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Partial consumption of medical face masks by a common beetle species

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The widespread distribution of microplastics (MPs) in the environment has motivated research on the ecological significance and fate of these pervasive particles. Recent studies have demonstrated that MPs may not always have negative effects, and in contrast, several species of Tenebrionidae beetles utilized plastic as a food source in controlled laboratory experiments. However, most studies of plastic-eating insects have not been ecologically realistic, and thus it is unclear whether results from these experiments apply more broadly. Here, we quantified the ability of mealworms (Coleoptera: Tenebrionidae) to consume MPs derived from polypropylene and polylactic acid face masks; these are two of the most commonly used conventional and plant-based plastics. To simulate foraging in nature, we mixed MPs with wheat bran to create an environment where beetles were exposed to multiple food types. Mealworms consumed approximately 50% of the MPs, egested a small fraction, and consumption did not affect survival. This study adds to our limited knowledge of the ability of insects to consume MPs. Understory or ground-dwelling insects may hold the key to sustainable plastic disposal strategies, but we caution that research in this field needs to proceed concomitantly with reductions in plastic manufacturing.

1. Introduction

The global reliance on plastics has resulted in plastic pollution in every ecosystem on Earth [1]. Microplastics (MPs), defined as plastic particles smaller than 5 mm, permeate aquatic and terrestrial landscapes and travel through food webs via consumer–resource interactions [2]. The widespread distribution of MPs has spurred research on how these ubiquitous particles affect ecological traits such as organism life history and survival [3–5]. Reviews of empirical studies have concluded that the fitness effects of MPs are organism specific and vary by plastic polymer, shape, and concentration [6].

Researchers have also begun to explore whether organisms can biodegrade plastic particles into non-toxic compounds. Larvae of the moths *Galleria mellonella* and *Plodia interpunctella* (both Lepidoptera: Pyralidae) can degrade polyethylene (PE) [7,8]. Larvae of the beetles *Zophobas atratus* and *Tenebrio molitor* (both Coleoptera: Tenebrionidae) have been shown to consume and metabolize a suite of plastic polymers including polypropylene (PP), polystyrene (PS), polyurethane, polyvinyl chloride, polyethylene terephthalate, as well as the plant-based polylactic acid (PLA) [9–17]. While the exact enzymatic mechanisms underlying plastic degradation by beetle larvae are unclear, shifts in the microbiome in the presence versus absence of plastics suggest an important role of gut microbes [16,18].

Despite the recent influx of studies investigating the ability of insects to digest plastics, several knowledge gaps remain. The two common methods of presenting the plastics to insects are (i) to place large numbers of the larvae

directly onto an intact piece of plastic [13,15,16,19] or (ii) to use pristine MPs of one polymer type purchased directly from a manufacturer [9,14,17]. In nature, insects have a choice of resources to consume and rarely will they be exposed to plastics that have not been modified by additives such as stabilizers, flame retardants, and antioxidants [20]. Thus, experiments that feed insects MPs made from common plastic products, ideally mixed with other food items, are needed to simulate conditions in nature. Additionally, we have limited data on the ability of insects to consume PP or PLA. PP accounts for almost 20% of globally produced plastics [21] and PLA is one of the most common plant-based plastics produced today [22]. Previous studies have demonstrated the ability of *T. molitor* larvae to ingest high-purity forms of PLA [18] and PP [9], but studies on the digestibility of PP- or PLA-based commonly manufactured products are lacking. Here, we test the ability of *T. molitor* to digest PP and PLA-based medical face masks when mixed in a matrix of a known food type (wheat bran).

2. Methods

(a) Study organisms and rearing conditions

In nature, mealworms (*Tenebrio molitor* Linnaeus) are scavengers and decomposers, found in decaying forest vegetation, primarily eat fungi, and are thought to go through one generation per year [23,24]. In laboratory conditions, mealworms can go through 8–22 instars before pupating and take < 1 year to almost 2 years to develop from egg to adult [23]. Mealworms are also highly resistant to starvation. Cotton & St. George [23] reported that out of 50 mealworm larvae, some were still alive six to eight months later despite receiving no food or water. Mealworms for this study were purchased from a pet supply store (Noah's Pet Ark, Vancouver, Canada). All mealworms were maintained on a diet of 100% wheat bran at the store and at the supplier (Noah's Pet Ark, personal communication). All mealworms were approximately 2 cm long (approx. 60 mg) and after purchasing, were maintained in the laboratory on Rogers Food brand (Rogers, British Columbia) wheat bran until the start of the experiment. Although the exact instar of the larvae is unknown (estimated: 4–5 instar), both experimental and non-experimental larvae pupated after two additional moults. Mealworm size and rearing conditions used here follow those used by studies that tested the ability of the same species to consume other types of plastics [10,12,18,19].

