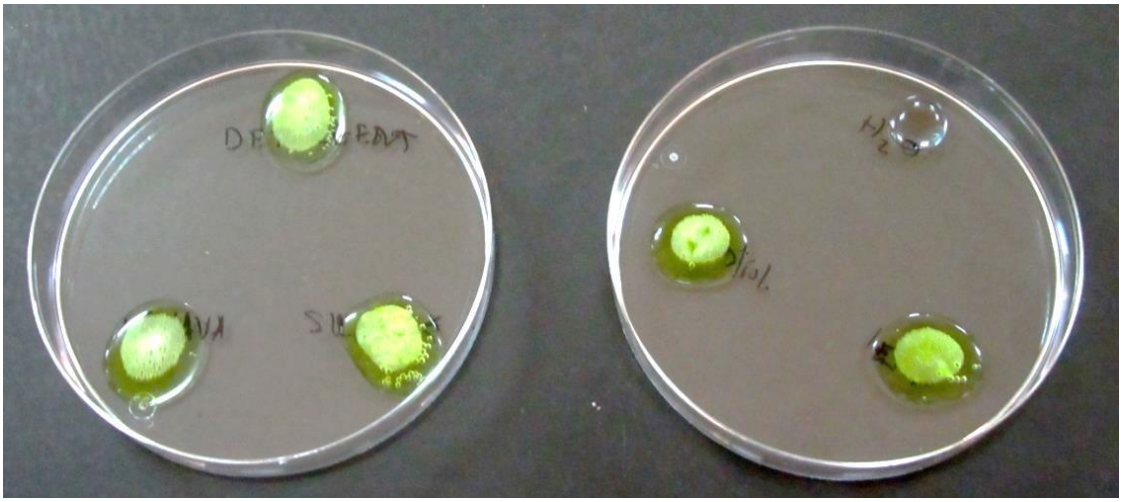

Figure 3. Visualization of catalase activity in leaf extracts from *Vigna unguiculata*. The activity of catalase was detected by the continuous bubbling of the mix due to the generation of oxygen, as a consequence of the breakdown of hydrogen peroxide, catalyzed by the enzyme.

These experiments were designed for advanced High School students who already have Biology/Biochemistry notions, and was a pilot project to test its feasibility. Based on the preliminary results obtained, it can be easily implemented in the classroom and will be used as a model for future research.

Acknowledgements

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Exoplanets and the limits of life

[1]
Introduction: The Astrobiology Project 2017-2018

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In the 2017-2018 edition of Ciencia BaSe, we have initiated a joint project with an ambitious aim: designing experiments for high school students to learn about the limits of life as we know it, and how to find exoplanets that might hold life forms of similar characteristics to those on Earth. The methodology combined the exploration of online resources to identify exoplanets and check their physicochemical properties and "wet lab" experiments to test the survival of different microorganisms under a range of conditions. Students were divided in six groups and each group was responsible for searching in exoplanet databases, reading adequate bibliography and defining potential candidates to hold life similar to that on Earth. Each group was also assigned one particular bacterial species to investigate. Experiments were carried out at the high school laboratory and results were compiled, shared and discussed by all the students.

Given that each group decided to prepare an independent report, but the microbiology results make more sense if presented in a comparative way, we have opted for a somewhat unusual format: Each group's report is presented with the Summary, Results and Discussion sections. Texts have been compiled as presented by the students (with some minor edition), but extracting the actual data, which are included in Figures and Tables following the six reports and a common Material and Methods section. The "my own ideas" section is then presented at the end.

Exoplanets and the limits of life

[2]

Finding a home outside the Solar System for some terrestrial bacteria: *Escherichia coli*

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SUMMARY

The objective of our investigation has been to search for a posible home for the bacteria that we have worked with in other planets outside the Earth. We have looked among exoplanets with the most similar characteristics to those of our planet to see if these could or could not be possible candidates to host life for our bacteria. For this end, we have exposed the bacteria to different environments, and we have studied the following factors:

- **The temperature limits in which they can live.**
- **The response of our bacteria to heavy metals.**
- **The acidity with which they can live.**
- **Its growth in different atmospheres.**

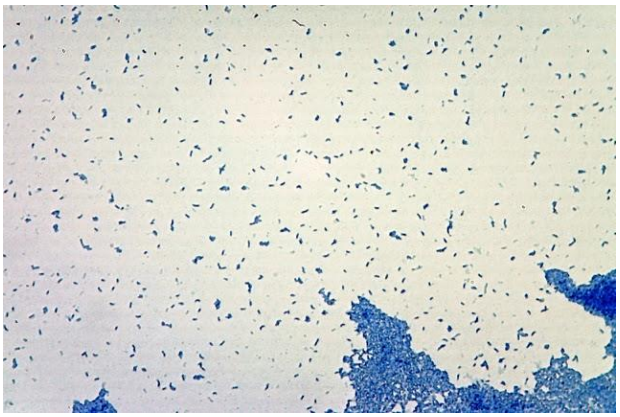
The bacteria with which our group has worked has been *Escherichia coli*.

Keywords: *Escherichia coli*, exoplanet, earth, universe, life forms, viability, resistance, heavy metals, temperature, growth, pH, atmosphere, UV radiation.

INTRODUCTION

Everyone knows that our planet will not last forever (and even more if we take care of it like we actually do) so apart from trying to raise awareness to improve its condition, in our project we're looking for another planet to colonize when things get complicated.

The main objective is to test how much our bacteria can resist to the tough conditions we will find in another planet. That's why in this project we mainly focus on exoplanet conditions in order to find a suitable one (like its possible atmosphere, pH, temperature…), and testing them in our bacteria, which were selected by their properties. Will they be able to withstand it?



E. coli observed after staining with methylene blue (x1000)

RESULTS

Metal Tolerance

Our project started with the Resistance Test: it consisted in making the microorganisms live with different concentrations of the heavy metals mentioned previously. We inoculated them with the help of sterilized toothpicks. The process had to be made with care so that we didn't damage the environment of the Petri Dishes. The results show that *E. coli* can resist medium concentrations of heavy metals (Figure 1 and Table 1).

pH tolerance

In this one, we had to put the bacteria in Petri Dishes where they were exposed to different pH intensities. We observed them after 24 hours and after 48 hours. As we can see in Figure 2 and Table 2, our bacterium has a great resistance to high pH conditions, which allows it to live in acid environments of the digestive tract.

Growing at different temperatures

We tested the maximum of temperatures our bacteria could grow at after 24h and 48h in a range between 6°C and 55°C. We can see that *E. coli* is able to live at the majority of the temperatures that were tested, but its better growth is at medium to high ones.

Growth in different atmospheres

The objective was to see how much the microorganisms could resist in different atmospheres. We placed them in pipes that were filled with LB medium (the same as Petri Dishes) and some preparated culture drops. For the CO2 atmosphere we used dry ice. *E. coli* is able to live in all of the atmospheres.

Studying bacterial growth in a simulated exoplanet soil

After receiving Brittany Hills' talk about Mars, we simulated other planets' (which we can find in our Solar System) soil and conditions exposing bacteria to UV raditaion. We used sterilized beach sand in pipes and culture drops. Later we subdued them to 15 sec UVA and UVB radiation and left them for three days in the darkness.

DISCUSSION

The results regarding this bacteria have been the following:

- The optimal growth temperature is 18°C-37°C after 48 hours.
- Regarding the response according to the heavy metals (working with proportions between 1mM and 10mM).
    - Copper: *Escherichia coli* grows optimally with 1mM of copper.
    - Iron: *Escherichia coli* grows optimally with 1mM of iron.
    - Zinc: *Escherichia coli* grows with 1mm of zinc.
    - (In all three cases there is no growth with 10mM.)
- pH tolerance (studied in a range between ph3 and ph9):

- *Escherichia coli* shows more tolerance with a pH= 7.

- Growth in different atmospheres:
  - *E. coli* grows in CO_2 atmosphere, and grows optimally in aerobic conditions and microaerobic conditions.

We also wanted to find the exoplanets that could be a candidate to host bacterial life, specifically our bacteria's one. With that in mind, we search for a exoplanet that:

- Has a temperature interval between 30-40°C.
- It doesn't matter if it has oxygen or not because *E. coli* can survive in anaerobic conditions.
- Doesn't have a high concentration of heavy metals.
- An acid environment.

