Feeding preference of insect larvae to waste electrical and electronic equipment plastics

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A list of abbreviations

Abbreviations	Full name
BW	beeswax
E-waste	Electronic waste
FT-IR	Fourier transform infrared spectroscopy
HIPS	high impact polystyrene
PE	polyethylene
PF	phenol-formaldehyde resin
PP	polypropylene
PS	polystyrene
PU	polyurethane
PVC	polyvinyl chloride
RPU	rigid polyurethane
SEM	scanning electron microscope
SR	survival rates
WEEE	waste electrical and electronic equipment
WHIPS	waste high impact polystyrene
WPS	waste polystyrene
WRPU	waste rigid polyurethane
XRF	X-ray fluorescence

Abstract

Waste electrical and electronic equipment (WEEE) plastics not only pollute the environment, but are challenging to treat in an environmentally friendly manner. Biodegradation by insect larvae is potentially an eco-friendly method to treat WEEE plastics, but information about the feeding preference of insect larvae to WEEE plastics is lacking. In this study, a total of nine WEEE and pristine plastics were chosen to feed larvae of the following two insect species, i.e. *Galleria mellonella* and *Tenebrio molitor*. *G. mellonella* larvae significantly favor corresponding pristine plastics compared to two types of WEEE plastics, waste rigid polyurethane (RPU) and waste polystyrene (PS). One possible explanation is the increased chlorine or metals in the WEEE plastics measured using X-ray fluorescence spectrometer analysis. Scanning electron microscopy and Fourier transform infrared spectroscopy show that the destruction of physical structures and changes in surface functional groups were found in the two types of WEEE plastics in the larval frass, implying that the larvae partly biodegraded the plastics. Meanwhile, the powdered waste high impact polystyrene plastics (WHIPS) were ingested, but not the lumpy ones, indicating that the consumption by *G. mellonella* larvae is improved by the WHIPS physical modification. In addition, *G. mellonella* larvae presented the following decreasing preference for pristine plastics under individual-plastic-fed mode: RPU > phenol–formaldehyde resin > polyethylene (PE) > polypropylene> PS ≈ polyvinyl chloride; this is possibly due to differences in physical properties and chemical structures of the plastics; feeding preference of the larvae under multiple-plastics-fed mode is relatively consistent to that under individual-plastic-fed mode. Interestingly, the consumption by *G. mellonella* larvae of PE is higher than that of PS, while *T. molitor* larvae showed the opposite trend, implying that insect larvae have different plastics preference. The findings provide insights into biodegradation of WEEE plastics by insect larvae.

Keywords: E-waste; Waste Electrical and Electronic Equipment Plastics; Insect Larva; Biodegradation; Feeding preference; *Galleria mellonella*; *Tenebrio molitor*

1. Introduction

Waste electrical and electronic equipment (WEEE) is a mixture of materials and components of scrapped electrical and electronic appliances (e-waste), such as obsolete computers and cell phones (Li et al., 2019). About 300 million computers are scraped in the USA, 1 billion cell phones are discarded in Europe, and 50 million tons of the WEEE is produced per year worldwide (Luhar et al., 2019). WEEE plastics are essential components of e-waste, serving as housing, casing, insulation, inner shelving, liner, and accounting for approximately 3.5%-49.1% (w/w) of the total weight of e-waste (Wu et al., 2020). Common WEEE plastics include high-impact polystyrene (HIPS) (Martinho et al., 2012), polypropylene (PP), polystyrene (PS; Wu et al., 2020), polyethylene (PE; Martinho et al., 2012), polyurethane (PU; Tantisattayakul et al., 2018) and polyvinyl chloride (PVC; Yang et al., 2013). Additionally, phenol–formaldehyde resin (PF), a thermosetting plastic, is often used in electrical insulators and plastic wear (Ramesh et al., 2007). The WEEE plastics often contain multiple dangerous toxic additives, such as heavy metals (e.g. lead, mercury, cadmium, and hexavalent chromium) and brominated flame retardants (e.g. polybrominated biphenyls, polybrominated diphenyl ethers, or tetrabromobisphenol A; Charitopoulou et al., 2020). Improper management and superficial treatment of the WEEE plastics lead to pollution of the natural environment and threaten human health by releasing the additives (Petridis et al., 2017). The harmful additives negatively impact human health by the damaging physiological system and causing cancer (Charitopoulou et al., 2020). Therefore, harmless treatment and disposal of WEEE plastics have far-reaching significance to environmental and health crises (Akram et al., 2019).

Plastic biodegradation is a more effective way to fight plastic pollution than other treatment methods such as landfill, incineration, physical recycling and chemical recovery due to its low costs and environmentally friendly impacts (Azubuike et al., 2016). A few studies have reported the biodegradation of plastics by microorganisms, such as bacteria (Bardaji et al. 2019) and fungi (Magnin et al., 2019; Osman et al. 2018), of which some utilize the plastics as carbon or nitrogen sources (Sheth et al., 2019). Osman et al. (2018) reported that at 30 ℃, *Aspergillus* sp. Strain S45 reduced the mass of PU films by 15%-20% (about 37.5 - 50 mg) over four weeks. Bardaji et al. (2019) found that in three months, 30% of PE (4.5 mg) was biodegraded by a *Paenibacillus* sp. at 37 °C after pretreatment with ethanol and bleach. However, the efficiency of plastic biodegradation by microorganisms is often relatively low, even with preconditioning or pretreatment processes (Krueger et al., 2015; Peng et al., 2019; Yang et al., 2014). Therefore, this process of applying microorganism is not conducive to the actual treatment of WEEE plastics.

Recently, insect larvae have been receiving attention due to their better processing ability and more robust applicability for biodegradation of plastics than microorganisms (Peng et al., 2019; Yang et al., 2015). The growth conditions of insect larvae are not as strict as those of microorganisms (Zhu et al., 2021), making them more suitable for practical applications. Yang et.al (2018) reported that up to 84 % of the PS fed to *Tenebrio molitor* larvae is consumed in 32 days (Yang et al., 2018b). Brandon et al. (2018) found that *T. molitor* larvae converted 49.0% ± 1.4% of the ingested PE into CO_2. Meanwhile, *Galleria mellonella* larvae were also used to degrade the plastics (Yang et al., 2014; Zhang et al., 2020). Bombelli et al. (2017) reported that a larval homogenate of *G. mellonella* smeared on PE films caused a mass loss of 13% (about 92 mg) in 14 h. The potential of this degradation mechanism is that the insect larvae could chew and eat the plastics as their only food source, rapidly degrading and mineralizing the ingested plastics to CO_2 and lowering molecular weight compounds after passage through the intestinal tract (Brandon et al., 2018; Wu et al., 2019; Yang et al., 2020). Gut microbial symbiont presence has been demonstrated to play an essential role in the biodegradation of ingested plastics in the gut of insect larvae (Brandon et al., 2018; Yang et al., 2020) and cause plastic depolymerization with the release of broken down substances to sustain the growth of insect larvae in the absence of other food (Yang et al., 2018b). However, the above studies are often based on pristine plastics, and not waste plastics.

