High School Students for Agricultural Science Research

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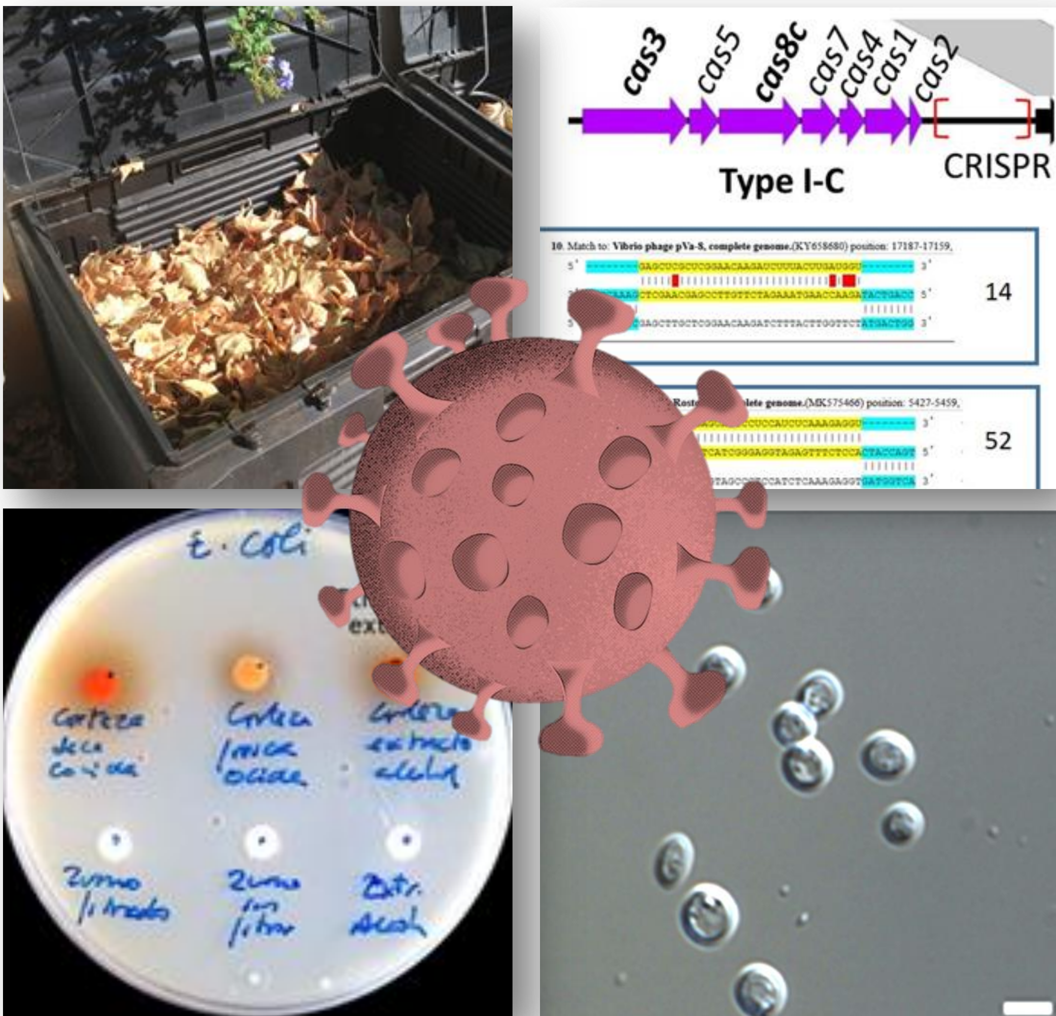

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Biological insights of Estación Experimental del Zaidín (EEZ) bio-waste composting

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Highlights

- Composting is a feasible methodology for bio-waste recycling.

- A significant loss of bio-waste dry weight was found during composting.

- Compost had more bacterial population than a soil.

- Composts did not have any phytotoxicity and could be used in agriculture.