Knowing this and a lot more of characteristics, we can propose our exoplanets. We selected them by searching on some websites that our teacher gave us (The Extrasolar Planets Encyclopaedia and Open Exoplanets Catalogue):

- **Gliese 667C F**

A possible candidate for some hypothetical type of life, such as our bacteria. It's a planet whose size is almost three times greater than our planet. It's close to the Earth, with a distance between them of 23.6 light-years, and it's in the habitability zone of its system. Its orbital period is 39 days. Regarding its temperature and assuming that its atmosphere was similar to that of Earth, it's estimated to have a somewhat lower temperature, 259°K. The star around which it revolves is called Gliese 667 C, part of a triple star system, so if we were situated on the surface of this planet we could observe three "suns", Gliese 667 A, Gliese 667 B and Gliese 667 C , the three stars of this system. In addition to Gliese 667 C F, other planets have been detected with characteristics also similar to those of Earth, and therefore, near the zone of habitability. Its atmosphere has not been detected yet so we do not know if it can have H_2O or other essential elements for terrestrial life. If the living conditions are similar and the atmosphere is also similar to Earth it is possible that bacteria or other simple organisms could have developed acquiring a way of life, that we do not know if it would be similar or not to ours.

Another series of exoplanets that we can highlight are:

- **Kepler-438b**

It's the confirmed exoplanet more Earth-like, with a similarity index of 88%. It belongs to the system Kepler-438, located at 472.9 light years of the solar system. Orbits a red Dwarf (Kepler-438). The stars of this type, with a size and luminosity very inferior to those of the sun, have a very active dynamic, they have a small habitability zone very close to it, and they are very long lived. This planet, which completes its orbit in just 35 days, remains in the habitable zone of the system. Such a small orbit implies a high exposure to the effects of its star, both to stellar winds and to gravitational interaction. Kepler-438b has a mass 1.27 times greater than Earth and a radius 1.12 times higher. Its surface average temperature

(considering a terrestrial-like atmosphere) is 37.45°C. It is considered to be the best candidate to host extraterrestrial life:

1. The closeness with its star, thanks to which its orbit and its rotation would be synchronized, so it would have a diurnal and a nocturnal hemisphere, divided by an area of the motionless twilight.
2. Its average temperature, which would be 37.45 °C. Considering the 14 °C of the terrestrial average, the climate of the exoplanet seems to be much warmer which could turn it into a deserted planet. (which could culminate in an uncontrolled greenhouse effect, similar to that of Venus.)
3. And its atmosphere, where it would be possible to find high concentration of inorganic oxygen even if the planet has no life.

- **Kepler-186f**

It is a planet that orbits a red dwarf star (Kepler-186) and the last of the five planets of its system. It is the first planet with a similar size to the Earth found in the habitability zone of a star although it is located in the most external side. It has a similar orbit to Mars with the Sun and a orbital period less than 129,9 days. This planet has got a temperature of -46°C average (assuming an atmosphere similar to the Earth one, because if its atmosphere were more dense, its temperature would be greater). Its similarity index to the Earth is of 64%, like Mars. Although it orbits a red dwarf star, it could be far enough so that the anchor effect does not occur, and the planet could rotate. Its gravity is expected to be similar to that of the Earth. The planet is approximately 11% larger in the radius than the Earth, giving a volume of approximately 1.37 times that of the Earth (between 0'87 and 2'03 times greater). Life possibilities are estimated to be similar to our planet.

Of the previously named exoplanets, Kepler-438b, is the only one found in the catalogue of habitable exoplanets of the laboratory of planetary habitation, that we were able to see in our teacher's blog. We propose it as our main candidate because it has similar characteristics to Earth, for example: its temperature it's the same that our bacteria finds perfect to live.

Acknowledgements

It's important to note that this whole project has been useful for us because it allowed us to work with people in the scientific area, whom received us with affection and plenty of wishes to teach us. We thank Manuel Espinosa for his job in demonstrating us microbial life forms and the conditions where they could flourish. We also thank Emilio García (although we didn't work with him) he delivered very interesting speeches. In addition we thank Antonio Quesada, we were learning with him during the entire year and he gave us the opportunity of participate in this job, making his classes more pleasant and going over the practice field. Lastly we will mention the Experimental Zaidín Centre, Astrophysics' Institute and our High School. Thanks to them we were able to realize the entire project and we're grateful for that.

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Exoplanets and the limits of life

[3]
Bringing *Bacillus megaterium* to space

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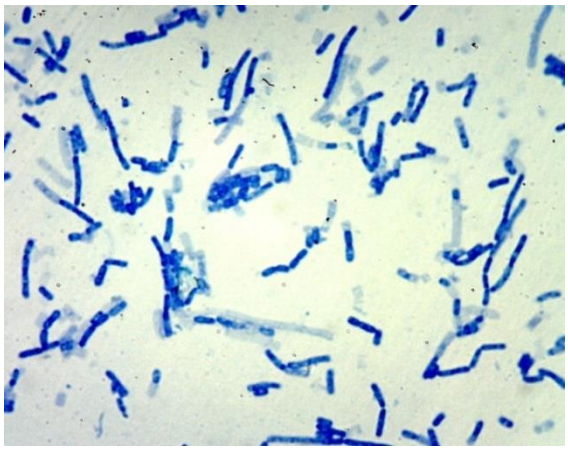
SUMMARY

With this project we try to search for possible exoplanets that accomodate our bacteria. For that we have carried out a series of experiments and we have studied the results of those experiments, and also the possible conditions of life of *Bacillus megaterium*. Later, we have used the database "The Extrasolar Planets Encyclopedia" comparing the characteristics of the Earth with the ones from the differents exoplanets and from the ones from our bacteria. With all that information we have concluded that Trappist 1-d can be a good candidate for *B. megaterium*

INTRODUCTION

Is there life outside our planet? If there is, Is it similar to the life we know? If it isn't, How do we find it? Thanks to the data that we have today, we can neither confirm nor deny existence of life out of this world, furthermore we really don't have a definition of life so we really don't know what we are looking for. Basing us on the characteristics of the bacteria *B. megaterium* and the in the ones from the earth, we submit them to a series of experiments for studying their possible conditions for life. Also, we focus on the search of exoplanets that can host *B. megaterium*. We have done four different experiments to study the types of life that can happen in different exoplanets and with that information discuss if the bacteria *B. megaterium* can survive



B. megaterium stained with methylene blue. Magnification 1000X

RESULTS

We performed a series of experiments to observe the different behaviours of the bacteria in different growth media. We started incubating our culture during 6 days, on plates containing metals like copper, iron and zinc, to see the tolerance to these elements. The obtained results of this experiment were the following ones:

1) Growth with different concentrations of copper: *B. megaterium* grows well at concentrations of 1 mM and 5 mM. However at concentration of 10 mM it does not grow.
2) Growth with different concentrations of iron: *B. megaterium* grows at concentrations of 1 mM and 5 mM but in 10 mM it doesn't grow.
3) Growth with different concentrations of zinc: *B. megaterium* only grows at a concentration of 1 mM and very poorly.

In conclusion, we haven't observed growth in concentrations of heavy metals equal or above 20 mM.

Regarding the second experiment, in which we submitted the bacteria to different temperatures and values of pH, we obtain the next result:

1) Growth of *B. megaterium* at different temperatures (6ºC, 18-22ºC, 37ºC, 45ºC, 55ºC): at 24 hours we have observed growth at 18-22ºC, 37ºC, and 45ºC. At 48 hours: we have observed growth at 18-22ºC, 37ºC and 45ºC. At 6ºC and 55ºC there is no growth.
2) Growth of *B. megaterium* at different pH values: at 24 hours we only find growth at pH=7 and at pH=9 the growth is limited. In pH=3 and pH=5 there is no growth. At 48 hours there is more growth at pH=7 and at pH=9, and no growth at low pH.

In the third experiment we studied the growth of our microorganism in different atmospheres: aerobic environment, atmosphere rich in CO_2 and in microaerobic conditions. We can appreciate that there is bacterial growth in all environments but in more abundance in the microaerobic one.

And fourth and last experiment we simulated the surface of an exoplanet as an habitat for our bacteria, with sterile beach sand that had been incubated and has been radiated. In relation to the radiation of ultraviolet rays in 320 nM we obtained an optical density of 0.36 and in 256 nM (UVB) we obtain an optical density of 1.54.

