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# Treatment of biowaste commingled with biodegradable bioplastic films using Black Soldier Fly larvae: Generation and fate of micro-plastics

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ABSTRACT

The use of Black Soldier Fly (BSF) larvae is emerging as a promising alternative for biowaste (i.e. food waste) treatment, generating larval biomass and process residues, suitable for use as animal feed and fertilizer, respectively. In line with an increasing use of starch-based bioplastics in food packaging, the presence of these biopolymers and associated biodegradable microplastics (BMPs) in food waste is expected to rise. Knowledge of the generation of BMPs and their fate in the BSF treatment process is scarce, or indeed, completely lacking in the case of small-sized BMPs (<50 μm). The present study aims to investigate the generation and potential accumulation of BMPs in BSF larvae process. Food waste mixed with starch-based bioplastic films was fed to larvae and BMPs of two particle sizes (inferior to and exceeding 10 μm in diameter) were monitored over time in rearing substrate and larval biomass. BMPs concentrations in substrate were compared with larvae-free control tests. The presence of larvae favoured the generation of BMPs. Concentrations of smaller-sized BMPs (<10 μm) increased by approximately 172% in the final substrate, and accumulated in the larval biomass with a peak exceeding the initial larval concentration by over 1000% just before prepupation, which is the typical stage they are collected when used as animal feed. These results indicate a potential risk of soil contamination by BMPs when final substrate is used as fertilizer and a risk of biomagnification phenomena when larvae are used as animal feed.

## 1. Introduction

Biowaste represents a significant fraction of municipal solid waste which should play a more relevant role in Circular Economy (Cossu et al., 2020; Stegmann and Liu, 2024).

Resource recovery from this fraction, both in terms of energy and material recovery, is generally practiced using conventional processes such as anaerobic digestion and composting (i.a. Sobieraj et al., 2022).

A promising alternative is offered by the use of Black Soldier Fly (BSF) larvae (*Hermetia Illucens*), which combines good treatment efficiencies with the generation of valuable resources such as larval biomass and stabilised residues, suitable for use as animal feed and fertilizer, respectively (Surendra et al., 2020).

Biodegradable plastics are widely used as an alternative to conventional plastics for various applications (such as food packaging, shoppers and bags for biowaste collection), due to their lower carbon footprint and claimed biodegradability during the conversion processes (Rosenboom et al., 2022). As a result, the biowaste mass undergoing treatment processes is significantly commingled with bioplastics, which in Italy constitutes 1% of biowaste (Centemero, 2017).

However, the effective biodegradation of these polymers in the natural environment is disputed (Liao and Chen, 2021; Lavagnolo et al., 2023), while more rapid fragmentation than conventional plastics has been observed, leading to a higher generation of biodegradable microplastics (BMPs), (i.a. Fan et al., 2022; Wei et al., 2021).

The effective degradation of biodegradable bioplastics in bioconversion processes has been investigated both in composting (i.a. Ruggero et al., 2020) and in BSF Larvae process (Grossule et al., 2023a). In

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both cases the presence of these biopolymers did not negatively impact the biodegradation process; while the complete degradation of the bioplastics was not achieved.

In line with standards set by International Standards Organization (ISO) on composting (Kale et al., 2007), a bioplastic can be labelled as compostable if, among other requisites, it undergoes fragmentation to less than 2 mm in size within 90 days. As a consequence, the issue of potential generation of bioplastic particles inferior to 2 mm, is generally overlooked.

Edo et al. (2022) is the only study claiming the absence of issues related to the generation of microplastics (MPs) from biodegradable plastics. They investigated five different composting facilities and did not find any presence of BMPs in any compost sample. In this study however, the use of a 25 μm filter for sample filtration limited the detection of MPs to particles exceeding 25 μm, thereby limiting the significance of their observation. In fact, MPs generated during the plastic degradation processes are found in greater abundance as their size decreases (i.a. Wei et al., 2021) and the primary contribution to environmental and biological toxicity comes from MPs smaller than 10 μm (Oliveri Conti et al., 2020). Therefore, analytical methodologies for detecting MPs and nanoplastics are crucial to avoid an underestimation of their quantification (Zuccarello et al., 2019).

