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Does the presence of heavy metal and catechol contaminants in organic waste challenge the physiological performance of the bioconverter *Hermetia illucens*?

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Abstract

The increased human activities and the worldwide population growth are constantly increasing the production of solid wastes. Over the years, waste management has thus become a prominent issue for several companies and municipalities, and several engineering techniques have been developed over the years in order to convert wastes into other solid materials of fuels. Yet, several techniques are important contributors to environmental pollution, and biological-based solutions have thus become progressively very popular. In particular, insect-based conversion of organic wastes represent eco-friendly tools, and the growth and development of insect species such as the black soldier fly have been tested and improved for a large diversity of organic wastes. However, organic wastes, including food wastes, may contain several pollutants such as heavy metals and catechol which could affect the bioconversion efficiency by incurring physiological costs that would be undetectable at the organismal level, *i.e.* have null to little effects on the life cycle of *Hermetia illucens*. In this context, assessments of antioxidant capacities can provide a rapid and low-cost evaluation of the capability of insects to handle exposure to heavy metals and catechol. Here, we aimed at measuring the physiological responses of the black soldier fly *H. illucens* grown on food wastes (kitchen, fruit or vegetable wastes) contaminated by cadmium, iron, lead or catechol. Biomarkers of oxidative stress (concentrations of hydrogen peroxide and protein carbonyls), total antioxidant capacity, non-enzymatic (ascorbic acid amounts) and enzymatic-based (activities of superoxide dismutase and polyphenoloxidase) were measured from the gut of the larvae. We found no evidence of deleterious impacts of food waste contamination by catechol or heavy metals on *H. illucens*. In most experimental treatments, the array of physiological endpoints we measured for evaluating the degree of oxidative stress experienced by the larvae remained similar to controls. Possible physiological effects were reported for cadmium and catechol only, which tended to increase the oxidation of proteins and hydrogen peroxide in the larvae. Finally, and importantly, our results suggest that the nature of the food waste can equally affect the physiological responses of the insect.

Keywords

Bioremediation; Heavy metal contamination; Oxidative stress; kitchen waste; vegetable waste; fruit waste

1. Introduction

The worldwide population growth, which ranges from 1 to 2% since the 1960s (Roser et al., 2013), and the booming economy and urbanization have significantly increased the production of solid wastes (Bingemer and Crutzen, 1987; Tarmudi et al., 2009; Nanda and Berruti, 2021). As a result, waste management has become a hot topic over the years, as inappropriate collection, disposal and recycling highly increase the risks of social and environmental issues (reviewed in Ferronato and Torretta, 2019). Owing to the large diversity of solid wastes, several engineering and/or biological techniques are implemented for their management, including combustion, composting (fermentation), pyrolysis, conversion into fuels (Mahmoud et al., 2022) or other solid materials (Chen and He, 2012; Kiran et al., 2014; Bilal et al., 2019; Yaashikaa et al., 2020). Importantly, the solution used for waste management must be technically and financially feasible, in addition to be eco-friendly.

Even if the sources and proportions of solid wastes vary from one country to another, and among urban areas, household wastes account for 55-80% of the total solid waste production (Abdel-Shafy and Mansour, 2018). In the 28-UE countries, for instance, food waste production has been estimated to 100 million tons / year, of which 45% are coming from households (Timmermans, 2015). The per capita household food wastes amounts to 82 kg in Germany, 99 kg in France, or 110 kg in Great Britain (Waste 2012). Food waste treatment has long relied on the use of anaerobic digestion, or composting alternatives. Yet, these procedures are based on fermentation processes which are contributing to greenhouse gas emission, thus increasing the ongoing climate change problem (Ramos-Elorduy, 2009; Meyer-Rochow and Chakravorty, 2013). This context has stimulated a range of investigations aiming at finding alternative waste management procedures, and at improving the recycling stream of solid wastes.

Insect-based bioconversion of food wastes has become very popular in the recent years (Gasco et al., 2020; Ravi et al., 2020; Kim et al., 2021; Lim et al., 2022), and typically represents an eco-friendly biological technology for waste management. Several insect species have been proved efficient models for sustainable food waste management, including the yellow mealworm *Tenebrio molitor*, the codling moth *Cydia* pomonella, the housefly *Musca domestica*, and the black soldier fly *Hermetia illucens* (see Fowles and Nansen, 2020, for a review). The large diversity of organic wastes the black soldier fly can consume – be it dry or wet – its short life span (Fowles and Nansen, 2020), voracity and high biomass conversion ratio (Surendra et al., 2020), makes it a valuable tool for food waste management. Moreover, larvae of the black soldier fly outperform other technologies in terms of costs, ecological footprints, and overall efficiency (Singh and Kumari, 2019), and have thus become a popular model all around the world for the management of food wastes.

