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Effects of microplastics and salinity on food waste processing by black soldier fly (*Hermetia illucens*) larvae

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Abstract

Background: The black soldier fly (*Hermetia illucens*) is gaining attention as an efficient decomposer of food waste. However, recalcitrant compounds such as plastics mixed into food waste may have negative effects on its growth and survival. Moreover, its efficiency of food waste degradation may also be affected by plastics. In addition, salt (NaCl) can also be present in high concentrations, which also reduces the efficiency of *H. illucens*-mediated food waste treatment. In this study, we assessed the growth of black soldier fly larvae (BSFL) reared on food waste containing polyethylene (PE) and polystyrene (PS) and NaCl. The weight of BSFL was measured every 2–4 days. Survival and substrate reduction rates and pupation ratio were determined at the end of the experiment.

Results: The total larval weight of *Hermetia illucens* reared on food waste containing PS was greater than that of the control on days 20 and 24. However, the survival rate was lower in the group treated with 5% PS, as was substrate reduction in all PS-treated groups. The weight of BSFL reared on food waste containing PE was lower than that of the control on day 6. PE in food waste did not affect the survival rate, but the pupation ratio increased and substrate consumption decreased with increasing PE concentrations. Regardless of the plastic type, the addition of NaCl resulted in decreased larval weight and pupation ratio.

Conclusions: Larval growth of black soldier fly was inhibited not by plastics but by substrate salinity. Additional safety assessments of larvae reared on food waste containing impurities are needed to enable wider application of BSFL in vermicomposting.

Keywords: *Hermetia illucens*, Waste management, Food waste, Decomposition ecology, Organic waste, Microplastics, Salinity, Polystyrene, Polyethylene

Background

The amount of food waste generated has increased as human population has risen, and this is considered a global issue that may threaten ecosystems and human health (Göbel et al. 2015). Many waste treatment methods such as landfill aeration and incineration are currently used, but these contaminate the environment by emitting harmful gases (Yang et al. 2013; Liu et al. 2017). Ocean dumping is another contributor to pollution of marine ecosystems (Choi et al. 2009) and thus it was prohibited in South Korea as of 2012 under the London convention on the Prevention of Marine

Pollution by Dumping of Wastes and Other Matter (Min and Rhee 2014). In addition, landfilling of organic waste was banned in the EU as of 2005 (Davidsson et al. 2008). As an alternative to disposal using these conventional methods, food waste can be recycled into valuable products such as compost and animal feed (Ahn et al. 2019). Although food waste has been regarded as an attractive resource for recycling due to its fermentable and environmentally safe nature (Marchettini et al. 2007), release of wastewater during the recycling process and high maintenance costs and energy use are major obstacles (Park et al. 2018; Kim et al. 2012). Therefore, there is an urgent need for the development of sustainable waste treatment technologies.

Vermicomposting has been gaining attention as a practical and attractive alternative for treatment of food

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waste (Elissen 2007; Lim et al. 2016). Among the various insects used in vermicomposting, the black soldier fly (*Hermetia illucens*) is regarded as an effective converter of organic wastes including food waste. It has been reported that black soldier fly larvae (BSFL) effectively decomposes food waste because of its strong mouthparts and relatively high gut enzymatic activity compared to other fly species (Kim et al. 2011; Tomberlin et al. 2002). Furthermore, pupae of *H. illucens* is composed of approximately 40% protein, 30% fat, and 9% chitin, and thus can be subsequently used as an animal feed (Newton et al. 2005; Newton et al. 2008; Cummins Jr et al. 2017; St-Hilaire et al. 2007). In addition, Wang and Shelomi (2017) reported that pupae of black soldier fly are suitable for use as animal feedstuff as they do not accumulate pesticides and mycotoxins. BSFL can also be used as nutritional supplements for animals and other valuable sources (Newton et al. 1997; Li et al. 2011a; Li et al. 2011b).

Although organic fractions are the main constituents of food waste, inorganic materials such as salt (NaCl) can be present in high concentrations and this can reduce the efficiency of food waste treatment mediated by BSFL (Kwon and Kim 2016). Considering that food waste generated in South Korea have salinity of approximately 0.7 to 1% (Hong et al. 2006; Lee et al. 2005; Park 2012), it is important to thoroughly determine the effects of salinity on the growth and performance of BSFL.