(b) Source of microplastics

We used VIRALOC eco and VIRALOC face masks (PADM Medical; Winnipeg, Canada) to make PP and PLA MPs. We tested the composition of both mask types using NMR spectroscopy. The main polymer in the VIRALOC mask was confirmed to be PP (electronic supplementary material, figure S1a) and that of the VIRALOC eco mask was PLA (electronic supplementary material, figure S1b).

We created MPs by melting the face masks (figure 1*a*,*b*) in a vented Fisher brand laboratory oven at approximately 180°C and grinding the cooled melted face masks using a heavy-duty spice grinder (LeiJieYin Brand, model 750). We removed the ear loops and the metal nose piece before melting. We also trimmed approximately 1 cm from both short edges of the face masks and unfolded the pleats before melting. We sieved the ground mask particles through a 250 µm metal sieve and kept the particles smaller than 250 µm (figure 1*c*).

(c) Mealworm food

We created a food mixture consisting of wheat bran, MPs and gelatin [18]. For each plastic type, we first mixed 1 g ground plastic with 9 g wheat bran. We prepared Knox brand unflavoured gelatin (ED Smith Foods, Ontario) according to the package instructions and poured 25–30 ml of the liquid gelatin into each bran–plastic dry mix. This amount of gelatin created a thickened and relatively homogeneous mixture of MPs and bran. We mixed the solutions and divided each MP–bran mixture evenly into twelve 250 ml glass beakers (figure 1*d*). We placed 20 mealworms into each of eight Bran + PP beakers and 20 mealworms into each of eight Bran + PLA beakers. We left four beakers of each mixture free of mealworms. We also made a mixture containing 10 g bran and approximately 2 ml gelatin. Two millilitres of gelatin were needed to turn the bran-only treatment into a mixture of similar consistency to the Bran + PP or Bran + PLA mixtures. We divided this bran-only treatment relatively evenly into eight beakers and added 20 mealworms to each beaker. Treatments, replicates and mealworm numbers are summarized in table 1. All beakers were kept at 22°C and in 24 h dark in a Panasonic MIR−254 incubator.

We quantified the number of MPs per milligram of food mixture (table 1) by dissolving the food mixtures from the eight mealworm-free beakers (four Bran + PP, four Bran + PLA) at the end of the experiment. These treatments did not receive any mealworms, and thus no MPs were consumed. We added 6 ml tap water to each beaker and gently mixed the food mixture until it dissolved. We poured the dissolved food mixture into a Borogov counting chamber and counted the number of MPs under a dissecting microscope (Zeiss Stemi 508, Oberkochen, Germany).

(d) Data collected

Throughout the experiment, we recorded the number of surviving individuals (mealworms and pupae), the weight of the food mixture and the weight of the surviving larvae, every 2–3 days. We removed pupae from the beakers to prevent cannibalism by larvae. By day 21, the majority of the food mixture had been consumed. Some beakers contained small hardened pieces of