While food wastes are largely made by food residues, they may also contain several additional organic residues, as for instance waste tableware, plastic fragments, or towels. Also, in some municipalities, food wastes are stored into biowaste bags before collection, and these bags have been reported as significant containers of heavy metals (Huerta-Pujol et al., 2010). Heavy metals are naturally present in the environment (volcanoes and erosion, for example), in addition to have an anthropogenic source. Anthropogenic contribution in the transfer of heavy metals to the environment ranges from electronic waste dispersion, unsafe management of medical wastes, industrial production and release of fertilizers, battery production, mining, pesticides, textiles, dyes, or painting (Fulekar et al., 2009; Dixit et al., 2015). The geochemical cycle of heavy metals can further maximize their accumulation in the environment, and this contributes to the destruction of ecosystem balances and loss of biodiversity (D'amore et al., 2005). Several other contaminants can also accumulate in the environment, including pesticides, pharmaceutical, hazardous wastes, or organic contaminants (Kassir et al., 2012; IDA, 2014; Vij, 2015), and vegetables and fruits can thus get contaminated by cadmium, lead, chromium, or iron (Melai et al., 2018; Sharma and Nagpal, 2020). Additional contamination may even occur during the processing and treatment stages of food and food wastes (Thakali et al., 2021). Thus, the potential presence of several classes of contaminants must be taken into account when assessing the insect-based management of food wastes.

The effects of heavy metals on living organisms have been studied intensively over the years (Cosio and Renault, 2020; Soliman et al., 2022), and evidence of the deleterious effects these pollutants have on biodiversity has accumulated. In contaminated soils, plant development may become impossible, as heavy metals inhibit the uptake of nutrients and plant growth, they impair enzymatic activity, induce oxidative stress and damages to macromolecules, ultimately leading to genotoxicity (Rasmussen et al., 2000; Olaniran et al., 2013; Fashola et al., 2016). In insects,heavy metals damage macromolecules, generating protein carbonylation, lipid peroxidation, or DNA strand breaks which are induced by increased levels of oxidative stress (Abdelfattah et al., 2017; Youssef et al., 2017). As a result, assessments of antioxidant capacities can provide a rapid and low-cost evaluation of the capability of the insects to handle exposure to a range of environmental stressors (Lalouette et al., 2011; Lawniczak et al. 2013; Renault et al., 2016), including exposure to heavy metals.

As mentioned above, food wastes can contain a range of contaminants other than heavy metals, as for instance catechol. While little catechol amounts can be found in fruits and vegetables, this compound is also synthesized as precursor of flavors and pesticides, and can reach high levels owing to its capacity to quickly solubilize in aqueous solutions, becoming toxic to living organisms. With the growing importance in finding eco-friendly solutions for food waste management, we are urgently needing information on the effects of food wastes contaminated by heavy metals and catechol on

insects. If the insects can handle such contaminated wastes, they may be valuable candidates for bioremediation and elimination of the excess of heavy metals and catechol from food wastes at lower costs as compared with the currently used techniques, such as chemical oxidation reaction, adsorption processes, and electrochemical techniques (Ahluwalia and Goyal, 2007; Wu et al., 2010; Siddique et al., 2015).

In the study, the physiological responses of the black soldier fly *H. illucens* grown in presence of contaminants - in the form of heavy metals and catechol - in food waste was evaluated. To that aim, 5th instars were used, as this often corresponds to the developmental stage which is considered in laboratory studies examining the impacts of contaminants on gut content and structure of *H. illucens* (Bonelli et al., 2020; Tanga et al., 2021; Zhineng et al., 2021). Larvae were reared on different types of food wastes (kitchen, fruit, or vegetable wastes), which may supply the insect with a range of compounds having pro-oxidant activities, or inducing oxidative stress (vegetable wastes with vegetables containing phenolic compounds). Food wastes were also contaminated with heavy metals or catechol. Seven days after the larvae were exposed to those experimental conditions, the concentration of H_2O_2, protein carbonylation, ascorbic acid, SOD activity, PPO activity, reducing power ability, antioxidant ability and antiradical activity DPPH was measured from the gut of the larvae. As heavy metals were formerly reported to have little effects on the growth and development of the black soldier fly larvae, we hypothesized that they would have low impacts on the diverse components of the antioxidant system we considered. Also, by focusing on the physiological effects of catechol and heavy metals at the gut level, an important biological compartment at play during biorecycling processes of organic wastes, we are first suggesting that the monitoring of oxidative stress responses could represent valuable metrics for assessing the effects of contaminants in this important insect biorecycler.