DISCUSSION

In our specific case, we have investigated two exoplanets, which at first seemed to have the ideal characteristics for the survival of our bacteria (*Bacillus megaterium*). These exoplanets are: Trappist 1-d y KIC 10255705b. We received help from the application "The Extrasolar Planets Encyclopedia" in which we introduce data that is similar to Earth and studied differents exoplanets that the application gave to us, having also into account the

characteristics of *B. megaterium* obtained from the experiments. After studying the characteristics of the diverse exoplanets we came to the conclusion that the most suitable exoplanets are Trappist 1-d and KIC 10255705b. Afterwards we compared the characteristics of one and the other with those of Earth We concluded that Trappist 1-d could become a good place to live, however, KIC 10255705b lacks the existence of H_2O, something indispensable for life, which would prevent our bacteria from growing in it. The characteristics that make it so ideal are: orbital period 4,049 days; radius of 0,0689 RJ; it presents H_2O molecules and has a temperature of 288 K (15ºC). Its star has a distance of 12.1 pc; temperature of 2550K and metallicity of 0.04; with respect to Earth: average temperature of 15ºC; radius of 6371 km; orbital period 365 days; H_2O among many other molecules. Its star (the Sun) has a temperature of 5778 K.

Acknowledgements

We are very happy to have been part of this very interesting project, since it was not a job, like any other, but for a few months we got into the role of a scientist, has made everything much more interesting and exciting. We want to thank Manuel Espinosa and Emilio García for coming to our class and inform us about the experiments that we were going to do in the future, also thanks to our teacher Antonio Quesada for giving us the opportunity to be in this project, we couldn't forget in our appreciations our high school for contacting the CSIC and last but not least to the CSIC for lending us the materials that we needed for the project to come through. In reference to the group of schoolmates that participated in this project, we didn't have any kind of problem and we all collaborated really well.

References

For doing this project we took information form http://biolabzv.blogspot.com/ , we also got help from Manuel Espinosa and our teacher Antonio Quesada, and we found the exoplanets in http://exoplanet.eu/ and http://openexoplanetcatalogue.com/

Exoplanets and the limits of life

[4]
Search for a suitable planet for *Halomonas sp.*

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SUMMARY

In this investigation, we want to prove if the strain of *Halomonas* we have worked with will be able to survive in an exoplanet. We have done some experiments were we recreate some of the conditions that are considered to influence the growth of bacteria, with the purpose of knowing the tolerance of *Halomonas sp* to that conditions as tolerance to temperature, pH, metals, UV radiation and different atmospheres. With the obtained results we have taken the following conclusions: *Halomonas sp* needs an exoplanet with a temperature around the 18ºC minimum and 45ºC maximum, a pH minimum at 5 a maximum of 9, they will not be able to survive in a planet with a high concentration of metals, they can survive in aerobic atmospheres and with high concentration of CO_2 and they can endure wavelength of 256 nm.

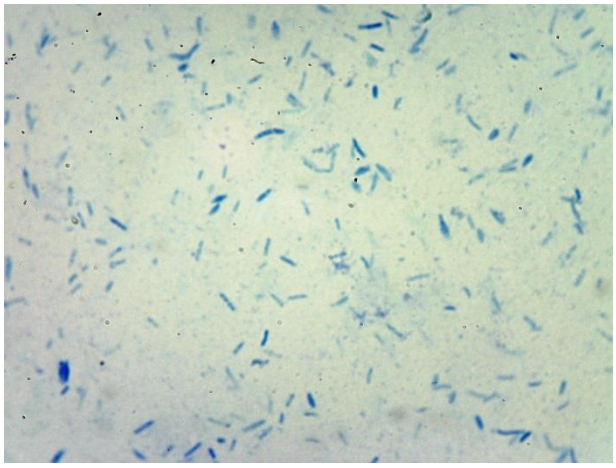
Keywords: *Halomonas*, exoplanet, atmosphere, pH, UV radiation, metals, extraterrestrial life, temperature

INTRODUCTION

The study of bacteria in adverse conditions is important because they are the most extreme living organisms, so if they can resist that conditions, they will be able to live in other planets and it allows us to know if we can found extraterrestrial life in those planets. We have started our investigation with the indications that we were given, based in other researches [1].

The problem of finding life outside the Earth is that we don't know how can life arise, but we know where life can proliferate based in the most extreme Earth conditions [2]. We know many important conditions and, although our bacteria is not so extreme like others, it has interesting peculiarities as its ability to grow in saline enviroments [3]. The strain of *Halomonas* we have work with need a planet with not extreme temperatures, a pH similar to the Earth, the planet cannot have a high concentration of metals in its composition, *Halomonas sp* can grown in aerobiosis, microaerobiosis and CO_2 atmospheres. They can also endure wavelengths of 256 nm.

Halomonas strain visualized in the microscope after staining with methylene blue (x1000)



RESULTS

Variant best results (growth)

Temperature **22ºc - 37ºc**

Different atmospheres **aerobiosis and microaerobiosis, co_2 atmosphere**

Ultraviolet radiation **d.o. 256nm: 2,06**

Tolerance to different metals: **Cu 1mm Fe 1mm Zn 1mm**

Tolerance to different ph levels: **ph 7- ph 9**

The *Halomonas* strain cultivated in temperature under 18ºC could not grow, and neither over 45ºC, the ideal temperature is around the 18º-45ºC.

Halomonas could growth in all the different atmospheres: aerobiosis, atmosphere with high concentration of CO_2, microaerobiosis.

Under the ultraviolet radiation in conditions like the ground of Mars they grow, but it is possible that the time of radiation was not enough.

Halomonas only could growth in a concentration of 1 mM of Cu, over this value they did not develop colonies. In Fe they could grow at 5 mM, over this they could not develop colonies. In Zn it only could grow in 1 mM concentration.

Halomonas grows with problems in a pH= 5. Colonies developed optimally in pH=7 and 9. At pH=3 there was no growth.

DISCUSSION

The problem with *Halomonas* is that they do not survive in some conditions, in many of the experiments they did not show any increase in their growth, but they could survive in a high level of UVA rays (or maybe the time of UV exposition wasn't enough). So searching an exoplanet for them was difficult although they can survive in places with a high level of salt [3]. According to some facts as the temperature, mass and radius of the planet and the

metallicity, temperature, mass and radius of the star we have chosen TRAPPIST-1e [4] [7] as the habitable planet for the strain of *Halomonas* we wave worked with. It has a temperature of 251°K, so it maybe has zones with a comfortable temperature as it happens with the Earth, that allows the planet having liquid water [5]. Its size is similar to Earth, 0.91 radius and 0.77 mass. Its star, Trappist-1, doesn't emit much UV radiation because the temperature is low, 2550K. TRAPPIST-1e is the most reasonable option.

Although it could have lost a big amount of water it is not relevant for its habitability because the percentage of water was very high and now the planet is in the habitability zone so it might not lose more water[6].

Other interesting planet is Proxima Centauri b, this planet is located in the red star Proxima Centauri which is the closest star to the Sun, located about 4 year light is the most near exoplanet discovered, this planet is located 0.05 AU of his star that makes this planet be anchored by tide , with one side of its hemisphere permanently facing the star, while the opposite side is in eternal darkness. The temperature is around the 234°K, in the hemisphere permanently facing the star the temperature should be so hot and in the other hemisphere so cold making all the water in this plane should be evaporated or iced, but there may be a strip between both sides with an ideal temperature to keep liquid water,and if it has a atmosphere this metaphorical living zone could be more bigger. We do not know much more about this planet but it is an interesting candidate because it is the nearest to us.

Acknowledgements

We thank Manuel Espinosa for spending his time teaching us how to culture the bacteria, the Chemistry department for letting us to use their facilities and our teacher for showing us a different way of learning. We thank Antonio Quesada our teacher for his in this project and all the facilities that he gives us to work. We thank Emilio Garcia and the others members of "La mesa redonda" for the spectacular speech that they did to all Zaidin students. We thank all our classmates for their help in some moments. We thank I.E.S. Zaidín-Vergeles for making this possible.

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Exoplanets and the limits of life

[5]
Looking for candidates to live in exoplanets: *Pseudomonas stutzeri*

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SUMMARY

We only know that there is life on Earth, but can any type of life exist in another place in the universe? Through subjecting our candidate bacterium, *Pseudomonas stutzeri* to diferent environments (temperature, radiation…) we tested where it could live. We lay out some candidates, such as exoplanet *Trappist 1-d*, that are really interesting due to their similar conditions with Earth. What implications would have the existence of life in other planets? Are we a peculiarity in the universe or a unequivocal tendency of matter for a higher complexity?