Summary

Bio-waste is defined as the biodegradable fraction of the waste produced at domestic, commercial or local levels, which include garden and park waste, food and kitchen wastes from households, restaurants or similar. It is characterised by an important content of water and an easily biodegradable organic matter, being mandatory a treatment before its disposal. Composting is a feasible methodology for bio-waste management, in which the organic matter is transformed by the own microorganisms presented in the raw materials. In this research, the feasibility of the bio-waste composting generated by Estación Experimental del Zaidín (EEZ) was studied. For that, two composting procedures (composting pot and bin) were assayed, and the degradation of the organic matter during the process, the relationship between temperature, moisture and bacterial population and the compost phytotoxicity were evaluated. Data confirmed 40% of dry weight loss of initial bio-waste during the process, probably related to CO_2 release during the organic matter degradation. Also, a relationship between temperature, moisture and bacterial population was found, being compost an organic material with more bacterial abundance compared to a soil. Finally, the maturity test of the obtained composts confirmed an absence of phytotoxicity, being ready for their use in agriculture.

Keywords: Bio-waste, composting pots, composting bin, organic matter, temperature, moisture, bacteria.

INTRODUCTION

Bio-waste is defined as the biodegradable fraction of the waste produced at domestic, commercial or local levels [1]. It includes green garden and park waste, food and kitchen waste from households and restaurants, and similar wastes produced at processing food plants [2]. The bio-waste production is variable in time and its main characteristics are high content of water and organic matter easily biodegradable, being mandatory a treatment before its disposal [3].

Composting is a feasible methodology for the bio-waste management. It is defined as a controlled bioxidative process, in which the organic waste is transformed by the own microorganisms of the raw waste [4]. Several phases can be observed during composting according to temperature evolution and microorganisms activity [5]: mesophilic phase (<40 ºC), thermophilic phase (40-60 ºC), cooling (60-40 ºC) and maturation phase (ambient), respectively. The bioxidative process (mesophilic and thermophilic phases) is the most active stage, where an important organic matter degradation, CO_2 emissions and microbial activity take place [5].

Microorganisms are one of the most important factor of soil biology [6, 7]. They constitute its living part and are responsible for the dynamics of soil transformation. Microorganisms modify soil properties and nutrient cycles, and can improve plants growth and development. Nowadays, bio-fertilizers or biostimulants based on soil microorganisms are commonly use in agriculture, due to they promote plant nutrition and growth. Also, composts can be a valuable source of plant growth-promoting microorganisms [8].

The aim of this project was to study the feasibility of bio-waste composting, with emphasis in its biological aspects. For that, the degradation of the organic matter during the process, the relationship between temperature, moisture and bacterial population, and the composts maturity were evaluated. Also, the bacterial diversity was assessed by microscopy.

MATERIALS AND METHODS

This study was carried out at the facilities of the Estación Experimental del Zaidín (EEZ), a research center of the Spanish Council for Scientific Research (CSIC) sited in Granada (Spain). The composting experiments were done at the EEZ Campus, an area of 2250 m^2 located in the city center, with several buildings and Departments including a restaurant, green zones and also, a botany garden.

1. Bio-waste.

The organic wastes used in this research were chipped tree prunings, fresh cut grass and food waste, all of them easily available at EEZ (Figure 1). The chipped tree prunings, mainly from *Platanus orientalis* and *Platanus occidentalis*, and the fresh cut grass were provided by the EEZ Gardening Service, and the food waste was supplied by the Restaurant staff.

Figure 1. Chipped tree prunings (a), fresh cut grass (b) and food waste (c) used for composting.

2. Composting.

Two different composting procedures were assayed: composting pots and a composting bin.

2.1. Composting pots.

In this experiment, eight 25 L-pots (Figure 2) were filled with a mixture containing equal volumes of chipped tree prunings (10 L) and fresh cut grass (10 L). Food waste was manually cut into small pieces with scissors and added to pots according to the following treatments (Figure 2):

- C (Control): No food waste was added.
- D1: 600 g of food waste was added.
- D2: 1200 g of food waste was added.
- D3: 2000 g of food waste was added.

Two replicates per treatment were done. The composting pots were placed in a greenhouse bench and once a week, were turned and checked for their humidity. The experiment started at 19 of December 2019 (T0) and finished at 26 of February 2020 (T1). At each time, a representative sample of 200-300 g was randomly taken for further analysis.

2.2. Composting bin.

In this experiment, a 450 L-composter (Figure 3) was filled with a mixture containing equal volumes of chipped tree prunings (200 L) and fresh cut grass (200 L), which was turned using a hand compost turner. The experiment started at 19 of February 2020 and one week later, the compost temperature rose up until thermophilic range (> 45 ºC). Then, a representative sample of 200-300 g was randomly taken from different bin locations for further analysis.