Nowadays, the major concern with MPs is related to bio-accumulation, biomagnification, and leaching phenomena from plastic additives (Cole et al., 2011). These issues pose significant risks to both ecosystems and, ultimately, human health (Oliveri Conti et al., 2020; Ferrante et al., 2022; Pulvirenti et al., 2022; Najahi et al., 2022). And many studies demonstrated that BMPs pose similar or even stronger negative effects on ecosystems compared to conventional MPs (Qin et al., 2021).

The present study aims to investigate the fate of BMPs when treating biowaste commingled with biodegradable bioplastics with BSF larvae process, by evaluating their generation and accumulation within the larval biomass and rearing substrate. As testing materials, food waste and starch based biopolymers were selected as reference biowaste and bioplastics.

To obtain significant results for evaluating the relevance of environmental and biological toxicity of BMPs, two different BMPs particle sizes have been considered: particles inferior to and exceeding 10 μm in diameter. Lower-sized BMPs (<10 μm) were analysed for the first time in the BSF larval process, by using an innovative methodology (set by some of the authors of the present manuscripts, Oliveri Conti et al., 2020) capable of achieving comprehensive detection of MPs and nanoplastics with a Limit of Detection of 0.1 μm (patent identification code: PCT/IB2019/051,838, March 7, 2019).

## 2. Materials and methods

### 2.1. Overall research program and objectives

Treatment of food waste commingled with starch-based bioplastic films using BSF larvae was simulated under lab-scale conditions (L test). As a control, a parallel test without larvae was conducted, involving the treatment of bioplastic-commingled food waste under aerobic conditions (C test), in order to better appreciate the effect of larvae process on BMPs generation.

Each test was executed in triplicate for accuracy and reliability.

The potential risk of BMPs accumulation and magnification was investigated by monitoring.

- the generation and accumulation of BMPs in rearing substrate (food waste) during BSF larval degradation process (L tests), compared with controls (C tests);
- BMPs accumulation in larval biomass.

BMPs were monitored in terms of particle quantification and size determination, taking into account two particle sizes: <10 μm and >10 μm in diameter.

Both environmental conditions and larval development throughout the degradation process were monitored to better understand, discuss and interpret the results.

Finally, variation of microplastics size was monitored in both rearing substrate and larval biomass thought the test.

The test setup and operation, previously detailed in Grossule et al. (2023a), is summarized below for the sake of completeness.

### 2.2. Preparation of the testing material

The testing material in each reactor was obtained by mixing 7 kg of food waste as rearing substrate, 0.049 kg of starch-based bioplastic film and 0.2 kg of wood branches as structured material.

The rate of biodegradable plastics in the mixture (0.7% w/w) was set on the basis of values from full scale composting plants in Italy (Centemero, 2017).

The bioplastic film was prepared by cutting Mater-Bi® supermarket bags into approximately 5 × 5 cm pieces. Mater-Bi®, labelled as compostable, is a thermoplastic composed of 20% starch, 70% Poly-Butylene-Adipate-co-Terephthalate, and 10% additives (Elfehri Borchani et al., 2015).

The structured material in the mixture was aimed to increase the porosity of the final testing material to facilitate air circulation. Graphical description of the testing material composition is given in Fig. 1.

### 2.3. Experimental design

The experiments were conducted in 20 L glass batch reactors, each sealed with a mesh net to prevent oviposition by other flies. The reactors were insulated and equipped with a temperature probe and an aeration system, ensuring a flow rate of 60 L/h into the testing material (as recommended by Palma et al., 2018), (Fig. 1). The experiment took place in a thermally insulated room, maintaining environmental conditions consistent with recommendations of Grossule and Lavagnolo (2020): a temperature range of 25–30 °C and a Light/Dark photoperiod of 18/6 h (Grossule et al., 2020, 2021, 2022, 2023b).

At the beginning of the experiment, all reactors were filled with the same testing material (7.249 kg). In the larvae test (L), approximately 7650 5-day-old larvae were added at a quantity determined as considering an optimal substrate feeding rate of approximately 61 mg/d/larva, and expected larval development time of around 15 days (Diener et al., 2009). The resulting larval density was 5 larvae/cm$^2$, which is equivalent to the maximum tolerated density suggested by Parra Paz et al. (2015). Daily monitoring involved manually turning the testing material and recording its temperature. Furthermore, moisture and pH of substrate were monitored at each substrate sampling, being amongst the most significant factors implicated in control of biodegradation of bioplastics (Grossule et al., 2023a). Sampling of rearing substrate and larvae occurred every five days until 50% of larvae had progressed to the prepupal stage, and again at test conclusion, when all larvae had reached the prepupal stage, identifiable by skin darkening. Larvae were washed and individually weighed. Both substrate and larvae samples were analysed for BMPs, including particle quantification and determination of mean diameter values for two size ranges: <10 μm and >10 μm. Test set up, operation and sampling were performed with a view to avoiding possible external microplastics contamination by using glass reactors, stainless steel utensils (for mixing, sampling and analysis) and glass sample containers.

