High School Students for Agricultural Science Research

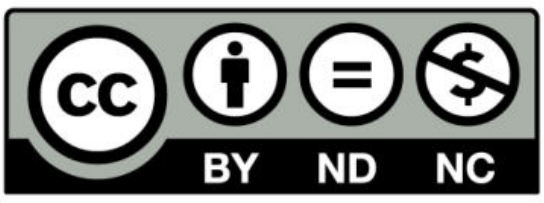
September 2024 **Volume 13**

High School Students for Agricultural Science Research

Volume 13 **September 2024**

ISSN: 2340-9746

Published in Granada by Estación Experimental del Zaidín. CSIC



Index

Moisture and mixture recipe strongly influence the abundance and biodiversity of invertebrates of bio-waste composting

Claudia Barros Wilkinson[1], Ezequiel Fernández Fernández[1], Estrella García Romero[1], Carla Gómez Álvarez[1], Natalia Mota Salceanu[1], Elsa Vallberg Fernández[1], M. Mar Vázquez Vázquez[1], Rubén Rodríguez Ramírez[2], Sara Pérez González[3], Raquel A. Juárez-Martos[3], Germán Tortosa[3#]

[1]*I.E.S. Los Cahorros, c/ Los Neveros, 12, 18193 Monachil, Granada, Spain*

[2]*Ariadna Estrategia Ambiental SL*

[3]*Nitrogen Metabolism in Rhizospheric Bacteria Group (NITRORHIZ). Estación Experimental del Zaidín (EEZ), CSIC, c/ Profesor Albareda, 1, 18008 Granada, Spain.*

#Corresponding author: german.tortosa@eez.csic.es

Summary

Invertebrates are one of the most abundant and ubiquitous groups of organisms on our planet. They have an important ecological and environmental role as they are involved in numerous processes of agricultural interest. An example is composting, a biological technology used to transform organic waste into fertiliser or compost. Consequently, the main objective of our research was to study the biodiversity of invertebrates in compost in order to understand the composting process itself. In this study, two compost piles were evaluated: A trapezoidal pile made with only wood chips (Pile 1) and a 600-L composting bin, made with food scraps and wood chips (2:1 volume to volume ratio; Pile 2). The main differences between compost piles were the moisture (30 and 70 % in Piles 1 and 2, respectively), the salinity (ten-fold higher in Pile 2 respect to Pile 1) and their recipes, which affected the biodegradability of the organic matter (Pile 2 > Pile 1). As expected, the total number of invertebrates was higher in Pile 2 (1534 ± 368 invertebrate per kg of dry compost) compared to Pile 1 (184 ± 24). Also, Pile 2 diversity was apparently higher respect to Pile 1. Spiders, mites, beetles, flies, true bugs and ants were photographed in Pile 1, while spiders, pseudoscorpions, woodlice, mites, beetles, earwigs, flies, ants, silverfishes and land snails were found in Pile 2. Only pseudoscorpions, woodlice, earwigs, silverfishes and land snails were detected in Pile 2, being good bio-indicators of the biological process of composting. According to our results, it can be concluded that water content and organic matter biodegradability of the organic wastes strongly affected the abundance and diversity of compost invertebrates.

Keywords: Ants, beetles, earwigs, flies, food scraps, land snails, mites, pseudoscorpions, silverfishes, spiders, true bugs, wood chips, woodlice.

INTRODUCTION

Invertebrates are one of the most abundant and ubiquitous groups of organisms on our planet [1]. From a taxonomic point of view, their classification is complex. Some interesting phyla are Arthropoda, Mollusca and Annelida. In the first one, we can find the chelicerates (arachnids), hexapods (insects), myriapods and crustaceans. In the second and third ones, mollusks (snails) and annelida (earthworms) are representative. All of them have a very important ecological and environmental role as they are involved in numerous processes of agricultural interest such as plant pollination, feeding other organisms or pest control, among others [2]. They are also decomposers of soil organic matter and improvers of its physical, chemical and biological properties.

Soils physical, chemical and biological properties are essential for their proper ecological functioning. Soil biology is known to modulate nutrient cycles and to improve soil structure [2]. The soil food web concept explains the relationship between soil living organisms. From a nutritional point of view, they can be classified into two general categories: decomposers of organic matter, or predators.