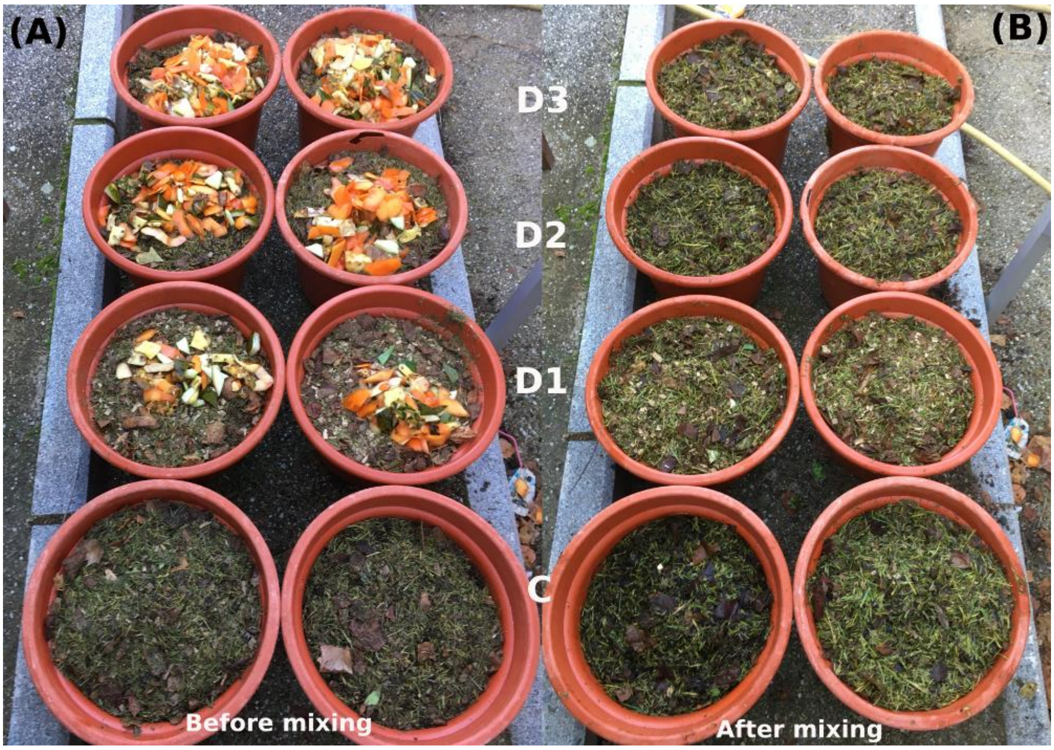

Figure 2. Composting pots before and after adding and mixing food waste according to each treatment: C (Control, no addition), D1 (600 g of food waste), D2 (1200 g of food waste) and D3 (2000 g of food waste).

Figure 3. The 450 L-composting bin and the hand compost turner used in this research.

3. Analysis.

3.1. Moisture.

Moisture was determined as the difference between wet and dry weights. Briefly, a representative sample of wet bio-waste (T0) or compost (T1) (100-200 g) was randomly taken and weighed in a precision balance. Then, samples were dried in an oven during 3 days at 70ºC until constant weight. Water content of each sample were calculated as followed:

$$\text{Moisture (\%)} = [(\text{wet weight} - \text{dry weight})/\text{wet weight}] \times 100$$

Composting pot wet weights were estimated just before sampling, at T0 and T1, with a manual portable luggage scale.

3.2. Temperature.

Compost temperature was randomly measured at different composting bin locations by using a digital thermometer with a probe (Figure 4).

Figure 4. Digital thermometer with a probe used to measure compost temperature.

3.3. Bacteria isolation and growth.

For bacteria isolation, 1-3 g of wet compost were aseptically placed in a 50 mL sterile tube. 30 mL of saline solution (NaCl 0.9%, w/v) was added to the tube and mixed by vortexing during 5-10 min. After that, the extraction was sedimented by gravity during 10 min.

For bacterial growth, 30 µL of the above extraction was added to Petri dishes, containing a general growing solid media for bacteria (TSA medium). The inoculation was done by adding 2 mm-glass balls to Petri dishes and by manually shaking during 1 min (Figure 5). Then, glass balls were removed and Petri plates were incubated at 28 ºC during 24 h. After growth, bacterial morphology was checked by using a light microscopy.