2. Materials and methods

2.1. Insect rearing

Groups of 100 adult flies were reared under controlled conditions (60% RH; L:D 12:12) at 37 °C in cages (30x30x40 cm, LxlxH). This rearing condition decreased developmental time significantly without impairing hatching and survival rates (Chia et al., 2018; Fazli Qomi et al., 2021). Then, pools of 900 offspring larvae were made and reared in the same experimental conditions, except that they were maintained in the dark in plastic boxes (15x30x20 cm, Lxlxh). Larvae were supplied with fresh vegetables, fruits and meat (ratio 1:1:1). For the purpose of the study, fifth instars (the species has six larval instars, see De Smet et al., 2018) of the black soldier fly *Hermetia illucens* were obtained from this rearing colony, which was established at the Entomology Department (Faculty of Science of Cairo University, Cairo, Egypt).

2.2. Exposure of the insects to food wastes and organic pollutants mixed with soil samples

Soil samples were obtained from the garden of the Entomology Department (Faculty of Science, Cairo University). First, soil samples were split into two batches (i) samples from one batch were spiked with heavy metals (2% solution of cadmium, iron or lead), or catechol, or organic compound used for pesticide production, and (ii) soil samples from the second batch served as controls (non-contaminated soil). Second, the contaminated soils were mixed with organic wastes in a 2:0.5 ratio (organic waste:soil, mass:mass). Organic wastes were made of fruit, vegetable or kitchen wastes that were obtained from the household of the Giza government (Egypt) in charge of cooking and cleaning the mass catering rooms.

Fifth instars of *H. illucens* were transferred to one of three batches: (i) 150 larvae were maintained at optimal rearing conditions (non-contaminated soil, and ideal nutritional media made of 1% of albumin, glucose, glycine and cholesterol gel), (ii) 150 larvae were fed with organic wastes (fruit, vegetable or kitchen wastes) mixed with non-contaminated soil and (iii) 600 larvae were fed with organic wastes (fruit, vegetable or kitchen wastes), and mixed with one of the four types of contaminated soil (Cd, Fe, Pb, or catechol) (see Supplementary information S1 for a detailed description of the experimental design). The larvae were maintained at these experimental conditions for 7 days, in the dark (R.H.: 60%, Temperature: 37 °C).

At the end of the exposure period, the 5^{th} instars of *H. illucens* were collected, and 50 individuals from each experimental condition were dissected in potassium phosphate buffer (pH= 7.0) at 4 °C to obtain gut tissues. The samples were further stored at -20 °C until being processed for the below-described physiological measurements.

2.3. Biomarkers of oxidative stress

The concentration of hydrogen peroxide and protein carbonyls, which represent valuable biomarkers of oxidative stress in insects, were measured from the gut tissues of 5^{th} instars of the black soldier fly. The concentration of hydrogen peroxide (H_2O_2) was determined spectrophotometrically according to Junglee et al. (2014). Briefly, 0.5 mg of sample were obtained by pooling gut tissues from 50 larvae (N = 3 replicates for each experimental condition). The samples were homogenized at 4 °C for 10 min (60 spikes / min) in 1 mL of potassium phosphate buffer (10mM; pH=7.0) containing 0.25 mL Trichloroacetic acid (TCA) (0.1% (w:v)), 0.5 mL KI (1 M) (one-step buffer: extraction and colorimetric reaction combined). After homogenization, the samples were centrifuged at 12,000 × g for 15 min at 4 ˚C. The absorbance of the samples was read at 240 nm, and H_2O_2 concentration was expressed as ppm using a calibration curve.

Amounts of protein carbonyls were determined following the procedure described by Levine et al. (1990). After gut tissue isolation, samples were homogenized in 5 mL of ice-cold phosphate buffer (containing 0.25 mL Trichloroacetic acid (TCA) (0.1% (w:v), 0.25 mL triton x-100 (1%)). Then, the samples were centrifuged at 2000 × g for 10 min at 4 °C. Afterwards, an aliquot of 800 µL of the supernatant was transferred to a clean microtube, and a volume of 200 µL of 10 mM 2, 4-dinitrophenyl hydrazine (DNPH) prepared in 2 M HCl was added. The samples were incubated for 30 minutes at room temperature, and further precipitated with 1 mL of 10% Tricholoroacetic acid (TCA). The pellet was washed four times with an ethanol/ethyl acetate (1:1) mixture, and dissolved in 1 mL of sodium phosphate buffer. Finally, the absorbance was measured at 366 nm, and the concentration of protein carbonyls was expressed as OD/mg protein.

2.4. Assessment of antioxidant capacity

The antioxidant capacity of the gut tissues of 5th instars exposed to the different experimental conditions was assessed by measuring (i) the total antioxidant capacity, (ii) the non-enzymatic and (iii) the enzymatic antioxidant capacities of the samples.