Distinct from pristine plastics, WEEE plastics often include pollutants, such as metals, Freon, brominated flame retardants, and polycyclic aromatic hydrocarbons (Dement'ev et al., 1991; Mao et al., 2020; Zhang et al., 2020). The effect of pollutants on WEEE plastic biodegradation by insect larvae such as *G. mellonella* larvae is unknown. Therefore, the feeding preference of the insect larvae toward the WEEE and pristine plastics deserves to be explored. Meanwhile, WEEE plastics often coexist, and contain more than 15 different polymers (Wang and Xu, 2014; Yang et al., 2013). However, the information on the feeding preference of insect larvae to multiple plastics with different physicochemical properties under the co-existence is lacking.

In this study, larvae of two insect species (*G. mellonella* and *T. molitor*), and nine types of plastic (three WEEE plastics: WRPU, waste polystyrene [WPS] and waste high-impact polystyrene [WHIPS]; six pristine plastics: RPU, PF, PE, PS, PP, PVC), were used to investigate the feeding preference of insect larvae. The six plastics are chosen according to their common and special physicochemical properties. Namely, RPU and PF are typical thermosetting plastics with amino (-NH) and hydroxyl (-OH) groups, PS is a thermoplastic plastic with benzene ring groups, and PVC holds the chlorine element. Scanning electron microscope (Azzam et al.) and Fourier-transform infrared spectroscopy (FT-IR) were applied to characterize the plastics' micromorphology and surface functional groups and corresponding frass, respectively. XRF was used to analyze element composition of the WEEE and pristine plastics.

2. Materials and methods

2.1 Sources of the larvae and plastics

G. mellonella and *T. molitor* larvae (Fig. S1a and b) were purchased from a breeding farm in Yancheng, Jiangsu, China. *G. mellonella* larvae were mature at 25-30 mm in length and 310-370 mg in mass; *T. molitor* larvae were mature at 20-25 mm in length and 55-110 mg in mass. The mature larvae had excellent consumption of and a strong effect of gnawing at plastics (Ellis et al., 2015). All the larvae were starved for three days before the experiment.

WRPU, WPS and WHIPS plastics were collected from an insulation material of waste refrigerator, a WEEE insulation material and an engineering plastic of WEEE shell, respectively, at an environmental protection company in Shanghai, China (Fig. S1c, d, and e). Pristine RPU, PF, PE, PS, PP, and PVC plastics were purchased from a plastic supplier in Shanghai, China. The WRPU, WPS, and all the pristine plastics were foamed plastics to avoid physical differences. Before the experiments, the plastics were cut into cubes with a side length of 10 mm (Fig. S1f), and then soaked in 75% alcohol (v/v) for 30 s, washed with sterilized water in triplicate, and subsequently dried at 30 °C under sterilized conditions for two days to remove the impurities on their surfaces. The sterilized and dried plastics were stored in a desiccator for the following experiments (Zhang et al., 2020).

2.2 Feed preference tests

Feeding preference for WEEE and pristine plastics Two kinds of WEEE plastics (WRPU and WPS) and corresponding pristine plastics (RPU and PS) were used to investigate the feeding preference of *G. mellonella* larvae. According to our previous study (Zhu et al., 2021), 2.0 g of each plastic was placed in a petri dish and used as a carbon source, to feed 40 similarly sized individuals of *G. mellonella* larvae. Then, the larvae were incubated at 30.0 ± 1.0 °C and kept in 70% ± 5% humidity and darkness for seven days. The plastics mass loss and larvae survival rates (SR) were measured each day. The remains of dead bodies were removed immediately. After the experiment, the larvae were cleaned with 0.3~0.6 MPa compressed air by an WM-606 air spray gun (Weima pneumatic tools series flagship store, Foshan city, China) to remove the impurity sticking to the larvae surface, and then transferred to new containers. After the larvae were cultivated for 24 h under the starvation conditions, the larvae frass were collected, and then stored at -20 °C for further analysis. Larvae fed on beeswax (Oguttu et al.) instead of plastics under the same experiment conditions were used as the control (Yang et al., 2015). All experiments were carried out in triplicates (Yang et al., 2021).

Feeding preference for WEEE plastics before and after physical modification The WHIPS plastics were compared to investigate the effect of plastic-size reduction on the feeding of *G. mellonella* larvae. The above WHIPS cubes with the size of 10 mm were broken by the crusher, and then sieved through a 120-mesh sieve (Zhang et al., 2020), and thus the smaller plastics with less than 125-μm in diameter were gained. Both of the original WHIPS cubes and sieved particles were used to feed the larvae, respectively, as the only carbon source. The other experiment conditions were identical to the above experiment about feeding preference for WEEE and pristine plastics.

Feeding preference for the plastics under multiple-plastics-fed mode Six pristine plastics (RPU, PF, PE, PP, PS and PVC) were used to investigate the effect of different plastic combinations on the feeding of *G. mellonella* larvae. A total of 30 pieces of the plastics were randomly collected for all the groups. Thirty pieces of each plastic were used for individual-plastic-fed groups. Five pieces of each plastic were applied for combination b1 with all six plastics. Ten pieces of each plastic were used for the c1 and c2 combinations: RPU, PF and PE were used for c1 combination; and PP, PS and PVC were used for c2. Fifteen pieces of each plastic were used for d1-d7 combinations with two plastics, which consisted of RPU and PF; RPU and PE; RPU and PP; PE and PP; PE and PS; PP and PS; and PS and PVC, respectively. The plastics used in the c1-c2, and d1-d7 combinations were chosen according to different plastic consumptions by the larvae in the b1 combination. Two controls for the SR measurements were conducted through BW-fed or un-fed instead of plastic-fed under the same experiment condition (Yang et al., 2015). The other experiment conditions were identical to the above experiment about feeding preference for WEEE and pristine plastics.