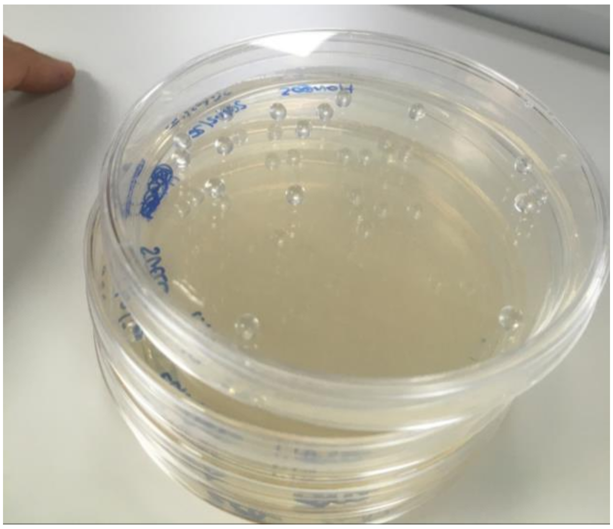

Figure 5. Bacterial inoculation of Petri dishes with 2 mm-glass balls.

3.4. Compost maturity.

Compost maturity was assessed with a seed germination test after adding a compost water extract. Briefly, 1 g of wet compost were placed in a tube containing 20 mL of tap water. Tubes were mechanical shaken during 2 h and were centrifuged at 5,000 *g* during 10 min . After that, 1 mL of the supernatant was added to a Petri dish, containing a slide of paper and 10 seeds of cress (*Lepidium sativum*) (Figure 6). Then, cress seeds were grown at room temperature in darkness during one week.

In order to compare compost phytotoxicity, a set of cress seeds were germinated with only tap water (control assay) and another set with a water extraction of a soil located close to the composting bin.

Figure 6. Compost maturity assessed with cress (*Lepidium sativum*) seeds germination test after adding a compost water extract.

Results and Discussion

Organic matter degradation during composting.

Figure 7 shows the weight of composting pots at the beginning (T0) and at the end (T1) of the experiment. According to these data, pots dry weights at T0 were ranged between 1.2-to-1.7 kg and two months later (T1), were close to 0.9 kg. These reductions represented losses of 42, 29, 43 and 41 % of dry weight for C, D1, D2 and D3 composting pots, respectively (Figure 8). Part of these losses might be explained due to the release of some gases like CO_2 during the degradation of the organic matter produced by microorganisms activity during composting [9].

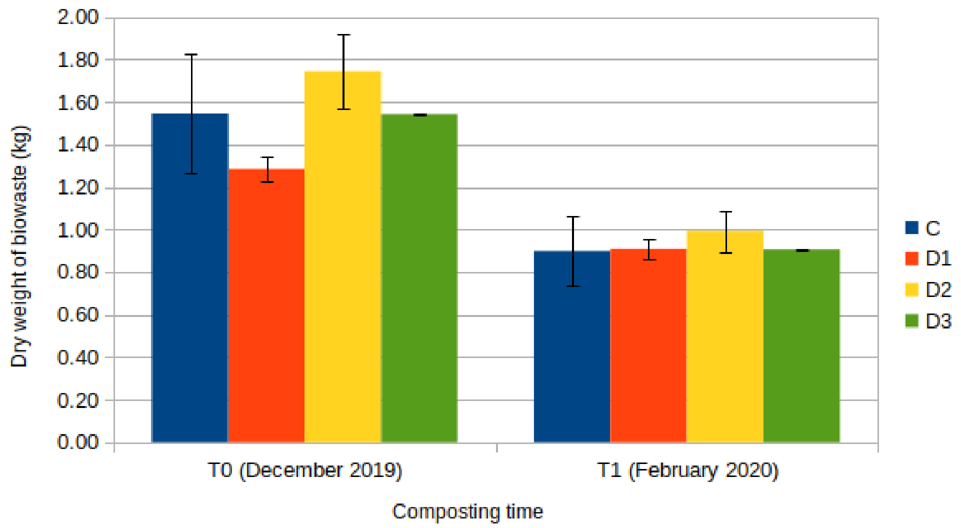

Figure 7. Dry weight of composting pots at the beginning (T0) and at the end (T1) of the experiment. Treatments are: C (Control, no addition), D1 (600 g of food waste), D2 (1200 g of food waste) and D3 (2000 g of food waste).

