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REVIEW

Effects of undesired substances and their bioaccumulation on the black soldier fly larvae, *Hermetia illucens* (Diptera: Stratiomyidae)–a literature review

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Abstract Black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is predominantly reared on organic wastes and other unused complementary substrates. However, BSF may have a buildup of undesired substances in their body. The contamination of undesired substance, e.g., heavy metals, mycotoxins, and pesticides, in BSF mainly occurred during the feeding process in the larval stage. Yet, the pattern of accumulated contaminants in the bodies of BSF larvae (BSFL) is varied distinctively depending on the diets as well as the contaminant types and concentrations. Heavy metals, including cadmium, copper, arsenic, and lead, were reported to have accumulated in BSFL. In most cases, the cadmium, arsenic, and lead concentration in BSFL exceeded the recommended standard for heavy metals occurring in feed and food. Following the results concerning the accumulation of the undesired substance in BSFL's body, they did not affect the biological parameters of BSFL, unless the amounts of heavy metals in their diets are highly exceeding their thresholds. Meanwhile, a study on the fate of pesticides and mycotoxins in BSFL indicates that no bioaccumulation was detected for any of the target substances. In addition, dioxins, PCBs, PAHs, and pharmaceuticals did not accumulate in BSFL in the few existing studies. However, future studies are needed to assess the long-term effects of the aforementioned undesired substances

Highlights
- The contamination in BSF occurs during the feeding process in the larval stage.
- Heavy metal types accumulate in BSFL and exceed the recommended concentration.
- Target substances of pesticides and mycotoxins were undetected in BSFL.
- Effects of the accumulation of the undesired substance on BSFL.

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on the demographic traits of BSF and to develop appropriate waste management technology. Since the end products of BSFL that are contaminated pose a threat to both human and animal health, their nutrition and production process must be well managed to create end products with a low contamination level to achieve a closed food cycle of BSF as animal feed.

Keywords Contamination · Heavy metals · Pesticides · Mycotoxins · Waste management · Animal feed

Introduction

Bioaccumulation is the increase of the concentration of a substance or element in an organism during its development through uptake from the environment (Wang, 2016). Undesired substances or contaminants that can be accumulated in an organism include heavy metals, pesticides, and mycotoxins. Bioaccumulation of undesired substances may influence the development, growth, and health of an organism (Proc et al., 2021). Hereby, the safety and quality of a derivative end product are affected through the contamination as well.

Non-essential heavy metals are metallic elements that at low levels of exposure may enhance adverse health effects on animals and humans (Jan et al., 2015) and present no benefit in organisms (Singh et al., 2011). These include cadmium, lead, arsenic, and mercury. Just as non-essential heavy metals, pesticides such as herbicides, fungicides, disinfectants, and insecticides may cause acute or chronic effects on health (Roberts & Reigart, 2013). Mycotoxins, on the other hand, are considered the most risk full food contaminant due to the substantial harm they do to health and food security (Smith et al., 1994).

The amounts of undesired substances allowed in food and feed products are regulated by the competent authorities of each country. One of the biggest barriers to the acceptance of insect farming is the limited knowledge of their safety as food and feed (Imathiu, 2020). The accumulation of contaminants in insects relies on the type and development stage of the insect, the substrate used for rearing, and the production or farming method (EFSA, 2015).

At the moment, in the era of sustainable development of various areas of industrial application of useful properties of insects, the most commonly used and studied insect is black soldier fly (BSF) (Raman et al., 2022; Tomberlin & van Huis, 2020). The BSF, *Hermetia illucens* (L.), is a large American fly from the order Diptera and family Stratiomyidae, which is considered to be a native of North and South America (Rozkošný, 2023, but currently, it is widely distributed throughout the world. The insect is one of the few invertebrate species capable of developing year-round in pure culture in a closed space of artificial conditions, which allows the species to be used for biotechnological purposes (Siddiqui et al., 2022a). It is well recognized that the utilization of insects, notably BSF, plays a crucial role in resolving difficulties related to large amounts of organic waste dispersed in the world. It has gradually been used to treat organic waste as it is considered to be an affordable and environmentally beneficial procedure (Siddiqui et al., 2022b; Singh & Kumari, 2019; Suckling et al., 2021). In recent decades, increased focus has been placed on the critical function that BSF larvae (BSFL) play in organic waste conversion (Lu et al., 2021). The production of BSFL is increasing due to its health and environmental benefits (van Huis et al., 2013). BSFL are organisms that can feed on decaying matter such as organic solid municipal waste, manure feces, and fecal sludge and thereby result in high-quality biomass for feed and food purposes (Jamaludin et al., 2021; Lalander et al., 2013a, b; Wang & Shelomi, 2017).

Regulation (EU) No 2017/893 removed the requirement that "products of animal origin must be sourced from a registered slaughterhouse" for reared insects. Regulation (EU) No 2017/893 brought about one of the most significant modifications in 2017 with regard to

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insects used as animal feed. By amending Regulations (EC) No 999/2001 and (EU) No 142/2011, this legislation made it legal to feed aquaculture animals and seven insects, including BSF. Insect rearing facilities, where the insects are typically also "slaughtered," could not adhere to the requirements specific to slaughterhouses.

Nevertheless, BSFL are entomo-remediators and entomo-extractors because they may accumulate various elements in their body (Proc et al., 2021). Hence, the use of BSFL as feed and food can be more complicated and may have a detrimental effect on the environment.

Pollutants such as heavy metals and mycotoxins are typically present in organic waste, which through consumption may remain in BSFL and thereby enter the feed and food chain creating a risk for both animal and human health. The amount and type of contamination depend on the diet and duration of exposure (Van Der Fels-Klerx et al., 2018). Some heavy metals that potentially accumulate in BSFL include cadmium, copper, arsenic, and lead; of greatest concern for BSFL are cadmium and arsenic (Zhang et al., 2021). Studies on BSFL report no accumulation of pesticides as well as mycotoxins (Purschke et al., 2017). The contamination's effect on the larvae is claimed not to affect their development. Nevertheless, the presence of undesired substances, specifically heavy metals, in BSFL may endanger the market entry of end products. The consumption of contaminated end products could cause adverse health risks for animals and human beings. The production method and the diet of the insects must be controlled to produce insects with a low contamination degree. This literature review is an analysis of the substances that can be found in BSFL and their effects on the larvae and derivate end products. Various potential rearing substrates are discussed to guide the audience towards good production practices. Since BSF can be deliberately used for waste treatment due to its ability to accumulate waste substances, this review also focuses on the issue of bioaccumulation in the context of feed and food.

