

Environmental Biotechnology: Biodegradation of Microplastics with Larvae of *Tenebrio Molitor* and *Galleria Mellonella*

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Plastics have become one of the serious problems with a significant negative impact, especially in the sea, due to their long biodegradation time. This is further aggravated when the size of microplastics is reduced because of the exposure to mechanical and temperature effort. One of the places where they are deposited by the dynamics of the sea waves are the beach areas. The research presents as a method of environmental biotechnology, the use of larvae of *Tenebrio molitor* and *Galleria mellonella* for the biodegradation of microplastics sample collected from the Azul beach in Ventanilla. The investigation began by characterizing the microplastics, these being identified as polyethylene terephthalate, polyvinyl chloride, and expanded polystyrene. The microplastics were then subjected for periods of 5, 10 and 15 days to larvae of *Tenebrio molitor* and *Galleria mellonella*, verifying that at 15 days there was a higher level of biodegradation of these, being 54.2% with 30 larvae of *Tenebrio molitor* for the Expanded Polystyrene and 34.4% with 30 *Galleria mellonella* Larvae for Polyvinyl Chloride. It is concluded that the use of these larvae turns out to be a viable and important way as an alternative for the degradation of microplastics and with the advantage of being friendly to the environment.

1. Introduction

The problem related to population growth is the production of solid waste, being "plastic pollution" one of the most relevant, which with the prolonged degradation time generates small particles, less than 5 mm called "microplastics", which contain contaminants due to the additives that have been used in their preparation (cosmetic industry, hygiene, etc.), that turn out to be threats to the oceans and marine species (Venkatesh et al., 2021). It is known that these plastics go hand in hand with social development and often represent 11% of the waste generated in middle-income countries (Solano Johanna K. et al., 2019), for this reason some governments have proposed measures reduction in the use of plastic products. In other cases, there was a need to recycle through classification and decomposition through crushing, washing, drying, granulation and extrusion, as was done in a municipality in the Czech Republic (Tocháček et al., 2021). Other alternatives apart from pyrolysis that is applied to polymers reinforced with carbon fiber, chemical processes are being studied through solvents that help recover both the fiber and the matrix; however, one of the drawbacks is the selectivity of the inputs due to the variety of composition of these materials (Seiler Elisa et al., 2021).

One of the ways to value plastics is through thermal degradation, which gasification and pyrolysis are used in the absence of oxygen and at a temperature variation range of 200 to 700 °C, being able to obtain bio oil, biogas and bio coal (Casazza et al., 2019). Another alternative is the use of microorganisms to speed up the process of plastic degradation, because, as already mentioned, plastic bags take a thousand years to decompose, with speeds ranging from 100 µm/year in water environments and 300 µm /year in soil (Chamas et al., 2020).

The microplastic degradation process through more organic processes was achieved through a bioassay process that lasted 60 days, polystyrene (Sycopor) biodegraded between 5% and 8%, compared to that of low-density polyethylene that it only reached 0.5% and 1.5%. This process was carried out by the larva *Tenebrio molitor*, under thermal conditions between 25 and 27°C and with humidity of 70 to 80% (Álvarez estepa D.N. and Botache Laguna L.M., 2020). Another study in this sense was the use of the *Galleria mellonella* larva that

consumed polyethylene and, according to the analysis carried out on the larva, micrometric particles were detected in its digestive tract cavities, indicating that they degrade the microplastic (Agnes et al., 2021); however, more research is needed to be certain whether this species has the ability to biodegrade polyethylene or PE (Bombelli Paolo et al., 2017).

An investigation for the degradation of polyvinyl chloride (PVC), 100 *Tenebrio molitor* larvae were used for 16 days, who were given microplastic powder whose size was between 70-150 μm , at an average temperature of 25 ° C, as a result, the presence of 34.6% PVC polymer was found in the organic residues of the larvae (Peng Bo-Yu et al., 2020). Other micro plastics used with this type of larva were remains of polystyrene (PS), polyurethane (kitchen sponge-PU) and polyethylene foam (PE), which after a process of exposure to the larvae for 58 days, produced a reduction of the mass of these plastic remains of 46.5%, 41.0%, 53.2% and 69.7% (Bulak Piot et al., 2021). In another test, the *Tenebrio molitor* and *Tenebrio Obscurus* larvae were used, they found a rapid degradation of polystyrene (PS) that could reach between 60-70% between 12-24 hours after an adaptation process between 1-2 weeks. In addition, it was appreciated that what was ingested transmitted energy to the larva for its vital activities, but not for growth (Shanshan Yang and Weimin.Wu, 2020). Also in a study, the action of PS degradation with the *Galleria mellonella* larva was investigated, using PS microspheres at 0.5 mg/larva and PS powder at 2.5 mg/larva, finding that there was a depolymerization of the styrene oxide microplastic and 4-methylphenol due to the metabolic pathways of the larva that allowed biodegradation through the enzymes of *Galleria mellonella* (Shuai Wang et al., 2022). With the larvae indicated above, a trial was carried out with a duration of 21 days, 150 larvae and using a co-diet improved with beeswax or wheat bran, to seek the highest consumption of polyethylene (PE) and polystyrene microplastics (PS), reaching a decrease of 0.88 and 1.95 g, affecting the plastic biodegradation and the physiological properties of the larvae (Yu-Lou et al., 2020).