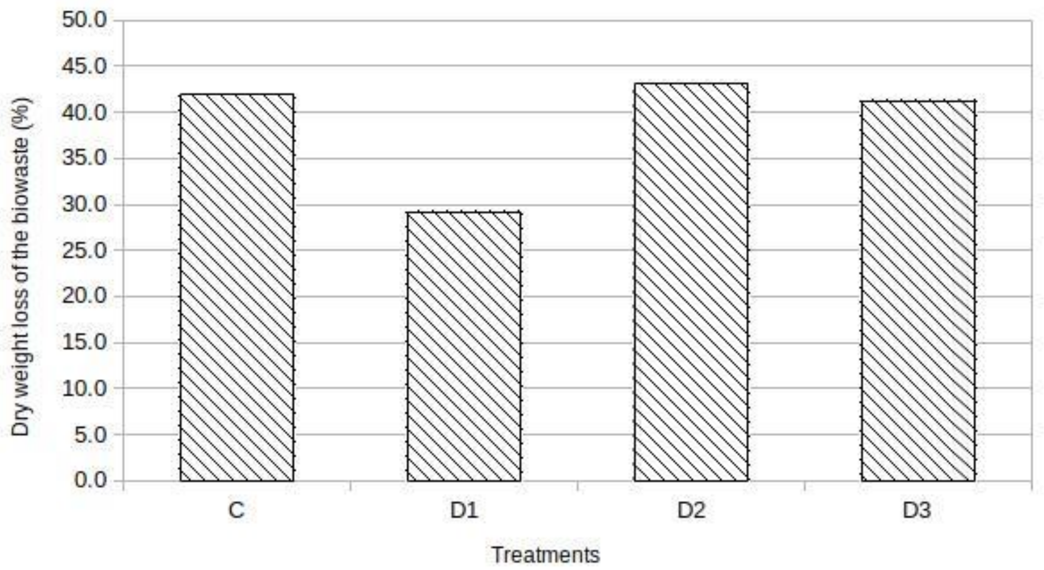

Figure 8. Dry weight loss (%) of composting pots during the experiment. Treatments are: C (Control, no addition), D1 (600 g of food waste), D2 (1200 g of food waste) and D3 (2000 g of food waste).

Relationship between temperature, moisture and bacterial population of compost.

As mentioned before, composting is a biological methodology for the organic waste treatment [4]. During this process, an important organic matter degradation occurs, especially due to the metabolic activity of a complex microbial population presented in the raw materials [5]. In order to know the role of microorganisms during composting, temperature, moisture and bacterial population were checked in a composting pile at thermophilic phase (the most active), and compared with a soil located close to the composting bin. According to Figure 9a, compost temperature was close to 50 ºC, 5-fold higher than the soil. This result confirms that compost was biologically active [5]. Also, the compost moisture was consistently higher than water content found in the soil (Figure 9b), being water content an important factor for the microbial development. As expected, bacterial population in compost was much notable than in the soil (Figure 9c). These findings suggested that a direct relationship between temperature, water content and bacterial population was found in the compost, being biologically more active than the soil.

Finally, some of the isolated bacteria were observed under a light microscope. As it can be shown in Figure 10, bacterial colonies had different morphology (forms and shape), which confirm that the compost biodiversity was relevant [5].