Feeding preference of two insect larvae One WEEE plastic (WRPU) and five pristine plastics (RPU, PF, PE, PS, and PVC) were used to compare the feeding preferences of two insect larvae (*G. mellonella* and *T. molitor*). As individual weights varied between species, 40 *G. mellonella* larvae and 100 individuals of *T. molitor* larvae were randomly collected, to ensure the groups' total weights were similar. their different individual weights. The culturing time was 20 days, and the plastics' mass loss was measured every two days during the first 8 days, and every four days during the last 8-20 days. The other experiment conditions were same as the above experiment about feeding preference for WEEE and pristine plastics.

2.3 Analysis and characterization methods

The larvae SR was measured by counting the number of living larvae. The consumption of the larvae to the plastics is estimated based on the mass loss by weighing residual plastics. The element composition of the pristine and WEEE plastics was measured by XRF spectrometry (XRF-1800, Shimadzu Ltd, Kyoto, Japan). The micromorphologies of the pristine plastics, WEEE plastics and BW, and their corresponding frass were determined using a JSM-6700F SEM (Royal Dutch Philips Electronics Ltd, Eindhoven, Netherlands) at 30 V. For this, the samples were dried at 80°C for 24 h, and coated with sputtering gold to increase their electrical conductivity (Peng et al., 2019). The FT-IR spectra of the samples were measured using a NICOLET 6700 FT-IR Spectrometer (Thermo Fisher Scientific Corporation, MA, USA) over the range of 4000-400 cm^{-1} (Kim et al., 2020), resolution of 4 cm^{-1} and scanning step of 1 cm^{-1}. Before the FT-IR analysis, the dried samples were crushed and sieved through a 200 mesh (Brandon et al., 2018).

3. Results and discussion

3.1 Feeding preference of *G. mellonella* larvae to different kinds of pristine and WEEE plastics

The SR and mass loss of WRPU, RPU, WPS, and PS plastics and BW by *G. mellonella* larvae are shown in Fig.1. The SRs of *G. mellonella* larvae in the WRPU- and WPS-fed groups ranged from 92.5%-98.5% after one week, suggesting that the two WEEE plastics are suitable as growth substrates for *G. mellonella* larvae. After a week, the cumulative mass losses of the WRPU and RPU plastics are about 0.70 and 1.16 g, respectively. The average consumption by the larvae of the WRPU and RPU plastics is 2.53 and 4.14 mg / (larva · d), respectively (Fig. 1a). The cumulative mass losses of the WPS and PS plastics are about 0.27 and 0.33 g, respectively. The average WPS and PS plastic consumption by the larvae is about 0.96 and 1.18mg / (larva · d), respectively (Fig. 1b). The results show that the consumption by *G. mellonella* larvae of the WRPU and WPS plastics is lower than that of the corresponding pristine plastics, implying that the larvae prefer the pristine plastics to the WEEE plastics. One possible reason is that odor-causing volatiles are released from the WEEE plastics due to the desorption of adsorbed polluting substances on their surface and the exudation of additive substances in the plastics (Demets et al., 2020). The volatiles may act as behavioral antagonist, and reduce the appetite of the larvae (Kwadha et al., 2019). As shown in Table 1, Cl contents in the WRPU and WPS are higher than that in the RPU and PS, respectively, probably resulting from the adsorption of the Freon on the plastic surface as the waste refrigerator foaming agent (Dement'ev et al., 1991).

The metal contents in the WEEE plastics is also much higher than that in the corresponding pristine plastics, probably relating to the fact that the WEEE plastics are liable to absorb heavy metals due to their environmental aging and wear (Li et al., 2021; Liu et al., 2020). The increase in the pollutants of the WEEE plastics may produce an enhanced adverse effect on the gut microbes in the larvae (Caravelli et al., 2004; Jacquioda et al., 2018), causing a lower consumption of the waste plastics than that of corresponding pristine plastics. Therefore, the larvae SR in the WPS-fed group is significantly lower than that in the PS-fed group (Fig. 1b).

In addition, the consumption rate and SR of *G. mellonella* larvae fed WPS and PS are lower than those of larvae fed WRPU and RPU. The possible reason is that compared with the WPS and PS, the WRPU and RPU contain carbon and nitrogen source and lower metal contents (Peng et al., 2019), which may supply better extensive nutrients and improve the larvae growth. These results provide important data for studying the biodegradation of waste plastics by insect larvae.

3.2 Physicochemical changes in the WEEE plastics before and after ingestion.

Physical changes in the WEEE plastics, pristine plastics, and corresponding frass were analyzed, to investigate their differences in terms of biodegradability after being ingested by *G. mellonella* larvae. As shown in Fig. S2, numerous plastic fragments are found in the plastics after being gnawed by the larvae, implying that the plastics are strongly gnawed by *G. mellonella* larvae. Compared with the pristine RPU and PS from Fig. S2b and d, the fragments from the corresponding WRPU and WPS plastics are higher, probably relating to an increase in the aging degree of the WEEE plastics during their application. As shown in Fig. 2b, b$'$, d, and d$'$, the RPU and PS feedstock have unbroken honeycomb-like cellular structures compared with the WRPU and WPS feedstock from Fig. 2a and c, complementing and confirming the occurrence of the WEEE plastics aging. Compared with the plastic feedstock, the morphologies of the corresponding plastic fragments from Fig.2a$'$, b$''$, c$'$, and d$''$ have different degrees of destruction, implying that the *G. mellonella* larvae destroy the physical structure of the plastics through gnawing (Brandon et al., 2018; Yang et al., 2020). However, no noticeable difference is found in the morphology of the fragments from the WEEE and their corresponding pristine plastics, indicating that *G. mellonella* larvae have a similar gnawing effect on the WEEE and pristine plastics.

Meanwhile, the frass from WRPU, RPU, WPS, and PS are very different from the BW frass, but have similar characteristics that the plastic-like materials are still discerned (Fig.2e'), and severely corroded in the frass (Fig.2a'', b''', c'' and d'''). In addition, the structures of the WPS and PS plastics seem less damaged than that of the WRPU and RPU plastics (Fig.S2), corresponding to the previous results of lower consumption of *G. mellonella* larvae to the WPS and PS. The results indicate that *G. mellonella* larvae destroy the physical structure of the plastics, possibly through the gnawing and biodegradation effect of the intestine (Brandon et al., 2018; Lou et al., 2020).