Composting is a biological technology that can be used to transform organic wastes into fertiliser or compost. An example is bio-waste, which is essentially formed by garden and park cuttings, and food and kitchen residues from households and catering establishments [3,4]. Although microorganisms are the main protagonists of the composting process, invertebrates start to appear when the composting temperatures are around 20-35 ºC. In this situation, a food web emerges, in which earthworms, termites, insects and mites, among others invertebrates take importance.

One of the challenges of composting science is to understand invertebrates' abundance and diversity and its evolution throughout the process [5]. According to that, the main objective of our research was to study the invertebrate's population in compost in order to understand the composting process itself. To achieve that, we establish a new methodology for invertebrate sampling and to analyse their biodiversity. Also, we measured the basic properties of compost and we proposed some invertebrates as bio-indicators to know the evolution of the composting process.

MATERIAL AND METHODS

1. Composting performance and compost characterization

The experimentation was carried out at the household waste recycling center of Monachil (Granada; Spain: 37.12460, -3.56191). In this facility, a municipal composting program was performed by the Environmental Department. Two organic wastes were usually collected and treated by composting: wood chips from municipal gardens, and food scraps from Monachil residents, provided by a local selective waste collection program.

Two composting systems were evaluated in this study (Figure 1):

- Pile 1: A trapezoidal pile, made with only wood chips
- Pile 2: A 600-L composting bin, made with food scraps and wood chips (2:1 volume to volume ratio)

During the experimentation, some compost characteristics were analyzed: temperature was evaluated by introducing an electric probe inside the piles [3]. Moisture content was calculated

after drying compost in an oven at 105 ºC during 24 h [4]. pH and electrical conductivity (EC) were measured using a portable pH meter (pH PCE-PHD 1-PH, PCE Instruments™) after 1:20 (w: weight to volume) aqueous extraction.

Figure 1. An overview of the two composting piles used in this study: An open trapezoidal pile (A) and a composting bin (B). The former (Pile 1) contained only wood chips (C) and the latter (Pile 2) bio-waste (wood chips+food waste, D).

2. Sampling and analysis of invertebrates´ abundance and diversity

In order to evaluate the invertebrates' abundance, a non-destructive sampling procedure was developed: A compost core (round 100-200 g) was obtained by using an Eijkelkamp™ Peat sampler, which was introduced until 30-50 cm inside each compost pile (Figure 2A). After that, the compost sample was carefully sieved at 0.9 cm with a garden riddle (Figure 2B). The resulting material was placed on a large white plastic tray and then, a visual invertebrates counting was performed during 1 minute by, at least, six people (Figure 2C). Finally, part of sieved material was used to analyse compost pH, salinity and moisture, and the rest, was returned into compost piles. Data were expressed as the total number of invertebrates (TNI) per kg of dry compost.

Figure 2. Experimental procedure of invertebrate sampling: Compost core sampling by using an Eijkelkamp Peat sampler (A), compost sieving at 0,9 cm (B) and visual invertebrates counting (C).

Several photographs and videos of invertebrates were taken during sampling using Android and iPhone mobiles equipped with portable optical lens (x10). Digital photographs were downloaded and the invertebrate's diversity was then checked by using the iNaturalist website (https://www.inaturalist.org/).

3. Experimental procedure

The experimentation was carried out from January to April 2024 and three samplings were done: the first one was used to optimize the sampling procedure and invertebrate's photographs, the second one to optimize invertebrate counting, and the third one, to collect data. Samplings were done in triplicate in each composting pile, which means 6 samplings per day (18 in total).

RESULTS

The main characteristics of the two compost are shown in Table 1. Both piles were managed differently, according to their composting system. Pile 1 was an open trapezoidal pile, which was frequently aerated with a composting turner machine. On the other hand, Pile 2 was a composting bin and aerated with a manual compost turner every 3 weeks. The recipes of both piles were also different: Pile 1 was made only with wood chips, meanwhile Pile 2 was composed with wood chips and food scraps at 2:1 volume to volume ratio. This proportion affected water content, being 30 % in Pile 1 and 3 times higher (70%) in Pile 2. The pH values in both piles were quite similar due to wood chips, but not the salinity. Pile 2 presented 4.22 ± 1.22 µS cm^{-1}, one fold higher than Pile 1 (0.43 ± 0.13 µS cm^{-1}).

Table 1. Main physical and chemical characteristics of Pile 1 and Pile 2 composts.