In addition to salinity in food waste, microplastics that can enter the food waste stream through various pathways may also lower the efficiency of food waste treatment by BSFL. It has been reported that microplastics, which are defined as plastic fragments that are less than 5 mm in length, are highly persistent contaminants and may inhibit the survival and development of organisms (Huerta Lwanga et al. 2016; Moore 2008; Ziajahromi et al. 2018; Al-Jaibachi et al. 2019; Herrero et al. 2015). When discarded into the environment, microplastics can cause detrimental effects to insects, such as malformation and inhibition of metabolism and reproductive function (Lee et al. 2013; Von Moos et al. 2012; Wright et al. 2013). More specifically, studies have demonstrated that polyethylene (PE) microplastics adversely affect survival, growth, and adult emergence in dipteran flies of the genus *Chironomus* (Ziajahromi et al. 2018; Silva et al. 2019). Conversely, it was also reported that microplastics had no significant effects on mortality and growth in the *Culex* mosquito (Al-Jaibachi et al. 2019). On the other hand, the effects of microplastics on BSFL growth are largely unknown. Therefore, it is imperative that we study the impact of microplastics and salinity as potential limiting factors on BSFL for its use in food waste treatment.

In this study, we determined the effects of microplastics and salinity in food waste decomposed by BSFL. The larvae and prepupae of *H. illucens* are generally used as a decomposer and later an animal feed (Diener et al. 2011), respectively, and thus parameters such as survival rates were examined in the larval stage. Among diverse types of plastics, we examined the effects of PE and polystyrene (PS) microplastics because of their large production and prevalence. The effects of PE and PS microplastics and NaCl on the development and decomposition efficiency of food waste by BSFL were investigated by rearing BSFL in food waste to which PE and PS in powder forms and NaCl have been added, and determining the weight gain, growth in length, survival rate, pupation ratio, and substrate reduction rate of BSFL.

Methods

Pretreatment of food waste

Food waste from meals was collected from several schools for 7 days and homogenized. The mixture was dried and ground using food-waste processing machine (SP-PUW100, Pyeng Kang Bio IT Mechatronics Co., Ltd., Iksan, Korea). The resulting powdered food waste mixture was sieved through a 2-mm sieve. Salinity data were obtained using 5 g samples of food waste ($n = 3$) mixed with water and allowed to settle at 25 °C for 24 h. The supernatant was separated and its salinity was measured using a salinity meter (SB-2000 Pro, HM DIGITAL Inc., Seoul, Korea). The salinity of the food waste was 0.13%. The moisture content of the food waste was adjusted to 60% for feeding trials.

BSFL

Two- to three-day-old BSFL were purchased from Entomo Co., Ltd. (Cheongju, Korea) and used in our study. The insects were hatched at the company and fed there for 2 to 3 days on liquid feed containing crude protein 3.75%, crude fat 3.33%, crude fiber 0.64%, and crude ash 1.65%. Approximately 3000 instar larvae were transported to the lab in a refrigerated box at 4 °C. During transport and after arrival, they were starved for about 24 h to minimize the effect of their pre-experimental diet.

Installation of growth chamber and cages

The growth chamber for insect breeding was made of acrylic plates (700 mm × 500 mm × 450 mm). As shown in Fig. 1a, a fan (DWV-10DRB, Korea) with a hole on the opposite side was installed to ventilate the chamber. To enable the movement of experimental equipment in and out of the chamber, a door (370 mm × 450 mm) was installed. A water bath (DH-WCB00106 Circulation Bath Water) was used to maintain the temperature of the chamber. Its hose was connected and fixed to the

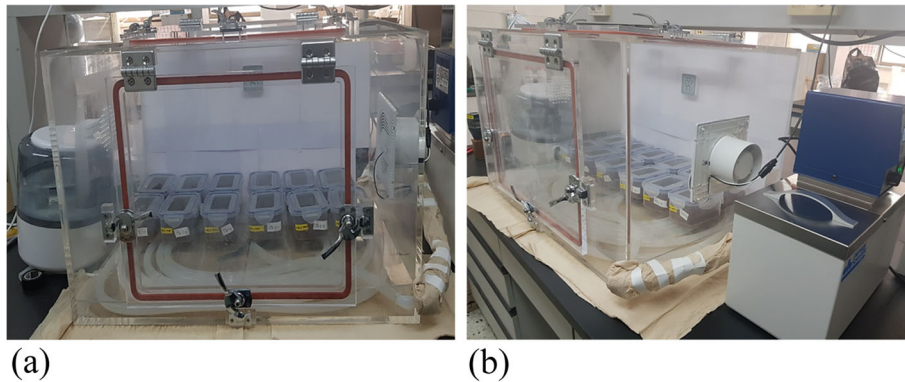


Fig. 1 Growth chamber used for rearing BSFL on food waste treated with PS and PE microplastics and NaCl. **a** Front and **b** side view of acrylic growth chamber (700 × 500 × 450 mm) in which small lightweight plastic containers (135 mm × 102 mm × 68 mm) were placed