Organic waste treatment through black soldier fly larvae

BSFL may offer solutions in waste management. This insect is fit to digest a plethora of organic substrates, non-withholding food waste of heterogeneous composition (both vegetal and meat), sewer sludge, and manure. The substrates employed vary strongly depending on the income setting. This chapter aims to provide an overview of organic waste treatment systems per income setting (according to the World Bank list of economies) and how this relates to substrate types.

Low- and middle-income countries

Solid municipal waste in low- and middle-income countries is represented by their high amounts of organic matter, with concentrations varying between 50 and 80% (Wilson et al., 2015; Joly & Nikiema, 2019; Hoornweg & Bhada-Tata, 2012). Being of organic nature, various biorefining options exist such as composting, biomass gasification, or rendering (Ellacuriaga et al., 2021; Jayathilakan et al., 2012; Takata et al., 2013). However, waste collection and management services are often poorly implemented in low- and middle-income settings, leading to uncontrolled disposal and burning (Lohri et al., 2017; Wilson et al., 2015). For instance, only 25% of 1600 tonnes of household waste in 2013 was collected in Sidoarjo (Java), only 10–15% of India's agro-processing waste is utilized, and 55% of Costa Rica's goes unrecycled (Diener et al., 2009a; Joly & Nikiema, 2019; Rindhe et al., 2019). Uncollected organic wastes and their leachates contaminate surface and groundwater, attract pests and disease vectors, emit GHG, and pose an immediate health risk to flora/fauna/population. These problems mount as low- and middle-income settings trend towards increased urbanization, population, and consumption (Voegeli & Zurbrügg, 2008). Appending to the solid municipal waste issues, many low- and middle-income settings often deal with poor sanitation or lack thereof (Morella et al., 2008). Open defecation and improperly discarded fecal sludge contaminate surface and groundwater leading. Vis-à-vis, Wolf et al. (2014) associated sewer implementations with great reductions in diarrhea cases. Many studies were performed where BSFL were fed with sludge. Lalander et al. (2013a, b) managed human fecal sludge with BSFL. The result showed that BSFL played a significant role in the reduction of *Salmonella* spp. in feces. Schmitt et al. (2019) also assessed the growth and safety of BSFL that were fed on solid aquaculture waste.

BSF is a saprophagous organism, meaning that it feeds on decaying matter. BSFL readily digests a plethora of organic substrates (Diener et al., 2009b). Organic solid municipal waste, manure, feces, and fecal sludge can be reduced and decontaminated via BSF digestion (El-Dakar et al., 2021; Lalander et al., 2019; Sarpong et al., 2019). Furthermore, BSF is not regarded as a disease vector and drives off pests, e.g., common houseflies (Banks et al., 2014; Lalander et al., 2019; Diener, 2010; Furman et al., 1959). The resulting larval biomass offers input for high-quality end products, such as feed, food, biodiesel, and soil amendments (Klammsteiner et al., 2020; Li et al., 2011a; Wang & Shelomi, 2017). In Taiwan and Vietnam, numerous studies on biodiesel production utilizing BSFL have been carried out (Kim et al., 2021). In Vietnam, BSFL was transesterified using solvents, such as methanol, to generate biodiesel (Nguyen et al., 2018). An enzyme-based method was studied in Taiwan to produce biodiesel from BSFL, considering that conventional techniques require a substantial amount of extractants and a prolonged extraction period (Su et al., 2019). Additionally, Malaysia also investigated the production of biodiesel using BSFL to alleviate food shortages, since fatty acid methyl ester content in BSFL is similar to fatty acid methyl ester content obtained from local plants (Wong et al., 2018).

The successful rearing of BSF on various organic waste substrates such as food waste, fruits and vegetables, poultry feed, solid aquaculture wastes, and fecal sludge (Schmitt et al., 2019; Kim et al., 2021) in low- and middle-income settings has repeatedly been proven (Nana et al., 2019; da Silva & Hesselberg, 2020). However, caution should be exercised towards sources of heavy metals, particularly cadmium and lead, as these are typically accumulated in the larval tissue and hinder development (Diener et al., 2011, 2015a, b; van der Fels-Klerx et al., 2016). Lopes et al. (2011) inventoried heavy metal concentrations in fecal organic wastes. They found that sludge contained the highest concentrations out of all fecal categories included. Sludge contained almost 20 times higher cadmium and lead concentrations than manure (Lopes et al., 2011).

Aside from the biological aspect, authors often signal the need for research into upscale strategies (Nana et al., 2019; Singh & Kumari, 2019). Particularly, the biological aspects of large-scale egg production have not been extensively studied (Čičková et al., 2015; Singh & Kumari, 2019). Furthermore, small-scale applications often employ low-tech solutions, requiring a lot of manual work. To allow for large-scale processing, automation methods need to be studied to manage labor costs (Chia et al., 2019; Nana et al., 2019; da Silva & Hesselberg, 2020).

The economic feasibility of large-scale BSF waste processing has repeatedly been stated (Joly & Nikiema, 2019; Zurbrügg et al., 2018). However, as reported by Čičková et al. (2015), these assessments are often based on extrapolations, assumptions, and generalizations that hamper the power of these conclusions. Indeed Joly (2018) reported the use of such simplifications. It is to be expected that operational costs will be lower through the workings of the economy of scale principle. However, the case regarding high- and upper-middle-income countries could be different.