Subsequently, chemical changes in the WEEE and pristine plastics, and corresponding frass were analyzed using FT-IR, shown in Fig. 3. In general, the spectra of WRPU and WPS feedstock, and the corresponding frass are similar to those of RPU and PS feedstock, and the corresponding frass, implying that the larvae have similar biodegradation characteristics to the WEEE and pristine plastics. Therefore, taking WEEE plastics as an example to state, compared with the WRPU feedstock, the corresponding frass have considerable changes in the three following regions of the spectra: 3330-3250, 1710-1550 and 900-500 cm^{-1} (Fig. 3a). These changes include (1) an increase in the hydroxyl related to the H-bonded N-H and –OH at 3296 cm^{-1}, suggesting the improvement of the hydrophilicity of the WRPU; (2) a transformation from free carbonyl bond in the main chain of WRPU at 1704 cm^{-1} to H-bounded carbonyl bond at 1644 cm^{-1}, indicating the formation of hydrogen bonds between the N-H and C=O due to the breakdown of the polymer chains; and

(3) an attenuation in the C=C and C-H peaks of benzene rings at about 1595, 814 and 765 cm^{-1}, implying the cleavage of the benzene ring (Oprea et al., 2010). The possible reason is that *G. mellonella* larvae mainly act on hard segments of the WRPU composed of carbamate bonds (Oprea et al., 2010). However, no noticeable change occurs in ether bonds at about 1071 cm^{-1}, possibly because soft segments of the WRPU composed of the ether bond are resistant to biodegradation. Compared with the WPS feedstock, the changes in the spectra of the corresponding frass present in the two following regions: 2500-3500 and 1400-1710 cm^{-1} (Fig. 3b). These changes include: (1) an increase in the -OH at 3269 cm^{-1}, suggesting the enhancement of the hydrophilic properties on the plastic surface (Lou et al., 2020); (2) disappearances of C=C bond at 1400-1710 cm^{-1} relating to benzene ring groups, and a decrease in =CH bond at 692 cm^{-1}, implying the cleavage of the benzene ring (Yang et al., 2018b); and (3) an appearance of C=O at 1637 cm^{-1} and C-O at 1030 cm^{-1}, indicating the oxidation of the WPS (Peng et al., 2019; Sekhar et al., 2016; Yang et al., 2014). The two plastics show the different degradation products based on FT-IR spectra data, attributed to the differences in chemical composition and the structure of WRPU and WPS. The former mainly contains carbamate bonds, and the latter mainly contains benzene rings and carbon chains.

On the other hand, in contrast with the WEEE and pristine plastics, the spectra of the BW feedstock have similar characteristics to those of the BW frass, including C-H compounds at about 2914 cm^{-1} and 2849 cm^{-1} representing hydrocarbon substance, C=O bonds at about 1739 cm^{-1} and 1649cm^{-1} relating to carbonyl groups, C=C groups at about 1463 cm^{-1}, C-H materials at about 1175 cm^{-1}, and C-O bonds at 1031 cm^{-1}. The considerable difference confirms that the WRPU, RPU, WPS and PS are ingested and partly biodegraded by the *G. mellonella* larvae.

The above results indicate that the physical structure and chemical composition of the WEEE plastics are changed after being ingested by *G. mellonella* larvae, due to the biological hydrolysis and oxidation of the plastics (Pellizzi et al. 2014). It deserves further investigation of the degradation pathway of the WEEE plastics through the gut of *G. mellonella* larvae and the role of gut microbes during the process. In addition, it is also suggested to study the effect of plastic chemical composition on the WEEE biodegradation.

3.3 Feeding preference of *G. mellonella* larvae to WEEE plastics before and after physical modification

Some WEEE plastics, such as WHIPS, are too hard to be ingested by insect larvae. In this study, lumpy and powdered WHIPS before and after physical modification were used to feed *G. mellonella* larvae to investigate the feeding preference of the larvae to the hard WEEE plastics with or without physical modification. As shown in Fig. S3a, there were no signs of damage on the lumpy WHIPS plastics after being fed to *G. mellonella* larvae. However, powdered WHIPS (Fig. S3b) is ingested by *G. mellonella* larvae and transformed into the larval frass (Fig. S3c). As shown in Fig. 4, the cumulative consumption of *G. mellonella* larvae to the powdered WHIPS reaches up to 2.49 mg / larva after seven days. The results imply that the physical decrease of the size of the plastics improves the ingestion and mineralization of *G. mellonella* larvae to the WEEE plastics (Ipser et al., 2019). The possible reason for the preference of *G. mellonella* larvae is that small-size WEEE plastics more easily enter the larval gut through their mouthparts. Therefore, changing the physical morphology of some hard or lumpy plastics is necessary, expanding the range of larvae capable of ingesting plastics. However, cumulative consumption of *G. mellonella* larvae to the powdered WHIPS is still lower than that of WPS foamed plastics, suggesting that the physical properties of the WEEE plastics play an essential role in their biodegradation by insect larvae.

3.4 Feeding preference of *G. mellonella* larvae to different plastic combinations

A variety of waste plastics usually coexist in the WEEE. Therefore, it is deserving of investigating the feeding preference of *G. mellonella* larvae in the co-existence of multiple plastics.

As shown in Fig. S4, *G. mellonella* larvae in the plastic-fed groups have SR of 93.75%-97.5% at the end of the experiment, which is higher than the un-fed group (85.0%), but lower than the BW-fed group (98.8%). The results indicate that the larvae have high survival and the plastics are feasible growth substrates for *G. mellonella* larvae. As shown in Fig. 5a, mass losses of the plastics in groups of individual-plastic-fed present a decreasing order as follows: RPU>PF>PE>PP>PS≈PVC. This implies that the consumption by *G. mellonella* larvae is different among the plastics, possibly due to their different physicochemical properties. RPU and PF possess poor abrasion resistance and toughness and contain urethane groups of nitrogen nutrient and hydrophilic hydroxyl groups, respectively, which are easily ingested and biodegradable (Liu et al., 2019; Tang et al., 2020). However, PE, PP, and PS hold stable carbon chain structures and simplified chemical groups, making them challenging to biodegrade by gut microbes in the larvae (Magnin et al., 2020; Peng et al., 2019). Lou et al. (2020) also reported that PS is difficult to degrade, as only a few bacteria and fungi can colonize PS films, or, if they can, they degrade PS at a minimal rate, and the larval consumption of the PS is lower than that of PE. In addition, as shown in Table S1, excessive density (291 kg / m^3) of PVC may prevent the degradation of the larvae (Yang et al., 2018a), and the presence of chlorine in PVC may also produce an adverse effect on the gut microbes in the larvae (Caravelli et al., 2004), leading to the lowest mass loss of all six plastics.