2.4.1. Total antioxidant capacity

The total antioxidant capacity was assessed with three distinct methods. First, the total antioxidant capacity was measured by determining the reducing power concentration of the samples according to the methodology described by Oyaiza (1986). Samples were mixed with 1 mL 0.2M pH=6.6 phosphate buffer and 0.5 mL 1% potassium ferricyanide, and incubated in a water bath at 50 ˚C for 20 min. Then, 1 mL 10% of TCA was added to each extract, which was centrifuged at 2000 g for 10 min at 4 °C. The absorbance was measured at 480 nm after the adding of 1.5 mL of 0.1% ferric chloride to the reaction mixture.

Second, the total antioxidant capacity of the gut tissues was measured according to the procedure described by Prieto et al. (1999). Gut tissues were mixed in a solution containing 0.25 mL 0.6 M sulfuric acid, 0.5 mL 28 mM sodium phosphate, and 0.25 mL 4 mM ammonium molybdate, and incubated at 95 ˚C for 90 min. The absorbance was measured at 695 nm.

Third, the α,α-diphenyl-β-picrylhydrazyl (DPPH) antioxidant activity was determined according to Blois (1958), and reported the capacity of a sample to inhibit the production of reactive oxygen species (expressed as a percentage). The DPPH assay was based on the measurement of the scavenging capability of the sample. A volume of 0.5 mL 0.5M DPPH was added to different amounts of gut tissues (0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mg gut tissue/mL solution), and the homogenates were incubated for different times before measuring absorbance at 525 nm. The nitrogen atom contained old electrons which were reduced by delivery a hydrogen atom from antioxidants to hydrazine (Contreras-Guzman

and Srong, 1982). The total protein concentration of samples was determined spectrophotometrically according to the method of Bradford (1976). All these experiments were done with three replicates.

2.4.2. Non-enzymatic antioxidant capacity

Ascorbic acid amounts were measured by grinding 0.5 mg of gut tissues in 1.5 mL 10% TCA. The samples were centrifuged at 2000 ×g for 10 min at 4 °C. A volume of 1 mL of the supernatant was collected, and 0.5 mL of dinitrophenyl hydrazine and 0.25 mL of 65% ice cold H_2SO_4 were added to each sample. After incubation for 30 min at 30 ˚C, the absorbance of the samples was measured at 520 nm.

2.4.3. Enzymatically-based antioxidant capacity

The activity of the superoxide dismutase (SOD) was measured following the procedure of Misra and Fridovich (1972). The reaction mixture contained 1.5 mL sodium carbonate buffer (200 mM; pH 10.0), 0.5 mL EDTA (10 mM), 0.5 mL of the supernatant of the sample homogenate, and 0.5 mL freshly prepared epinephrine (15 mM). The absorbance was measured at 480 nm. SOD activity was expressed as OD/µg protein/min.

For polyphenoloxidase (PPO) activity, we followed the method of Kumar and Khan (1982). The reaction mixture contained 1 mL potassium phosphate buffer (0.1 M, pH 6.0), 0.5 mL 0.1 M catechol and 0.5 mL of enzyme extract. The purpurogallin that was formed was read at 495 nm. PPO activity was expressed as OD/min/mg protein.

2.5. Statistical analyses

Statistical analyses were performed with IBM SPSS Statistics for Windows (Version 17.0. Armonk, NY: IBM Corp.). As the data were not meeting the homoscedasticity requirements, non-parametric tests were carried out with k independent Kruskal–Wallis tests. These non-parametric tests were run for comparing the effects of the experimental conditions on the amounts of H_2O_2, protein carbonyls, ascorbic acid, DPPH antioxidant, reducing power, total antioxidant capacities, and the activities of the enzymes superoxide dismutase, and polyphenoloxidase. Data were expressed as means and standard deviations. Correlations between the concentration of each sample and the DPPH antioxidant assay were performed with Pearson's regression analysis using multiple regression models.

3. Results

3.1. Biomarkers of oxidative stress

The amounts of hydrogen peroxide (H_2O_2) ranged from 0.2 to 0.4 ppm in most experimental conditions **(Figures 1Ai-iii)**. Higher amounts were measured in the gut of the larvae that were exposed to the mixture 'kitchen wastes x soil contaminated by cadmium'; the lowest amounts were recorded from larvae grown on 'vegetable waste x soil contaminated with Fe'. Relative to controls, a significant decrease in H_2O_2 concentration was measured from gut tissues of larvae maintained on 'vegetable wastes x Cd-contaminated soil', and 'fruit wastes x Fe-contaminated soil'. Conversely, H_2O_2 concentration was the highest in larvae fed on vegetable wastes, 'vegetable wastes x Pb-contaminated soil' and 'kitchen wastes x Cd-contaminated soil' **(Figures 1Ai, iii)**. Overall, a similar pattern was observed for protein carbonyls **(Figures 1Bi-iii)**.