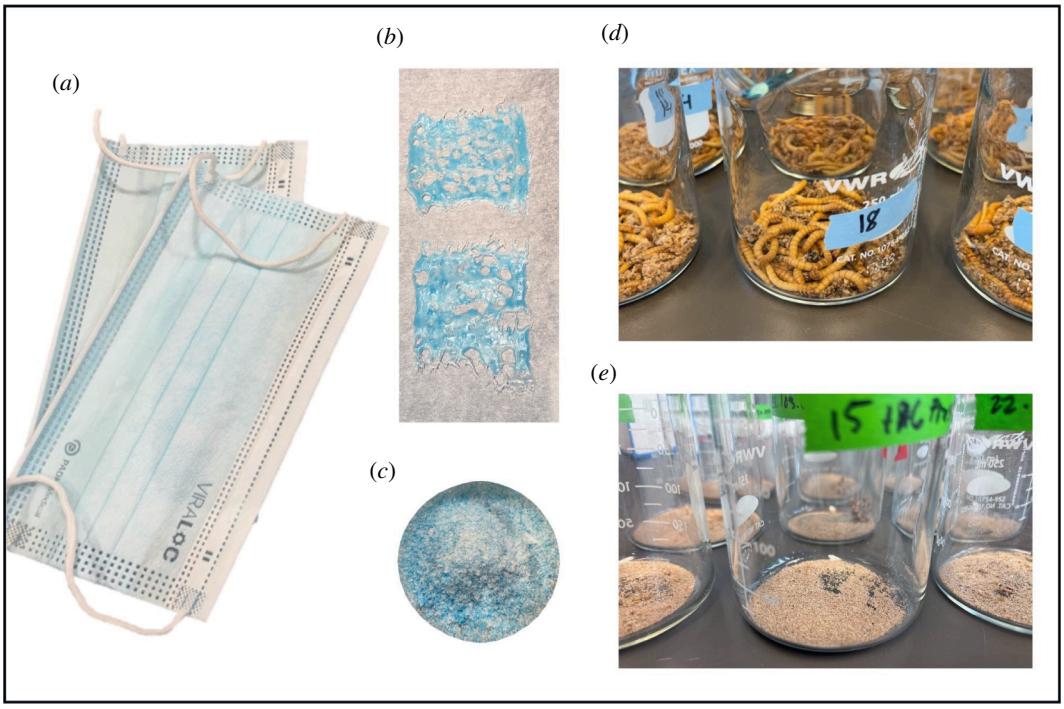

Figure 1. Photos of (*a*) the polypropylene (PP) face masks used in the study, (*b*) two individual face masks after melting and (*c*) multiple face masks after melting and grinding. (*d*) Twenty mealworms were placed in each replicate 250 ml beaker and fed a mixture of face mask and wheat bran, or just wheat bran. After most of the food mixture was eaten, (*e*) mealworms were frozen and mealworm frass (shown in the photo) was dissolved and searched for egested microplastics.

Table 1. Summary of treatments and replicates. MW, mealworm; PLA, polylactic acid; PP, polypropylene.

treatment	number of replicates	starting number of mealworms per beaker	average plastic particles per milligram food mixture at the beginning of the study
Bran + MW	8	20	0
Bran + PLA	4	0	5.2
Bran + PLA + MW	8	20	5.2
Bran + PP	4	0	5.4
Bran + PP + MW	8	20	5.4

food mixture that had remained untouched for several days, and thus we assumed these pieces were inedible. On day 23, after counting all individuals, we left the mealworms in the beakers for approximately one additional week to egest any materials remaining in their digestive tract [25]. On day 30, we removed and froze all mealworms and left the frass in the beaker to quantify any egested MPs (see §2e).

To quantify the number of MPs in the Bran + PP/PLA + MW treatments at the beginning of the experiment, we first calculated the weight loss of the food mixture due to evaporation. To do this, we quantified the weight change of the food mixture from treatments that did not contain mealworms. At the end of the experiment, the Bran + PP treatment was 30 ± 1% (mean ± standard deviation) of its original weight and the Bran + PLA treatment was 29 ± 1% of its original weight. Because the evaporation rate was similar between these two treatments, we used 0.295 to correct for weight loss due to evaporation in the Bran + PP/PLA + MW treatments.

We calculated the number of starting MPs per beaker in the Bran + PP/PLA + MW treatments using the following formula: (mixture weight (mg) on day 1) × (0.295) × (average MPs of PP or PLA per mg) (table 1). We calculated the number of MPs left in any uneaten food mixture using: (mixture weight (mg) on day 23) × (average number of PP or PLA per mg). We calculated the total number of MPs consumed per mealworm using: ((starting number of MPs – final number of MPs)/average number of mealworms in the 23 day experiment). Finally, we calculated the per cent of MPs consumed as: ((final number of MPs/initial number of MPs) × 100).

(e) Particles excreted

To quantify approximately how many MPs were excreted in mealworm frass (figure 1*e*), we dissolved 100 mg of frass from each beaker in 2.5 ml tap water. We counted the MPs using the same method used to count MPs in the food mixtures. Peng *et al.* [25] reported that it took mealworms approximately 4 days to excrete all frass (and plastics) from the digestive tract. Thus, no frass likely remained in the mealworm digestive tracts when we began counting MPs from the frass (experiment day 30, 7 days post cessation of feeding). We did not examine whether MPs were found inside mealworms.