High- and upper-middle-income countries

Eurostat estimates that municipal waste treatments steadily decrease the utilization of landfills. However, usage of incineration doubled between 1995 and 2019 (Eurostat, 2021). Europe, unfortunately, includes large amounts (76–102 Mt in 2008) of food and gardening waste in the mixed municipal solid waste, logically leading to increased incineration and landfilling (European Commission, 2008). This is an underutilization of these organic biodegradable wastes that could be composted or digested to useable constituents. Undermining its previous waste-to-energy strategy, one incentive of the European Commission's new Circular Economy Action Plan proposes taxing landfill and waste incineration practices (European Commission, 2020). Moreover, the recent enforcement of carbon exchanges (e.g., the European Climate Exchange) provides an additional economic incentive for stakeholders (such as waste processors) to save carbon credits (Circular Organics, 2021). Nevertheless, landfilling and incineration practices are still conventional. Suitable innovative replacements should be found.

BSF proposes a solution to conventional waste management practices. For instance, waste processing via BSF emits less GHG than composting (Mertenat et al., 2019; Pang et al., 2020). Mertenat et al. (2019) concluded 47-fold lower GHG emissions, comparatively. Both reduced substrate and BSF biomass offer valuable products in the form of feeds, biofuel, and soil amendments. According to Diener et al. (2015a, b), various developed regions are erecting large-scale facilities that utilize BSF for the digestion of upwards of 100 tonnes of waste per day. As has been stated in the case of low- and middle-income settings, little is publicly known on the subject of upscaling. This is also true for BSF processing in developed countries, but because of another reason. Naturally, this information is not disseminated as these are proprietary systems that need to maintain a competitive edge. However, the legislation does define some constraints on where these facilities have to work within.

The European Commission has established Regulation (EC) No. 999/2001 guidelines for the control, prevention, and elimination of certain transmissible spongiform encephalopathies. Usage of animal protein as feed for production animals has henceforth been banned through this regulation. Over the subsequent years, particular feeds in combination with production animals have been permitted, for instance, insect protein for aquaculture animals (2017/893). Legal applications of BSF have very recently broadened even further. As of August 2021, it is permitted to feed BSF to poultry and pigs. This surely creates new market opportunities for insect rearers. It should be noted that with the advent of Commission Regulation 2021/1372, various other animal feeds are also permitted as poultry and pig materials. These other animal meals could be very competitive, and these often have lower prices than insects. Commission Regulation 2021/1372 allows for the feeding of animal derivatives to poultry and porcine. Yet, it is not allowed to feed insects such materials. Since insects are regarded as "farmed animals," meaning that they are also subject to the appropriate use of animal by-products (1069/2009). Therefore, it is not allowed to feed animal by-products to insects, which hampers the utilization of substrates such as manure and organic municipal wastes.

Australia, Canada, China, Mexico, South Africa, and the USA have also begun to allow the use of BSFL for feed production (Gold et al., 2018a; Lähteenmäki-Uutela et al., 2017). However, currently, BSFL cannot be grown on non-feed substrates in the listed countries. For example, BSFL is reared on chicken manure, and then, feeding the larvae to chickens, fish, or humans is currently prohibited in the USA (Cummins et al., 2017). Existing legal restrictions on the use of BSFL waste as livestock feed in the EU and the USA mean that BSFL is rather to process nutrients from organic waste into another form, e.g., compost or biodiesel; thus, they cannot currently be legally used to create closed nutrient cycles. It is reported that BSFL feeds on a huge number of types of organic materials and is already being used for small-scale waste disposal using substrates, for example, distillers grain (Webster et al., 2016), food waste (Popa & Green, 2012), rice straw (Zheng et al., 2012), fecal sediment (Lalander et al., 2013a, b), manure (Yu et al., 2009), animal by-products, and kitchen waste (Nguyen et al., 2015). BSFL accumulates lipids from organic waste, which are converted into biodiesel (Wang et al., 2017). Henceforth, many authors have concluded that BSFL can make a significant contribution to the sustainable development of aquaculture as a partial or complete replacement due to their ability to convert organic waste into larva biomass containing a high protein efficiently (Magalhães et al., 2017).

Research on the treatment of organic waste by BSFL and the effectiveness of their bioconversion has become a trend in Asian countries. Particularly in China, the demand for food of animal origin increases as the population increases, which leads to an increase in livestock breeding. Liu et al. (2019b) studied the rate of conversion of organic waste and volatile fatty acids, as well as the fecal characteristics of the soil when composting various livestock feces using BSFL. Pet excrement was fed to BSFL and then composted over 9 days. BSFL's composting resulted in a 22% reduction in organic matter and 14% reduction in nitrogen, as well as an 80% increase in volatile fatty acid accumulation. Accordingly, BSFL's composting is an effective method in upper-middle-income countries, for example, China, because it increases the maturity of feces and the quality of the products obtained as a result of composting (Kim et al., 2021). There were attempts to use BSFL as well as microorganisms in China, to obtain biodiesel from solid waste from restaurants and rice straws (Zheng et al., 2012). The authors found that 2000 BSFL fed on rice straw mixed with restaurant solid waste can produce 43.8 g of biodiesel. The resulting biodiesel fuel complied with the EN 14214 standard published by the European Committee for Standardization, which outlines the testing procedures and requirements for fatty acid methyl esters. The production of biodiesel using BSFL has also been proposed by other researchers from China. Li et al. (2011a, b) obtained 36 g, 58 g, and 91 g of biodiesel by extracting crude fat from 1000 BSFL fed for 10 days on the droppings of cattle, pigs, and chickens, respectively. These results propose that BSFL can substitute expensive food biomass in biodiesel production. Furthermore, Korean studies declare that the better decomposition of BSFL organic substances, compared with many other species of flies, is associated with their higher enzymatic activity using amylase, lipase, and protease (Kim et al., 2011; Park & Yoe, 2017).