3.2. Assessment of total antioxidant capacity

The effects of the experimental treatments on the total antioxidant capacity were assessed with three distinct methods. Distinct patterns were observed when the measures considered the reducing power concentration **(Figures 2Ai-iii)** or the total antioxidant capacity **(Figures 2Bi-iii)**. In the latter method, the total antioxidant capacity was often equivalent among the experimental groups, with the exception of larvae fed with fruit wastes, as compared with controls **(Figures 2Bi-iii)**. Conversely, reducing power concentration was decreased significantly in most groups, except in larvae maintained on 'vegetable wastes x Fe- or Pb-contaminated soils', and 'kitchen wastes x Pb-contaminated soils' **(Figures 2Ai-iii)**.

To monitor the changes in the inhibition percentage of DPPH over time according to the amount of gut tissues, a kinetic study was performed **(Supplementary Information S2 and S3)**. A strong and significant positive correlation was found between the concentration of gut tissues of 5th instars of *H. illucens* and DPPH inhibition percentage **(Table 1)**. Moreover, GEE analysis reported the existence of a significant effect of the type of food wastes, and of the type of pollutants, on the DPPH inhibition percentage **(Table 1, Supplementary Information S4, Figures 2Ci-iii)**. These kinetics also showed that an incubation time of twenty minutes was suitable for assessing DPPH inhibition percentage. Using this incubation time, we reported that the percentage of inhibition of ROS production was progressively increased with increasing concentrations of gut tissues, and reached a plateau at concentrations of gut tissues of 0.20 mg / mL, except for larvae fed on 'wastes x Pb-contaminated soil' **(Supplementary Information S2 and S3 4)**.

DPPH inhibition percentage did not differ significantly among larvae fed on non-contaminated and contaminated vegetable wastes **(Figure 2Ci)**, while it was significantly increased in larvae maintained on fruit wastes mixed with contaminated soil **(Figure 2Cii)**. Finally, DPPH inhibition was increased in larvae maintained on 'kitchen wastes x Fe-contaminated soil' and 'kitchen wastes x catechol-contaminated soil' **(Figure 2Ciii)**.

3.3. Non-enzymatic and enzymatically-based antioxidant capacities

The highest concentration of ascorbic acid was measured from gut tissues of larvae maintained on Fe^{2+}, Cd^{2+}, and from larvae fed with vegetable, fruit, and kitchen wastes. Relative to controls, levels of ascorbic acid were decreased in the gut tissues of larvae maintained on vegetable wastes contaminated with Cd^{2+} and Pb^{2+}, on fruit wastes contaminated with Pb^{2+}, and on kitchen wastes contaminated with Cd^{2+}, Fe^{2+}, Pb^{2+} and catechol. Overall, GEE analysis revealed that all experimental conditions affected ascorbic acid concentrations (S**upplementary Information S4**), and the highest increased in ascorbic amount relative to controls was reported for vegetable wastes contaminated with Fe^{2+} **(Figure 3)**.

Superoxide dismutase (SOD) activity was increased significantly when larvae were maintained on 'fruit wastes x Cd- or catechol-contaminated soils' **(Figures 3Bi-iii)**. The adding of Fe resulted in the decrease of SOD activity whatever the nature of the wastes. When wastes were mixed with contaminated soil, polyphenol oxidase (PPO) activity was significantly increased as compared with controls **(Figures 3Ci-iii)**.

4. Discussion

The use of insects for recycling wastes is under consideration since several decades (DeFoliart, 1975; Nguyen et al., 2015; Ojha et al., 2020; Abdelfattah et al., 2021; Abdelfattah and Lim, 2021; Cammack et al. 2021), and is increasingly attracting entrepreneurial companies. Insects, and more particularly larvae of *H. illucens* (Purkayastha and Sarkar, 2021), can consume large amounts of organic wastes for achieving their life cycle, with substrate reduction ranging from 40 to 60%, and associated bioconversion values ranging from 5 to 20% for a range of kitchen, fruit and vegetable wastes (reviewed in Surendra et al., 2020). As a result, the potential offered by *H. illucens* as a composting agent is under deep investigations. The rearing procedure and the nature of the wastes (Myers et al., 2008, Zhou et al., 2013, Surendra et al., 2020) affect substrate conversion. Yet, conversion efficiency, and the associated bioconversion, can be improved significantly by a range of factors (Surendra et al., 2020). For instance, the addition of *Bacillus subtilis* to the poultry manure can increase bioconversion by *ca.* 14%, and can increase manure reduction from 35.8 to 40.5% (Xiao et al., 2018). Genetic manipulation of the fly can also improve substrate reduction (Zhan et al., 2020). Importantly, organic waste can contain a number of persistent pollutants, including heavy metals which were formerly reported to have null to little effects on the life cycle of *H. illucens* (Diener et al., 2015), even at high concentrations. Yet, those contaminants could elicit physiological responses which are undetectable at biological scales. In the present work, we fed larvae of *H. illucens* with different food wastes, of which some were contaminated with catechol or heavy metals, and examined the cascading effects these exposures had on the the antioxidant responses of