As shown in Fig. 5b, c, and d, the mass loss order of the corresponding plastics in the multiple-plastics-fed is similar to that in the individual-plastic-fed groups, whether they are fed through six-plastic, three-plastic or two-plastic combinations. The results show that the *G. mellonella* larvae randomly ingest all the plastics in the combinations, and have similar feeding preferences to the plastics whether individual-plastic- or multiple-plastics-fed. Lou et al. (2020) also reported that the *G. mellonella* larvae do not only select to ingest BW or bran in the mixture of plastics (PS or PE) and the BW or bran, but the consumption to the preferred food, such as BW , bran or RPU in the mixture materials is the largest in all the ingested food. The results suggest that various plastics are fed to the larvae together, which improves the practical application possibility of biodegradable plastics.

3.5 Feeding preference of two insect larvae (*G. mellonella* vs *T. molitor*) to

WRPU

The above *G. mellonella* larvae show the feeding preference to the different WEEE and pristine plastics, and then *G. mellonella* and *T. molitor* were compared to establish the difference in feeding preference of the insect larvae to plastics. As shown in Fig. 6, the total mass losses of the WEEE and pristine plastics in the *G. mellonella* larvae groups are higher than that in the *T. molitor* larvae groups under their similar total weight. After eight days, the consumption of the plastics by *G. mellonella* larvae is almost unchanged due to the pupation of the larvae. The result indicates that the consumption of the plastics by *G. mellonella* larvae is higher than that by *T. molitor* larvae. It corresponds to Billen et al.'s (2020) findings that the consumption of PS or PE by *G. mellonella* larvae is higher than that of *T. molitor* larvae (mealworms). The possible reason is that *G. mellonella* larvae have a larger volume, higher activity, higher food intake and further-developed gut system (Cassone et al., 2020; Wojda, 2017), leading to their higher survival and diet to the plastics (Matyja et al., 2020). As shown in Fig. S5, the WRPU cubes in the *G. mellonella* larvae group possess many holes and generate some fragments at the end of the experiment, but in the group of *T. molitor* larvae, the cubes are not damaged. The result complements and confirms that *G. mellonella* larvae have a higher ingesting activity to the plastics than *T. molitor* larvae. Both *G. mellonella* and *T. molitor* larvae show the highest consumption of RPU and the lowest consumption of PVC in all the plastics. These results imply that the two larvae groups possibly have similar intestinal microflora. However, some differences between *G. mellonella* and

T. molitor larvae gnawing at the plastics can also be observed. *G. mellonella* larvae consumption preference of the PE is higher than that for the PS, but the *T. molitor* larvae have the opposite results, corresponding to the results of Brandon (2018). Additionally, the *G. mellonella* larvae consumption preference for the PF is much higher than that for the PS, but the consumption preference of the *T. molitor* larvae to the PF and PS is similar. These results indicate that the two larvae groups have different preferences for the plastics, possibly due to their different intestinal microflora, which needs further investigation.

4. Conclusions

G. mellonella larvae exhibit a lower consumption preferences for the WRPU and WPS plastics than corresponding pristine plastics, possibly due to their higher Cl or metal contents in the plastics. SEM and FT-IR analyses of the plastics and the corresponding frass show that the WEEE plastics are ingested and partly degraded by the larvae. *G. mellonella* larvae ingest the powdered WHIPS, but not the lumpy one, implying that a physical decrease of the size of the plastics improves larvae consumption of the WEEE plastics. *G. mellonella* larvae consume more RPU than PF, followed by PE, PP, PS and PVC, whether larvae were individual-plastic- or multiple-plastics-fed, implying that the larvae randomly ingest all the plastics in the presence of multiple plastics, and their consumption difference may result from their specific physical properties and chemical structures. In addition, *G. mellonella* larvae have higher consumption ability and preferences for the WEEE plastics than *T. molitor* larvae. The possible reason is that the *G. mellonella* larvae have higher gnawing activity, a further developed gut system, and distinguished gut microflora. Further investigation of functional characteristics of the larval gut microbe is required to understand the mechanism affecting the feeding preference of the insect larvae to the WEEE plastics.

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Figure

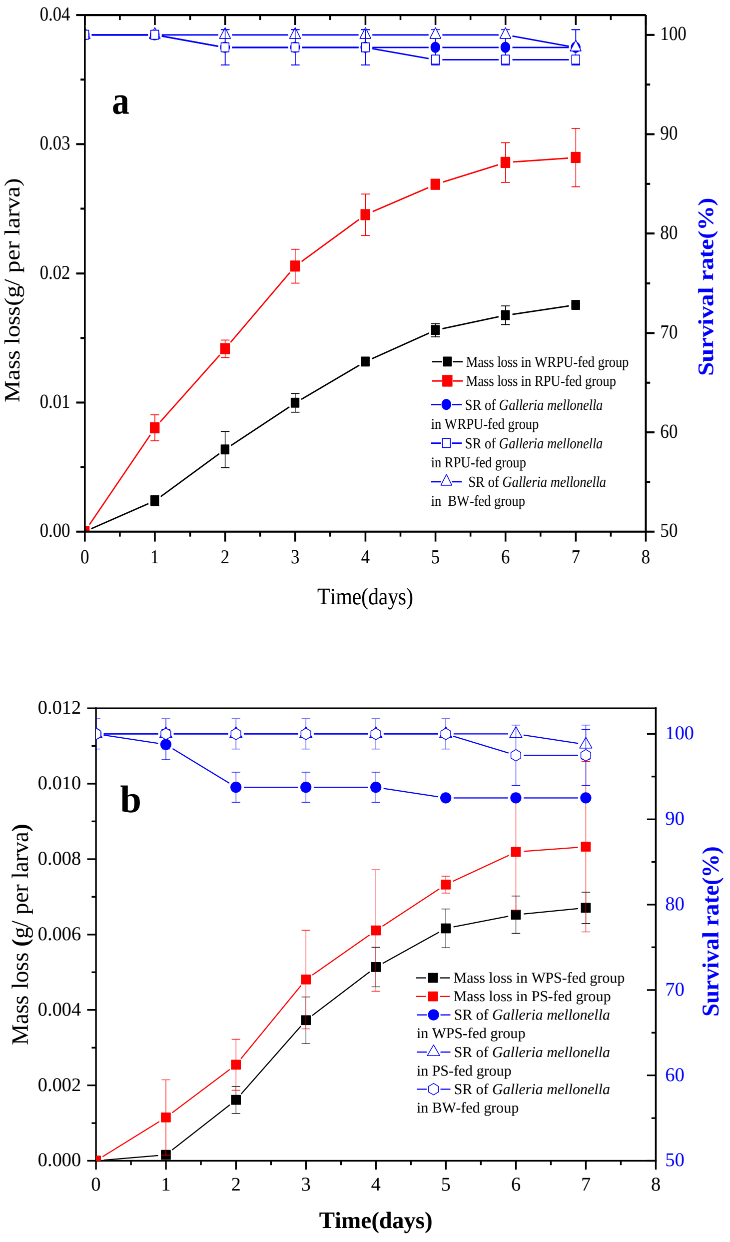
Fig. 1. Cumulative consumption and survival rate of *G. mellonella* larvae fed with WRPU and RPU (a), WPS and PS (b), and BW. WRPU, waste rigid polyurethane; RPU, rigid polyurethane; WPS, waste polystyrene; PS, polystyrene; BW, beeswax.