bottom of the chamber with a cable tie. Air temperature and humidity were monitored using a thermo-hygrometer (MHO-C201, China) installed on the wall of the chamber. The temperature at the chamber base was measured using an infrared thermometer (Sunche-DT8380H). The chamber air temperature and humidity during the experiment were maintained between 27 and 28 °C and 50% or greater, respectively. The temperature of the base of the chamber was maintained between 28 and 29 °C. Small, lightweight plastic containers (135 mm × 102 mm × 68 mm) were used as breeding cages, as shown in Fig. 1a, b. Each lid had a rectangular opening to allow air to move out of the cage and was screened to prevent the larvae from escaping.

Plastics

PE and PS are the primary constituents of disposable bags and containers, and as they are widely used in food packaging (Pascall et al. 2005), they can occur in food waste. Additionally, these plastics are fragmented into micro-size during food waste processing (Rist et al. 2018). It is well known that microplastics that are 10–500 µm in size are major source of plastic pollution (Wu et al. 2017). Hence, 400–500 µm microplastics were used in our study. More specifically, powder forms of PS (HILENE, Korea) and low-density PE (Fisher Scientific, USA) that are 500 and 400 µm in diameter, respectively, were used.

Experimental design

PS and PE powders, as specified above, were each mixed with 80 g food waste at 5%, 10%, and 20% (all w/w). These concentrations that fall within a broad range were chosen for our study because there are no previous studies that have examined the effects of microplastics in food waste on the growth of BSFL, and thus the response to a wide range of microplastic concentration needs to be determined. The control treatment consisted

of 80 g food waste without the addition of plastics. Each experiment consisted of an experimental cage containing treatment or control feed and 100 larvae, carried out in three replicates. The larval weight of *H. illucens* was measured, as described below, for 24 days. Water was supplied every 2 to 3 days to prevent the cage from drying out. Larval survival rate, pupation ratio, and substrate reduction were measured at the end of the experiment.

In addition, as food waste may have a high salt content, further treatments consisting of 20% w/w PE or PS plus 1%, 2%, or 3% NaCl were evaluated. NaCl concentration higher than 4% has been reported to increase the mortality of BSFL (Kwon and Kim 2016), and thus NaCl concentration only up to 3% was treated to the food waste in our study. BSFL reared on food wastes containing 20% PE or PS was used as the control for this series of experiments. Conditions and measurement protocol were the same as those for the main series described above. All measurements in both series were carried out in triplicate.

Measurement of growth rate, survival rate, and pupation ratio

Larval weight

A total of 10 BSFL were randomly selected from each cage every 2 to 4 days. The food waste residues on the bodies of these larvae were removed by thoroughly rinsing them with distilled water and drying on paper towels. Subsequently, the larvae were weighed and returned to the appropriate cages. The mean weight for each experiment was calculated.

Survival rate

The survival rate was determined for each trial according to the following formula.

$$\text{Survival rate of BSFL} = \frac{\text{Survivors}}{\text{Sample}} \times 100$$

Sample : number of larvae used

Survivors : number of remaining larvae and prepupae

Pupation ratio

A count of pupae surviving each experiment was performed; then, the pupation ratio was calculated using the following formula.

$$\text{Pupationratio}(\%) = \frac{\text{Prepupae}}{\text{Survivors}} \times 100$$

Survivors : number of remaining larvae and prepupae

Prepupae : number of prepupae

Substrate reduction rate

The substrate reduction rate was calculated using the following formula:

$$\frac{W_1 - W_2}{W_1} \times 100$$

W_1 : amount (g) of substrate at the start of trial

W_2 : amount (g) of substrate at the end of trial

Statistical analyses

Statistical analyses were carried out using SAS version 9.4 (SAS Inc., USA) to test the effects of treatments on the growth of BSFL. One-way analysis of variance (ANOVA) was used to evaluate the effect of treatments on larval weight, survival rate, pupation ratio, and substrate reduction. Significant differences of treatment groups were accepted at $\alpha = 0.05$. Where the effects were statistically significant, Tukey's honest significant difference test was conducted to determine which means differed within a group ($p < 0.05$).