Keywords: *Pseudomonas stutzeri*, Trappist 1-d, Trappist 1-e, Keppler 62-e, exoplanets.

INTRODUCTION

The conditions on Earth for life to emerge were very special. That is why finding planets with similar conditions is hard. We can take a different focus to facilitate work: we can search for organisms in our planet that can survive in the conditions of other planets, to see how compatible these are with life.

RESULTS AND DISCUSSION

In the first results we can clearly observe the limits of survival in the presence of heavy metals, since above 15 mM none of the strains tested is able to grow. Even at 10 mM most bacteria had reduced growth, except *Pseudomona putida*..

The results indicate that Zn is the most toxic metal of those tested since even at 5 mM only three species were able to grow, including *Pseudomonas stutzeri*. Iron would be the most permissive for life. Therefore we should look for a planet containing low concentration of zinc.

When bacteria were exposed to different temperatures it was clear that their optimal conditions are in a range between 18-37ºC, although some like *P. stutzeri* can grow well up to 45ºC. This does not mean they cannot survive outside these values, but with difficulty. It is worth noting that *Bacillus subtilis* can grow at higher temperatures than the rest.

The analysis at different pH showed that alkaline environments were more apt for bacterial growth, while acidic pH values limited colony development.

In the case of the different atmospheres, growth was detected in all cases, suggesting metabolic flexibility. Although *P. stutzeri* is aerobic, it can grow in microaerobiosis and in the presence of increased CO_2, which does not seem to be particularly toxic for this strain (2,3).

Finally, after 15 seconds of irradiation with UVA and UVB, growth was observed in all cases. We can conclude that a short, direct exposure to this radiation is not lethal to bacteria in sand. Probably a prolonged indirect exposure with an atmosphere that absorbs part but not all of the radiation can have a mutagenic potential in the long term, making it a relevant factor.

Once compiled all the information, it is clear what are the requisites for candidate planets to hold life:

• Average temperature between -20 and 80°C. Temperature fluctuation over the year varies; even though the average temperature is low for liquid water, in some months maximal temperature could make it viable, as on Earth where the average temperature is 15°C but has very different environments ranging from -30°C to 100°C.

• A low energy radiation from the star, with a peak in the visible spectrum or lower.

• A concentration of metals similar to Earth or even slightly higher.

• An atmosphere with oxygen in the case of *P. stutzeri*.

Los planetas que cumplan estos requisitos posteriormente pueden ser contrastados con el índice de similitud con la Tierra (IST) (1).

The only planets with all the requisites are the TRAPPIST. Particularly two: TRAPPIST-1 d, with a mass and radius similar to Earth, its average temerature is like that of Earth: 15°C, the metallicity of its star is 0,04Fe/H and its maximum radiation emission is 1136 nm (infrared radiation). On the other hand, TRAPPIST-1 e, has a mass 600 times higher than Earth but a similar radius and an average temperature of -22°C. Considering that temperature has a wide range of oscillation during the year, at some times it could be adequate for life, although both planets have an orbital period of 4 and 6 days respectively. Besides, the high density of the planet would imply a strong gravitational force and high pressure.

Both planets could be habitable and hold liquid water. Although the information that they may have too much water might difficult our purpose of searching for or even introducing life in them (6). We know that Mars had water in the past and it lost it (7), so, how long would it take to lose enough water to make it habitable? Could there have been life in Mars while it had water? And in that case, could it have survided until today even in the harsh conditions of Mars? And the most important and disconcerting question, could Earth follow the same path and lose so much water as to become non-habitable? (2,3,4,5).

Another candidate planet is *Kepler 62-e*, with an average tempearature of 28.45°C and supposing it has an atmosphere similar to Earth. The maximum radiation emission of its star is in the visible range and its metallicity is -0.2, slightly lower than that of the Sun. It has 83% similarity with Earth, based on the IST (8), so it could be a good candidate. However, some

measurements show that the planet is covered by an ocean. Possibly, simple organisms could develop on its surface since we know life in our planet originated in the oceans (2,4).

As a final conclusion and reflection on the project, we have to make balance. The main objectives were to find candidate bacteria able to survive in extreme conditions (or conditions similar to Earth) found in other planets and try to respond to the question of life potentially existing in another place in the universe. The first question has ben successfully solved. We have a candidate to live in an exoplanet like TRAPPIST y *Kepler 62-e*, *Pseudomonas stutzeri*. This has limitations beyond just experimentation and theory, like how to send life to other places in the Universe and keeping it there, but it has expanded our view about life. The second question is impossible to solve. It is not in our hands to know the existence of life in other worlds. Maybe not life as we know it but based on other basic molecules or elements like silicium. Only time will tell the limits that human beings can reach and our capacity to know the surrounding reality.

Acknowledgments

We thank for this experience Manuel Espinosa, microbiologist from Estación Experimental del Zaidín (CSIC); Emilio García, astrophysicist from Instituto de Astrofísica de Andalucía (CSIC); Antonio Quesada, teacher in Instituto Zaidin Vergeles.

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Exoplanets and the limits of life

[6]
Is there life beyond Earth? *Bacillus subtilis*

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SUMMARY

The objective of this project has been to investigate about life outside the Earth and the different places where it could be developed. To carry it out we have worked on different bacteria, with their own characteristics, and we have subjected them to different conditions to check if they are compatible and can survive and develop in different exoplanets. By performing different tests with different conditions we have seen that our bacteria have developed, but not in all the conditions that we had proposed. This allows us to understand that our bacteria can live in exoplanets that have other properties than Earth.

INTRODUCTION

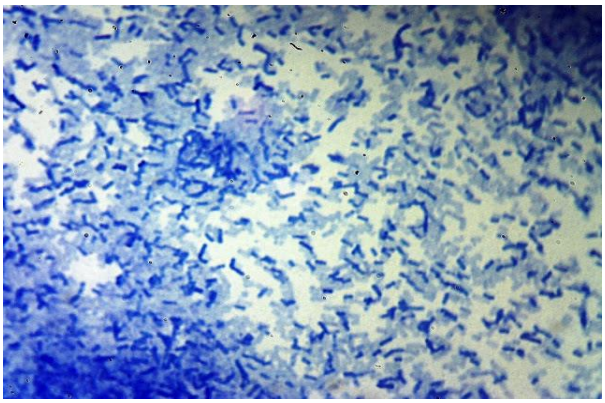
To carry out this project, our group has studied *Bacillus subtilis*. This bacterium has been subjected to four experiments detailed below, to know if it could be a candidate to live in any of the 3588 exoplanets found so far. The experiments were:

-Grow the bacteria in different concentrations of heavy metals.

-Test the survival capacity of bacteria in different temperatures and pH values.

-Study bacterial growth in different atmospheres.

-Study the effect of radiation on bacteria in soil.



B. subtilis stained with methylene blue. (1000x)

RESULTS

In the first experiment, where we grew bacteria in a medium with different concentrations of Cu, Fe y Zn, we found that in Cu 1mM and 5mM they can grow without problems, but they cannot survive at 10mM. The same result was obtained with Fe. However, in the case of Zn, *Bacillus subtilis* grows poorly at 1mM and it cannot survive at 5mM or 10mM (Table 1)

Bacillus subtilis grows at all the temperatures tested, except at 6°C. The same happens at different pH except pH=3. When exposed to different atmospheres, *Bacillus subtilis* can grow without problems. And finally, after being exposed to radiation they grow forming clumps.

DISCUSSION

Once we have the results of our experiments, we look for exoplanets compatible with these characteristcs. These are KEPLER-90h y KIC10255705b.

KEPLER-90b	KIC 10255705b
Mass→ 0,8 mJ	Mass→ 1,1 mJ
Temperature→292 K	Temperature → 295 K
Metallicity → 0,12	Metallicity → 0,13

Thanks to these data we can know in which exoplanet our bacterium can survive. In KEPLER-90h there are possibilities since temperature is not too high. The star's metallicity indicates that it is a gas planet. We believe these two planets could be candidates for *Bacillus subtilis* to survive, since they are in the habitability zone, their stars are similar to our Sun and their conditions could be are close to those of Earth.

Acknowlegements

We want to thank our teacher, Antonio Quesada from I.E.S. Zaidín Vergeles, and the scientists Manuel Espinosa from Estación Experimental del Zaídin and Emilio García from Instituto de Astrofísica, for their attention and dedication in this project.