Fig. 2. Micromorphology of WPU, RPU, WPS, PS, and BW, and the corresponding frass by SEM. a, WRPU feedstock; a’, WRPU fragment; a”, WRPU frass; b and b’, RPU feedstock; b”, RPU fragment; b’”, RPU frass; c, WPS feedstock; c’, WPS fragment; c”, WPS frass; d and d’, PS feedstock; d”, PS fragment; d’”, PS frass; e, BW feedstock; e’, BW frass; WRPU, waste rigid polyurethane; RPU, rigid polyurethane; WPS, waste polystyrene; PS, polystyrene; BW, beeswax;

Fig. 3. FT-IR spectra of WEEE, pristine plastics and BW feedstock and the corresponding frass. a) feedstock and corresponding frass of WRPU, RPU and BW; b) feedstock and corresponding frass of WPS, PS, and BW; WRPU, waste rigid polyurethane; RPU, rigid polyurethane; BW, beeswax; WPS, waste polystyrene; PS, polystyrene.

Fig. 4. Cumulative consumption by *G. mellonella* larvae of the WHIPS and powdered WHIPS. WHIPS, waste high impact polystyrene

Fig. 5. Consumption by *G. mellonella* larvae in the groups with only-one kind (a), (b) six-kinds (b), three kinds (c), two kinds (d) of the plastic. RPU, rigid polyurethane; PF, phenolic resin; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.



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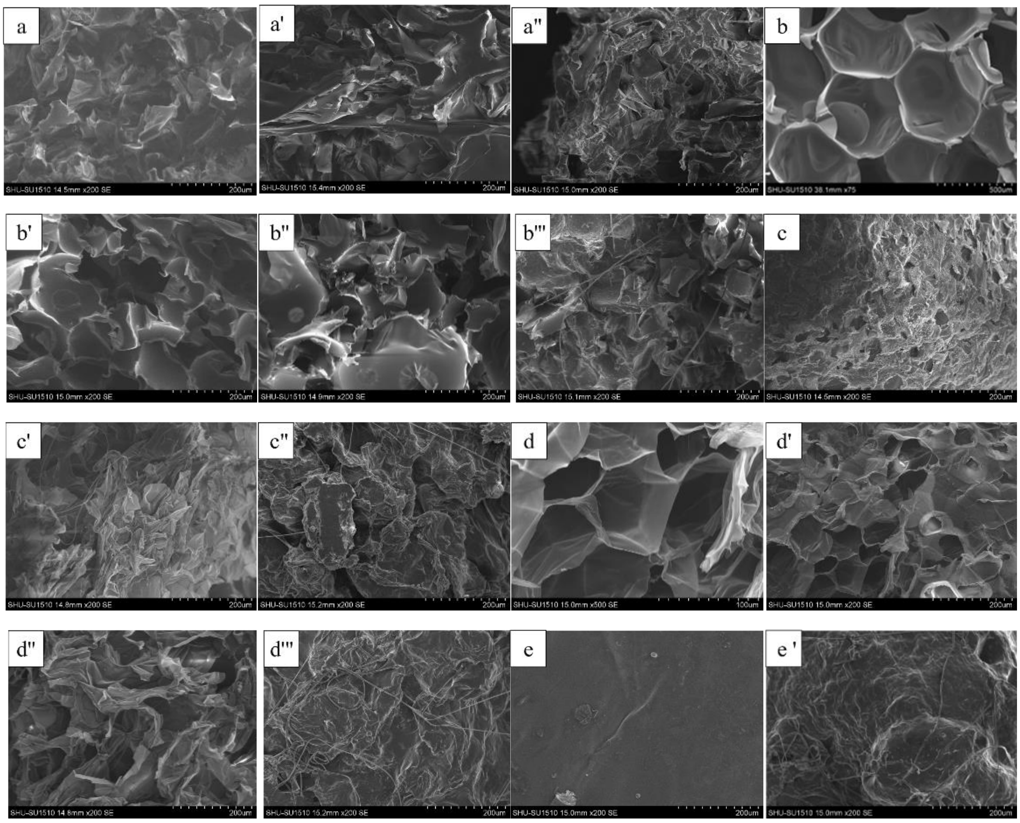