Results

Larval growth on food waste containing PS and PE

Figure 2a shows the weight of BSFL reared on the PS-containing food waste substrates PS20% (20% w/w PS added), PS10% (10% added), and PS5% (5% added). The weight of BSFL reared on PS20% was 17% greater ($p = 0.0298$) on day 20 and 11% greater ($p = 0.0250$) on day 24 compared to the control. In addition, on day 24, the weight of BSFL reared on PS10% was 11% greater than that of the control. Figure 2b shows the weight of BSFL reared on PE-containing food waste substrates PE20% (20% w/w PE added), PE10% (10% added), and PE5% (5% added). The weight of BSFL reared on PE10% was 21% lower than that of the control ($p = 0.0075$) on day 6.

However, thereafter, no significant difference between PE groups was observed.

Survival, pupation ratio, and substrate reduction rate of BSFL reared on food waste containing PS and PE

The survival rate of BSFL reared on PS5% was significantly lower than that of control insects ($p = 0.0335$) (Fig. 3a). Substrate reduction was significantly lower in the PS-treated groups than in the control, regardless of the concentration of the microplastics ($p = 0.0045$). No differences in the survival rate were observed between PE-treated groups and control (Fig. 3b). However, the pupation ratio was approximately doubled in the PE10% and PE20% groups, compared to the control ($p = 0.0025$). Substrate reduction also decreased as the concentration of PE increased ($p < 0.0001$).

Larval growth on food waste containing PS and PE plus NaCl

Figure 4a shows the larval weight of *H. illucens* reared on the PS and NaCl-containing food waste substrates PS20+1 (20% w/w PS with 1% NaCl added), PS20+2 (2% NaCl), and PS20+3 (3% NaCl), collectively PS20+. From day 1 to day 8, no differences in the BSFL weights between the PS20+-treated groups and controls were observed. However, from days 12 to 24, statistically significant differences in the BSFL weights were observed between these groups. Specifically, on day 12, the weights of the BSFL in all PS20+ groups were lower than that of the control ($p = 0.0014$); this was also the case on day 20 ($p = 0.0037$). On day 15, the weights of the BSFL reared on PS20+3 were 32% lower than the control BSFL weight ($p = 0.0015$); on day 24, it was 27% lower ($p = 0.0002$).

Figure 4b shows the larval weight of *H. illucens* reared on the PE and NaCl-containing food waste substrates PE20+1 (20% w/w PE with 1% NaCl added), PE20+2 (2% NaCl), and PE20+3 (3% NaCl), collectively PE20+. The weights of the BSFL reared on PE20+ substrates were significantly different from that of the control at all measurements except for those on days 1 and 8. Specifically, on days 4 and 6, the weights of the BSFL reared on PE20+2 and PE20+3 were 23% ($p = 0.0075$) and 35% ($p = 0.0157$) lower than that of the control, respectively. On day 12, the weights of the BSFL reared on PE20+2 were 29% less than that of the control ($p = 0.0003$). On days 15, 20, and 24, the weights of the BSFL reared on PE20+3 were 24% ($p = 0.0042$), 20% ($p = 0.0336$), and 20% ($p = 0.0028$) lower than that of the control, respectively. Figure 4c, d shows the larval length of *H. illucens* reared on the PS and PE and NaCl-containing food waste substrates. Larval length of *H. illucens* was shorter in all treatments groups when compared to the control.