Effect of undesired substances on growth, development, and survival of black soldier fly larvae

To achieve an economically feasible large-scale breeding program, BSFL is preferably reared on organic waste that cannot be further processed for feed and food production due to safety concerns for livestock and humans (van der Fels-Klerx et al., 2020). Some of the organic wastes used to feed BSFL are kitchen waste, livestock manure, decaying fruits and vegetables, crop waste, and former foodstuffs (e.g., expired use-by-date products) (Liu et al., 2019a; van der Fels-Klerx et al., 2020; Wang & Shelomi, 2017). However, these organic wastes usually are contaminated by various persistent undesired substances due to agrochemical applications,

Table 1 Effect of pesticides, heavy metals, and mycotoxins on the performance of BSFL

Undesired substances	Effects on BSFL	References
Residual pesticides		
Chlorpyrifos	No significant effect	Meijer et al. (2021)
Chlorpyrifos-methyl[a]	No significant effect	Purschke et al. (2017)
Cypermethrin	Reduce the larval body mass and survival only at high concentrations (≥ 0.3 mg/kg)	Meijer et al. (2021)
Imidacloprid	Stimulate the larval growth	Meijer et al. (2021)
Pirimiphos-methyl-	No significant effect	Purschke et al. (2017)
Propoxur	No significant effect	Meijer et al. (2021)
Spinosad	Reduce the larval body mass and survival only at high concentrations (≥ 2.0 mg/kg)	Meijer et al. (2021)
Tebufenozide	No significant effect	Meijer et al. (2021)
Heavy metals		
Arsenic (As)	No significant effect	van der Fels-Klerx et al. (2016)
Cadmium (Cd)	No significant effect, but high concentrations (≥ 50 mg/kg) slightly prolong the larval development	Diener et al. (2015b), van der Fels-Klerx et al. (2016), Wu et al. (2020)
Copper (Cu)	No significant effect	Wu et al. (2020)
Lead (Pb)	No significant effect, but high concentrations (≥ 125 mg/kg) slightly prolong the larval development	Diener et al. (2015b), van der Fels-Klerx et al. (2016)
Zinc (Zn)	No significant effect, but high concentrations (≥ 2000 mg/kg) slightly prolong the larval development	Diener et al. (2015b)
Mycotoxins		
Aflatoxin B1	No significant effect	Bosch et al. (2017), Meijer et al. (2019)
Aflatoxin B2[a]	No significant effect	Purschke et al. (2017)
Aflatoxin G2[a]	No significant effect	Purschke et al. (2017)
Deoxynivalenol	No significant effect	Camenzuli et al. (2018)
Ochratoxin A	No significant effect	Camenzuli et al. (2018)
Zearalenone	No significant effect	Camenzuli et al. (2018)

[a] The substance was applied simultaneously with other substances during the experiment

industrial disposals, and microorganism activities (Bryden, 2012; Houbraken et al., 2016; Onakpa et al., 2018; van der Fels-Klerx et al., 2020). Undesired substances are often found in moderate to high levels in organic wastes, including residues of pesticides, heavy metals, and mycotoxins (Charlton et al., 2015; Houbraken et al., 2016; Purschke et al., 2017; Meijer et al., 2019). Many studies have reported the various effects of ingestion of these undesired substances on the growth, development, and survival of BSFL (Table 1). Interestingly, BSFL seems to be well adapted and able to tolerate the presence of most undesired substances in their diets.

Residual pesticides

Pesticides are widely used in modern agriculture to prevent yield losses caused by phytophagous insect infestations and to maintain the product's quality (Popp et al., 2013; Lee et al., 2019). However, the excessive application of pesticides will lead to higher residues that persist in crops, feed, and food (Bajwa & Sandhu, 2014; Purschke et al., 2017). The effect of various pesticides on the growth and survival of BSFL has been explored by some researchers (Meijer et al., 2021; Purschke et al., 2017). BSFL mass-reared on cornflour mixed with chlorpyrifos, chlorpyrifos-methyl, and pirimiphos-methyl (concentration of each pesticide was 0.4 mg/kg) did not show any reduction in their growth and survival (Purschke et al., 2017).

Recently, a more thorough study has been conducted to evaluate the performance of BSFL maintained on artificial diets spiked individually with various pesticides (i.e., chlorpyrifos, cypermethrin, imidacloprid, propoxur, spinosad, and tebufenozide) (Meijer et al., 2021). The study indicated that feeding on diets spiked with chlorpyrifos, propoxur, and tebufenozide at 0.05 mg/kg did not negatively affect the larval body mass and survival. Similar outcomes were also observed even when the same pesticides were added up to ten times their original concentrations. In contrast, the presence of spinosad at 2.0 mg/kg and cypermethrin at 0.3 mg/kg significantly reduced the larval body mass and survival, but not when their doses were lowered to 0.2 and 0.1 mg/kg, respectively. Interestingly, larvae fed on diets spiked with imidacloprid weighed more than the control, both when the imidacloprid was given at 0.1 mg/kg and 1.0 mg/kg (Meijer et al., 2021).

Therefore, it is evident that BSFL showed variation in susceptibility in response to the type and concentration of pesticides. However, long-term studies on the effect of low or sub-lethal concentrations of pesticides on life-history traits of BSF are urgently needed. Previous studies evidenced the adverse lifetime consequences when insects are continuously exposed to a pesticide at sub-lethal concentrations, such as slower growth and developmental rate, lower survival, and reduced fecundity of their next generations (Müller, 2018). Nonetheless, exposure to a pesticide at sub-lethal concentrations may also induce hormesis in insects. Hormesis is a phenomenon in which sub-lethal concentrations of a pesticide positively alter the metabolism and behavior of insects exposed, leading to better performance and fitness of the insects (Cutler, 2013; Müller, 2018).

Heavy metals

Industrial waste disposal and agricultural intensification contribute to the increase of heavy metals in soil (Yang et al., 2018). Crops grown in polluted soil absorb heavy metals in a large amount and accumulate them in their tissues (Raskin et al., 1994), thus posing hazardous effects to herbivores and organisms at higher trophic levels. Over the years, a large body of knowledge on the effects of heavy metal exposure on the biological parameters of insects has been published (Ali et al., 2019a; Luo et al., 2020). In caterpillars (lepidopteran larvae), the addition of heavy metals such as cadmium (Cd), lead (Pb), and zinc (Zn) into their diets significantly reduce their growth and survival, as well as inhibits their development (Ali et al., 2019b).