### 2.4. Analytical procedure for microplastics identification and quantification

Larvae samples were washed with deionized water to eliminate all

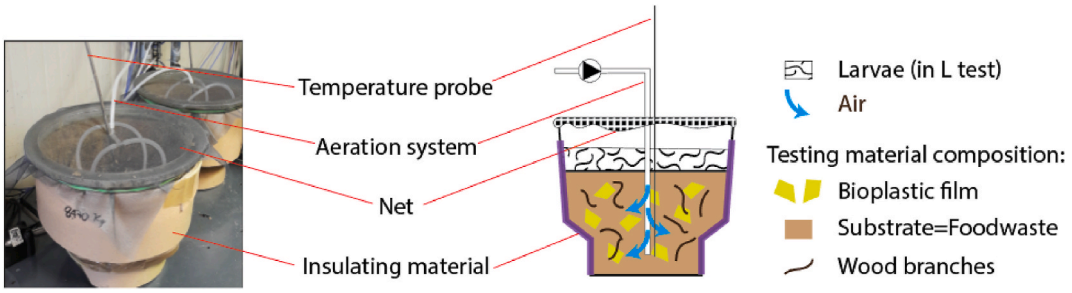


**Fig. 1.** Testing reactors arrangement. 20 L glass batch reactors, each sealed with a mesh net to prevent oviposition by other flies. The reactors were insulated and equipped with a temperature probe and an aeration system. At the beginning of the tests, all reactors were filled with the same testing material (7.249 kg), including biodegradable plastics, food waste and wood branches. L = larvae tests including larvae, while no larvae were added to control tests (C tests) (Modified from Grossule et al., 2023a).

epibiota and any adhering particles. Washed larvae and rearing substrates were processed for MPs extraction according to the patented method (International Application No. PCT/IB, 2019/051,838, Italian patent n. 102,018,000,003,337–07 March 2018, European patent n.3788344 of the July 20, 2022) at the University of Catania. A more detailed description of this newly patented methodology can be found in previously published studies (Oliveri Conti et al., 2020; Ferrante et al., 2020; 2022). Briefly, a 0.2 g aliquot of sample was weighed, placed in a clear glass container and subjected to MPs extraction using the patented method with acid, employing a graphite block digestion instrument operated at ~80 °C for 90 min. Following mineralization, an aliquot of organic solvent was added to each sample, and the mixture was briefly vortexed. After centrifugation, the lower phase was carefully transferred into a new glass tube. The resulting extracts were dispersed onto 25 mm diameter aluminium stubs and then coated using pure gold using a 108 Auto Sputter Coater (Cressington Ltd., UK), before undergoing particle detection and quantification using a Scanning Electron Microscope (Cambridge Instruments Mod. Stereoscan 360) coupled to an Energy-Dispersive X-ray detector (EDX; Diffractometer Rigaku Miniflex). Analysis permitted the determination of shape as well as discrimination of MPs from other organic materials.

The patent facilitated comprehensive analysis of sample stubs. Observation of the stubs enabled the total number of MPs to be calculated using MICROPLAST software version 1.0 (included in the patent) and determination of MPs diameter. For each sample, results were reported as the number of particles per gram of dry weight (d.w.) and average radius of particles.

To prevent background noise and cross-contamination from clothing and plastics during sample preparation and experiments, the following preventive actions were implemented: (i) glassware and metal equipment were washed before use with filtered high grade water and acetone; (ii) particle-free nitrile gloves were used, coupled with clean cotton-based laboratory coats; (iii) experiments were conducted in a clean room under a horizontal laminar flow cabinet with controlled access; (iv) sample containers were closed with a glass lid or aluminium foil when not being handled.