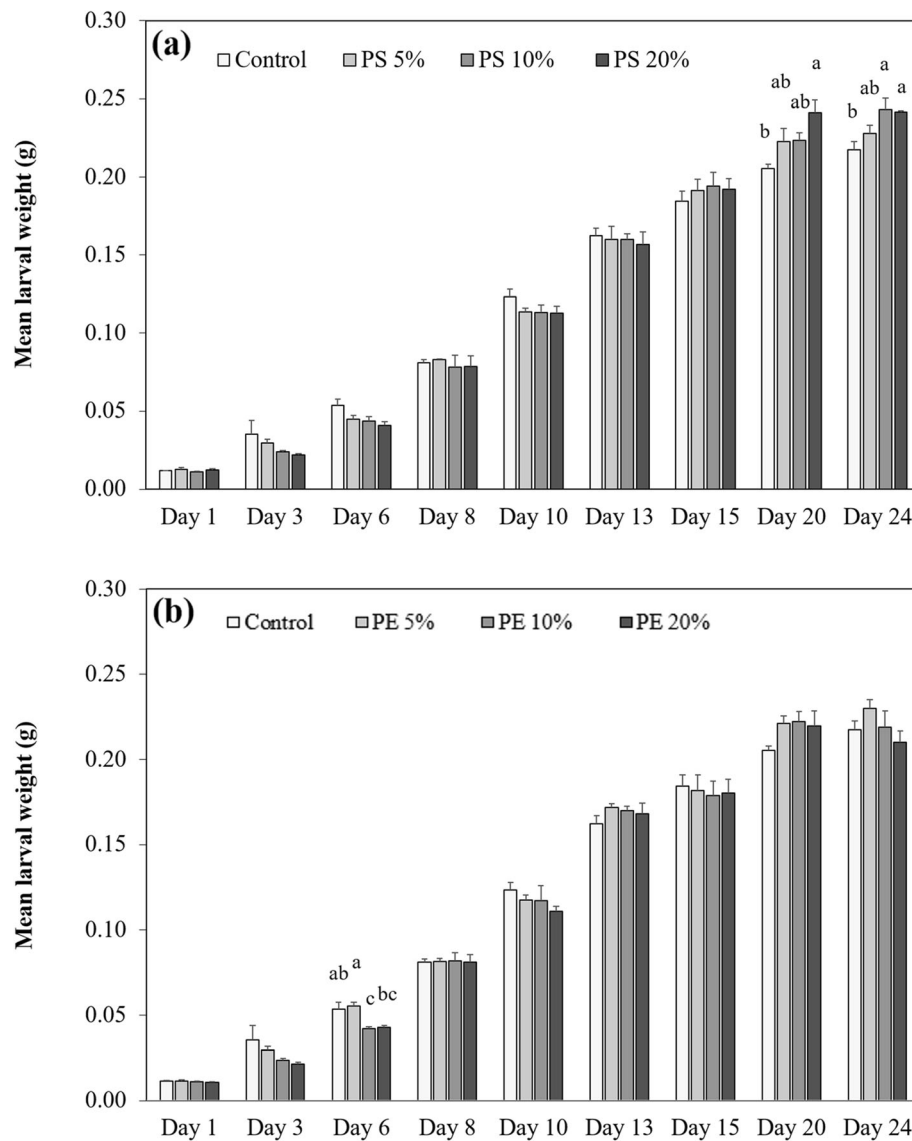


Fig. 2 Effect of microplastic treatments on larval weight. The weight of larvae reared on food waste treated with **a** polystyrene (PS) and **b** polyethylene (PE) over 24 days. The percentage PS or PE (w/w) is indicated on the legend of each figures. Letters **a**, **b**, and **c** indicate a significant difference ($p < 0.05$) between treatments

Survival, pupation ratio, and substrate reduction rate of BSFL reared on food waste containing plastics and salt

Figure 5a shows the survival, pupation ratio, and substrate reduction rates of BSFL reared on PS20+ substrates. No statistically significant differences in the survival rate were observed between the treatment groups and control; however, the pupation ratio of the BSFL reared on PS20+2 was significantly lower ($p = 0.0018$) than that of the control. In addition, substrate reduction decreased with increasing NaCl concentrations ($p < 0.0001$).

As shown in Fig. 5b, the pupation ratio in the PE20+2- and PE20+3-treated groups were significantly lower than that of the control. The substrate reduction rate was

significantly lower ($p = 0.0012$) in the same two groups compared to the control.

Discussion

In this study, we investigated the effects of PS and PE microplastics and salinity on BSFL. Our results show that the PS and PE microplastics in the food waste did not affect the survival rate of BSFL. However, a higher pupation ratio and a lower substrate reduction rate were observed in the BSFL reared on food waste treated with PS and PE microplastics, compared to the control. Previous studies have been reported for the decomposition of diverse organic wastes including fruit