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Exoplanets and the limits of life
[7]
Can *Pseudomonas putida* be an alien species in exoplanets?

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SUMMARY

To know if bacteria can survive in exoplanets, several tests had to be made, subjecting them to different temperatures, pH, radiation, and atmospheres. With these tests, data were obtained to search for an exoplanet. Pseudomonas putida can survive in environments with a pH between 5 and 10, a temperature between 18 and 45°C, atmospheres with various oxygen proportions, and UV irradiation. We also tested the survival in different concentrations of metals. Once these experiments were done, we searched for exoplanets with suitable characteristics. The best candidate is exoplanet Trappist 1-e.

Keywords: Atmosphere, Earth, UV radiation, Mars, pH, growth medium, soil, *Pseudomona putida*, temperature, exoplanets.

INTRODUCTION

Will it be possible to take life to other planets? To answer this question, we have studied different bacteria, among them *Pseudomonas putida*, to observe how they respond to different stimuli and environmental changes: Temperature, pH, metals, different atmospheres, and survival in sand after UV radiation. The importance of all this is life in exoplanets similar to Earth, existing there or brought in. We have studied this possibility in Trappist 1-e, a planet similar to Earth. For that, we have read many articles and taken into account several parameters to find a planet where our bacteria could survive. These parameters were: Habitability zone, temperature of its star, gases in its atmosphere, presence of water, size, mass and similarity to Earth.

RESULTS

Pseudomonas putida can survive in environments at temperatures between 18 and 45°C, with a pH between 5 and 10, an atmosphere in aerobiosis, microaerobiosis, or rich in CO_2, and irradiation at 320 nm or 256 nm. It can survive in metal concentrations of 5mM.

DISCUSSION

The planet we have chosen for *Pseudomonas putida* is Trappist 1-e. This planet fulfills some conditions, such as temperature. It is in the habitability zone of its star.

Acknowledgements

We thank IES Zaidín Vergeles for this great opportunity; Antonio Quesada for his efforts, patience and preseverance; Manuel Espinosa for his help in this activity and providing the material needed; Emilio García for his collaboration in the project and information to help in it. We have ejoyed the experience and we have learned outside the books and class routine, getting into a very complex and interesting research.

References

Information for this project was obtained from the blog: biolabzv and the webpage for planet search: exoplanet.eu

http://www.nationalgeographic.com.es

http://www.europapress.es

Exoplanets and the limits of life

MATERIAL AND METHODS

Microorganisms

Bacillus megaterium. It is a big bacterium that can reach 4 µm of length by 1,5 µm of width. It is a gram positive bacterium widely spread that normally lives in aerobic environments. You often can find it in couples or chains. When the situation is dangerous they form spores that are structures of resistance.

Bacillus subtilis is a rod-shaped gram positive bacteria. It is considered a facultative anaerobe. It is motile, with flagella. When environmental conditions are extreme, they form resistance bodies called endospores, which germinate once conditions return to normal. It is a model microorganism for genetic studies and has been used in astrobiology studies in assays where martian environments have been simulated. It usually lives in the upper layers of soil although it has also been found as commensal in human intestine.

Escherichia coli. It is a gram negative bacteria belonging to enterobacteriacea family. It is a facultative anaerobe, which means that it can grow in environments without oxygen. It moves by flagella. We can find it in human and animal digestive tract but some strains may be pathogenic; so its ideal temperature to grow is 37ºC. E. coli is a model organism used in molecular genetics.

Halomonas sp. *Halomonas* is a genus of halophilic (salt-tolerating) proteobacteria. They grow in the range from 5 to 25% NaCl. Members of *Halomonas* are rod-shaped bacteria, generally 0.6-0.8 µm by 1.6-1.9 µm. They move using flagella. They grow in the presence of oxygen, although some have been reported to be able to grow without oxygen. *Halomonas* species have been found in a broad variety of saline environments including estuaries, ocean, and saline lakes.

Pseudomonas putida. It is a gram negative rod-shaped saprotrophic soil bacteria. It is not a pathogenic species for human beings. It has a very diverse metabolism, including the ability to degrade organic compounds, so it has been used in bioremediation. *P. putida* form biofilms when it colonizes plants roots.

Pseudomonas stutzeri. It is a gram-negative bacillus motile by flagella. Its size is 1-3 µm length, 0.5 µm width. Its metabolism is strictly aerobic and lives in soil. It is a denitrifying bacterium with high capacity to degrade organic compounds, making it useful for bioremediation. Some strains interact with toxic metals.

Culture medium

LB (Lysogeny broth) is a nutrient-rich medium for the growth of bacteria that contains peptides and casein peptones, vitamins, trace elements and minerals. Formulation per one liter: 10 g peptone, 5 g yeast extract, 5 g sodium chloride. LB agar medium requires 12 g agar.

Methods

Tolerance to heavy metals. We tested if bacteria could survive in presence of high concentration of heavy metals. For this experiment we prepared Petri dishes with LB culture medium and we added salts of zinc, iron and copper to concentrations of 1 mM, 5 mM, 10 mM, 20 mM, 50 mM. Bacteria were inoculated in Petri dishes with the help of a sterile toothpick. They were incubated in a heater at 28ºC. Bacterial growth was studied day after day for a week.

Tolerance to temperature. Inoculated Petri dishes were inoculated with bacteria and were incubated at different temperatures. Plates were cultivated in a freezer and in a heater with different temperatures for a week.

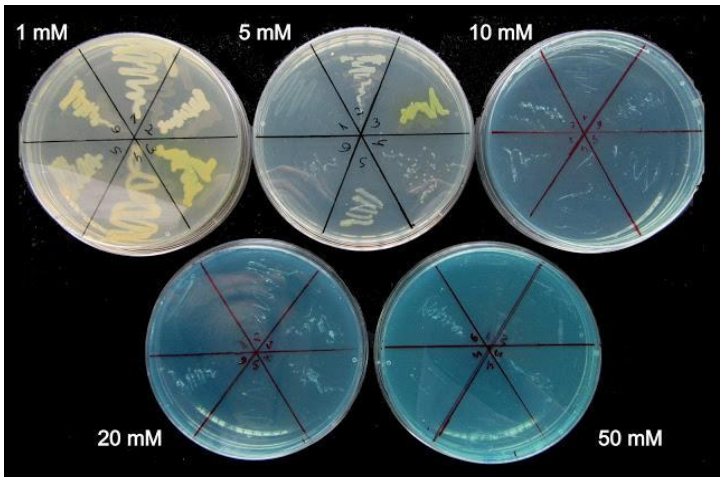
Tolerance to pH. We tested if bacteria were able to survive in plates at different pH values: pH=3, pH=5, pH=7 and pH=9. LB medium was added with chlorhydric acid or sodium hydroxide in order to get the correct pH.

Effect of different atmospheres on bacterial growth. Tubes with liquid medium were sterilized and prepared with different atmospheres and then were inoculated with bacteria. One of them, in contact with air, had aerobic atmosphere (21%) of oxygen; tubes with microaerobic atmosphere (low concentration of oxygen) were prepared filling them to the top with liquid medium and closing them hermetically. High concentration CO2 atmosphere was prepared adding to the tubes with liquid culture medium some pieces of dry ice; when they sublimated they were inoculated and hermetically closed. Bacterial growth was studied by changes in turbidity.

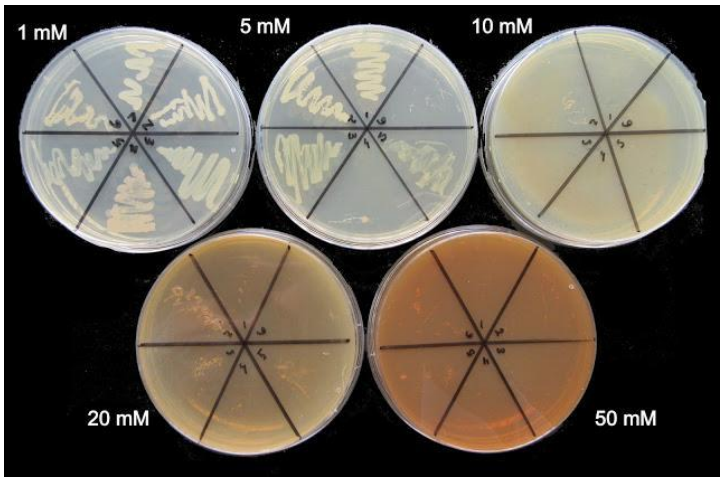
Exposure to UV radiation in simulated exoplanet soil. Tubes with sterilized beach sand were inoculated with few drops of liquid culture of the different bacteria. Samples were irradiated for fifteen seconds with UV radiation (320 nm and 256 nm). Microorganisms were incubated for three days at 30ºC in darkness. Afterwards, 10 ml of sterile NaCl solution (0,9%) were added to the soil, tubes were agitated and let decant. From the supernatant 20 µl aliquotes were taken and sown on agar plates or tubes with sterile liquid medium. After three days the appearance of colonies was studied and the growth in liquid medium was analyzed by measuring turbidity in a spectrophotometer at 600 nm.