Three reagent blanks were run with each batch of samples, and no measurable MPs were detected in these blanks, demonstrating that any potential contamination resulting from sample treatment was insignificant. Recovery of MPs was calculated using samples fortified with 3 μm dark red polystyrene microparticles purchased from Merck – Sigma Aldrich, Germany, with a recovery range of 88–103%.

## 3. Results and discussion

### 3.1. Environmental condition of degradation process and larvae growth

In order to better understand, discuss, and interpret the potential generation of microplastics and fate in larval biomass and substrate residues, it is crucial to consider both environmental conditions present throughout the degradation process, and larval development. These factors were previously detailed in Grossule et al. (2023a) and are summarized here for the sake of completeness.

The biodegradation of bioplastics is driven by the following key factors: temperature, moisture, and pH. High temperature and moisture-rich environments play a crucial role in accelerating hydrolysis reactions and microbial growth rates (Kale et al., 2007). Specifically, starch-based polymers undergo hydrolysis under alkaline conditions (Sakkara et al., 2020). Fig. 2A illustrates the variation in average temperature, moisture content and pH, in both L and C tests.

Mesophilic conditions were established in both L and C tests. However, from the fifth day, L test exhibited higher temperature, reaching up to 45 °C, attributed to heat release from larval metabolism and friction generated by movement (Bonelli et al., 2019). Higher moisture content and pH were generally observed in L tests compared to C tests. Alkaline conditions were primarily due to the excretion of alkaline urine by BSF larvae which contributed to the overall increase in substrate pH (Ma et al., 2018). Environmental conditions established during the larval degradation process were more favourable for bioplastic degradation, characterised by higher moisture, temperature, and pH levels compared to those observed in the C test (Grossule et al., 2023a), thus suggesting a higher potential for BMPs generation in L tests. Additionally, shear stress generated by the movement of larvae on the plastic film surface might have further contributed to the disintegration of particles. Indeed, larvae demonstrated a tendency to shift the plastic films towards the surface, as depicted in Fig. 3.

Larval growth is illustrated in Fig. 2B in terms of variations in average larval weight and prepupation percentages. The larvae achieved a maximum average wet weight of approximately 187 mg and a 50% prepupation after 15 days, completing prepupation at day 26th.

According to Lievens et al. (2023), the ingestion of microplastics (53–63 μm in size) by BSF larvae is influenced by the size of the larvae's mouth, which, in turn, correlates with larval growth. Specifically, as larvae grow larger, their mouths increase in size, resulting in a higher intake of microplastics. However, as stated by Heussler et al. (2023), microplastics accumulate exclusively in the larval gut, facilitating the excretion of accumulated microplastics before reaching the pupation stage, when the gut is emptied, or during fasting periods (Lievens et al., 2023). Consistently, an increase in BMPs concentration in larval biomass was expected during the first 15 days, followed by a decrease once the pupation stage started. However, there is still a considerable gap in our knowledge of the fate of smaller microplastic sizes (<50 μm).

### 3.2. Biodegradable microplastics generation and accumulation in substrate

Fig. 4 illustrates the variation of BMPs content (# particles/gDM, DM = Dry Matter) in substrates of L and C tests, and in larvae biomass, considering two particle sizes: inferior to and exceeding 10 μm in diameter. The time at which 50% prepupation occurred is also