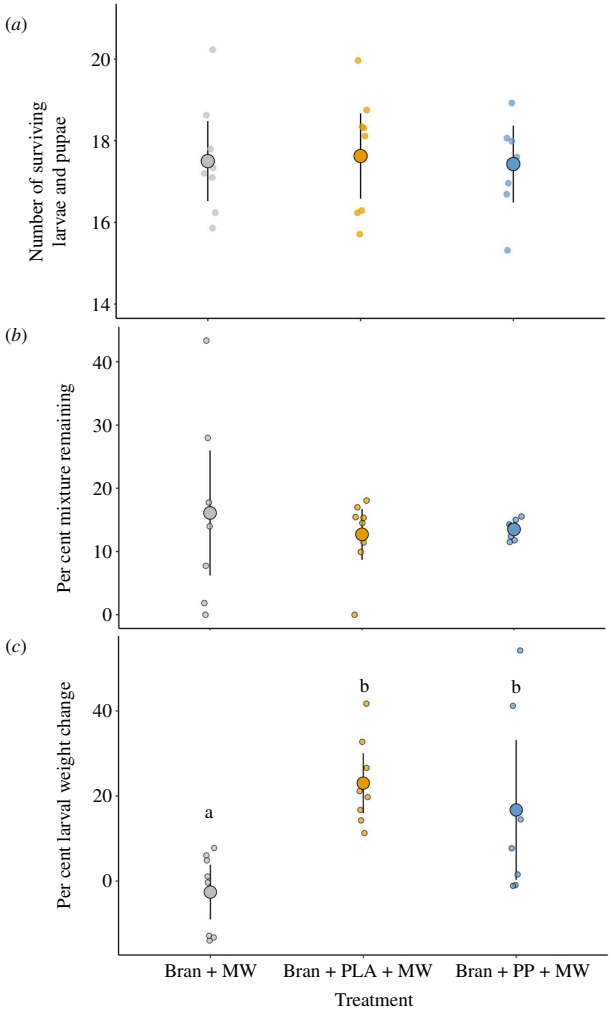

Figure 2. (*a*) The number of surviving mealworms and pupae, (*b*) per cent food mixture remaining at the end of the experiment and (*c*) average per cent weight change per larvae (per beaker) from the beginning to the end of the experiment. Letters denote significant differences from Tukey HSD post hoc tests between treatment groups. For all figures, larger coloured dots denote the mean treatment value. Black lines extending from the mean represent the 95% confidence interval. Smaller dots are the raw data points. Treatments are colour-coded: grey = Bran + MW; orange = Bran + PLA + MW, and blue = Bran + PP + MW. MW, mealworm; PLA, polylactic acid; PP, polypropylene.

(f) Data analysis

In total, we assessed the following traits: (i) survival (number of remaining live mealworms on day 23 + total number of pupae produced); (ii) number of pupae produced; (iii) per cent food mixture remaining (food weight on day 23/weight on day 1 × 100, accounting for evaporation); (iv) per cent change in average larval weight per beaker ((final average weight per larva – initial average weight per larva)/initial average weight per larvae × 100); (v) MPs consumed per mealworm; (vi) per cent MPs consumed overall; and (vii) MPs egested per mg frass. We used analysis of variance (ANOVA) to examine whether there was an effect of plastic treatment (levels: no plastic, PLA masks, PP masks) on mealworm survival, the number of pupae produced, and the average per cent weight change per larva (per beaker). The effect of plastic treatment on 'per cent food mixture remaining' was analysed using a Kruskal–Wallis non-parametric test because the variances among treatments were unequal. We used ANOVA to examine whether the number of MPs consumed per mealworm, the per cent MPs consumed, and the number of MPs egested differed between the plastic types (levels: PLA, PP). All treatments and replicates per treatment are listed in table 1. For tests where we used ANOVA, we used residual plots and q–q plots to check that the data met the assumptions of ANOVA. Homogeneity of variances was tested using Levene's test [26]. No data were transformed. Tukey post hoc tests were conducted when necessary. All analyses were conducted in R v. 4.3.0 [27], and raw data are available in Dryad [28]. Analyses with p values < 0.05 were considered to be statistically significant.