The effects of heavy metals exposure through diets on BSFL have also been evaluated by several studies (Diener et al., 2015b; van der Fels-Klerx et al., 2016; Purschke et al., 2017; Cai et al., 2018; Wu et al., 2020). Unlike caterpillars, BSFL showed a high tolerance to exposure to heavy metals. Incorporation of arsenic (As) (1 to 4 mg/kg), Cd (0.5 to 1 mg/kg), and Pb (2.5 to 10 mg/kg) in diets did not affect the body mass and survival of BSFL (van der Fels-Klerx et al., 2016). Another study also reported no significant difference in body mass between BSFL reared on Cd-contaminated diets at 10 to 80 mg/kg and the control. Similarly, the body mass was not affected when the BSFL were reared on copper (Cu)-contaminated diets at 200 to 800 mg/kg. Moreover, feeding on the Cd and Cu-contaminated diets did not reduce their survival (Wu et al., 2020). However, high doses of Cd (50 mg/kg), Pb (125 mg/kg), and Zn (2,000 mg/kg) seemed to prolong the development of BSF (eclosion from egg to prepupa); the BSF development ranges from 19.3 to 20.7 days in heavy metal-treated groups compared to 18.4 days in the control (Diener et al., 2015b).

Therefore, it could be hypothesized that heavy metal contaminations do not significantly affect the biological parameters of BSFL unless the amounts of heavy metals in their diets are highly exceeding their thresholds. Nevertheless, researchers have postulated that high concentrations of boron (B), mercury (Hg), nickel (Ni), and Pb may inhibit the growth of BSFL, while Cd, chromium (Cr), Cu, Hg, and Zn potentially reduce their survival rate.

Mycotoxins

The secondary metabolites produced by various species of fungi that possess toxicity to animals and humans are defined as mycotoxins (Wild & Gong, 2010; Bryden, 2012). *Aspergillus* spp. and *Fusarium* spp. are found to be the ubiquitous fungal species in pre- and post-harvest products and are responsible for causing most of the mycotoxin contaminations of the products (Udomkum et al., 2017; Leni et al., 2019). The most frequent mycotoxins found in high levels (very toxic) are aflatoxin B1 and ochratoxin A produced by *Aspergillus* spp. and deoxynivalenol and zearalenone produced by *Fusarium* spp. (Bosch et al., 2017; Camenzuli et al., 2018; Leni et al., 2019; Meijer et al., 2019). Exposure and ingestion of these mycotoxins are detrimental for some insect species (de Zutter et al., 2016; Llewellyn & Chinnici, 1978; Niu et al., 2009). For instance, *Sitobion avenae* (Hemiptera: Aphididae) and *Helicoverpa zea* (Lepidoptera: Noctuidae) suffered slower development and increased mortality when they were exposed to mycotoxins (de Zutter et al., 2016; Niu et al., 2009). Nevertheless, other insects such as *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), *Drosophila melanogaster* (Diptera: Drosophilidae), and BSF have been known to develop strategies to compensate for the presence of mycotoxins in their diets by metabolizing the ingested mycotoxins into harmless compounds (Berenbaum et al., 2021; Camenzuli et al., 2018).

Previous studies have reported that BSFL has a high mycotoxin tolerance (Bosch et al., 2017; Camenzuli et al., 2018; Meijer et al., 2019; Purschke et al., 2017). According to Bosch et al. (2017) and Meijer et al. (2019), the diets spiked with aflatoxin B1 at 0.01 to 0.5 mg/kg did not affect the body mass and survival of BSFL. Similarly, simultaneous ingestion of aflatoxin B2 and aflatoxin G2 did not alter the growth and survival rates of BSFL (Purschke et al., 2017). In addition, individual incorporation of deoxynivalenol, zearalenone, and ochratoxin A, into diets at a concentration of 0.1, 0.5, and 5 mg/kg, respectively, did not affect the growth, survival, and development of BSFL (Camenzuli et al., 2018).

Monitoring the concentration of contaminants in BSF

Bruno et al. (2021) monitor the bacterial concentration in BSFL injected with different concentration of *Escherichia coli*/*Micrococcus luteus* mix (from 104 to 109 CFU/ml) for up to 72 h. The study finds that the bacterial dosage provided had an inverse relationship on the survival of the larvae. Particularly, larger bacterial dosages (10^5, 10^6, 10^7, and 10^8 CFU/ml) gradually decreased the number of living larvae over time (72%, 64%, 60%, and 16%, respectively, after 72 h), whereas 100% of the larvae injected with 10^4 CFU/ml bacteria remained alive 72 h after the infection. At the maximum bacterial concentration (10^9 CFU/ml), all the insects perished in less than 24 h. In light of these findings, larvae were infected in all subsequent experiments by injecting 10^5 CFU/ml of the bacterial mix because this was the lowest concentration that could reduce the welfare of the larvae without producing a significant amount of mortality, allowing us to track the immune response over time.

Protective mechanisms of BSF against undesirable compounds

The innate immune system of BSF is well developed, and it is separated into cellular defense responses and humoral defensive responses (Muller et al., 2021). The primary cellular and humoral processes, as well as their activation mechanisms, are substantially conserved (Bruno et al., 2021). In this insect, the cellular

Table 2 Comparison of the contaminated substrate, pesticide concentration on larvae, and maximum residue level (MRL) for contaminants