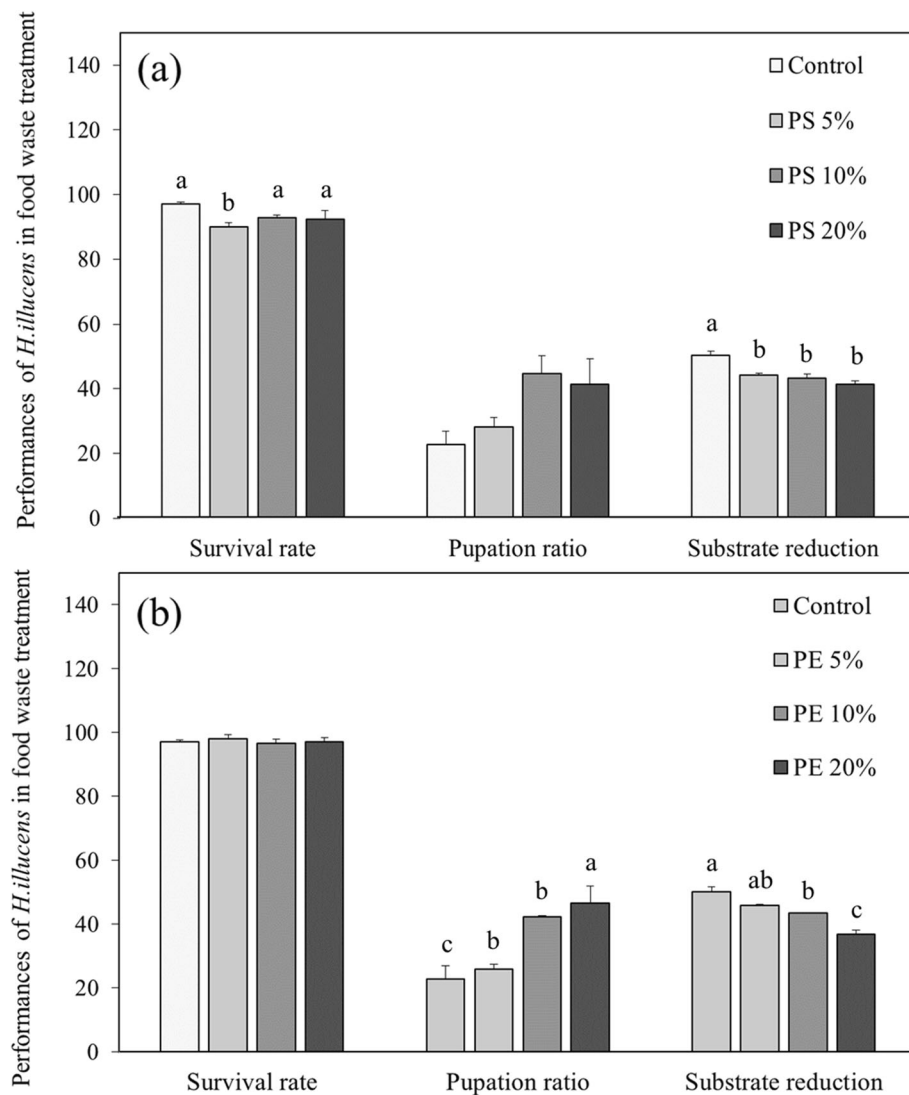


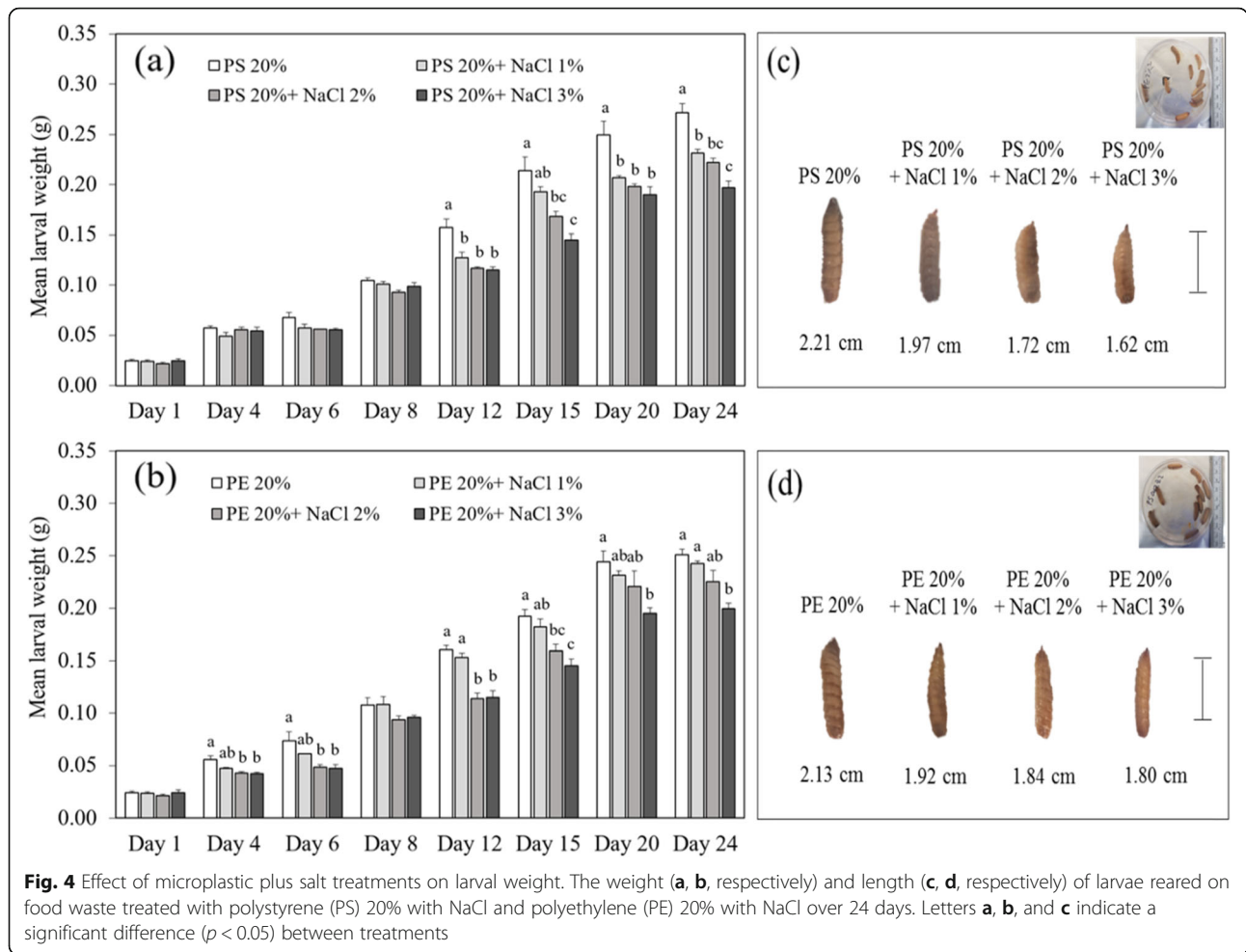
Fig. 3 Survival, pupation ratio, and substrate reduction rates (mean ± standard error; $n = 3$) of *H. illucens* larvae reared on food waste containing **a** PS and **b** PE. Letters **a**, **b** and **c** indicate significant difference among treatment groups