3. Results

The number of surviving *T. molitor* (larvae + pupae) did not differ among food treatments (figure 2*a*; $F_{2,20} = 0.04$, $p = 0.96$). The average number of survivors across all treatments was 17.5 (87.6%) ± 1.34 s.d. There was no difference in the number of pupae produced among treatments ($F_{2,20} = 0.5$, $p = 0.61$). There was also no overall difference in the per cent of food mixture remaining among the three treatments (figure 2*b*, Kruskal–Wallis $\chi^2 = 0.19$, d.f. = 2, $p = 0.9$; avg. food remaining: 14% ± 0.09 s.d.). Larvae that

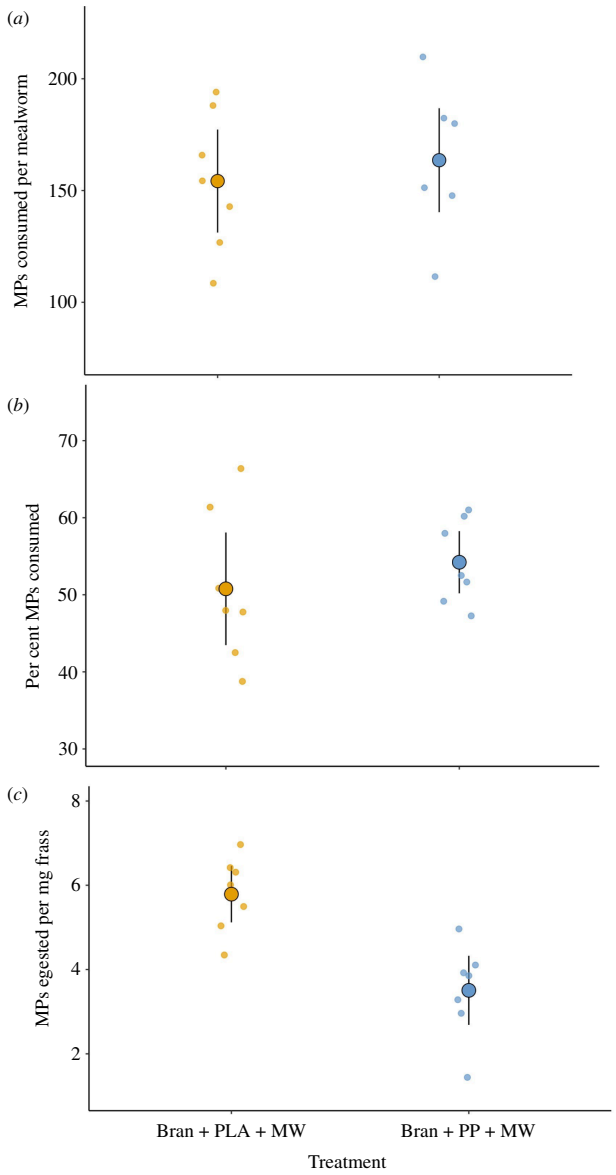

Figure 3. (*a*) The number of microplastics (MPs) consumed per mealworm; (*b*) per cent MPs consumed by the end of the study and (*c*) per cent MPs egested per mg frass. Larger coloured dots denote the mean treatment value. Black lines extending from the mean represent the 95% confidence interval. Smaller dots are the raw data points. Treatments are colour-coded: orange = Bran + PLA + MW, and blue = Bran + PP + MW. MW, mealworm; PLA, polylactic acid; PP, polypropylene.

consumed the PLA or PP diets gained significantly more weight than larvae that consumed just bran (figure 2*c*; $F_{2,20} = 6.6$, $p = 0.005$; Tukey HSD: PLA versus Bran $p = 0.006$, PP versus Bran $p = 0.048$, PLA versus PP $p = 0.69$).

Mealworms consumed similar numbers and percentages of PP or PLA MPs (MP number: figure 3*a*; $F_{1,12} = 0.31$, $p = 0.59$; overall mean ± s.d. = 158.9 ± 30.4; particle percentage: figure 3*b*; $F_{1,12} = 0.66$, $p = 0.43$, overall mean ± s.d. = 52.5% ± 7.9). Mealworms fed the PP food mixture egested approximately 40% fewer MPs versus those fed the PLA food mixture (figure 2*c*; $F_{1,12} = 0.17.86$, $p = 0.001$).