Substances	Concentration in feed (mg/kg)	Concentration in larvae (mg/kg)	MRL[a] (mg/kg)	References
Azoxystrobin	0.50	1.50 ± 0.10	ND	Lalander et al. (2016)
Carbamazepine	0.16	0.30 ± 0.08	ND	Lalander et al. (2016)
Chlorpyrifos	0.50	0.01 ± 0.00	0.05	Meijer et al. (2021)
Chlorpyrifos- methyl	0.40	< LOQ 0.00	ND	Purschke et al. (2017)
Cypermethrin	0.10	0.08 ± 0.03	0.03	Meijer et al. (2021)
Imidacloprid	1.00	0.01 ± 0.00	0.10	Meijer et al. (2021)
Pirimiphos-methyl	0.40	0.00 ± 0.00	ND	Purschke et al. (2017)
Propiconazole	0.35	> 1.70	ND	Lalander et al. (2016)
Propoxur	0.50	< LOQ 0.00	0.05[b]	Meijer et al. (2021)
Roxithromycin	0.50	0.90 ± 0.09	ND	Lalander et al. (2016)
Spinosad	0.20	0.01 ± 0.00	2.0	Meijer et al. (2021)
Tebufenozide	0.50	0.01 ± 0.00	0.05[b]	Meijer et al. (2021)
Trimethoprim	0.29	0.80 ± 0.10	ND	Lalander et al. (2016)

ND not determined

[a] According to Directive 2002/32/EC and Regulation 396/2005/EC

[b] *LOQ* lower limit of analytical determination

and humoral responses have distinct kinetics: following the immunological assault, phagocytosis and encapsulation are quickly initiated, whereas humoral components act later. Antibiotic-active compounds are necessary for survival in decomposition settings because they provide a strong barrier against undesirable compounds (Bruno et al., 2021). Antimicrobial peptides (AMPs) are naturally occurring antibiotics that may either kill or stop the growth of a variety of microorganisms, particularly those that target the cell envelope (Alencar-Silva et al., 2018). The BSFL's capacity for high expression of AMPs and other substances with activity against hazardous chemical resistant can also be taken into consideration when looking at the possible bioactive substances that can be recovered from the larvae (Almeida et al., 2020).

Analysis of the bioaccumulation of undesired substances in black soldier fly larvae

During waste processing treatment, different types of organic waste can be used for feeding BSFL from industries, markets, restaurants, domestic food waste, animal droppings, and human feces. Yet, the persistent pollutants, e.g., heavy metals, commonly contaminating the organic waste may accumulate in the immature stages of BSF (i.e., larvae and prepupae) and then enter the food chain. Other contaminants found on the BSF body during the rearing process using organic waste are pesticides and mycotoxins. The final product of the organic waste treatment process using BSF is the use of its protein contained in larvae as animal feed. Therefore, the contaminations should be studied and analyzed to whether it applies to the limit on recommended standards (Table 2).

Residual pesticides

Researchers point out that either wild harvested or reared insects have a similar probability of chemical contamination through the feeding process (Marone, 2016). In their study, Purschke et al. (2017) reported that bioaccumulation of pesticides in BSFL (chlorpyrifos, chlorpyrifos-methyl, and pirimiphos-methyl) could not be detected. Though the concentration of pesticide was still detected below its initial, Meijer et al. (2021) investigated six active ingredients of pesticides, i.e., chlorpyrifos, propoxur, cypermethrin, imidacloprid, spinosad, and tebufenozide. These active compounds were present in the larvae in concentrations that were either very low or below the lower limit of analytical quantification (LOQ) (Table 3) compared to the concentration in the substrate, thus implying that bio-accumulation did not occur. The level of quantification measurement is determined under the European Commission Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed and Regulation (EC) No. 396/2005 on maximum residue levels (MRLs) of pesticides in or on food and feed of plant and animal origin. MRL refers to the highest legal concentration of a pesticide residue in or on food or feed that is determined under this regulation relating to the good agricultural practice and the lowest amount of consumer exposure required to protect susceptible consumers.

Heavy metal contamination

The heavy metal from contaminated waste enters BSFL bodies through the feeding process. It enters the waste stream in several ways, through environmental background emissions from surrounding land, water, and air or improper treatment and management of heavy metal waste (Diener et al., 2015b). Fly larvae are similar to other insects that gain nutrients for their metabolic requirements from the feeding process (Cohen, 2005). While contaminants are absorbed by terrestrial organisms through a feeding mechanism (biomagnification), aquatic organisms accumulate pollutants through diffusion (bioconcentration). Both bioconcentration and biomagnification are referred to as bioaccumulation. Bioaccumulation factor (BAF) determined by measuring the exposure of pollutant in organism from pollutant in feed (Diener et al., 2015b).

A study by Proc et al. (2020) showed that heavy metal contamination occurred in all development stages of BSF during the waste treatment process. During waste treatment process using BSFL, the larvae were fed using substrate. One of the common feeding techniques to maintain the production of larvae was using chicken feed as substrate and complement material of organic waste diet. Bioaccumulation of heavy metals, such as (Cd), (Pb), and (Zn) spiked in BSFL fed with chicken feed, revealed that cadmium accumulation in larvae and prepupae was higher than the initial concentration in the substrate, while lead and zinc accumulation was not detected (Diener et al., 2015b). Moreover, Wu et al. (2020) also reported that the concentrations of (Cd) and (Cu) accumulated in the body of BSFL fed with animal manures were dramatically increased following the exposure doses. The study by Purschke et al. (2017) showed that (As) was found in the larvae and the residual substrate in the same quantity as the initial substrate. Meanwhile, the accumulation of (Hg) in the larvae was not detected. The results varied in several studies where the contaminant was given in certain doses to analyze the impact of heavy metals on the body of BSFL (Table 3). Concentration of the heavy metals accumulated in BSFL was different determined by the diets, heavy metal types, and exposure concentrations (Wu et al., 2020).