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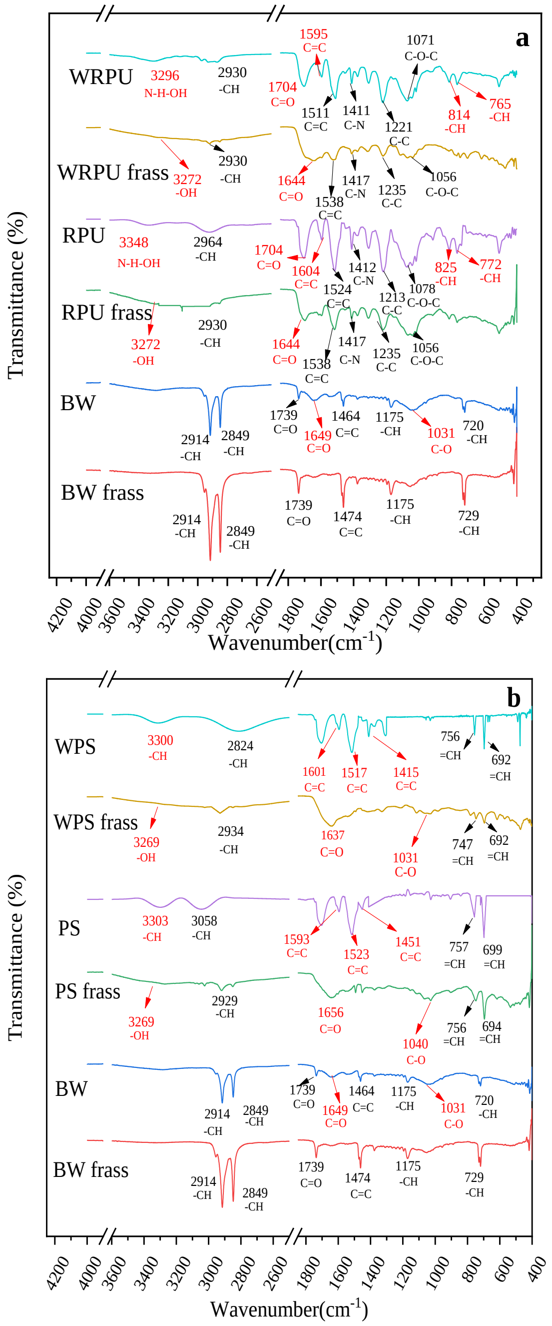

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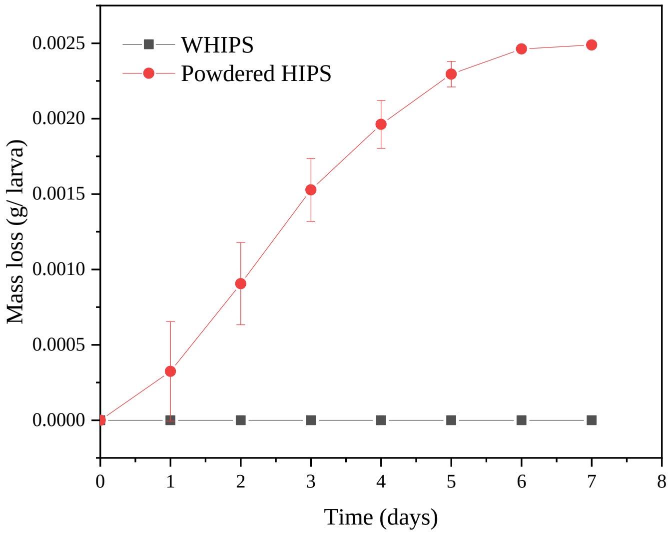


Fig. 4. Cumulative consumption by *G. mellonella* larvae of the WHIPS and powdered WHIPS.

WHIPS, waste high impact polystyrene

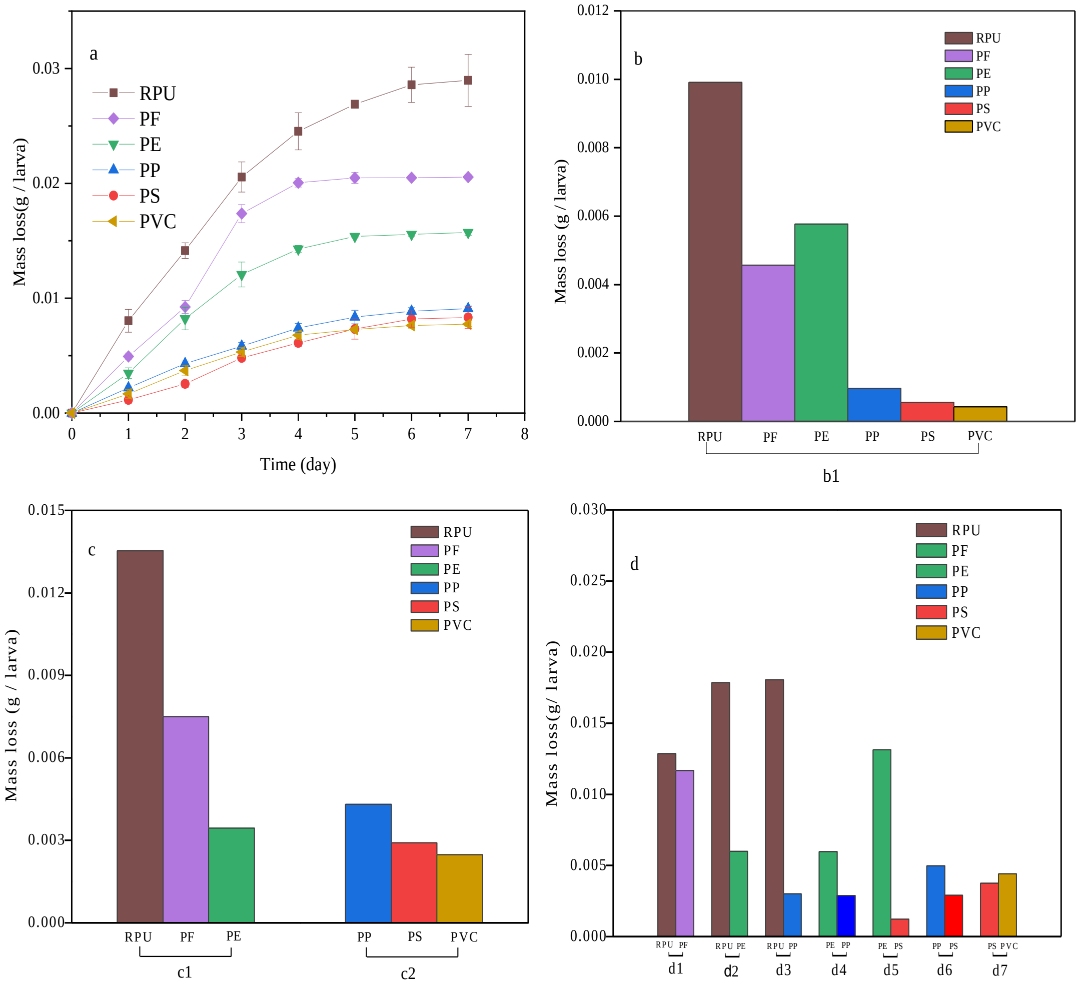


Fig. 5. Consumption by *G. mellonella* larvae in the groups with only-one kind (a), (b) six-kinds (b), three kinds (c), two kinds (d) of the plastic. RPU, rigid polyurethane; PF, phenolic resin; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.