Selection of exoplanets. Two databases were managed in order to select exoplanets with conditions compatible with the survival of our microorganisms: The Extrasolar Planets Encyclopaedia (http://exoplanet.eu) and the Open Exoplanet Catalogue (http://openexoplanetcatalogue.com). We have selected exoplanets in the hability zone of its star, with measured or estimated temperatures between 230 K and 373 K, star maximum radiation emission in the visible spectrum or infrared (calculated from Wien Law, knowing the star temperature), star metallicity similar to the Sun's and size and mass of the planet similar to Earth. Special attention has been paid to exoplanets with detected water in their atmospheres.

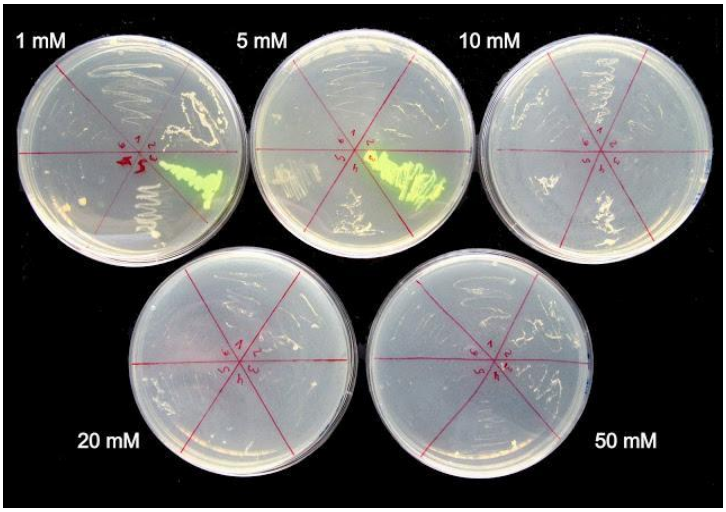
Figures and Tables



LB + $CuCl_3$



LB + $FeCl_3$



LB + $ZnCl_3$

Figure 1. Bacterial tolerance to increasing metal concentrations.
1) *B. subtilis*; 2) *B. megaterium*; 3) *P. putida*; 4) *P. stutzeri*; 5) *E. coli*; 6) *Halomonas sp.*

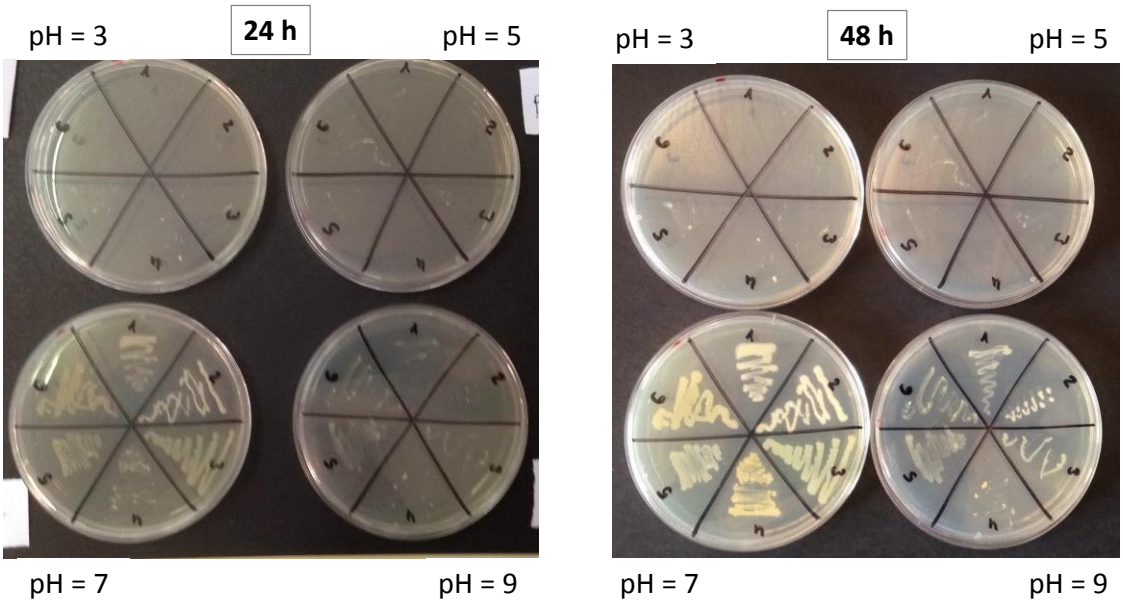


Figure 2. Growth at different pH values after 24 and 48 h. Numbers are as in Figure 1

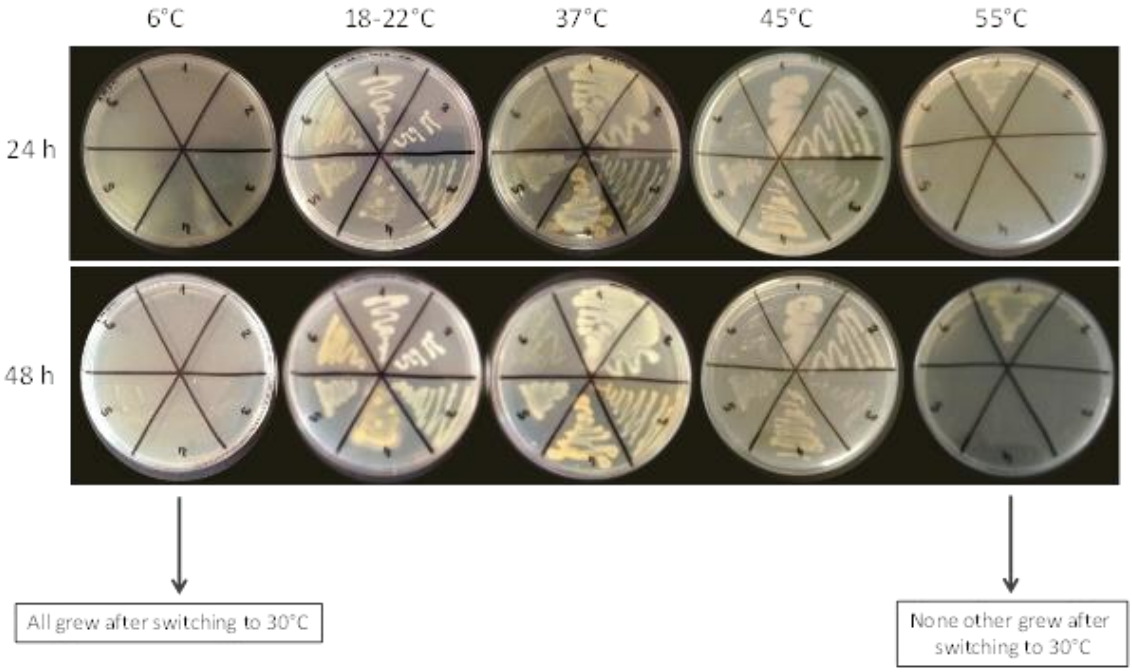


Figure 3. Effect of temperature on bacterial growth on plates. Numbers are as in Figure 1