Characteristics	**Pile 1**	**Pile 2**
Composting system	Open trapezoidal pile	600-L composting bin
Recipe	Wood chips	Wood chips and food scraps (2:1 volume to volume ratio)
Moisture (%)	30.4 ± 1.3	72.5 ± 3.6
pH	8.6 ± 0.5	8.3 ± 1.2
Electric Conductivity (µS cm^{-1})	0.43 ± 0.13	4.22 ± 1.22
Biodegradability	Low	High
Visual presence of invertebrates	No	Yes

Initially, invertebrates were not detected in Pile 1 after a preliminary visualization. On the contrary, in Pile 2 they could be easily detected running on the top of the compost mixture. After sampling, as expected, the number of invertebrates were directly related to water content. The TNI in Pile 1 was 184 ± 24, meanwhile Pile 2 presented 1534 ± 368, close to one fold higher (Figure 3).

Concerning biodiversity, a total of 9 different invertebrates were photographed in Pile 1 and 12 in Pile 2 (Table 2 and 3, respectively). In the former, they belong to Aranae (spiders), Trombidiformes (mites), Coleoptera (beetles), Diptera (flies), Hemiptera (true bugs), and Hymenoptera (ants) orders (Table 2), meanwhile Aranae (spiders), Pseudoscorpionida (pseudoscorpions), Isopoda (woodlice), Trombidiformes (mites), Coleoptera (beetles), Dermaptera (earwigs), Diptera (flies), Hymenoptera (ants), Zygentoma (silverfishes) and Stylommatophora (land snails) orders were found in the latter (Table 3). Only pseudoscorpions, woodlice, earwigs, silverfishes and land snails were detected in Pile 2, being mites, pseudoscorpions, woodlice and earwigs the most abundant (50 to 70% of the total invertebrates detected).

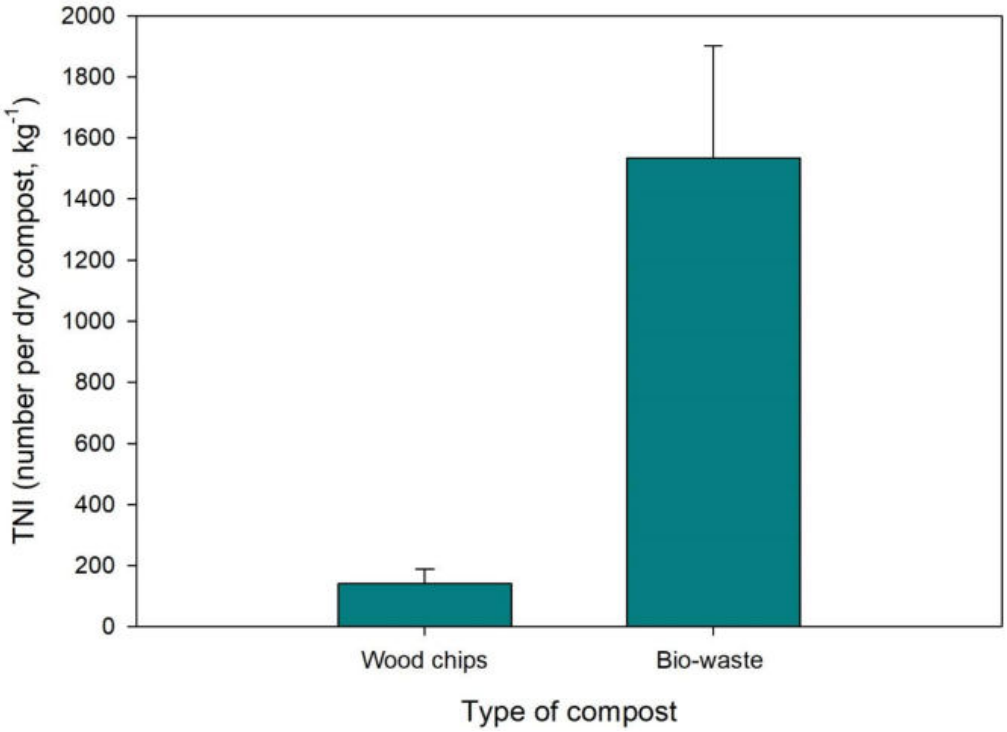


Figure 3. Total number of invertebrates (TNI) in wood chips (Pile 1) and bio-waste (Pile 2) composts. Data are expressed as total number of invertebrates per dry compost (kg^{-1}).