With the existing scientific literature on the subject, the research carried out aimed to make use of environmental biotechnology to verify the biodegradation of microplastics obtained on a beach on the Peruvian coast using *Tenebrio molitor* and *Galleria mellonella* larvae. The results will serve to propose an alternative for environmental management of the existing plastic waste in the place.

2. Materials and Méthods

2.1 Materials

For the development of the research, the sample of microplastics was obtained from the Costa Azul beach of Ventanilla Callao (Figure 1a), through a simple probabilistic sampling, following the collection process after separating them from the sand by sieving 2 mm, 2.5 mm, and 5 mm (Figure 1b), managing to collect polystyrene (Tecnopor), polyethylene (plastic bags) and polyethylene terephthalate (beverage containers), collecting 10 grams for each of them. In addition to this, six glass tanks (30 cm x 30 cm x 20 cm) were built and used to place the larvae and the polymers as shown in Figure 1c.



Figure 1: Place of collection, sifting of microplastic samples and containers for larvae

2.2 Characterization of microplastics

The microplastics collected (polyethylene, expanded polystyrene and polyethylene terephthalate) on the Costa Azul beach were characterized by mechanical and traction analysis, finding that the polyethylene terephthalate

presents, on average, a greater elongation and maximum stress; while the expanded polystyrene registered greater thickness as presented in Table 1.

Of the polymers analyzed, it was found that polyethylene terephthalate also recorded the highest values in area and maximum strength, while polyvinyl chloride had the greatest width.

Regarding the tonality of the color that characterizes each selected polymer, these range from transparent (polyethylene terephthalate and polyvinyl chloride) to white (expanded polystyrene and polyvinyl chloride).

Table 1: Characterization of microplastics

Type	Width (mm)	Thickness (mm)	Initial Length (mm)	Final Length (mm)	Área (mm ²)	Maximun Force Kg (N)	Maximun Stress Kg/mm ² (Mpa)	Elongation (%)	Color o hue
Polyvinyl chloride	7.5	0.03	25.4	103.0	0.23	0.19 (1.84)	0.83 (8.18)	308.6	White, transparent, red, black
Polyethylene terephthalate	7.44	0.21	25.4	315.10	1.56	5.26 (51.63)	3.37 (33.10)	1,140.5	White
Polystyrene	6.45	3.05	25.4	38.1	19.67	1.30 (12.79)	0.07 (0.65)	50.0	Transparent

Likewise, the bending test was carried out on polystyrene starting from a sample 3.05 thick, 6.45 mm wide, with a length between supports of 120 mm, a bending stress of 0.64 Mpa was obtained.

2.3 Characterization of the larvae

In relation to the larvae used in the research, these were acquired from the National Service for Environmental Sanitation (SENASA), in a batch of one thousand, between *Tenebrio molitor* and *Galleria mellonella* larvae. The first one in its larval stage of approximately 20 to 25 days, white color and approximately 0.74 cm long (Figure 2a). Regarding the second type of larvae, they were also acquired in their larval stage of approximately 1 to 2 weeks, mustard-colored and approximately 0.49 cm long (Figure 2b).

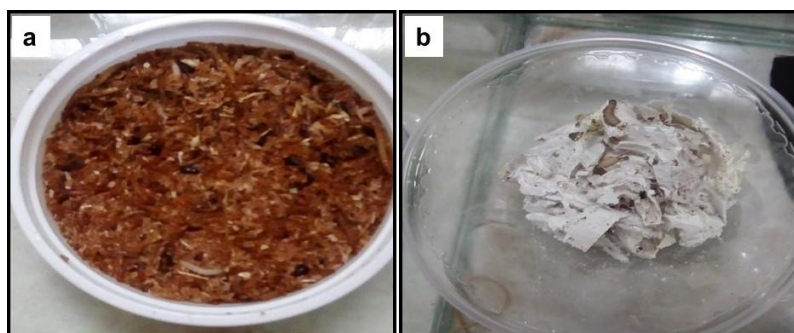


Figure 2: a) *Tenebrio molitor* larvae and b) *Galleria mellonella* larvae, acquired

2.4 Preparation of reservoirs for larvae

In Figure 3, we can see the deposits prepared to house the larvae with the presence of the polymers, the larvae initially followed an adaptation process and adequate to guarantee survival during the fifteen days that the experiment lasted, in such a way that the environmental conditions exist that allow the adequate development of the larvae of *Tenebrio molitor* and *Galleria mellonella*, for the degradation of the selected microplastics PVC, EPS, PET. In the study, 5 g of each type of microplastic was used, which an amount of thirty (30) larvae was added.