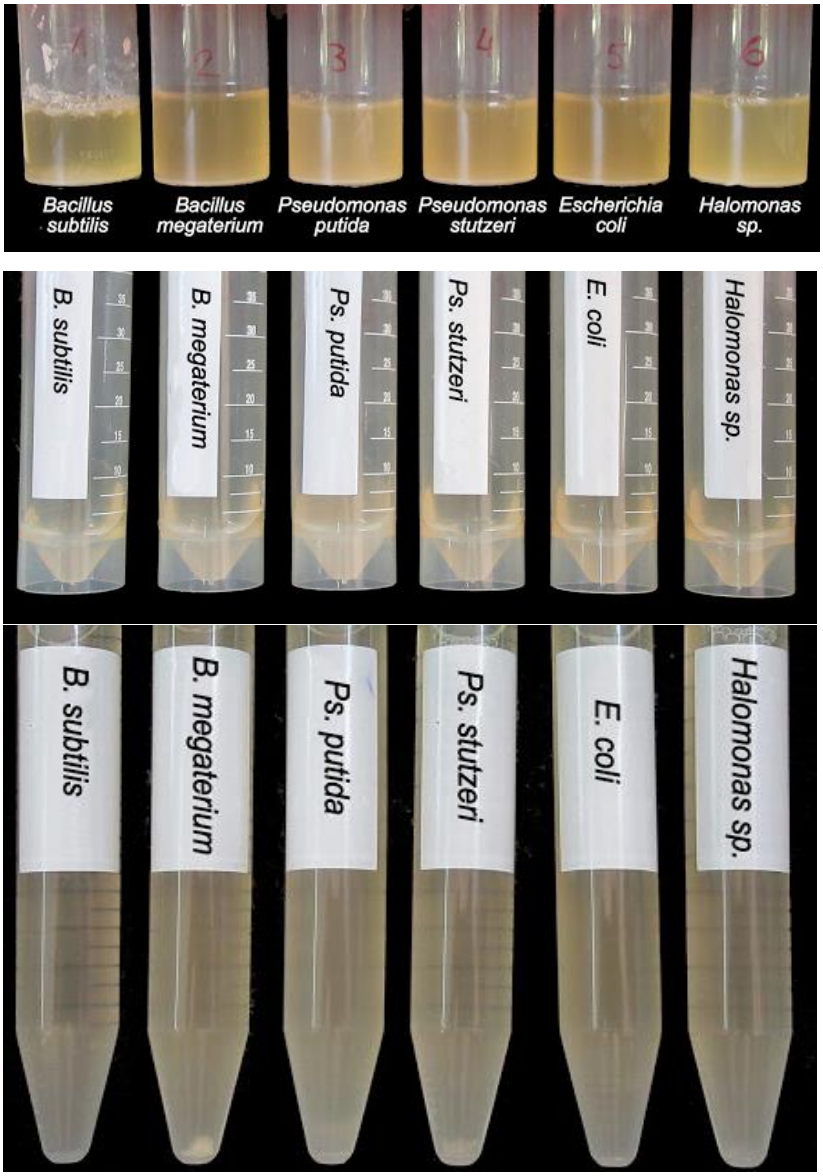


Figure 4. Growth in liquid medium in aerobic (top), high CO_2 (middle), and microaerobic (bottom) conditions

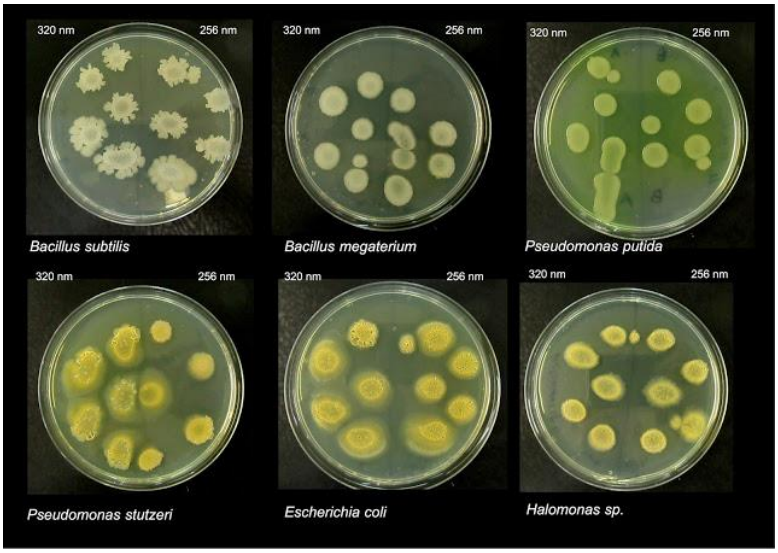


Figure 5. Growth on plates after inoculation of bacteria in sterile sand, irradiation with UV for 15 seconds and incubation in the dark for 3 days.

Table 1. Evaluation of growth in the presence of metals. The number of plus (+) symbols indicates the degree of growth. The minus (-) symbol indicates no growth.

	Cu			Fe			Zn		
	1 mM	5 mM	10 mM	1 mM	5 mM	10 mM	1 mM	5 mM	10 mM
Bacillus subtilis	+++	+++	-	+++	+++	-	+	-	-
Bacillus megaterium	+++	+++	-	+++	+++	-	++	-	-
Pseudomonas putida	+++	+++	-	+++	+++	-	+++	+++	+
Pseudomonas stutzeri	+++	++	-	+++	+++	-	+++	+	-
Escherichia coli	+++	++	-	+++	+++	-	+++	++	-
Halomonas sp.	+++	-	-	+++	+	-	++	-	-

Table 2. Growth in LB at different pH, after 24 and 48 h.

Bacteria	pH = 3	pH = 5	pH = 7	pH = 9
Bacillus subtilis	-	+	+++	++
Bacillus megaterium	+	-	+++	++
Pseudomonas putida	-	+	+++	++
Pseudomonas stutzeri	-	-	+++	+
Escherichia coli	+	+	+++	++
Halomonas sp.	-	+	+++	++

24h

Bacteria	pH = 3	pH = 5	pH = 7	pH = 9
Bacillus subtilis	-	+	+++	++
Bacillus megaterium	+/-	-	+++	++
Pseudomonas putida	+	+	+++	++
Pseudomonas stutzeri	-	-	+++	+
Escherichia coli	+	+	+++	++
Halomonas sp.	+/-	+	+++	++

48h

Table 3. Growth at different temperatures, after 24 and 48 h.

Bacteria	6º C	18-22º C	37º C	45º C	55º C
Bacillus subtilis	-	+	+++	+++	++
Bacillus megaterium	+	-	+++	+++	-
Pseudomonas putida	-	+	+++	++	-
Pseudomonas stutzeri	-	-	+++	+++	-
Escherichia coli	+	+	+++	++	-
Halomonas sp.	-	+	+++	+	-

24h

Bacteria	6º C	18-22º C	37º C	45º C	55º C
Bacillus subtilis	+/-	+++	+++	+++	++
Bacillus megaterium	-	+++	+++	+++	-
Pseudomonas putida	+	+++	+++	++	-
Pseudomonas stutzeri	-	+++	+++	+++	-
Escherichia coli	+	+++	+++	++	-
Halomonas sp.	-	+++	+++	+	-

48h

Table 4. Growth in liquid medium in different atmospheres

	Aerobiosis	Atmósfera CO_2	Microaerobiosis
Bacillus subtilis	+++	++	++
Bacillus megaterium	++	++	+++
Pseudomonas putida	+++	+++	++
Pseudomonas stutzeri	++	++	++
Escherichia coli	+++	++	+++
Halomonas sp.	++	++	++

Table 5. Turbidity (OD 600 nm) of suspensions recovered from sand after irradiation with UV light and incubation for 3 days in the dark.

	320 nm	256 nm
Bacillus subtilis	grows in clumps	grows in clumps
Bacillus megaterium	0.36	1.54
Pseudomonas putida	1.94	1.77
Pseudomonas stutzeri	grows in clumps	grows in clumps
Escherichia coli	1.18	1.37
Halomonas sp.	1.54	2.01

My own ideas

In this year we have been working in this Astrobiology Project. All the clases we were asked to do many things about it, but when it comes oyt to give an overall opinión about it, I think that it is the most difficult part. When we are given a project with such ideas as this one, it is difficult to have any opinion against it.
Anyway, if I had to say what I think I would express my satisfaction. Although it was a difficult topic, it didn't seem so complicated due to the continous explanations the CSIC researcher gave us when it was neccesary which made it highly understandable for us.
It was a great way of learning living how researchers work for myself. The project allowed us to manipulate with lab materials and tools and we also had to write a scientific essay, what I consider extremely useful for the future and a great way to sum up and to consolidate the knowledge we have learnt through this year.
When it comes to propose new ideas, I would like to know mor about the exoplanets we proposed and test more conditions about Mars because I find this planet quite interesting and accesible because of its near distance to the Earth.
Anyway, my overall opinion is quite good and I enjoyed it so much as a new form of learning.