DISCUSSION

The study of invertebrates can be a valuable tool to evaluate the composting process and also, compost characteristics [6]. In this study, we have studied two compost piles with several physical and chemical differences, which directly modulated the abundance and the diversity of compost invertebrates. The first difference was moisture. Water content represented only 30% in Pile 1, which was made with only wood-chips, and 70% in Pile 2, made with 2:1 proportion of wood chips and food scraps. This fact is explained due to the water content of food waste, which is usually high [7]. Water content is essential for invertebrate development, affecting its abundance and diversity, being higher in Pile 2 than Pile 1.

The second main difference was related to compost recipes. Pile 1 was only made with wood chips, meanwhile Pile 2 contained wood chips and food scraps. It is well known that food wastes are easily biodegradable, especially compared to wood brunches, which are rich in lignin and cellulose [8]. These two factors strongly affected the abundance and diversity of invertebrates in compost, being both higher in Pile 2 compared to Pile 1. According to our data, the more water content the compost had, the higher invertebrate abundance and diversity were. Similar results were found for diversity, being Pile 2 the compost in which more invertebrate types were detected.

Table 2. Main invertebrates identified in Pile 1 (only wood chips).

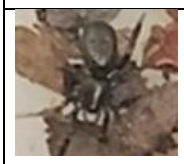
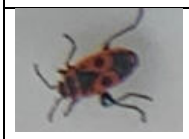
Photo	Common name (Spanish)	Common name (English)	Phyla	Subphyla	Class	Order	Is it predator?
	Araña	Spider	Arthropoda	Chelicerata	Arachnida	Aranae	Yes
	Ácaro rojo	Mite	Arthropoda	Chelicerata	Arachnida	Trombidiformes	No
	Escarabajo de los cereales de cuello estrecho	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	No
	Escarabajo encapuchado	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	No
	Escarabajo errante	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	Yes
	Escarabajo rojo de la harina	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	No
	Mosca	Fly	Arthropoda	Hexapoda	Insecta	Diptera	No
	Zapatero o chinche de campo	True bugs	Arthropoda	Hexapoda	Insecta	Hemiptera	No
	Hormiga negra de jardín	Ant	Arthropoda	Hexapoda	Insecta	Hymenoptera	Yes

Table 3. Main invertebrates identified in Pile 2 (wood chips + food scraps).

Photo	Common name (Spanish)	Common name (English)	Phyla	Subphyla	Class	Order	Is it predator?
	Araña	Spider	Arthropoda	Chelicerata	Arachnida	Aranae	Yes

	Pseudoescorpión	Pseudoescorpion	Arthropoda	Chelicerata	Arachnida	Pseudoscorpionida	Yes
	Ácaro rojo	Mite	Arthropoda	Chelicerata	Arachnida	Trombidiformes	No
	Cochinilla de humedad	Woodlouse	Arthropoda	Crustacea	Malacostraca	Isopoda	No
	Escarabajo errante	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	Yes
	Escarabajo de la cama o gusano de harina menor	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	No
	Escarabajo oscuro	Beetle	Arthropoda	Hexapoda	Insecta	Coleoptera	No
	Tijereta, tijerilla, cortapicos	Earwigs	Arthropoda	Hexapoda	Insecta	Dermaptera	Yes
	Mosca	Fly	Arthropoda	Hexapoda	Insecta	Diptera	No
	Hormiga	Ant	Arthropoda	Hexapoda	Insecta	Hymenoptera	Yes
	Pececillo de plata	Silverfish	Arthropoda	Hexapoda	Insecta	Zygentoma	No
	Caracol	Land snail	Mollusca		Gastropoda	Stylommatophora	Yes

According to their alimentary habits, compost invertebrates can be classified into two categories: decomposers and predators [5]. The former directly degrades the organic matter from wastes, and the latter, eats decomposers. In Pile 1, some decomposers (mites, some beetles and true bugs) and predators (spiders, some beetles and ants) were found. Beyond these invertebrates, in Pile 2 we also found woodlice and silverfishes as decomposers and pseudoscorpions, earwigs and land snails as predators. These results indicated that the relevance of the biological process was more active in Pile 2 compared to Pile 1, probably due to its higher water content and biodegradability of the organic matter.