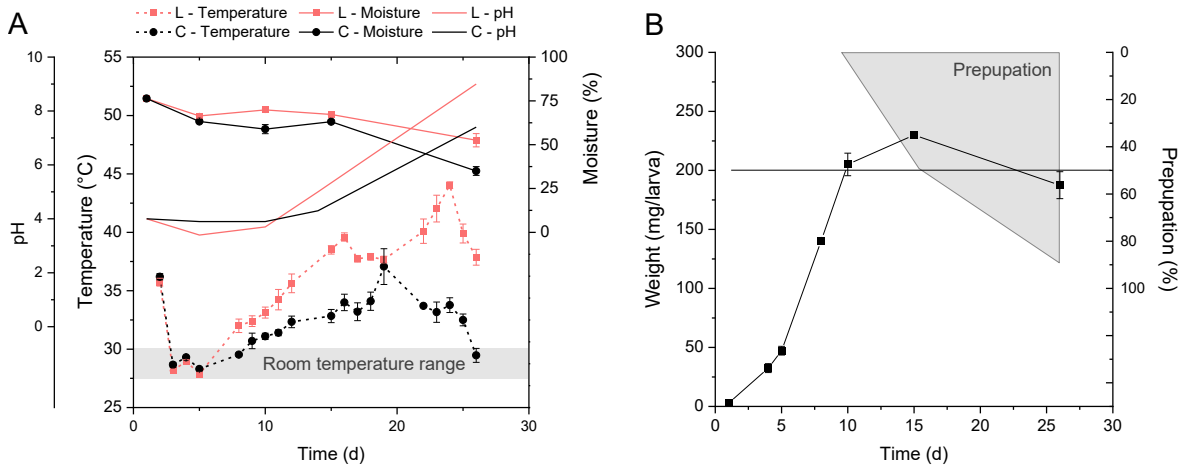


**Fig. 2.** Environmental conditions of degradation process and larvae growth in larvae and Control tests: average variation of temperature, moisture content and pH (A); average larval wet weight variation and prepupation % (B). (L = larvae test; C = control test); (Modified from Grossule et al., 2023b).

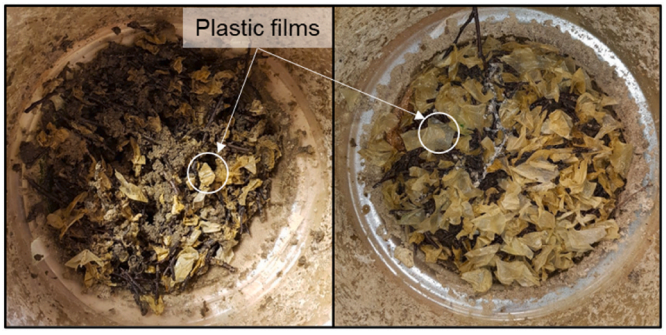


**Fig. 3.** Top view of the larvae test reactor after daily mixing and after one day of the process: BSF larvae tend to shift the plastic films towards the surface.

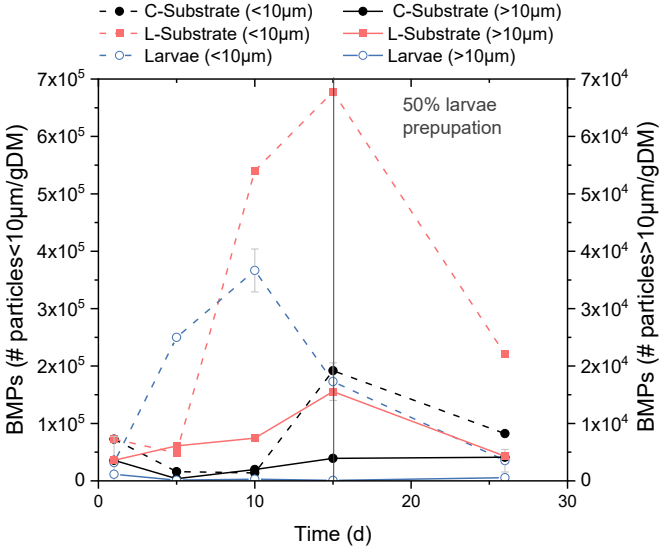


**Fig. 4.** Variation of BMPs content (# particles/gDM, DM = Dry Matter) in substrates of larvae and control tests tests and in larvae biomass, considering two particle sizes: smaller and bigger that 10 μm in diameter. (L = larvae test; C = control test) The time at which 50% prepupation occurred is also indicated.

indicated.

Regarding BMPs in substrate, in both tests, the concentration of lower-sized BMPs (<10 μm) was generally higher than that of bigger-sized particles by an order of magnitude, displaying an inverse proportionality of particle concentration to their size. These results are in line with literature relating to non-biodegradable MPs (Mhiret Gela and Aragaw, 2022; Oliveri Conti et al., 2020).

Considering the variation of particle concentrations over time, both size classes exhibited a general increase in concentration, reaching their peak on the fifteenth day, in both L and C tests. However, concentrations in L tests were approximately 3-fold higher compared to those in C tests. Specifically, at the peak, the concentration of lower-sized particles (# particles <10 μm/gDM) in L and C substrates was approximately $6.8 \times 10^5$ and $1.9 \times 10^5$, respectively, while the concentration of larger-sized particles (# particles >10 μm/gDM) was approximately $15.6 \times 10^3$ and $4.1 \times 10^3$ in L and C substrates, respectively. These findings confirmed the enhanced degradation conditions present in L tests which resulted in an improved BMPs generation.