and vegetable mixture and pig manure using BSFL, and the growth and development of BSFL differed depending on the substrates employed (Jucker et al. 2017; Liu et al. 2018; Meneguz et al. 2018); this is not surprising given that various substrates including food waste have heterogenous traits in their composition (Hossain et al. 2014). More studies are required to apply BSFL in processing of diverse types of food waste. In addition, considering that the larvae and prepupae of *H. illucens* are generally used as a decomposer and later an animal feed, toxicological studies such as bioaccumulation and generational transport are needed for confirmation of sustainability of food waste processing mediated by BSFL.

Our results revealed that the pupation ratio of BSFL was higher in microplastic-treated groups compared

to the control. Previous studies reported that various contaminants including poly aromatic compounds can act as an endocrine disruptor and lead to increased body weight of midge (Arambourou et al. 2019). Further study is needed to investigate whether PE or PS also can act as endocrine disruptor and thus affect the physiology of BSFL.

Lower larval weight and pupation ratio were observed for BSFL reared on substrates containing NaCl, compared to the control group. Considering that no adverse effects were observed in the BSFL reared on substrates that were identical except in their salt content, it is likely that BSFL are affected more by salinity than by microplastics. In a previous study, Kwon and Kim (2016) reported that behavioral inhibition of *H. illucens* was observed and larval growth was inhibited at over 3%



NaCl. In line with these results, the lower larval weight and pupation ratio observed in our study likely resulted from inhibition by NaCl.

On the other hand, no differences in the survival rates between larvae reared on salt-containing substrates and the relevant control groups were observed. Kwon and Kim (2016) reported that BSFL can be effective degrader of food waste in South Korea, because the food waste have 0.7 to 1% salinity on average (Lee et al. 2005; Park 2012). A study reported that two nematode species, *Heterorhabditis bacteriophora* and *Steinernema glaseri*, have high salinity tolerance and can survive on soil treated with 9.4 g/L NaCl. However, nematode survival was lower in soils treated with 18.0 g/L NaCl (Thurston et al. 1994). In addition, it was observed that weight of earthworm larvae was reduced at salt concentration of 0.2%, and the mortality rate started to increase at 0.8% (Guzyte et al. 2011). In our study, no adverse effects of NaCl on the survival rate of BSFL was observed in groups exposed to PS and PE microplastics and salt,

and this indicates that *H. illucens* has a relatively high salt tolerance and thus has high potential to be used for vermicomposting of food wastes with high salt concentration.