4. Discussion

Mealworms consumed approximately 53% of the MPs found in their food mixtures. The consumption of plastic particles led to increased mealworm weight gain and did not appear to affect development or survival. There was no difference in the consumption rate of PP versus PLA. Each treatment started with 1 g of finely ground (< 250 µm) PP or PLA divided among eight replicate beakers. Using this starting weight, we determined that throughout the experiment (approx. 20 days), 160 mealworms consumed 530 mg of plastic, equivalent to 0.165 mg PP or PLA per individual mealworm per day. For PLA, this consumption rate is lower than the feeding rates of 0.625 and 12 mg per larva per day reported by previous studies [17,18]. Both of these previous studies used pure PLA powder (Zhonglian Petrochemical Co.) and different bran-to-plastic ratios than those used here. Although further testing is needed, it is possible that the lower PLA consumption rate observed here was due to the use of PLA from a face mask rather than pure PLA powder directly sourced from a manufacturer.

The PP feeding rate observed in this study (0.165 mg PP per individual mealworm per day) was similar to the 0.13 mg PP per individual mealworm per day rate reported in a recent study [9]. However, other previous studies have demonstrated lower

consumption rates (0–0.05 mg per individual per day) of PP by mealworms [11,15,29]. It is unclear why there is considerable variation in PP consumption rate among studies. In a related study, Wang *et al*. [16] tested the ability of mealworms to consume the three constituent layers of face masks. The consumption rate of the outer, middle and inner layers was 0.001, 0.03 and 0.015 mg per individual mealworm per day, respectively. All PP studies reviewed here did not use a co-diet and mealworms were presented with only the PP product, although [9] also mixed pure PP with agar. Several studies have tested the ability of mealworms to consume PS foam or blocks. The feeding rates observed in the PS studies range from 0.05 to 0.24 mg per individual mealworm per day (12,13,15,19,30]).

We found MPs in *T. molitor* frass, demonstrating that although particles were ingested, a small fraction was egested. We hesitate to make strong conclusions about egestion rates because we did not use spectroscopy to examine the molecular weights of the egested plastic particles. A change in molecular weight may indicate that the plastic was partially degraded [11] and thus our counts would potentially overestimate the number of MPs in frass. *Tenebrio molitor* frass production is approximately 0.5 $g\,g^{-1}$ of food ingested [17,18,31]. Using this rate, the net plastic consumption rate of PP-based face masks is approximately 39.6% and that for PLA-based face masks is approximately 22.6%. It is also possible that egested plastics were too small to see under the microscope (< 50 µm). Previous studies observed 2–5 µm sized particles of PS and LDPE (low-density PE) in mealworm frass and concluded that mealworms both biodegraded and biofragmented plastics [32]. Lastly, we also did not dissect mealworms to examine how many MPs, if any, remained inside the insect. There were no remaining plastic particles in mealworms 4 days post cessation of plastic feeding in a previously published study [25], suggesting that our 7 day wait period was likely sufficient for mealworms to fully empty their gut contents.

Our study demonstrates that *T. molitor* larvae can consume PP and PLA MPs made from medical face masks. Our study is unique because we assessed whether *T. molitor* consumed plastics derived from a commonly used plastic product and when combined with a wheat-based diet. We suggest future studies explore whether closely related beetles or those found in similar ecological niches can also consume plastic particles. Tens of thousands of beetle species live in the understory or belowground, and these ecosystems accumulate large quantities of MPs [33]. Although it is unrealistic to depend on insects to solve the global plastic pollution crisis, we advocate for greater collaboration between ecologists and plastics engineers to widen the diversity of insect species assayed and to increase the ecological relevance of plastic ingestion assays. Understanding the mechanisms underlying plastic biodegradation by insects may reveal novel approaches for sustainable plastic manufacturing or disposal. Despite the potential for insects to aid in the global plastic pollution crisis, by our calculations, it would take 100 mealworms 138 days (approx. 4.5 months) to consume one face mask (approx. 2270 mg). During the COVID-19 pandemic, over 2 billion medical face masks were used daily in Asia alone [34]. The quest for sustainable plastic degradation strategies must go hand-in-hand with reductions in plastic manufacturing.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. All data are available from the Dryad Digital Repository [28].

Supplementary material is available online [35].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. S.G.: investigation, methodology, writing—review and editing; A.D.: conceptualization, methodology, writing—review and editing; N.K.: methodology, writing—review and editing; M.T.: conceptualization, formal analysis, funding acquisition, project administration, writing—original draft.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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