Table 3 Comparison of heavy metal-contaminated substrate, concentrations on larvae, and recommended standards for contaminants

Heavy metals	Initial concentration (mg/kg)	Concentration in larvae (mg/kg)	Recommended standards (mg/kg)	References
Arsenic (As)	3.00[b]	2.80 ± 0.40[b]	2.0[c]	Purschke et al. (2017)
Cadmium (Cd)	0.02[a]	7.00[a]	2.00[c]	Diener et al. (2015b)
Copper (Cu)	100.00	165.68	200.00[d]	Wu et al. (2020)
Lead (Pb)	0.05[a]	3.80[a]	10.00[c]	Diener et al. (2015b)
Mercury (Hg)	0.20[b]	0.10 ± 0.03[b]	0.10[c]	Purschke et al. (2017)
Zinc (Zn)	10.00[a]	NA	ND	Diener et al. (2015b)

NA not applicable, *ND* not determined

[a] Moisture adjusted to 60%

[b] Moisture adjusted to 45%

[c] European Commission Directive 2002/32/EC (European Parliament and of the on undesirable substances in animal feed)

[d] GB26419-2010 (Chinese standard on limited content of Cu in feeds)

Mycotoxins

Besides heavy metals and pesticides, mycotoxins are the most contaminant found on BSF rearing with organic materials. Constant mycotoxin contamination poses acute poisonings or induces carcinogenic, teratogenic, or mutagenic effects. Purschke et al. (2017) also reported that various mycotoxins (aflatoxin B1/B2/G2, deoxynivalenol, ochratoxin A, and zearalenone) pose a dangerous threat to particular monogastric (e.g., pig, poultry). The analysis result showed that mycotoxins were observed in extremely small amounts in both larvae and residual substances. Moreover, mycotoxins accumulation was not detected in the BSFL. Microbial activities in organic waste and larva gut enzymes perform decomposition of pesticides and mycotoxins. The reduction of these contaminants is related to the composting process. However, further study on this topic is required as these contaminants contain diverse compounds, and each has its unique properties affecting the decomposition process (Gold et al., 2018a). In addition, Camenzuli et al. (2018) reported that the impact of contaminated substrates by deoxynivalenol (DON), known as *Fusarium* toxins, zearalenone (ZEN), aflatoxin B_1, and ochratoxin A (OTA) in BSF that were identified to be below the maximum limit and accumulation, did not occur.

Other undesirable contaminants

In addition to heavy metals, Charlton et al. (2015) reported that the level contamination of 1140 different chemical contaminants, including pesticides and mycotoxins, was analyzed in BSF larvae, which are used as a source of protein for animal feed. The contaminants including veterinary medicines, dioxins, polyaromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) were found in BSFL reared on agro-industrial waste. The study showed that all the samples of PCB ICES6 values (0.05 to 4.28 μg/kg) were below the limit (see Table 4) allowed for animal feed by European Commission. Furthermore, the calculation of WHO-TEF in all samples recorded was lower than the limit (see Table 4) with values of 0.14 to 0.44 ng/kg. The values of PAH4 were reported between 0.28 and 9.28 μg/kg. However, there was no limit in EU regulations specified for animal feed. Additionally, previous studies revealed that mycotoxins, PCBs, PAHs, a few pesticides, and pharmaceuticals did not accumulate in BSFL (Bosch et al., 2017; Charlton et al., 2015; Lalander et al., 2016; Purschke et al., 2017).

Typical waste streams with potential risk for black soldier fly larvae

Due to its high feed plasticity, BSFL can be reared on many types of substrates, including a variety of industrial by-products, as well as municipal waste and manure, for example. Nonetheless, their safety regarding aspects such as pesticides, toxins, heavy metals, and microbiological contamination must be taken into consideration. The screening for the safety of these substrates is of greater importance due to different compositions depending on location and possible contaminants during the growth and/or processing of the materials.

High moisture waste streams can bring risks due to the easy microbiological spoilage, which can increase the number of pathological bacteria and toxins in the substrate. By-products might get contaminated during storage by pathological fungi, for example, *Aspergillus* sp., *Fusarium* sp., *Monascus* sp., and *Penicillium* sp., which generates some mycotoxins (Banu & Muthumary, 2005; Bhat & Reddy, 2017; Garon et al., 2006). Moreover, the microbial activities and temperatures can make other risk contaminants (e.g., pesticides) transform, contaminating the by-products with other chemical substances. According to a study

Table 4 Recommended standards for selected chemical contaminants

Dioxins, polychlorinated biphenyls (PCB), and polyaromatic hydrocarbons (PAH)		
Chemical contaminants	**Maximum level**	**References**
PCB ICES-6[a]	10 μg/kg	EC Directive 2002/32/EC amendment 277/2012/EC
WHO-TEF[b]	0.75 ng/kg	EC Directive 2002/32/EC

[a] CES-6 is a set of 6 PCBs, namely PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180

by Ekielski et al. (2018) with spent grains, a suitable by-product for BSFL rearing, they found that mepiquat and chlormequat used as pesticides can be exposed when roasting grains during the brewing process. Therefore, these components might be present in trace amounts in this substrate.

Regarding pesticides, some processing steps in the food industry can either help reduce or concentrate them in the food. Dehydration steps, for instance, can concentrate them (Li et al., 2011b). Meanwhile, some studies reveal that certain steps, e.g., boiling, juicing, and washing, can lower the levels of pesticide residues (Li et al., 2011b; Randhawa et al., 2007), indicating the necessity of observing the individual processing steps of all components derived from industrial by-products used in the BSFL diet.

Moreover, the heavy metal content in the soil is a key aspect of the safety assurance of the by-products, since the use of polluted urban streams water for irrigation contributes significantly to its concentration in the soil, which then can put these substances in the food chain, including the biological side streams. This is most important, in developing countries, which can benefit greatly from the use of insects such as the BSFL for waste management, where urban growth and expansion are usually accompanied by poor waste disposal and water contamination (Tomno et al., 2020). Consequently, understanding the risk of undesired substances bioaccumulating from various types of waste and their potential transfer to end products is crucial for the future sustainable development of industrial technologies for processing organic waste using BSFL.

Assessment of the risks of bioaccumulation of undesired substances in black soldier fly larvae to end products

The popularity of insects as livestock alternatives in the food and feed industry is increasing (van Huis et al., 2013). As insects for feed and food are farmed animals, their feed substrates must fulfill the requirements of legal frameworks such as those set by the European Union (European Commission, 2017). Levels of heavy metals, mycotoxins, or pesticide residues may not overpass the maximum amounts stated. Surpassing these amounts concerns public health and causes economic deficits. The health of animals and humans and the quality of the end product are directly influenced by undesired substances (Reddy & Reddy, 2015). Hence, animal feed and end products can generate physical, chemical, or biological risks to food safety (FAO & WHO, 2019). The ingestion of food with the maximum limit of residues does not impair health even if several residues are eaten simultaneously (BfR, 2013).