the individuals. We found no evidence of deleterious impacts of food waste contamination by catechol or heavy metals in *H. illucens*, with antioxidant responses remaining similar to controls in most experimental treatments. Possible physiological effects were reported for cadmium and catechol, which tended to increase the oxidation of proteins and hydrogen peroxide in the gut tissues of the larvae. In addition, our results suggested that the nature of the food waste equally affected the physiological responses of the insect, in particular vegetable and fruit wastes.

Several studies have reported that *H. illucens* can be used for sustainable waste management, and the species also has the potential for bioremediation of various toxins and pollutants (Bulak et al., 2018; Scala et al., 2020; Mahmood et al., 2021; Franco et al., 2022). *Hermetia illucens* can indeed develop on contaminated organic wastes, and remove, at least partially, the hazardous effects these pollutants can have for biodiversity and environment in general (Bulak et al., 2018; Scala et al., 2020; Mahmood et al., 2021; Franco et al., 2022). While there is evidence that the contaminants we added to food wastes can induce oxidative stress in a range of insect species (Abdelfattah et al., 2017; Yousef et al., 2017; Bilal et al., 2019; Gizaw et al., 2020; Zielińska et al., 2021), they had no major effects on the different components of the antioxidant system we considered in 5th larval instars of *H. illucens*. Our findings generally match the existing literature, with for instance lead showing little accumulation in the larvae (Diener et al., 2015). In the insect, it is possible that the effects of lead are inactivated by storing this metal into granules, or by binding to metallothionein, as formerly reported in other arthropod species (Wallace et al., 2000). Then, metal-rich granules can be transported and immobilized in the cuticle (Hare, 1992; Bergey and Weis, 2007), with molting allowing the depuration of accumulated heavy metals (Roberts and Johnson, 1978; Bergey and Weis, 2007). Also, increased excretion of heavy metals may have occurred, as reported in larvae of the gypsy moth (Jiang et al., 2018). The existence of such processes in *H. illucens*, which could contribute explaining the absence of effects of lead exposure on the antioxidant system, should be investigated in future studies.

Only cadmium, in mixture with kitchen wastes, tended to slightly increase the level of oxidative stress measured from the gut tissues of the larvae. Cadmium is one of the few heavy metals which is bioaccumulated by larvae of the fly (Charlton et al., 2015; Gao et al., 2017; Purschke, et al., 2017). This highly toxic heavy metal can be deleterious to living organisms (Neckameyer and Matsuo, 2008), especially dipterans such as *H. illucens* (van der Fels-Klerx et al., 2016). The severe toxicity of cadmium ions mainly results from its similarity with and Ca^{2+}, which conducts to the absorption of this metal by cells through Ca^{2+} channels, and finally leads to metal accumulation inside cells (Braeckman et al., 1999). Even if there is little information reporting the effects of heavy metals and quinones on the physiological responses (oxidative stress) of larvae of *H. illucens*, our general knowledge of the capacity of the species to handle heavy metals has increased quickly in the past year, in particular for Cd. Zhang et al. (2021)

characterized the metallothionein proteins that help larvae of the fly to withstand exposure to Cd. In parallel, exposure to high Cd amounts have been reported to enrich the gut microbia (e.g. *Brucellaceae*, *Enterobacteriaceae*, *Alcaligenaceae*, *Campylobacteraceae*, and *Enterococcaceae*) of the larvae (Wu et al., 2020). All of these changes are likely contributing to explain the low effect of Cd on the growth and development of *H. illucens* (Gao et al., 2017), on prepupal weight, development time, or sex ratio (Diener et al., 2015). Finally, in their literature review, Schrögel and Wätjen (2019) concluded that there is no indication of Cd, or other heavy metals, buildup inside insect tissues. In the present work, Cd-enriched food wastes did not trigger antioxidant responses in larvae of *H. illucens*, with the exception of Cd-contaminated kitchen wastes which resulted in an increase of H_2O_2 production, in turn increasing in PPO activity in the larvae grown on this media. Even if bioaccumulated in the insect, these findings suggest that Cd amounts were progressively released from one molt to another, or from one developmental stage to the next (See Lievens et al., 2021 for a review), and/or *via* excretion (Gao et al., 2017). Altogether, these mechanisms may greatly reduce the risk of physiological stress for the black soldier fly.