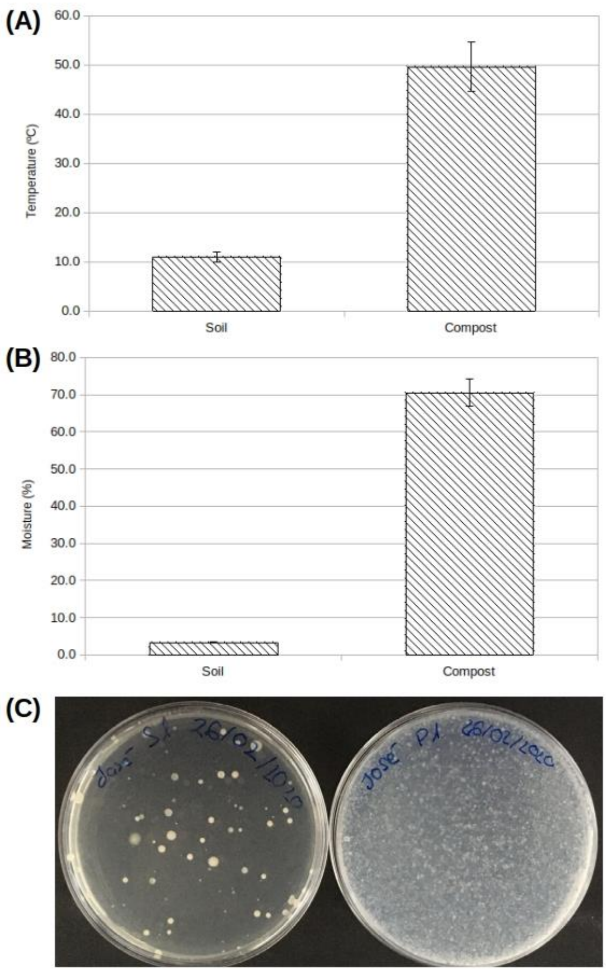

Figure 9. Temperature (A), moisture (B) and growth of bacterial population (C) in soil (left) and compost (right) at thermophilic phase.

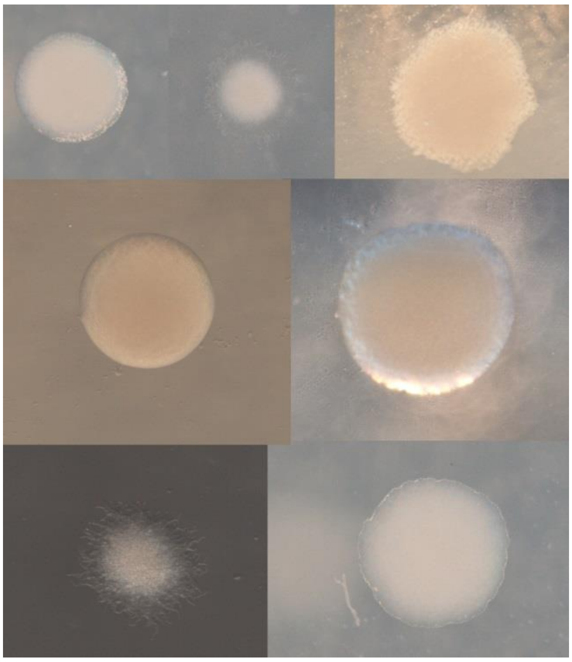

Figure 10. Different morphologies of bacteria isolated from thermophilic compost.

Maturity of composts.

The disposal of organic waste can produce an important impact in the environment, especially to soil and plants. In order to know compost maturity, phytotoxicty was assessed with a germination test based on cress seeds. According to Figure 11, no differences in seeds germination between tap water (used as control) and the compost water extract were found. Similar results were obtained when a soil water extraction was used. These findings indicated that composts at T1 had no phytotoxicity and could be used in agriculture as organic amendments or fertilisers (Figure 12).

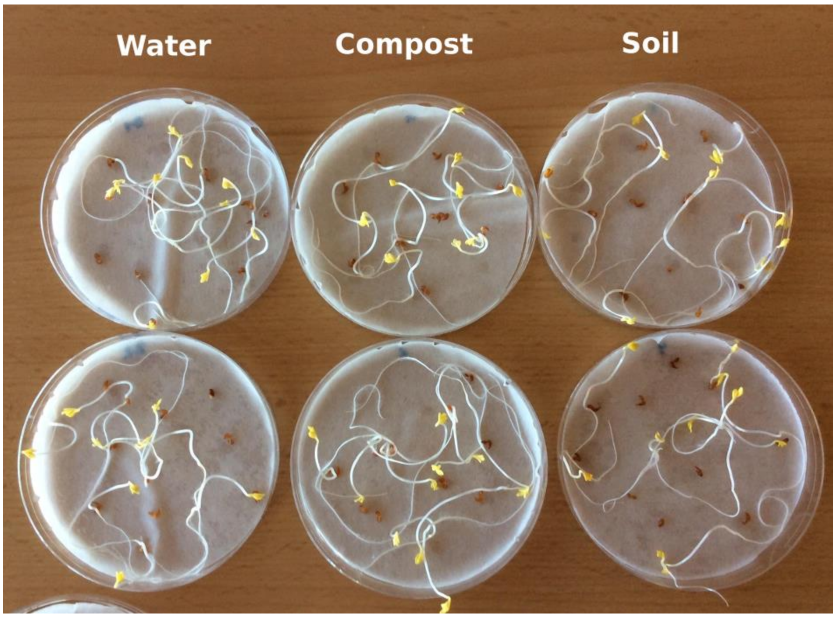

Figure 11. Cress (*Lepidium sativum*) germination with tap water (used as control), compost water extract and soil water extract, respectively.