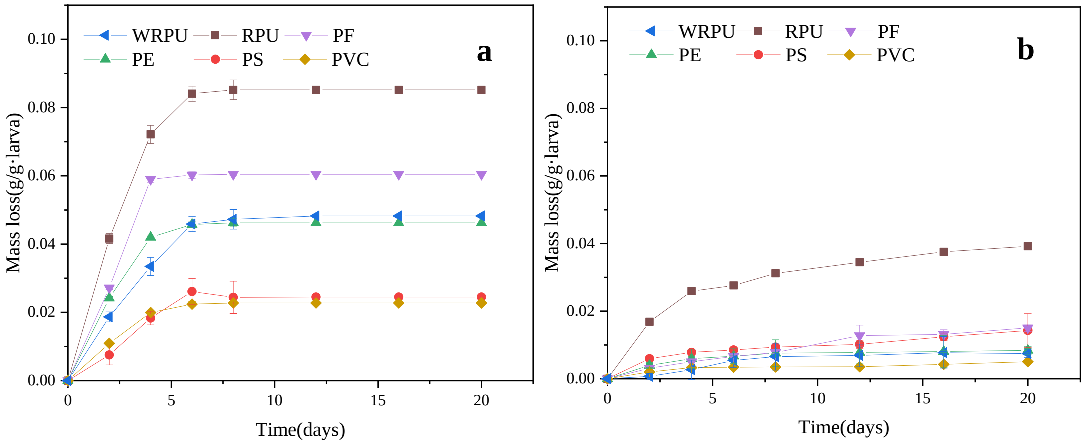


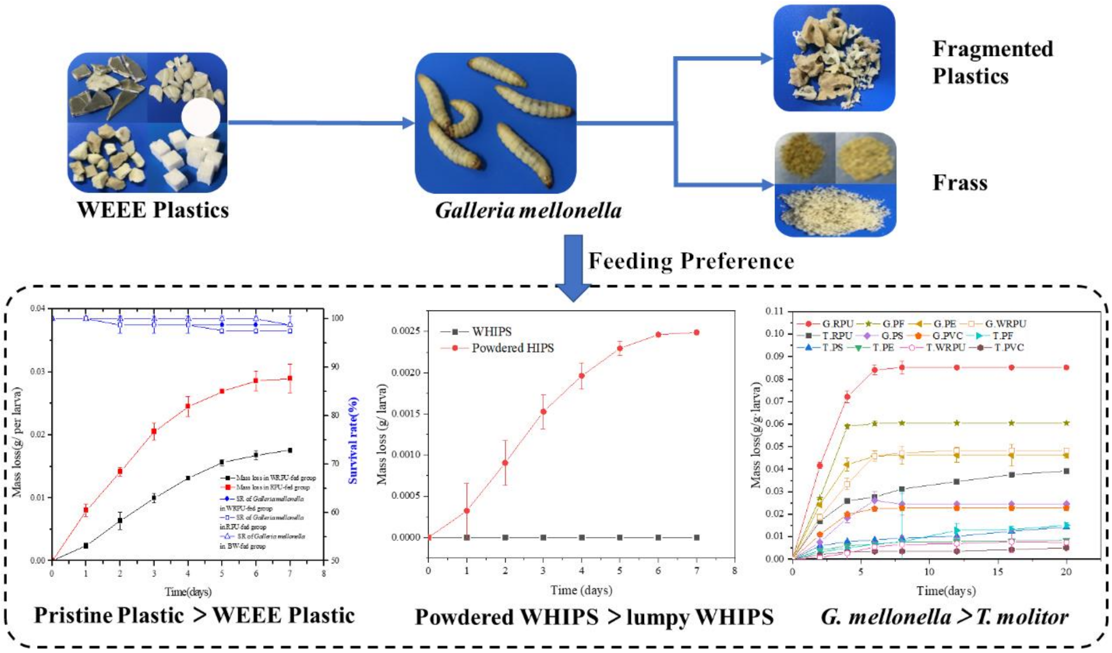
Fig. 6. Cumulative consumption by *G. mellonella* (a) and *T. molitor* (b) larvae of the plastics. WRPU, waste rigid polyurethane; RPU, rigid polyurethane; PF, phenol-formaldehyde resin; PE, polyethylene; PS, polystyrene; PVC, polyvinyl chloride.

Table 1 Elemental composition of the WEEE and corresponding pristine plastics through XRF

Samples	Elemental contents（wt.%）										
	C	N	O	Si	Cl	P	K	Mg	Al	Ca	Fe
WRPU	53.60±1.63	16.47±0.87	24.55±1.41	2.09±0.44	1.59±0.16	0.17±0.00	0.38±0.08	ND	0.32±0.01	1.46±0.18	1.34±0.38
RPU	55.95±2.68	15.65±0.11	24.84±0.88	0.55±0.34	ND	0.18±0.02	0.10±0.00	ND	0.07±0.04	0.49±0.04	0.23±0.05
WPS	71.77±0.48	ND	18.35±0.90	2.34±0.00	0.54±0.00	0.24±0.07	0.16±0.18	0.45±0.11	0.79±0.06	2.38±0.21	1.8±0.00
PS	81.03±3.34	ND	11.04±0.35	1.51±0.25	0.18±0.02	0.13±0.08	0.02±0.02	0.24±0.01	0.15±0.06	0.29±0.03	0.39±0.04

WRPU, waste rigid polyurethane; RPU, rigid polyurethane; WPS, waste polystyrene; PS, polystyrene; ND, not detectable.

Graphical abstract



Highlight

1. *G. mellonella* larvae prefer pristine plastics to corresponding WEEE plastics
2. The additives in WRPU and WPS may affect the ingestion of *G. mellonella* larvae
3. *G. mellonella* larvae ingest powdered WHIPS rather than the lumpy one
4. *G. mellonella* larvae have similar feeding preference in different combinations
5. *G. mellonella* and *T. molitor* larvae have different preference to the plastics