Figure 3: Experimental environment for polymer biodegradation with larvae of *Tenebrio molitor* and *Galleria mellonella*

3. Results and discussion

3.1 Physical characteristics of the larvae in the experiment

During the experimental phase, which involved the degradation process of the types of polymers (five grams) with a number of larvae (thirty), it was possible to observe the evolution of their physical conditions during the 15 days that the experiment lasted. The *Tenebrio molitor* (Figure 3a), with the PVC, starts with an average length of 0.74 cm, which is maintained for five days, after ten days it reaches 0.78 cm and at the end of the experiment it had 0.85 cm, determining a growth rate of 0.073 mm/day. For the EPS, the larva starts with 0.73 cm, which was maintained for five days, on the tenth day it reached 0.75 cm and on the tenth day it reached the size of 0.79 cm, which produced a growth rate of 0.04 mm/day. For the PET, the initial larva size was 0.71 cm, remaining for five days, to later reach 0.73 cm in ten days and remained the same length until the end of the experiment, corresponding to a growth rate of 0.013 mm/day.

The *Galleria mellonella* larva interacted with each of the polymers, its evolutionary growth was recorded: for PVC, the growth rate was 0.04 mm/day, for EPS a growth rate of 0.05 mm/day and for PET a growth rate of 0.05 mm/day was recorded, it was obtained a growth rate of 0.013 mm/day, a lower value compared to PVC and EPS, as can be seen in detail in figure 3b.

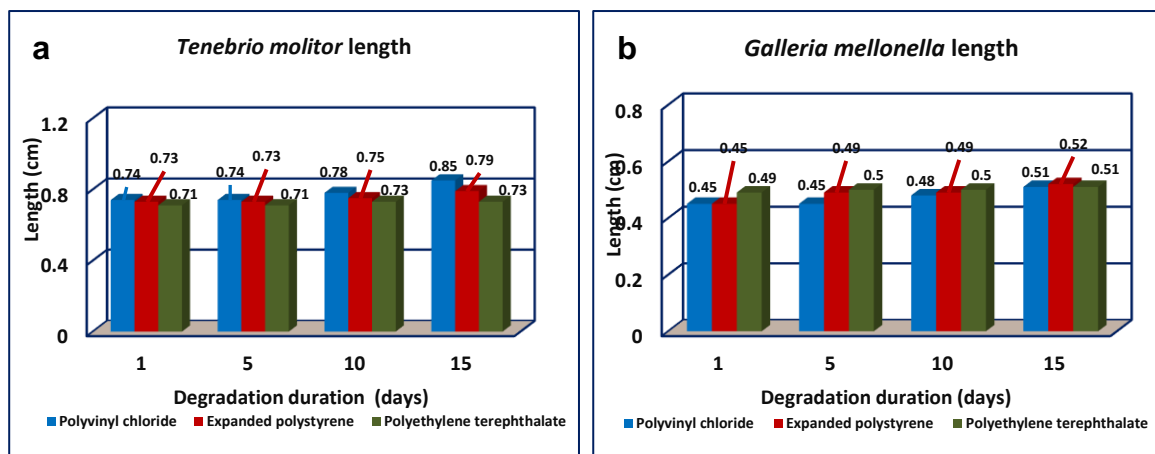


Figure 3a: Growth of *Tenebrio molitor* larvae against microplastic

Figure 3b: Growth of *Galleria mellonella* larva against microplastic

In relation to the weight reached by the larvae during the experimental phase for each of the polymers used, it was observed that: *Tenebrio molitor* (Figure 4a) reduced its weight by 30% (PVC), 85% (EPS) and 11% (PET) and for *Galleria mellonella* (Figure 4b), losses of 55% (PVC), 60% (EPS) and 20% (PET) were obtained, these results can be sustained with, what is indicated in the scientific literature where they are mentioned that the

consumption of microplastic contributes to generating energy for the survival of the larvae, but does not contribute to winging weight as mentioned by Yu-Lou et al. (2020) and Yang and Wu (2020).