Several pollutants are particularly frequent in the environment and in organic wastes (Dai et al., 2017; Masindi and Muedi, 2018; Zhou et al., 2021), meaning that the use of living organisms for the bioconversion of food waste must consider the array of pollutants' possible impacts on bioconverter insects (Musarurwa and Tavengwa, 2020; Awa and Hadibarata, 2020). Surprisingly, we have little knowledge of the effects of heavy metals and catechol on the physiology of larvae of *H. illucens*, even though this may provide us with valuable information on the possible pollutants' impacts that could be undetectable when monitoring development and growth. Indeed, while pharmaceuticals and pesticides are not bioaccumulated in larvae of *H. illucens* maintained on contaminated wastes (Charlton et al., 2015; Lalander et al., 2016), these pollutants had detectable effects on SOD (contaminated fruits) and PPO (contaminated kitchen wastes) activities, and reducing power capacities (contaminated vegetable, fruits and kitchen wastes). While these physiological responses likely limited the risks of reactive oxygen species-induced damages to macromolecules and tissues, it may latter have physiological costs for reproduction of *H. illucens*. It is also possible that larvae are less sensitive to catechol than adults, as these compounds can be used for exoskeletal stabilization and hardening after they are oxidatively conjugated with proteins (Kramer et al., 2001).

When larvae of *H. illucens* were fed with vegetables wastes, the level of oxidants and damages were both increased, as depicted by variations of H_20_2 and protein carbonyl amounts. Plants are known to produce a number of molecules (quinones, flavonoids, alkaloids, etc…) that can act as pro-oxidants, and increase the likelihood of oxidative stress. Although, post-harvested handling of crops contribute to the secretion of mycotoxins by pathogens (Meitha et al., (2020), potentially favoring the

production of ROS. Consistently, PPO activity was significantly increased in the gut tissues of *H. illucens* as compared with controls, providing an additional suggestion of the presence of flavonoids and polyphenols in the media made of vegetable wastes (Meitha et al., 2020), and subsequent eliciting of the antioxidant system. Conversely, fruit wastes tended to decrease the oxidative stress experienced by the larvae, as revealed by the lower H_2O_2 and protein carbonyl amounts. Only Cd-contaminated fruits, as discussed above, resulted in increased amounts of H_2O_2 and protein carbonyls, likely resulting from the ability of fruit wastes to adsorb Cd metal ions (Chen et al., 2018).

In conclusion, our findings provided additional lines of evidence of the strong ability of the black soldier fly to develop on contaminated food wastes. The array of measures we performed provide a comprehensive overview of the effects of different food wastes and pollutants on the antioxidant system from gut tissues of *H. illucens* larvae. We found that heavy metals and catechol had little effects on the physiology of the larvae, as depicted by the low impacts on the antioxidant system of gut tissues. These results revealed the ability of the species to detoxify heavy metals and catechols. Our study confirmed the suggestion that the insect has strong potential for handling polluted wastes and represents a promising model for entomoremediation. Yet, as it remains unknown if the pollutants are sequestrated in the exoskeleton or excreted, it is suggested that the use of black soldier fly grown on contaminated wastes as animal feedstuff should be avoided, unless the fate of the pollutant is known, as already recommended by Lohri et al., (2017) and Purschke et al., (2017)

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Compliance with ethical standards

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Disclosure of Potential Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Credit Author Statement

David RENAULT: Formal analysis; Funding acquisition; Writing - original draft; Writing - review and editing. **Eman A. ABDELFATTAH:** Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Writing - review and editing.

Research data for this article

The raw data of the article are available from the authors upon request.

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Figure 1. Biomarkers of oxidative stress: hydrogen peroxide (H_2O_2) concentration **(A)**, and protein carbonyl amounts **(B)** measured from gut tissues of 5th instars of *Hermetia illucens* fed on different types of wastes (vegetables, fruits, and kitchen), and contaminated with heavy metals (Cd, Fe, or Pb) or catechol. Values are expressed as means and standard deviations.

Mean values marked with different small letters depict significant differences among control and the different waste types (food wastes alone, food wastes with Cd, Fe, Pb, or food wastes with catechol) (Kruskal-Wallis revealed, $p < 0.05$). Mean values marked with different capital letters report significant differences among the different types of food wastes (vegetable, fruit and kitchen wastes) (Kruskal-Wallis revealed, $p < 0.05$).