Figure 12. Aspect of T1 compost used in the germination test.

CONCLUSIONS

1. Composting is a feasible methodology for the EEZ bio-waste treatment.
2. An important reduction in the dry weight of bio-waste occurred during the process, probably related to CO_2 release.
3. A direct relationship between temperature, moisture and bacterial population was found in a thermophilic compost.
4. Compost is more biologically active than a soil, showing an relevant bacterial diversity.
5. The composts had no phytotoxicity and could be used in agriculture.

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MY OWN IDEAS

José María Díaz

Teniendo en cuenta todas las actividades que hemos ido realizando a lo largo de todo el proyecto, en general, me ha encantado. Incluso teniendo en cuenta también toda la crisis del coronavirus todos los miembros del equipo hemos sido capaces de seguir adelante con el proyecto, y eso ha sido nuestra decisión, no era obligatorio en ningún momento, por tanto, es de lógica imaginar que todos seguíamos entusiasmados. La pregunta es la siguiente: ¿Qué nos motiva a seguir adelante? ¿Qué se podría cambiar para mejorar aún más?

Desde mi punto de vista, las veces en las que mejor me lo he pasado han sido los momentos en los que me veía a mi mismo sumergido en lo que podría ser un futuro próximo, es decir, una especie de flashforward a lo que significa en un futuro trabajar como un científico, ya sea de biologo, quimico etc… Esa sensación de la que estoy hablando solo me ha surgido 2 o 3 veces, las demás simplemente me sentía de otra forma en la que había alguien tomando las decisiones enfrente de mi, sin ser yo mismo autónomo. Nada me ha empujado a ir yo por mi cuenta hasta que vino el Covid, que hubo una especie de parón en el grupo y ya tuve yo que dar mis propias opiniones…

Con todo esto quiero decir que se nos debería dar aún más autonomía a la hora de investigar algo, fijar un objetivo claro que sea de principio a fin, y dejarnos a nuestro aire, a no ser que nos veamos encerrados, de esa forma seremos nosotros los que tomaremos las decisiones, como hacen los verdaderos científicos, que normalmente no tienen a nadie que sepa todo enfrente, y cuando acabe el proyecto y nos demos cuenta de que de verdad hemos sido nosotros los que manejabamos el ajedrez y no las fichas de éste se nos verá con otra cara.

Esa autonomía se puede conseguir si de verdad se reflexionan opciones, que las hay de sobra, pero eso no está en mi mano, prueben una sesión próxima de Piiisa a darles un objetivo claro al alumnado y que ellos mismos intenten sacar un resultado usando los conocimientos que han aprendido en la escuela, quizás de esta forma algunos aprendan de que el colegio sirve de verdad, y puedan ponerse las pilas por la motivación que les pueda surgir antes del fin de su carrera académica.

Dando por acabada ya la crítica constructiva, agradezco de corazón a todos los organizadores de este proyecto, que son capaces de crear tal actividad que puedas sentirte de verdad un científico, y eso es muy muy complicado de conseguir.

Yusuf Coletti

Según mi opinión este proyecto me ha parecido bastante interesante en aspecto de probar muchas herramientas y mucha práctica que aparte de la información, los datos recibidos, hemos aprendido como funciona todos los instrumentos y todas las nuevas maquinarias de los laboratorios aprendiendo básicamente el uso de las herramientas más simples y algunas complejas. La información que adquirimos seguramente se nos olvide al cabo de unos años a no ser que trabajamos en esto, pero la experiencia y el tiempo en este ámbito científico ser crucial para identificar si te gusta trabajar en este ámbito o prefieres trabajar en otro.

Aparte adquirimos conocimientos de como hacer un buen compost ya que, aunque parezca fácil tiene su grado de complicación. Yo, por ejemplo, he hecho un compost con mi padre en este confinamiento y gracias a lo básico aprendido en PIIISA ha salido muy bien.

Solo he visto un punto negativo y es la falta de conocimientos básicos sobre el proyecto ya que dedicamos un par de sesiones en aprender los conceptos básicos y a algunos como a mi no me han quedado del todo claro.

Pero por lo demás nos lo hemos pasado muy bien y Germán nos ha enseñado a la perfección los aspectos de la vida científica el día a día de un científico investigador. Además de presentarnos a grandes científicos de su departamento viendo de reojo sus respectivos experimentos.

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