Maia Morteyrú Cornejo, 1º bachillerato.

This project has been, as our teacher says, a good wat to keep learning science in spite of going out further our text book and our lessons.
I had never worked with bacteria before, so I have been able to learn so much with it. It is not tje first time I work with explanets; the time before was just watching exoplanets and how they move in simulations in a talk. This time we have gone further from that and even have found exoplanets with similar characteristics to the Earth; in fact this was one of the main objectives of our Project.
It has been also the first time I write a science essay, so it is very positive. No I know how its structure is and how to write them. Basically, it has been very beneficial for us.
Now, I know a lot mor about exoplanets, about the environments bacteria can resist. I could watch that homeostasis processes occurs in bacteria like Hallomonas. Even I have learned a little bit more of English, specifically, science vocabulary.

Delia Rodríguez López, 1º bachillerato.

En mi opinión ha sido una experiencia magnífica. Hemos podido trabajar como científicos. Desde la práctica se aprende mucho más que desde la teoría. El hecho de manipular material científico, trabajar con él, realizar diversos experimentos, es mucho más eficaz que si nos estudiamos toda esta teoría, procesos y demás y lo plasmamos en un examen. Después del examen vamos a olvidar la mitad de la información; sin embargo la práctica es algo que

siempre vamos a saber ya que lo hemos hecho nosotros, lo hemos podido observar y esos conocimientos duran para siempre.
Por otro lado, el hecho de trabajar con otros compañeros de clase hace que aprendamos a trabajar en grupo, a organizarnos y a dividirnos las tareas.
Creo sinceramente que la aportación de los materiales necesarios para la realización de este proyecto, cedidos por la Estación Experimental del Zaidín, ha sido fundamental para que se lleve a cabo. Por otro lado, sin nuestro profesor todo ello habría sido imposible ya que cualquier profesor no nos ofrece la oportunidad de realizar este tipo de proyectos.

Cristina Castro Jiménez, 1º bachillerato.

Durante este curso hemos experimentado con bacterias para conocer sus características y encontrar otro posible planeta donde pueda encontrarse algún tipo de vida similar a la de la Tierra. Empezamos sin experiencia alguna, algunos sin entender mucho de lo que iba a tratar este proyecto.
Ha sido bastante interesante; es algo que no es muy común hacer y se convierte en una oportunidad de aprender de una forma bastante agradable.
Con esto he conseguido entender cómo de compleja es la vida y que una poca variación del entorno influye hasta el hecho de que pueda desarrollarse o no.
Se han llegado a valorar parámetros como el pH, la temperatura, diversas atmósferas y la tolerancia a metales de seis tipos distintos de bacterias. Casi todas dieron mayoritariamente resultados muy similares, pero siempre alguna puede sorprendernos. Lo más interesante ha sido trabajar con los microorganismos, aprender a movernos con más soltura por un laboratorio y manipularlas. Lo más complicado para mi ha sido conformar el artículo científico con tantos datos y queriéndolo hacer agradable de leer y no muy pesado.
No creo que haya faltado mucho por hacer; la mayor parte de las propuestas se han comentado y hemos conocido un gran número de conceptos sobre la vida, tanto en la Tierra como la posibilidad de su existencia fuera de nuestro planeta.

Tania Delgado Ortiz, 1º bachillerato.

Durante el proyecto hemos estudiado varias bacterias con la finalidad de saber si sobrevivirían en exoplanetas similares a la Tierra. El proyecto ha estado muy bien; se aprende no solo a tratar a las bacterias sino a usar los materiales de laboratorio y a trabajar en él.
Los estudios llevados a cabo han sido muy satisfactorios, pero si hubiéramos tenido más tiempo tal vez podríamos haber realizado las ideas que cada grupo ha propuesto; por lo demás todo muy bien.
No ha sido difícil llevar a cabo el proyecto pues nos han explicado muy bien
lo que teníamos que hacer en cada caso, pero a la hora de observar los resultados ha sido complicado sabe cuánto habían crecido al verlas en las placas de cultivo ya que a veces unos

veíamos crecimiento y otros no. Ha sido una gran oportunidad y una experiencia muy productiva.
Me hubiera gustado llevar a cabo el proyecto propuesto por mi grupo que consistía en someter a las bacterias a atmósferas con diferentes características para observar si crecería o no. Las atmósferas serían de diferentes planetas similares a la Tierra en cuanto a su composición, pero tendrían más o menos gases o distintas proporciones de ellos.
Se han propuesto ideas muy interesantes pero debido al tiempo o la falta de materiales y medios no se han podido llevar a cabo.
Toda la información obtenida en los resultados de los experimentos y la que nos han dado en las distintas charlas nos ha ayudado mucho y nos ha aportado nuevos conocimientos.

Almudena Martín Martínez, 1º bachillerato.

Este proyecto nos ha ocupado durante gran parte del curso. Ha sido una gran forma muy distinta de trabajar y aprender ciencia. Yo me impliqué desde el principio contrastando y valorando datos y resultados; y he aprendido gracias a esto mucho sobre Biología y Astrobiología.
Las implicaciones que tendría encontrar vida en el universo, o encontrar candidatos terrestres a vivir en otros planetas son enormes; podríamos hablar de un fenómeno común y una tendencia natural de la materia a originar vida o, por el contrario, de un fenómeno único e irrepetible del cual nosotros somos el máximo exponente.
En cuanto al material y los métodos teníamos cierta limitación, aunque eso no impidió que realizáramos experimentos muy interesantes y educativos. Incluso propusimos nuestros propios experimentos y uno de ellos se llevó a cabo. Aunque creo que una vez recopilados los datos por separado deberíamos haber hecho un experimento final en el que recreáramos las máximas condiciones posibles.
Finalmente este proyecto ha sido una gran experiencia que no tendré la oportunidad de volver a repetir. Agradecimientos una vez más a los colaboradores de la Estación Experimental del Zaidín y del Instituto de Astrofísica de Andalucía y a nuestro maestro de Biología que se implica como ningún otro.

Marcos Molina Fernández, 1º de bachillerato.

Durante este curso hemos estado trabajando con múltiples bacterias; cada grupo tenía su propia bacteria, lo que le permitía especializarse y en mi caso también comprometerse con ella. Se ha creado un interés en mi en cada uno de los experimentos que se hacía de modo que quería saber si mi bacteria era la más apta para habitar otros mundos. He aprendido de forma positiva y palpable muchas cosas de las bacterias, cómo cultivarlas, a usar material de laboratorio y también sobre exoplanetas.
Propuse un experimento a mis compañeros de grupo que estaba basado en cuánta capacidad de concentración de sal podían resistir nuestras bacterias. Ya sabíamos que

Halomonas, la bacteria de nuestro grupo, sería la más resistente ya que es una bacteria halófila, pero desconozco cuánta es la diferencia con las otras bacterias. Este valor nos permitiría demostrar si algunas de las bacterias podrían sobrevivir e lugares muy salados. Uno que especialmente me llamó la atención son las supuestas salmueras que se pueden formar en las noches marcianas bajo el suelo; si en esos espacios con agua, gran concentración de sal, temperaturas no tan bajas y sin radiación se podrían desarrollar ambientes con vida.

Fernando Quirós González, 1º bachillerato.

Este proyecto me ha parecido muy interesante a a vez que importante ya que hay mucha información que he podido conocer que para mi era desconocida. Este proyecto me ha gustado porque me he implicado y he podido ver y conocer de primera mano cómo trabaja la gente profesional en estos terrenos, tanto en lo práctico como en lo teórico.
También me ha encantado poder cultivar yo misma las bacterias, algo que para mi era un proceso desconocido y que gracias a este proyecto he podido conocer en primera persona.
Para mi la mayor dificultad ha sido buscar y razonar por qué escogíamos un exoplaneta, ya que hay que tener en cuenta que algunos parámetros cuyo significado yo desconocía.
Por último quiero decir que volvería a repetir este proyecto, miles de veces, sin pensármelo ya que me ha aportado muchos valores y he podido conocer muchas cosas, desde cómo está compuesto un medio de cultivo hasta los parámetros que hay que tener en cuenta para poder saber y conocer si un exoplaneta es apto o no para que puedan sobrevivir y desarrollarse las bacterias.
Por último me gustaría agradecer a todas las personas que han contribuido a este proyecto y han hecho posible que se desarrollase.

Asunción Urquiza Castillo, 1º bachillerato.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