Figure 2. Total antioxidant capacity measured from gut tissues of 5th instars of *Hermetia illucens* fed on different types of food wastes (vegetable, fruit, or kitchen wastes), and contaminated with various heavy metal and catechol (Cd, Fe, Pb, and catechol). **(A)** Reducing power concentration, **(B)** Antioxidant ability assay (AAA), and **(C)** DPPH antiradical activity assay. Values are expressed as mean and standard deviations.

Mean values marked with different small letters depict significant differences among controls and larvae maintained on different food wastes (food wastes alone, food wastes with Cd, Fe, Pb, or catechol) (Kruskal-Wallis revealed, $p < 0.05$). Mean values marked with different capital letters report significant differences among the different types of food wastes (vegetable, fruit and kitchen wastes) (Kruskal-Wallis revealed, $p < 0.05$).

Figure 3. Non-enzymatic and enzymatically-based antioxidant responses measured from gut tissues of 5th instars of *Hermetia illucens* fed on different types of food wastes (vegetable, fruit, or kitchen wastes) and combined with heavy metals (Cd, Fe, Pb) or catechol. **(A)** Concentration of ascorbic

acid, **(B)** Activity of superoxide dismutase (SOD), and **(C)** Activity of polyphenol oxidase (PPO) (C). Values are expressed as means and standard deviations.

Mean values marked with different small letters report significant differences among control and larvae maintained on different food wastes (food wastes alone, food wastes with Cd, Fe, Pb, or catechol) (Kruskal-Wallis revealed, $p < 0.05$). Mean values marked with different capital letters depict significant differences among the different food wastes (vegetable, fruit and kitchen wastes) (Kruskal-Wallis revealed, $p < 0.05$).

Credit Author Statement

David RENAULT: Formal analysis; Funding acquisition; Writing - original draft; Writing - review and editing. **Eman A. ABDELFATTAH:** Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Writing - review and editing.

Highlights

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- Fruit wastes tended to decrease the oxidative stress of larvae of the black soldier fly
- Vegetables wastes increased the amounts of H_20_2 and protein carbonyls in larvae of BSF
- Catechol, which tended to increase the oxidation of proteins and hydrogen peroxide
- Cadmium in mixture with kitchen waste slightly increased oxidative stress of BSF
- Heavy metals and catechol had little, yet visible, effects on larvae of fly

Table 1. Pearson's correlation coefficient among DPPH activity from gut homogenates of 5th instars of *Hermetia illucens* and the different types of wastes (vegetables, fruits, and kitchen) alone or in combination with heavy metals (Cadmium: Cd; Iron: Fe; Lead: Pb) or catechol.

Treatment	Type of wastes	r	Equation	Type of equation	R^2
Control	**Vegetables**	0.944**	y= 30.6 ln(x) + 139.8	Logarithmic	0.96

	Fruits	0.584*	y= 26.7 ln(x) +126.2	Logarithmic	0.39
	Kitchen	0.943**	y=33.92 ln(x) + 144.2	Logarithmic	0.97
Wastes alone	**Vegetables**	0.926**	y=30.3 ln(x) +140.8	Logarithmic	0.97
	Fruits	0.926**	y=31.1 ln(x) + 141.7	Logarithmic	0.97
	Kitchen	0.868**	y=46.1 ln(x) + 162	Logarithmic	0.91
Wastes + cd	**Vegetables**	0.919**	y=30.5 ln(x) + 141.1	Logarithmic	0.96
	Fruits	0.931**	y=22.5 ln(x) + 129.4	Logarithmic	0.92
	Kitchen	0.912**	y=36.1 ln(x) +148.6	Logarithmic	0.96
Wastes + Fe	**Vegetables**	0.924**	y=24.2 ln (x)+ 132.2	Logarithmic	0.97
	Fruits	0.930**	y=22.6 ln(x) + 130.2	Logarithmic	0.97
	Kitchen	0.941**	y=20.3 ln(x) + 126.5	Logarithmic	0.95
Wastes + Pb	**Vegetables**	0.953**	y=39.4 ln(x) + 152.3	Logarithmic	0.97
	Fruits	0.977**	y=23.3 ln(x) + 130.2	Logarithmic	0.91
	Kitchen	0.949**	y=28.4 ln(x) + 137.6	Logarithmic	0.97
Wastes + catechol	**Vegetables**	0.896**	y=30.9 ln (x) + 141.9	Logarithmic	0.95
	Fruits	0.308	y=12.1 ln(x) + 106.9	Logarithmic	0.11
	Kitchen	0.892**	y=28.8 ln(x) + 139.3	Logarithmic	0.93