From Day 15 until the end of the test, BMPs <10 μm were found to have halved in C test, while BMPs >10 μm remained relatively constant. This can be explained by the simultaneous decrease in both temperature and moisture, leading to less favourable conditions of degradation. As a result, the additional generation of larger-sized BMPs and their further fragmentation into smaller BMPs were both prevented, maintaining a constant concentration in the substrate. On the other hand, smaller-sized BMPs produced earlier, underwent partial degradation, leading to a reduction in concentration.

In L tests, the peak concentrations on the fifteenth day coincided with the peak of average larval weight and 50% larval prepupation. As the prepupation process advanced, an increasing number of larvae displayed reduced feeding activity, metabolic rate, and movement (Cai et al., 2022), leading to a subsequent decrease in shear stresses on plastic films. As a result, no further generation of larger-sized BMPs occurred, while their fragmentation into smaller-sized BMPs may justify a reduction in concentration. A fast and significant decrease occurred in BMP <10 μm, enhanced by the highest temperature and pH values at the end of the test.

Complete degradation of BMPs in substrates of both L and C tests, taking into account both particle size categories, did not occur. Higher accumulation of BMPs was observed in larvae processing. Specifically, the final concentration of smaller-sized particles (# particles <10 μm/gDM) compared to initial concentration, increased by approximately 172% and 13% in L and C substrates, respectively. Meanwhile, the concentration of larger-sized particles (# particles >10 μm/gDM) increased by approximately 20% and 15% in L and C substrates,

respectively.

### 3.3. Biodegradable microplastics accumulation in larvae biomass

Regarding the BMPs concentration in larval biomass (Fig. 4), consistent with the size of BMPs generated in substrate of L test, the concentration of lower-sized BMPs (<10 μm) in larval biomass was higher than that of bigger-sized particles, by two orders of magnitude. The result is in line with the findings of literature reports whereby ingestion of microplastics is proportional to particle content in the substrate (Lievens et al., 2023). The same study suggested that accumulation of MPs is proportional to mouth size—dependent on larval age, observing larvae starting to take up MPs at the age of 10 days, when larvae mouth opening and MPs were of the same size (53–63 μm).

In the present study, the accumulation of smaller-sized BMPs (<10 μm) in larvae biomass was observed from the outset, as they were smaller than the larvae mouth opening (15–30 μm; Lievens et al., 2023) even at a young age (5 days old).

BMPs <10 μm accumulated in larval biomass until the tenth day when it reached the peak concentration of approximately $3.7 \times 10^5$ (# particles/gDM), representing more than half of particle concentration in the substrate at the same time. The peak was achieved before 50% larvae prepupation was reached (on the fifteenth day). As larvae progressed to the prepupation stage, they started to empty their guts (Heussler et al., 2023), resulting in a tenfold reduction in particle concentration in larval biomass to approximately $3.6 \times 10^4$ #particles/gDM by the end of the test. However, complete elimination of BMPs was not achieved, as reported by Lievens et al. (2023).

No significant accumulation in larger-sized BMPs (>10 μm) was observed: starting from approximately 1140 #particles/gDM, concentrations ranged between 550 and 110 #particles/gDM during the test.

### 3.4. Biodegradable microplastics size variation

Determination of the shape of microplastics (MPs) was performed based on SEM images (a few examples are provided in Fig. 5), in terms of particle diameter.

Fig. 6 illustrates the variation of average BMPs diameter (μm) in substrates of L and C tests, and larvae biomass considering two particle sizes: inferior to and exceeding 10 μm in diameter. The diameter of BMPs ranged between 1.5-4 μm and 12–20 μm, respectively, for smaller- and larger-sized particles in both substrates and larval biomass. A general decrease in particle size occurred in both L and C substrates throughout the test, with the exception of larger-sized particles in C tests, while no significant variation in particle size occurred in larvae biomass.