Spiders, mites, beetles, flies and ants are ubiquitous invertebrates [1, 2]. They are frequently found in soils, gardens and, also in composts [5]. According to our data, they were in both Piles but pseudoscorpions, woodlice, earwigs, silverfishes and land snails were only detected in Pile 2. Probably, these invertebrates cannot tolerate a dry ambient like Pile 1 had, with only 30% of moisture. For that, they could be good bio-indicators for the biological process of bio-waste composting.

Finally, it is important to note that this research is a preliminary study and results can be strongly affected by sampling bias. In order to reduce this negative effect, more research is needed. Nevertheless, it can be concluded that water content and organic matter biodegradability of the organic wastes strongly affected the abundance and diversity of bio-waste compost invertebrates.

Acknowledgements

Authors want to thank to Monachil city council, especially to Luis Gabriel Aróstegi Cabello, Blas Gómez Portillo and Mª Ascensión González Romero, Jesús Montañés López, their kindness and implication in our project. Also, to Paloma Pizarro Tobías for her revision of the written English.

References

[1] Invertebrates. Soil Ecology WIKI from the University at Buffalo. Available in: https://soil.evs.buffalo.edu/index.php/Invertebrates

[2] Griffiths HM, Ashton LA, Parr CL, Eggleto P. 2021. The impact of invertebrate decomposers on plants and soil. New Phytol, 231: 2142-2149. https://doi.org/10.1111/nph.17553

[3] Roldán-Segura C., Walkley C., Tortosa G. 2018. Recycling bio-waste by small-scale composting. High School Students for Agricultural Science Research, 7, 13-17. ISSN: 2340-9746.

[4] Díaz J.M., Moro C., Coletti Y., de la Torre A., de la Torre J., Rolland A., Ledesma D., Bedmar E.J., Tortosa G. 2020. Biological insights of Estación Experimental del Zaidín (EEZ) bio-waste composting. High School Students for Agricultural Science Research, 9, 14-26. ISSN: 2340-9746.

[5] Invertebrates of the Compost Pile. Cornell Composting Science & Technology. Available in: https://compost.css.cornell.edu/invertebrates.html

[6] Steel H., Wim B. 2012. Biodiversity of Compost Mesofauna and Its Potential as an Indicator of the Composting Process Status. Dynamic Soil Plant, 5, (2), 45–50. http://hdl.handle.net/1854/LU-1996013

[7] Tatàno F., Pagliaro G., Di Giovanni P., Floriani E., Mangani F. 2015. Biowaste home composting: Experimental process monitoring and quality control. Waste Management, 38, 72-85. https://doi.org/10.1016/j.wasman.2014.12.011.

[8] Larney F.J., Olson A.F., Miller J.J., DeMaere P.R., Zvomuya F., McAllister, T.A. 2008. Physical and Chemical Changes during Composting of Wood Chip–Bedded and Straw-Bedded Beef Cattle Feedlot Manure. J. Environ. Qual., 37: 725-735. https://doi.org/10.2134/jeq2007.0351

MY OWN IDEAS

Claudia Barros Wilkinson

I will always remember the day our teacher came into class talking about a scientific project, which left us all wondering what we would do for the rest of the course. When our scientists informed us that we would be working with invertebrates and compost piles, I was a little shocked as I expected anything but not that. I had thought it would be something inside a laboratory, with gowns and gloves; However, it turned out to be quite the opposite.

Our project consisted of analyzing the biodiversity of invertebrates in the compost, sampling and counting species. At first it didn't catch my attention and I found it boring, but throughout the different sessions, I changed my mind. Every time we discovered an unknown invertebrate, I became more eager to continue investigating.

What I liked most about this project by far was being able to closely analyze the invertebrates through the photos we took of them during sampling; and identify them, thus being able to learn about many species that I did not know about.

It also caught my attention the time we went to the laboratories of the Estación Experimental del Zaidín (EEZ) to prepare for the congress, which would be a few days later.