Conclusion

In this study, the effects of salinity and PS and PE microplastics in food waste on the growth and substrate reduction of BSFL were assessed. PS and PE contained in the food waste lowered the substrate reduction rate, whereas PE increased the pupation ratio. Regardless of the types of microplastics treated with NaCl, larval growth, pupation ratio, and substrate reduction rates were reduced by NaCl. However, no adverse effect of microplastics and NaCl on survival rate of BSFL was observed. Therefore, although the treatment efficiency may be lowered, it is likely that BSFL may be applied in the treatment of food waste containing microplastics and high salinity. Microplastics contained in food waste, however, may accumulate in BSFL during vermicomposting of food

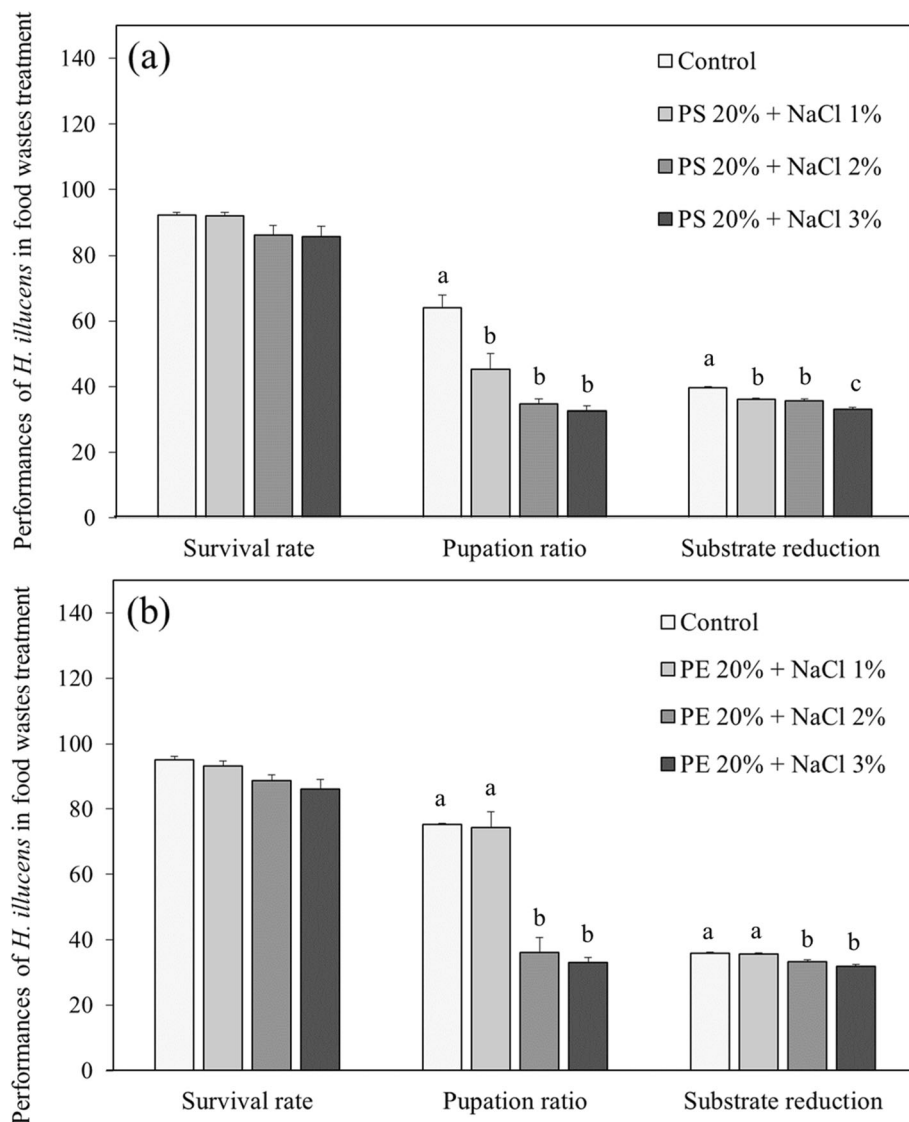


Fig. 5 Survival, pupation ratio, and substrate reduction rates (mean ± standard error; $n = 3$) of *H. illucens* larvae reared on **a** PS and **b** PE-containing food waste added with NaCl. Letters a and b indicate significant difference among treatment groups

waste. Considering that BSFL is used as animal feeds after food waste treatment, additional studies are needed to investigate long-term effects of microplastics on *H. illucens* such as bioaccumulation and generational transport.

Abbreviations

ANOVA: Analysis of variance; BSFL: Black soldier fly larvae; PE: Polyethylene; PS: Polystyrene

Authors' contributions

SC designed the study, performed the experiments and data analysis, and wrote the manuscript. CK participated in the design of the study and wrote the manuscript. MK conducted the experiments and reviewed the manuscript. HC conceived the study and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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