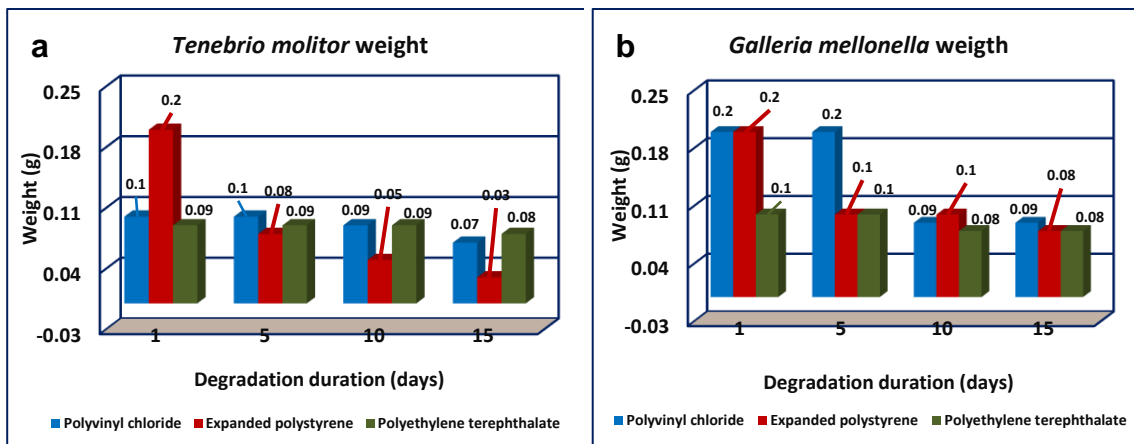


Figure 4a: Behavior of the weights of the larva *Tenebrio molitor* against the microplastic

Figure 4b: Behavior of the weights of the *Galleria mellonella* larva against microplastic

3.2 Degradation of microplastics

During the experimental process that was developed for 15 days, it was possible to know the level of degradation of the polymers, compared to the actions of the selected larvae, finding that: *Tenebrio molitor* (Figure 5a) generated a decrease in PVC by 10%, EPS in 54% and PET in 12%; while *Galleria mellonella* (Figure 5b) reduced PVC by 34%, EPS by 18% and PET by 2%, which leads us to indicate that the larva with the highest consumption capacity was *Tenebrio molitor* for EPS and PET; however, the *Galleria mellonella*, consumed more PVC, these results have also been reflected in the investigations of Álvarez and Botache (2020), as well as in the works of Rejasse et al. (2021), Peng Bo-Yu et al. (2020) and Bulak Piot et al. (2021).

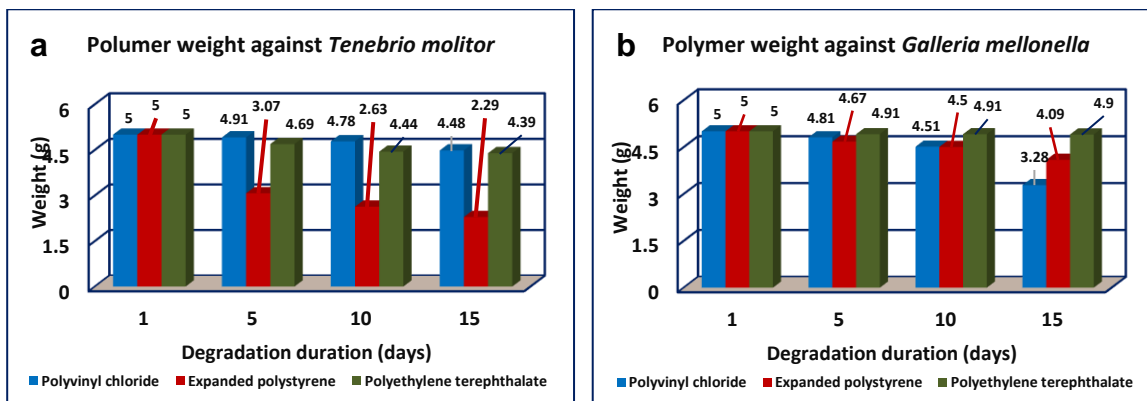


Figure 4a: Behavior of the weights of the larva *Tenebrio molitor* against the microplastic

Figure 4b: Behavior of the weights of the *Galleria mellonella* larva against the microplastic

4. Conclusions

Biodegradation of the polymers occurred, reaching an average mass reduction of 54% for EPS and 12% for PET using the *Tenebrio molitor* larva and 43% for PVC using the *Galleria mellonella* larva. In addition, it was found that the *Tenebrio molitor* larva experienced a growth of 15% when consuming the PVC and the *Galleria mellonella* a growth rate of 16% for EPS and 4% for PET, after an experimental process of 15 days. In this period, both larvae experienced weight losses of the order of 85% and 60% for *Tenebrio molitor* and *Galleria mellonella*, respectively, due to the consumption of EPS. Therefore, it is verified that, if there is the feasibility of

the application of this biotechnology to degrade polymers as an alternative solution to the problem of plastic waste, studies must be continued to make the method more efficient.

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