End products containing undesired substances face the prohibition of their entrance into the market due to health concerns (Schrögel & Wätjen, 2019). This represents an economic loss for the producer as well as a negative impact on the environment. Therefore, the contaminant levels must follow good practices. Safety policies and regulations regarding edible insects ensure a risk-free supply for the consumer; therefore, governments should prioritize the safety of this product category (Imathiu, 2020).

Pesticides have the potential to be hazardous to people and can have both short-term (acute) and long-term (chronic) negative health impacts (WHO, 2018). The extent and type of effect depend for example on the function of the pesticide. Similar to pesticides, heavy metals found in food affect human health, directly and indirectly. Their toxicological effects depend on their chemical form, the exposure route and amount, the duration of the exposition, age, gender, and the biological species (Caussy et al., 2003; Khan et al., 2015; Mahalakshmi, 2012; Tchounwou et al., 2004). Populations that ingest foods contaminated with metals are deficient not only in micronutrients but also in macronutrients, especially in fats, proteins, and minerals such as calcium and iron (Iyengar & Nair, 2000).

Heavy metals can react with carbohydrates, proteins, fats, and vitamins to different extents (Khan et al., 2015). The lack of vitamins may cause further physiological and pathological disorders. Overall, food containing heavy metals, for example, Hg, As, Cd, and Pb, are known to cause acute and chronic health effects in animals and humans (D'Souza & Peretiatko, 2002; Khan et al., 2009) such as cancer, nutritional deficiency, kidney failure, renal diseases, nervous system failure, and oxidative stress of cells (Wilk et al., 2016; Ali et al., 2019a) and engage genetically by binding with the DNA (Hossain & Huq, 2002). Heavy metals are non-biodegradable and possess a long half-life; therefore, they persist in the body of organisms (Ikeda et al., 2000) and the environment (Nabulo et al., 2006).

Cd presence in BSF product is of great concern to the feed industries. It has been often observed that BSF may exceed the cadmium MRL proposed by the EU. One study claimed as well that Pb can build up in BSF at levels greater than those found in the substrate.

Gao et al. (2017) and another claimed that BSF has the potential to accumulate As (Van Der Fels-Klerx et al., 2018). For the prevention of chemical risk in the BSF end product, environmental control and feed substrates of the insects need to be assessed (Tang et al., 2009). Other methods of prevention are watching out for contaminants during the processing steps between farming and consumption.

On the other hand, mycotoxins, the most impactful contaminants concerning public health and food security, are undesired substances produced by pathogenic and food spoilage molds (Smith et al., 1994). They seem to be of greater concern in developing countries especially in Africa and Asia, due to the wild harvesting of animals (Defoliart, 1995). Ensuring hygiene and applying regulations on farming and food production reduces the risk of the development of such organisms in food. However, many organisms can metabolize mycotoxins and therefore do not present a risk of carry-over to end products.

According to Van Der Fels-Klerx et al. (2018), there are no indications of insects as feed and food as toxic or reactive substances for consumption in Europe. The risk of the consumption of BSF is low as BSF cannot bioaccumulate pesticides (Lalander et al., 2016; Purschke et al., 2017; Wang & Shelomi, 2017), and no presence of mycotoxins and fungicides has been proven. No insects have been proven to accumulate mycotoxins (Schrögel & Wätjen, 2019). However, other insects apart from BSF can accumulate pesticides (Meijer et al., 2021). Leni et al. (2019) state that BSF can metabolize mycotoxins; however, it is not yet certain how.

Conclusions

The contamination of undesired substances such as heavy metals, mycotoxins, and pesticides in BSF mainly occurs in the larval stage. The contamination process mainly occurred during the feeding process on BSFL with organic wastes and other complementary substrates. The pattern of accumulated contaminants in the bodies of BSFL is varied distinctively depending on the diets as well as the contaminant types and concentrations. Heavy metals (Cd, Cu, As, and Pb) are reported to have accumulated in BSFL. Cd, As, and Pb concentration exceeded the recommendation standard (EC Directive 2002/32/EC). Yet, following the previous results of the accumulation of the undesired substance in BSFL's body, their effects on BSFL growth and development appear to be not significant. Meanwhile, a study on the fate of pesticides and mycotoxins in BSF larvae indicates no bioaccumulation was detected for any of the target substances. In addition, dioxins, PCBs, PAHs, and pharmaceuticals did not accumulate in BSFL in the few existing studies. Thus, the analysis of the available information showed a minimal level of risk of bioaccumulation and bioconversion of undesired substances in the BSFL. Consequently, the use of BSFL as an effective means of processing organic waste has a high potential for widespread distribution in all countries of the world that are faced with problems with environmental waste processing methods. However, during industrial implementation, it is necessary to develop quality and safety management systems for waste processing technology, which will take into account the conditions of processing and the type of waste (for example, the content of heavy metals and pesticides in waste and environmental parameters). Also at the moment, there is a need for future studies to assess the long-term effects of undesirable substances discussed in the article on the demographic characteristics of BSF. In case the absence of risks of dangerous factor's manifestation in the long term is confirmed, there will be a basis for revising legislation in the field of BSFL rearing and unlimited production of animal feed and feed additives based on them. Creating closed food cycles in which BSFL are cultivated on animal husbandry biowaste and used in animal feed due to non-toxic bioconversion will contribute to sustainable agriculture.

Author contribution Shahida Anusha Siddiqui: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, visualization, project administration, writing–original draft, writing–review and editing, supervision. Ito Fernando: writing–original draft. Khoirun Nisa': writing–original draft. Mohd Asif Shah: validation, resources. Teguh Rahayu: writing–original draft. Adil Rasool: validation, resources. Owusu Fordjour Aidoo: validation, writing–review

and editing. All authors have read, have understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the instructions for authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

Data availability The data that support the findings of this study are available from the corresponding authors upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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