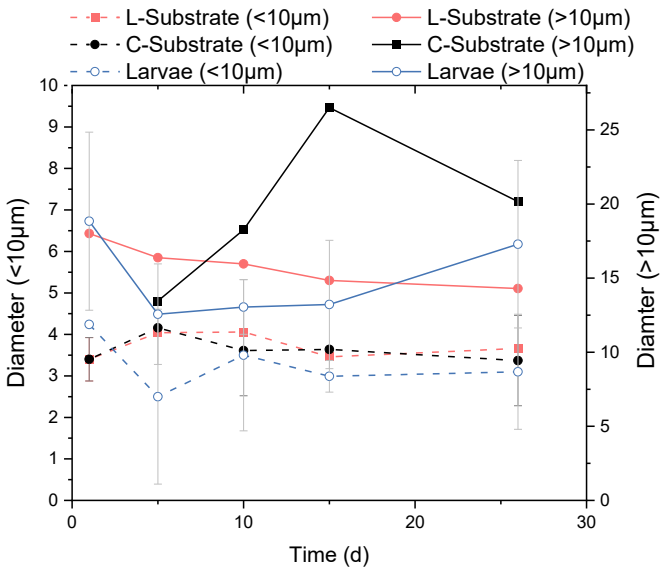


**Fig. 6.** Variation of average BMPs diameter (μm) in substrates of Larvae and Control tests, and in larvae biomass considering two particle sizes categories: inferior to and exceeding 10 μm in diameter.

## 4. Conclusions

Food waste contaminated by starch-based bioplastic film was treated using a BSF larvae process with the aim of investigating the generation of microplastics from bioplastic film (BMPs) and potential accumulation of the latter within the larvae biomass and rearing substrate. Two categories of particle size were considered: inferior to and exceeding 10 μm in diameter. Based on the results obtained, the following conclusions may be drawn.

a) The generation and fate of smaller-sized BMPs (<10 μm) were analysed for the first time in the BSF larvae process, exhibiting a higher concentration compared to larger-sized particles by an order of magnitude, suggesting their higher relevance in future studies.
b) The larval process displayed more favourable degradation conditions, enhancing the generation of and resulting in a 3-fold higher concentration for both size classes compared to control tests. These conditions were influenced by high temperature, pH, moisture, and shear stresses on plastic films exerted by larvae in L tests.
c) Higher BMPs accumulation was observed in larvae processing, suggesting potential risk of BMPs soil contamination when residues are used as fertilizer.
d) Smaller-sized BMPs (<10 μm) started to accumulate in larval biomass since the beginning of the test reaching a peak in concentration just before start of the prepupation stage, (exceeding the initial concentration by over 1000%), when larvae are typically

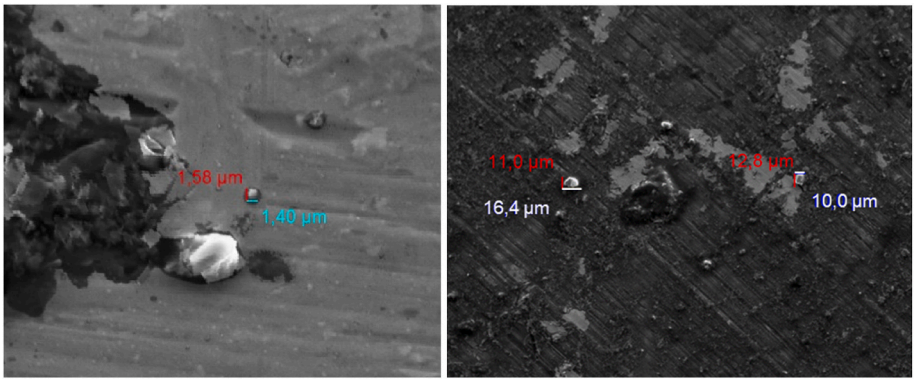


**Fig. 5.** Scanning Electron Microscope images of MPs with sizes inferior to and exceeding 10 μm in diameter.

collected for animal feed. This suggests a risk of biomagnification phenomena when larvae are used as animal feed.

e) The entity of these potential risks should be further investigated by monitoring the fate of BMPs in agricultural soil and throughout the food chain.

## CRediT authorship contribution statement

**Valentina Grossule:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gea Oliveri Conti:** Writing – original draft, Methodology, Formal analysis. **Paola Rapisarda:** Formal analysis. **Eloise Pulvirenti:** Formal analysis. **Margherita Ferrante:** Writing – review & editing, Supervision, Methodology. **Maria Cristina Lavagnolo:** Writing – review & editing, Supervision, Methodology.

## Declaration of competing interest

none.

## Data availability

Data will be made available on request.

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