Ezequiel Fernández Fernández

In my opinion, it has been an impressive project, quite educational and fun. The work of scientists is truly admirable. Thanks to this project we learned many new things about invertebrates (which was the fundamental pillar of our work), and other aspects related to biology. My experience doing invertebrate research work with the name "BICHOCOMPO" and with the help of the scientists German Tortosa and Ruben Rodríguez has been unforgettable. We have used material that I have never seen before, such as the sampling probes, one spring-shaped or another shovel-shaped that closed and opened, the pH meter, sieves, ... We found invertebrates that I had not noticed before, or I did not know their name, but thanks to this project I was able to find them out. This was the first time I have attended such an important scientific conference. I was a little nervous when I entered, because I had to present our work, and in case something went wrong. But when I started talking and explaining our work I was calmer and made it more natural. Other students, some older, some younger than us, also presented their projects and did very well, talking about various other topics about biology, each more interesting. In conclusion, it has been an experience that everyone should experience and appreciate and continue with this type of initiative in the future.

Estrella García Romero

In my opinion, when our teacher began to explain us our project, I did not like it, since working and analyzing invertebrates did not excite me too much.

When we started our work I still didn't like it very much since there were many insects, but every day that we went to the sampling, we counted the invertebrates and we sifted the compost, it seemed like a very interesting project with a great future.

My biggest fear was to present our work at the congress with more institutes since I had never done anything like it, I thought I would not be able to do it because of the nerves I had, but in the end we all did it very well and in my opinion it was one of the best projects that had been presented.

Thanks to this work and having had the opportunity to get to know the world of scientists, the way they work and how involved they were, I am clear that in the future I would like to study something related to the laboratory and invertebrates.

Carla Gómez Álvarez

The moment our teacher told us that we were going to have the opportunity to do a scientific project, I thought about being in the laboratory, wearing white coats and using the instrument of a scientist, so I was very intrigued waiting for the topic of the project.

However, I was very wrong, our project was to examine and analyze the invertebrates in the compost. Honestly, I have to say that I get a little bit disappointed, because we weren't going to go to the

laboratory and I didn't like the "bugs" to much, but what I didn't know, was that this project was going to be one of the most important thing of my last course of ESO.
To my surprise, the project turned out to be very interesting, entertaining and fun; in addition, possibly, I had the opportunity to do more things than I could have done in a laboratory.
Thanks to this project, I have learned many things, such as appreciating teamwork, knowing the way scientists work, etc.; in addition to all the information about invertebrates.
I believe that all students should have the opportunity to enjoy this experience, since it is an unforgettable experience that allows you not only to work with scientists, learn new things and help you decide about your future, but also have a good time.

Natalia Mota Salceanu

At the beginning, when they presented the project to us and told us that it was about compost, we all looked at each other with a look of "what is this?", because the first thing that came to our mind was how we were going to touch the garbage and that we could get bitten by spiders or see a worm, although none of this happened afterwards.
For me (I'm afraid of bugs), what put me off the most was that we were going to work with them, but later, as the project progressed and we carried out more sampling, I was eager to see what we found, how many of each type there were, if there was any species that we had not found before,...
Now, I remember when we all looked for a while and took photos of the tray where we put the compost, to see how many invertebrates we could see. Also when we had to use force with the probe to put it in the compost and take it out (that had its trick, to be honest), but little by little we gained interest and I would say even a taste for it.
In my opinion, it has been an experience that has helped me know what science is like in practice, since until now we only studied theory and did little practice. It has also helped me know how to work more as a team.
In conclusion, I would say that everyone who likes science should do it to really know what this "world" is like.

Elsa Vallberg Fernández

At first, when the teacher told us about the possibility to participate in a project, I thought it would be interesting and fun, but then when she mentioned that the project was to analyze invertebrates in compost, I was a little disappointed since I am not a big fan of bugs. But as the project has progressed, I have liked it more and I have learned new things.
I have enjoyed when we have been sampling in the compost piles, laughing together with our classmates, like, for example, when one of the classmates got compost on her when she pulled the sampling probe. I really enjoyed the day we all went to the Estación Experimental del Zaidín (EEZ), where a congress was being held a few days later. It seemed like an interesting place to visit, full of curiosities, and that made me want to learn more about the things that were exhibited there.
The day of the congress I was very nervous, but in the end everything went well and we presented the project with great success. To be honest, this project has been a rewarding experience and I recommend everyone to participate as you will learn new things and it can open new doors. I would love to participate in a project like this in the future, to continue learning new things. Thanks to this experience, I have learned how fun and interesting science can be and it has opened up new opportunities for me in the future.
Finally, many thanks to Germán Tortosa and Rubén Rodríguez for helping us during the process and giving us this opportunity to participate in the project.


eez
estación experimental del Zaidín